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Brown beer vinegar: A potentially functional product based on its phenolic profile and antioxidant activity

ELENA MUDURA[1], TEODORA EMILIA COLDEA[1]*, CARMEN SOCACIU[2], FLORICUȚA RANGA[2], CARMEN RODICA POP[2], ANCUȚA MIHAELA ROTAR[2] and ANTONELLA PASQUALONE[3]

[1]Department of Food Engineering, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania, [2]Department of Food Science, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania and [3]Food Science and Technology Unit, Department of Soil, Plant, and Food Science (Di.S.S.P.A.), University of Bari "Aldo Moro", Bari, Italy

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Abstract: The aim of the present study was to create a functional, enriched in polyphenols and free of alcohol product obtained by acetic fermentation of beer. Beer and vinegar were tested first for their phenolic content and antioxidant activity, by the Folin Ciocalteu and the free radical scavenging activity by the 1,1-diphenyl-2-picrylhydrazyl free-radical scavenging assay, respectively. Then, the separation and identification of the 30 phenolic compounds was realized by high-performance liquid chromatography coupled with positive electrospray ionisation and diode array detection (HPLC-DAD–ESI(+)-MS) analysis. Identification of the phenolic compounds data was realized based on the UV spectra of each compound. Based on a calibration curve (R^2 = 0.9985), the amounts of the phenolic compounds, expressed as mg cathechin equivalents (CE)/L, were calculated. The total phenolic content of the beer and vinegar samples determined using Folin–Ciocalteu reagent were of 428.9±1.58 and 661.5±7.69 mg GAE L^{-1}, respectively, which contributed to the high antioxidant activity in the vinegar sample of 82.18 %. Statistically significant differences were observed after acetic fermentation between each parameter ($p < 0.05$). Brown beer vinegar represents a rich source of polyphenols and phenolic derivatives, compared to beer. By its increased phenolic content and antioxidant activity, brown beer vinegar could be considered another source of valuable compounds to beer, which could also be of interest in special diets.

Keywords: brewing; acetic fermentation; polyphenols quantification.

* Corresponding author. E-mail: teodora.coldea@usamvcluj.ro
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INTRODUCTION

Vinegar is one of the fermented beverages used by consumers in their daily diet as a flavouring agent, as a preservative and as a healthy drink. Beer vinegar is made in two steps involving yeast for alcoholic fermentation,[1] followed by acetic acid bacteria for acetic fermentation.[2] As a perspective, food industry manufactures support the capitalizing of bioactive compounds from beer, such as polyphenols, vitamins, minerals, nitrogenous compounds, fibre and carbohydrates.[3] The profile of phenolic compounds found in beer is very complex due to their provenience and their transformations during the production process. Polyphenols are considered the main natural antioxidants in brewing raw materials and beer, of which 70 % come from malt, and 30 % come from hop (*Humulus lupulus* L.).[4] Important classes of phenolic compounds found in beer are hydroxybenzoic acids, cinnamic acids and flavonols.[5] The total phenolic content in beer ranges from 250 to 500 mg GAE L^{-1}, with dark beer containing the highest amount (489 mg GAE L^{-1}).[6] Significant contributions to the *in vitro* antioxidant activity of beer was attributed to (+)-catechin, (–)-epicatechin, and ferulic, syringic, caffeic and protocatechuic acids[7] extracted mostly from malt and in small amounts from hops.[8] Dietary phenolic compounds play a significant role as antioxidants *in vivo*.[9] Beer increases plasma antioxidant capacity in humans, thereby reducing chronic disease risk.[10]

The flavonoids in hops are considered derivatives of 2′,4,4′,6′-tetrahydroxy--3′-prenylated chalcone and the most important are xanthohumol, desmethylxanthohumol, dehydrocycloxanthohumol, and the flavanones isoxanthohumol, 8-prenylnaringenin and 6-prenylnaringenin.[11] These hops prenylflavonoids have a positive effect on the human health due to their antioxidant, anticancer effect, antimicrobial and anti-inflammatory properties. Hops have been considered to be an important source of substances having estrogenic effects. 8-Prenylnaringenin has been shown to be one of the most potent phytoestrogens identified so far.[12]

