sciendo
10.2478/aucft-2022-0002

SUITABILITY OF SELECTED WINTER CULTIVARS OF WHEAT FROM PRODUCTION OF WHEAT BEER

– Research paper –

Justyna BELCAR[*,1], Tomasz R. SEKUTOWSKI[2], Miłosz ZARDZEWIAŁY[1] and Józef GORZELANY[1]

[1]*Department of Food and Agriculture Production Engineering, Collegium of Natural Sciences, University of Rzeszow, St. Zelwerowicza 4, 35-601 Rzeszów, Poland*
[2]*Institute of Soil Science and Plant Cultivation, National Research Institute, Pulawy, Department of Weed Science and Tillage Systems, Orzechowa 61 St., 50-540 Wroclaw, Poland*

Abstract: The study investigated the feasibility of three winter cultivars of wheat – *Elixer*, *Rockefeller* and *Gimantis*, for production of wheat beer. Reference samples were produced from commercially available wheat malt. The findings showed the beer produced from commercially available wheat malt presented the best sensory and physicochemical parameters, including real final fermentation of 70.11%. The results related to the investigated wheat cultivars showed good quality properties in the beer produced from malts obtained from *Gimantis* cultivar (content of CO_2 – 0.58%), and from *Rockefeller* cultivar (alcohol content - 4.62% v/v). A similar relationship was observed in the sensory assessment. The findings related to beer produced from malt of *Elixer* wheat cultivar showed relatively low effectiveness of the production process (69.7%) and final beer fermentation (65.52%), and at the same time high contents of polyphenols (210 mg/dm^3) and antioxidant activity. Wheat malts produced from *Gimantis* and *Rockefeller* cultivars can effectively be used as a raw material in beer production; on the other hand, malt from *Elixer* cultivar, found with higher antioxidant contents, may be used as an additive to be applied in a small quantity in the mashing process in order to enhance the beer in terms of its potential health benefits.

Keywords: wheat beer, sensory assessment, antioxidant potential, beer quality determinants

INTRODUCTION

For several decades, brewers and consumers have been increasingly interested in beers in which other feedstock is used in addition to barley malt (Belcar et. al., 2020). The basic ingredients in beer production include water, malt and hops as well as yeast. These are the most standard materials used in beer making for hundreds of years. Wheat (*Triticum aestivum* L.) is one of the most widely grown crops in the world (FAOSTAT 2019). The food industry takes advantage of the demand for wheat, mainly the milling and baking industries. In the recent years Polish researchers have been investigating the feasibility of winter wheat cultivars in production of malt and beer.

The lack of rigorous specifications related to the relevant quality characteristics of wheat grain makes it difficult to identify wheat varietes suitable for brewing, as is the case with barley grain. Beer makers mainly take into account the protein content in wheat (with recommended value up to 11.0-11.5% d.w.) and most commonly choose winter cultivars characterised by floury endosperm associated with higher extract yield (Belcar et. al., 2020; Mascia et. al., 2014; Depraetere et. al., 2004). Wheat grain contains no husk which in the case of barley constitutes a natural filtering layer. Moreover, the former feedstock contains high molecular weight proteins (*e.g.* glutenin and gliadin) which negatively affect the wort filtration rate due to which technological additives, such as rice husk, are applied to improve the effectiveness of the process.

Wheat beers are products of top fermentation, and they are commonly made with 40 – 60% addition of unmalted wheat or wheat malt (Kunze, 2010). Interaction between the two types of malt (wheat