Beer is the major dietary source of prenylflavonoides. Dark beers present higher levels of prenylflavonoids because dark malts contain Maillard reaction compounds that inhibit the isomerisation of prenylflavonoids compounds with less bioactivity. A good yield of prenylflavonoids from hops to the final beer can be achieved by the usage of dark malt in the brewing process.[12]

Beer as a natural drink, moderately consumed, may become a source of many health-promoting compounds.[4,12,13]

However, the beneficial health effects of moderate beer consumption are strongly related to damages to health that could occur due to the alcohol, such as pancreatitis, diabetes and pancreatic carcinoma.[14]

The brewing industry generates large amounts of by-products and recent research has been directed towards the reuse of spent grains, brewers spent hops and brewers spent yeasts, from nutritional, economic and environmental points of

view.[15] Less attention has been given to waste or unsold beers that could be valorised through acetic fermentation. Beer vinegar is made from finished beers of all types, and the character of the beers carries through to the finished vinegar. Vinegar is an important source of bioactive compounds and could be obtained by traditional (surface processes) or submerged methods, with the former contributing to an enhancement of the level of flavour compounds, increasing both the quality and acceptance of the products, due to the prolonged duration required for the acetic fermentation.[16] Considering the interest in hop-based products related to health benefits, every year new products are launched on the market.

To the best of our knowledge, no paper has focused on an investigation of the phenolic profile of beer vinegar. Therefore, the aim of the present study was to characterize the most important functional properties of this product, namely the phenolic profile and antioxidant activity.

EXPERIMENTAL

Reagents

The reference catechin (min. 98 % purity) was provided by Sigma–Aldrich (Steinheim, Germany), gallic acid (min. 98 % purity) from Carl Roth (Karlsruhe, Germany), DPPH (2,2--diphenyl-1-picrylhydrazyl) from Sigma–Aldrich (Steinheim, Switzerland), methanol (min. 99.8 % purity) from Chempur, Piekary Śląskie, Poland, Na_2CO_3 of ≥ 99.9 % purity from Sigma–Aldrich and Folin–Ciocalteu phenol reagent from Merck.

Beer and vinegar processing

According to usual operative conditions developed in the Brewery Pilot plant of the UASVM Cluj-Napoca, Romania, the following experimental plan was replicated three times. Dark lager beer from 100 % all grain malted barley, *i.e.*, pilsen, caramel and roasted (Weyermann Specialty Malting Company, Bamberg, Germany) was produced on the pilot scale in the microbrewery of the Faculty of Food Science and Technology UASVM Cluj-Napoca, Romania. Hop pellets 90 type (Magnum and Perle cultivars) crop 2016, were supplied from Moragroind hops farm (Targu Mures, Romania). Two types of hops were used as pellets, Magnum cultivar for bitter taste, and Perle cultivar for flavour, which were added at the beginning and at the end of the boiling process, respectively, each in specific amounts (data not shown). A starter culture of fermentation yeast (*Saccharomyces carlsbergensis*) was received from a local brewery and was added at a dose of 0.5 L yeast per 10^2 L wort (1.5×10^7 yeast cell per L wort). Malt was ground in a motorized roller mill MAV3 (Tehnofavorit, Bontida, Romania) and grist was transferred to a saccharification kettle. The saccarification process was monitored by a controlled system (Centra, Cluj-Napoca, Romania) and the mash was transferred to a lauter tun. After separation, the wort was boiled for 90 min. The hopped wort was settled for clarification, refrigerated to 8 °C, pitched with slurry yeast and sent to the primary fermentation tank (Pierre Guerin, France). The primary fermentation lasted 7 days at 10 °C, then the process was set to secondary fermentation, for 14 days at 3 °C. The fermentation tank had 3 independent cooling areas, 3 temperature sensors, 1 pH sensor and tandem gas analyzer for dissolved O_2/CO_2 (Key Instruments, USA). The fermentation was monitored daily (data not shown) with an automatic FermentoStar analyzer type 3572 (Funke–Gerber, Germany) for ethanol and real extract. The original gravity and ethanol content of the finished beer were of 12.8 °P, and 6.5 vol. %, respectively. When the degree of fermentation reached 85 %, the beer

was filtered and transferred to the next stage of fermentation. Acetic fermentation was conducted by Orleans-type surface fermentation, in 1 L vessel capacity, with magnetic stirrer bar daily homogenisation for 10 min, and lasted 60 days at 25 °C, in the presence of naturally occurring *Acetobacter aceti* (at a count of 10^6 CFU mL^{-1}). Final chemical parameters of vinegar were 45 g/L acetic acid and 21.8 g/L dry extract. No thermal treatments were performed for beer or for vinegar. These experiments were performed in triplicate. Prior to storage, the beer was freed of CO_2 in an Erlenmeyer shaking flask. Both beer and vinegar samples were stored in a freezer at –20 °C immediately after completion of the fermentation process (alcoholic and acetic, respectively) until analysis.

Determination of total phenolic content (TPC)

Before each analysis, the samples were filtered. Measurement of the total phenolics content was performed using the Folin–Ciocalteu method,[17] with some modifications. Each 0.025 mL sample of brown lager beer or brown beer vinegar was mixed with 1.8 mL of distilled water and 0.12 mL of Folin–Ciocalteu reagent and immediately vortexed. After 5 min at room temperature, 0.34 mL of an aqueous Na_2CO_3 solution (7.5 %) were added and the mixture stood at room temperature for 2 h. The absorbance was measured at 750 nm on a UV––Vis spectrophotometer (PharmaSpec UV-1700, Shimadzu, Kyoto, Japan). A calibration curve was performed using different concentrations (0.0–1.0 mg mL^{-1}) of standard gallic acid solutions (R^2 = 0.9997). The concentration of TPC is expressed as mg GAE L^{-1} of sample.

Determination of the free radical scavenging activity by the 1,1-diphenyl-2-picrylhydrazyl free-radical scavenging assay

The scavenging activities of the beers and vinegars on the stable free radical DPPH were assayed using the standard method,[17] with some modifications. A beer or vinegar sample (0.01 mL) was transferred into a glass test tube with a screw cap, mixed with 0.09 mL distilled water and 3.9 mL methanolic DPPH solution (0.025 g L^{-1}). After 30 min incubation in the dark, the absorbance of the sample was measured at 515 nm against a methanol blank (A_0) using a double-beam UV–Vis spectrophotometer (PharmaSpec UV-1700, Shimadzu, Kyoto, Japan). Three readings per sample were taken. Positive and negative controls (0.5 mg mL^{-1} gallic acid and methanol, respectively) were treated in the same manner as the samples. The following equation was used to determine the DPPH free radical scavenging activity (*RSA*) expressed in %:

$$RSA = 100\frac{A_0 - A_1}{A_0}$$

where A_1 is the absorbance of DPPH free radicals in the sample.

High-performance liquid chromatography coupled with positive electrospray ionisation and diode array detection (HPLC-DAD–ESI(+)-MS) analysis

The samples were filtered through a 0.45 μm nylon filter and injected (10 μL) into the HPLC system. For the separation of the phenolic compounds, an Agilent 1200 HPLC system equipped with a diode array detector (DAD) coupled with a mass detector (MD) single quadruple Agilent 6110 (Agilent Technologies, CA, USA) was used. The separation of the phenolic compounds was realised on an Eclipse XDB C18 column (150 mm×4.6 mm, 5 μm) (Agilent Technologies, CA, USA). The mobile phases were water: 0.1 % acetic acid in acetonitrile (99:1, solvent A) and 0.1 % acetic acid in acetonitrile (solvent B), at a flow rate of 0.5 mL min^{-1}.[18] All runs were monitored at 280 nm. The following gradient was applied: % B: 0–2 min, 5 %; 2–18 min, 5–40 %; 18–20 min, 40–90 %; 20–24 min, isocratic, 90 %; 24–25

min 90–5 %. For MS fragmentation, the ESI (+) ionization model was used with the following conditions: 3000 V capillary voltage, at 300 °C, an 8 L min^{-1} nitrogen flow and *m/z*: 100–1000 full-scan. Two levels of energy were used to obtain 50 or 100 fragments in the range *m/z*: 100–1000 Da. The phenolic compounds data identification was realised based on the UV spectrum of each compound. Data acquisition and interpretation of the results were performed utilizing ChemStation, Agilent Technologies, CA, USA. Based on calibration curve (R^2 = = 0.9985), the amounts of phenolic compounds, expressed as mg catechin equivalents (CE) L^{-1}, were calculated.