Received: 10.11.2021
Accepted in revised form: 10.03.2022

[*] Corresponding author.
E-Mail address: justyna.belcar@op.pl

and barley) is linked with a need to introduce certain technological modifications in the brewing process. Furthermore, because of the varied chemical composition the final products present unique sensory profiles owing to the wide range of compounds responsible for the aroma and taste of beer, which are produced during the fermentation process (Ignatov et al., 2014). Sensory profile of beer is affected by such factors as yeast used, wort extract concentration, aeration as well as fermentation temperature (Verstrepen et al., 2003). Intensity of metabolic transformations in yeast during fermentation depends on the content of amino nitrogen in wort; the compound is associated with the formation of esters and higher alcohols, as well as undesirable substances such as dimethylsulphide (DMS) and diacetyl in the obtained beer (Depraetere et. al., 2004). The final product of the brewing process is characterised by such factors as intensity of haziness, stability of the delicate and smooth frothy foam, attractive aroma (*e.g.*:, a hint of vanilla, clove, banana or fresh fruit) and slightly bitter taste (Byeon et. al., 2021; Hu et.al., 2019; Wu et. al., 2015). The degree of haziness depends on the interactions and on formation of colloidal connections between (gluten) proteins and starch or polyphenols (Depraetere et. al., 2004). Stability of beer head is positively affected by ions of metal contained in the wort and by addition of hops; on the other hand, the limiting factors include lipids and high concentration of alcohol in wheat beer (Hu et. al., 2019). Wheat beers are found with relatively low concentrations of alcohol and high contents of bioactive antioxidants and nutrients. Antioxidant compounds, most importantly including polyphenols, protect human organism against oxidative stress, however they are highly sensitive to temperature, pH, oxygen level, light and yeast. During beer aging process they are responsible for changes in flavour (He et. al., 2012). Through their activity, antioxidants neutralise free radicals which attack and damage the structure of DNA, membrane lipids and proteins (Fogarasi et. al., 2015). In the recent years we can observe efforts to restore the traditional methods of beer making, mainly in craft breweries which draw practical information from the original art of brewing and combine the conventional approach with numerous advancements. In a way, this is a return to the basics in beer-making. The approach, whereby products are made using very simple methods, provides an impulse for changes taking place in the beer market. Craft beers present high sensory qualities, and the products are not subjected to filtration or pasteurisation. The purpose of the study was to produce wheat beer, at a microbrew scale, from malts obtained as a result of a micro-malting process applied to wheat grain of three winter cultivars; it also aimed to identify antioxidant and sensory properties of the products, and to examine their physicochemical characteristics, and finally to determine the potential of the wheat varieties as a feedstock used in beer production.

MATERIAL AND METHODS

Material

The research material consisted of three types of wheat malt obtained at the Department of Agricultural and Food Engineering at the University of Rzeszów in a process of micro-malting applied to grain of three winter cultivars of wheat, i.e., *Elixer*, *Rockefeller* and *Gimantis* (class C cultivars), produced in a rigorous field experiment conducted in the vegetation period of 2019/2020 in Jelcz-Laskowice (51°21´N; 17°35´W), in a facility administered by the Department of Herbology and Plant Cultivation Techniques of the Institute of Soil Science and Plant Cultivation State Research Institute in Wrocław. The control sample was made from commercially available wheat malt acquired from Viking Malt company in Strzegom (Poland). The malts were refined to the required particie size using a Cemotec disc mill (FOSS, Denmark). The wheat malt was mixed at 1:1 ratio with the commercial Pilzner-type barley malt obtained from Viking Malt company in Strzegom (Poland). Table 1 presents the characteristics of the wheat malts and the barley malt.

Production of wheat beer

Samples of American-style wheat beer were produced in a laboratory at the Department of Agricultural and Food Engineering at the University of Rzeszów. The brew kettle ROYAL RCBM-40N (Expondo, Poland; applied at 80% process efficiency) was filled with a 2.5 kg mix of crushed barley and wheat malt as well as 8.75 l of water (3.5 l of water per each kilogram of malt). The mash was heated up to 52°C and then maintained in this temperature and vigorously stirred for 15 minutes. Subsequently the mash was left to rest, the process temperature was rised up to 66°C and maintained for 1 hour (vigorous stirring was continued during the initial 30 minutes). After that time, the temperature was rised up to 72°C and maintained

for 30 minutes. Finally, the temperature was rised up to 78°C and maintained for 10 minutes. The process ended when a negative result of iodine-starch test was obtained. The mash was subjected to filtration and sparging with water at a temperature of 78°C. The wort was again placed in the brew kettle and heated up to a temperature of 100°C at a rate of 2°C/1min. The wort was boiled for a total 60 minutes; during this time hops were added in three doses. The first one, i.e., 3 g of *Citra* hops (with α-acid contents of 12.9%) was added after 20 minutes; the second – 9 g of *Amarillo* hops (with α-acid contents of 7.5%) after 45 minutes, and at the end of the boiling process 17.7 g of *Citra* hops were inserted to the wort. When the boiling process was completed, the hot wort was chilled down using a spiral immersion cooler and tap water as a cooling agent. After 17 minutes the temperature of the wort decreased to 20°C. The worts produced were found with extract content of 11.9°P. The cooled wort was transferred to a fermentation vessel, with a capacity of 30 l.