Statistical analysis

All the experiments were performed in triplicate. The data are reported as means ± standard deviation (*SD*). ANOVA analysis of variance was used to compare the mean values, using SPSS 19.0 statistical analysis (IBM, New York, USA) and the Tukey HSD test with a confidence interval of 95 or 99 %. A *p*-value below 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Total phenolic content and antioxidant activity

The total phenolic content of the beer and vinegar samples determined using Folin–Ciocalteu reagent were 428.9±1.58 and 661.5±7.69 mg GAE L^{-1}, respectively, which contributed to the antioxidant activity in the vinegar sample of 82.18 %. Similar values for the phenolic content were registered in other beers.[7,19,20] Both the content of phenolics and the antioxidant activity of the vinegar were in the range reported by other studies where balsamic vinegars were analyzed.[21,22] The brown beer had a 63.03 % antioxidant activity, which is also in accordance with previous studies.[20] After acetic fermentation of the beer, both TPC and antioxidant activity registered significant increases ($p < 0.001$). Previously, the antioxidant activity of beer was attributed especially to xanthohumol, cinnamic, caffeic and ferulic acids and gallocatechin.[15] Even though the Folin–Ciocalteu method is widely applied for quantification of total phenolics in different matrices, vegetable or beverages, some of the beer contents may interfere, such as Maillard reaction compounds or sulphites, For this reason, a complete evaluation of the phenolic profile *via* the identification and quantification of the individual phenolic compounds is recommended.[7]

Individual phenolic compounds

After the wort boiling phase, a large amount of polyphenols is lost in the cold break or later in the cold conditioning, and a little is lost during the alcoholic fermentation process of beer.[23] Due to the oxidative processes during acetic fermentation, the concentration of some of the hop-derived phenolic compounds decreased, mainly prenylflavonoids (Table I). In the present study, the sum of individual phenolic contents revealed an increase of 40 % after the acetic fermentation of brown beer, because of the increasing concentration of solid pro-

ducts due to water evaporation. Statistically significant differences were observed after acetic fermentation between each parameter ($p < 0.05$).

TABLE I. Individual phenolic contents in beer and vinegar expressed as mg CE L^{-1}; the values are expressed as the mean of three replicates. n – number of samples. $p \geq 0.05$, not significant

Compound	R_t / min	$[M–H]^+$	Beer ($n = 3$)	Vinegar ($n = 3$)	p
Protocatechuic acid *O*-glucoside	2.61	317, 155	7.57±0.05	7.42±0.03	<0.05
3-Caffeoylquinic acid	2.89	355, 181,163	26.44±0.13	40.01±1.13	<0.001
(4-Hydroxyphenyl)acetic acid	3.26	153, 136	7.53±0.27	11.84±0.02	<0.001
4-Vinylguaiacol	3.42	151	2.25±0.07	10.22±0.04	<0.001
Catechin 7 *O*-glucoside	3.65	453, 291	9.75±0.06	8.84±0.02	<0.001
4-Hydroxybenzoic acid	3.90	139	9.47±0.02	38.23±0.05	<0.001
(3-Hydroxyphenyl)acetic acid	4.24	153, 136	11.56±0.03	18.95±0.04	<0.001
Catechin 5 *O*-glucoside	4.61	453, 291	23.81±1.73	7.24±0.06	<0.001
Coumaric acid *O*-glucoside	5.19	327, 165	3.43±0.02	4.90±0.05	<0.01
Ferulic acid *O*-glucoside	5.58	357, 195	3.93±0.10	4.33±0.02	<0.001
Gallic acid	5.93	171, 153	2.87±0.16	5.72±0.04	< 0.01
Vanilic acid *O*-glucoside	6.46	331, 169	12.07±0.38	10.25±0.03	< 0.01
Gallocatechin	7.17	307	5.57±0.56	7.66±0.10	<0.001
Sinapic acid *O*-glucoside	8.56	387, 255	3.17±0.15	14.03±0.12	<0.001
Catechin *O*-diglucoside	9.05	615, 453, 291	4.08±0.09	8.41±0.04	<0.001
Kaempferol *O*-glucoside	9.83	449, 297	4.16±0.05	6.28±0.04	<0.001
Feruloylquinic acid	10.42	369, 195	10.81±0.11	6.60±0.15	<0.001
Chlorogenic acid	10.74	355, 181, 163	10.06±0.01	18.30±0.02c	<0.001
(+)-Catechin	11.17	291	4.64±0.23	7.89±0.04	<0.001
(–)-Epicatechin	12.30	291	4.08±0.05	7.78±0.12	<0.001
Caffeic acid	12.70	181, 163	8.76±0.16	10.58±0.08	<0.001
Sinapic acid	13.11	225	11.30±0.51	15.5±0.06	<0.001
Apigenin *O*-glucoside	13.71	433, 271	7.08±0.08	6.15±0.02	< 0.01
Quercetin *O*-glucoside	14.18	465, 303	7.77±0.11	7.05±0.06	< 0.01
Cohumulone I	14.64	349, 237	4.87±0.10	4.44±0.02	<0.001
Cohumulone II	15.73	349, 280	3.62±0.04	6.58±0.10	<0.001
8-Prenylnaringenin	16.74	341, 273	4.55±0.23	2.33±0.02	<0.001
6-Prenylnaringenin	17.81	341, 273	2.43±0.05	1.86±0.02	<0.001
Humulone	19.85	363	1.55±0.10	5.62±0.08	<0.001
Isohumulone	20.92	363	1.67±0.19	4.14±0.03	<0.001
Sum of individual phenolic compounds, mg CE L^{-1}			220.85	309.15	–

Phenolic compounds play their role in beer not only as health benefits, but also they participate in the colloidal and sensory stability of beer.[8] The bitter taste and astringency of beer is strongly related to the level of hydroxybenzoic and hydroxycinnamic acids.[24]

Despite all the reports indicating the overall protective action of polyphenols, gallic acid shows the greatest pro-oxidant potential with negative implications for the physical stability of beer.[25] The amount of gallic acid found in brown

beer (2.87 mg CE L^{-1}) was higher compared to that found in other previously described beers,[19] but similar to ones determined in other studies.[26,27] 4-Hydroxybenzoic acid was also previously reported in beer.[5,24]

Hydroxycinnamic acids are present in all plant matrices. Many health effects are related to a diet rich in these compounds, such as reducing the risk of cardiovascular disease, strong antioxidant activity, anticarcinogen.[28] A previous study reported 0.13–0.38 mg L^{-1} sinapic acid in commercial beer samples.[19] Still, the present results were higher for sinapic acid, 11.30 mg CE L^{-1} in brown beer, and the protective role of melanoidins from the coloured malt used could be an explanation. Sinapic acid became a compound of interest to researchers for its antioxidant, antimicrobial, anti-inflammatory, anticancer, anti-anxiety activities, being considered as superior to ferulic or caffeic acids.[29] It comes from malted barley, being formed during malting process, and its value is in the range 0.6–2.0 mg kg^{-1} dry weight.[30] The concentration of sinapic acid can increase during beer maturing, while it is sensitive to thermal treatment.[31] During acetic fermentation, the concentration of sinapic acid significantly increased to 15.5 mg CE L^{-1}. The same sensitivity was determined for caffeic acid. The fact that the concentrations of caffeic and sinapic acids increased during acetic fermentation proved that they are not oxygen sensitive; on the contrary, their concentrations increased by 20, and 40 %, respectively. Caffeic acid induces apoptosis in human cancer cells starting at a dose of 18 mg 100 mL^{-1}.[32] Moreover, caffeic acid is a metabolite of chlorogenic acid. In the intestine, chlorogenic acid, which was found in the present samples of 10.06 mg CE L^{-1} beer, and almost doubled in vinegar, is hydrolyzed into caffeic acid, which has a stronger antioxidant activity than that of its precursor.[33] The content of ferulic acid, found in its free form in beer and as feruloylquinic acid,[5] was determined to be 10.81 mg CE L^{-1} in the beer sample, but, due to its oxygen sensitiveness, was lower in the vinegar sample, 6.60 mg CE L^{-1}. Other hydroxycinnamic acid derivatives identified and quantified in beer and vinegar were coumaric acid *O*-glucoside, ferulic acid *O*-glucoside and sinapic acid *O*-glucoside. Hydroxycinnamic acid derivatives play an important role in the treatment of diabetes, obesity and metabolic syndrome.[34]

Beer is a rich source of hydroxyphenylacetic acids, along with cider, olive oil and wine,[5] and the present results proved their increasing content after acetic fermentation.