Following a rehydration process, Safale US-05 ($6x10^9$ viable cells/g) yeast was added. The fermentation process was carried out at a temperature of 18°C. Each day pH value was checked using a digital pH-meter (Hanna Instruments, Poland) and the content of alcohol was inspected with Balling-scale aerometer (Alla France, France) in order to monitor the course of fermentation. Content of alcohol was determined by taking 100 cm^3 of fermenting wort, placing a Balling-scale aerometer in it and reading the apparent extract content. Then, based on the difference between the original extract and the apparent extract read on the aerometer scale, it was used a appropriate calculator to determine the current alcohol content in the fermenting wort.

Table 1. Characteristics of the wheat malts and the barley malt

Parameter	Wheat malt (Elixer variety)	Wheat malt (Rockefeller variety)	Wheat malt (Gimantis variety)	Commercial wheat malt	Commercial barley malt
Extract yield [% d.w.]	86.00^{b}±1.22	85.80^{b}±0.83	87.10^{a}±0.91	82.00^{c}±0.78	80.00^{d}±1.07
Total protein [% d.w.]	11.60^{ab}±0.29	11.80^{a}±0.17	10.80^{c}±0.20	11.20^{bc}±0.11	11.40^{b}±0.24
Soluble protein [% d.w.]	4.71^{c}±0.08	5.17^{b}±0.05	5.85^{a}±0.14	4.81^{c}±0.09	3.75^{d}±0.06
Diastatic power [WK]	331.00^{a}±9.64	305.00^{b}±9.85	315.00^{b}±13.20	280.00^{c}±8.93	324.00^{ab}±9.55
pH	5.75^{b}±0.07	5.48^{d}±0.11	5.34^{e}±0.15	5.92^{a}±0.09	5.53^{c}±0.12
Final attenuation [%]	82.37^{a}±0.95	80.13^{e}±0.90	80.87^{d}±0.93	81.86^{c}±0.88	82.14^{b}±0.96

Data are expressed as a mean value (n = 3) ± SD; SD – standard deviation.
Mean values within a raw with different letters are significantly different ($p < 0.05$)

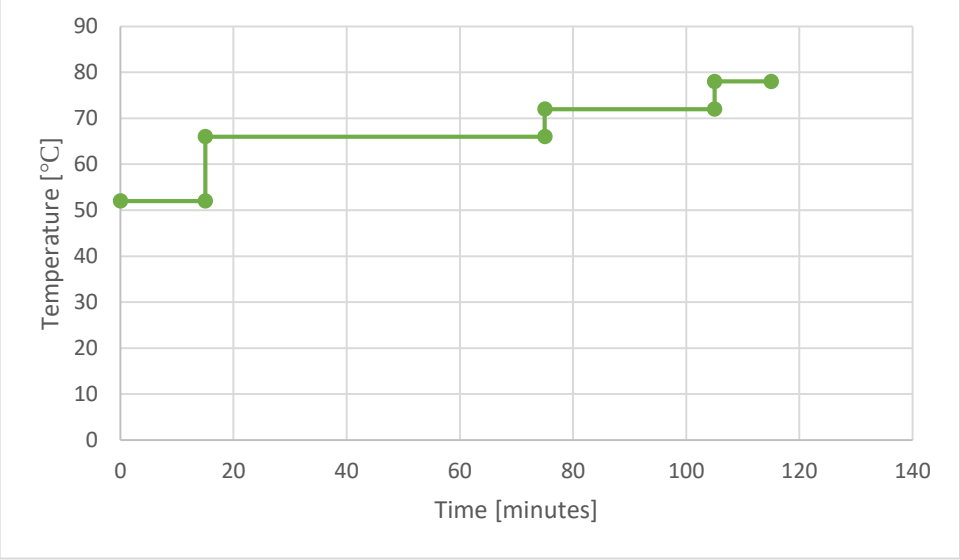

Figure 1. Infusion method applied in the mashing process

After the 14-day fermentation process, aqueous solution of sucrose (1,5 g in 0,5L bottle) was added and the beer was poured into bottles for refermentation to produce adequate level of carbonation. The beer was stored at a temperature of 17-19°C. The sensory assessment and physicochemical analyses were performed three months after the bottling. The wheat beer produced using the commercially available malt is marked as CB (Control Beer), whereas the samples made from the specific wheat varieties, *Elixer*, *Rockefeller* and *Gimantis* are marked as (EB – *Elixer* Beer), (RB – *Rockefeller* Beer) and (GB – *Gimantis* Beer).