Phenolic compounds play an important role in beer and to its haze stability, catechin and epicatechin being involved in both haze formation[23] and beer colloidal stability.[35] The catechins in beer come from malt, 80 %, and the rest from hop (*Humulus lupulus* L.). Due to their affinity for proteins, the catechins from barley (*Hordeum vulgare* L.) are known to be involved in the formation of beer haze, their role in beer stability becoming essential.[36] (+)-Catechin and (–)-epicatechin, known as contributors to beer bitterness, were previously identified in

beer samples.[4,5,8,27,37] Hop is a valuable ingredient in beer as it contains 14 % phenolic compounds and important amounts of catechin.[4] The contents of (+)--catechin and (–)-epicatechin ranged between 0.57–1.27 mg L^{-1} and 0.08–0.39 mg L^{-1}, respectively, in other beer samples.[19] In brown beer vinegar, these compounds were almost doubled, due to the concentration process. Derivatives of flavanols, catechin 7-*O*-diglucoside and gallocatechin, were also identified in beer.[8,36]

Flavonol derivatives, quercetin *O*-glucoside and kaempferol *O*-glucoside, were recently reported in beer.[5] Quercetin *O*-glucoside can be metabolized in the small intestine.[38] Through its anti-inflammatory and antioxidant properties, it also plays a role in preventing cardiovascular diseases. As chemopreventive agents, by their demonstrated biological activities, *i.e.*, antioxidant,[39] anti-inflammatory, anti-proliferative, pro-apoptotic and anti-antigenic, they positively interfere in all stages of carcinogenesis.[40]

Apigenin and its derivatives were previously reported in artichoke (*Cynara cardunculus* var. *scolymus* L.), the same family as hop (*Humulus lupulus* L.), *Cannabaceae*,[41] vegetables and other condimentary plants.[5] Their chemoprotective activity in unstabilised beer was demonstrated,[42] and in hop products, their cytotoxic activities.[43]

Frequently, hop is used in pellets form in the brewing process, as was the case in the present study, but can also be used as whole cones or extracts. The lupulin gland inside the cones contains hop bitter resins, essential oils, tannins and polyphenols, responsible for the flavour and bitterness of beers. The beneficial effects of bitter acids (α and β acids) can be summarized as potential anticancer activity, apoptotic effect, anti-inflammatory or protection against metabolic disorders (diabetes, cardiovascular diseases and metabolic syndrome). In addition, these valuable compounds are known for their antifungal and antibacterial activities, which impact beers preservation,[44] suggesting their further medical and food industry applications.[45] The content in α-acids is a very important attribute of hop because it determines their economical value, with implications in the brewing process when the constant quality of beers is imperative or simply when developing a new type of beer.[13] Unfortunately, many breweries tend to use lower hopping dosage rates (a reduction of approximately 30 % in the last decade) for economical reasons.[13] Additionally, bitter acids are recognized for their chemo-protective activities. For instance, humulone is known for its antioxidant, anti-inflammatory and antitumor promoting effects. The contents of α acids reported previously[13] ranged between 2.20–13.09 % of the total contents of bitter acids (8.52–20.35 %), of which between 18.48–21.00 % were represented by cohumulone in the Magnum variety hop pellets. In the brown beer vinegar sample, both humulone and isohumulone increased significantly to 5.62 and 4.14 mg CE L^{-1}, respectively.

During the brewing process, the content of bioactive compounds decreases due to process losses. The technological stages in beer making, *i.e*., boiling, must filtration, alcoholic fermentation, beer filtration and pasteurization, contribute to important losses of nutrients and aroma compounds. For example, prenylflavonoids are lost during wort boiling, hot wort separation, and during the fermentation, conditioning, and beer filtration. Bitter resins, represented by iso-α acids, are compounds that give the specific bitter taste to beer during wort boiling with hops. Iso-α acids are more soluble and bitter than α acids. Unfortunately, the isomerisation yield of α acids to iso-α acids is quite low, only 20–35 % of the initial content could be retrieved in beer, depending on the beer type, because the remaining did not isomerise or was lost during boiling, fermentation or beer filtration. Hops are a rich source of prenylflavonoids, 8-prenylnaringenin being the most active phytoestrogen currently known. Prenylflavonoids found in beer have different biological activities, *i.e*., antioxidant, anticarcinogenic, anti-inflammatory, estrogenic and antiviral. Still their amount in beer is strongly related to the applied brewing process, mainly by their insufficient extraction during wort boiling. It was demonstrated that the more intense the malt colour is, the higher are the yields of prenylflavonoids in beer, due to the protective role of Maillard compounds.[12]

The group of alkylmethoxyphenols was represented in the present study by 4-vinylguaiacol, which was reported to be involved in the characteristic aroma of beer.[5,46] It is formed during wort boiling, when ferulic acid releases 4-vinylguaiacol through decarboxylation by thermal decomposition, and during fermentation by an enzymatic reaction.[5] Previous studies recommended an amount of 2.2–3.5 mg L^{-1} was necessary for the pleasant aroma of beers, while an amount higher than 4 mg L^{-1} could contribute negatively to the sensorial quality of beers. In the present study, a value of 2.25 mg L^{-1} was found in the beer, which is in accordance with amounts found previously before in beer (0.02–2.7 mg L^{-1}).

CONCLUSIONS

Finally, it is to consider that brown beer vinegar represents a richer source of polyphenols and phenolic derivatives, compared to beer. The complex composition of brown beer vinegar could recommend its use in the treatment or prevention of several diseases affecting elderly or young people, where special nutrition and dietetics are required. By its increased content of phenolic and antioxidant activity, and being free of alcohol, this potentially functional product could be considered a better source of valuable compounds than beer, which could also be used in special diets. The strategy proposed could also be useful to add value to the liquid waste from the brewing industry.

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ИЗВОД

СИРЂЕ ИЗ ТАМНОГ ПИВА: ПОТЕНЦИЈАЛНО ФУНКЦИОНАЛНИ ПРОИЗВОД ЗАХВАЉУЈУЋИ СВОМ ФЕНОЛНОМ САСТАВУ И АНТИОКСИДАТИВНОЈ АКТИВНОСТИ

ELENA MUDURA[1], TEODORA EMILIA COLDEA[1], CARMEN SOCACIU[2], FLORICUȚA RANGA[2], CARMEN RODICA POP[2], ANCUȚA MIHAELA ROTAR[2] и ANTONELLA PASQUALONE[3]

[1]Department of Food Engineering, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania, [2]Department of Food Science, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania и [3]Food Science and Technology Unit, Department of Soil, Plant, and Food Science (Di.S.S.P.A.), University of Bari 'Aldo Moro', Bari, Italy

Циљ рада је био добијање производа ферментације тамног пива који је функционалан, обогаћен полифенолима и који не садржи алкохол. У пиву и сирћету је одређен фенолни садржај и антиоксидативна активност, користећи Folin–Ciocalteu реагенс, као и способност хватања слободних радикала, користећи 1,1-дифенил-2-пикрилхидразил тест. Раздвајање и идентификација 30 фенолних једињења је изведена користећи HPLC--DAD–ESI(+)-MS. Фенолна једињења су идентификована преко UV спектара. Користећи калибрациону криву (R^2 = 0,9985), израчунате су количине фенолних једињења и изражене као еквиваленти катехина (CE)/L. Укупни садржај фенола у пиву и сирћету, одређен Folin–Ciocalteu реагенсом, је био 428,9±1,58 mg GAE/L, односно 661,5±7,69 mg GAE/L, доприносећи великој антиоксидативној активности сирћета од 82,18 %. Статистички значајне разлике су нађене за сваки параметар након сирћетне ферментације (p < < 0,05). Сирће тамног пива је бољи извор полифенола и фенолних деривата од пива, што га, заједно са већом антиоксидативном активношћу, чини бољим извором корисних састојака и треба га узети у обзир у специфичним дијетама.

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