Sensory analysis

The sensory analysis was performed by a team of 10 experts, in a sensory analysis laboratory. Coded samples of beer were given to the assessors at random order. In the assessed beers, a sensory profile was applied where each of the quality indicators of wheat beer (beer head, clarity as well as taste and aroma) was matched with an appropriate quantifier reflecting the quality with numbers from 1 to 5, where 1 corresponded to the poorest quality, and 5 to the highest quality of the assessed indicator. Subsequently the assessors put the beers in order, from the product with the highest quality to the product with the lowest quality. The sensory profile was examined using one-way analysis of variance ANOVA (Tukey's test).

Assessment of fermentation process in wheat beers

Changes in pH value during the fermentation process (fermentation days 0-14) were determined using a digital pH-meter (Hanna Instruments, Poland), changes in the content of alcohol were determined using a Balling-scale aerometer (Alla France, France; content of alcohol was determined by taking 100 cm^3 of fermenting wort, placing a Balling-scale aerometer in it and reading the apparent extract content. Then, based on the difference between the original extract and the apparent extract read on the aerometer scale, it was used a appropriate calculator to determine the current alcohol content in the fermenting wort) and the changes in extract content were measured by determining the specific weight of wort/beer at a temperature of 20°C. All data were obtained in triplicate.

Analysis of the quality indicators in the wheat beers

The yield of the wheat beers was calculated as a percent ratio of the capacity of the produced beer to the capacity of the wort subjected to fermentation (at an assumed 80% process efficiency). The characteristics of the fermentation process were determined in accordance with EBC method 9.4, by calculating the following three values: apparent extract AE) measured as the specific weight of beer at 20°C after alcohol has been distilled from the beer; original extract (OE) measured as the specific weight of the wort at 20°C, before fermentation, as well as real extract (RE) measured as the specific weight of the beer at 20°C after fermentation has been completed. The parameters reflecting activity of yeast during fermentation, *i.e.*:, apparent attenuation (AA) and real attenuation (RA), were determined using the following formulas (Byeon et. al., 2021; Mascia et. al., 2014).

$$AA = \frac{(OE - AE)}{OE} x100 \quad (1)$$

$$RA = \frac{(OE - RE)}{OE} x100 \quad (2)$$

The produced beers were assessed for the contents of carbon dioxide (PN-A-79093-6:2000), bitter substances - in accordance with EBC method 9.8 (International Bitterness Units - IBU), diacetyl - in accordance with EBC method 9.24.2, and alcohol - in line with EBC method 9.2.3.The content of free amino nitrogen (FAN) was marked with EBC method 9.10.1, and the total polyphenol contents - in line with EBC method 9.11, and pH was measured in line with EBC method 9.35. The colour of the beers was measured in EBC units, in line with EBC method 9.6 (Analytica EBC, 2004). Titratable acidity of the wheat beers was determined by subjecting beer samples to titration with 0,1 M NaOH, with the endpoint at pH = 8.2 (Adadi et. al., 2017). All data were obtained in triplicate.

Antioxidant potential of the wheat beers

DPPH Test

The antiradical activity of the wheat beers was determined using DPPH radical, in mark with the method proposed by He et al., (2012). A 0.05 mmol/L solution of DPPH (2,2-diphenyl-1-picrylhydrazyl; Sigma-Aldrich; Steinheim, Germany) in ethanol was prepared for this purpose. A 7.8 cm^3 sample of the solution was put in a test tube, with 0.2 cm^3 of diluted beer, and incubated in darkness for 60 minutes at a temperature of 37°C; subsequently the absorbance at the wavelength λ=517 nm was examined using Vis V-5000 spectrophotometer (Shanghai Metash Instruments Co. Ltd., China). The blank contained distilled water instead of beer. The results were converted as trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Sigma-Aldrich; Steinheim, Germany) equivalent (mmol TE/L). All data were obtained in triplicate.

FRAP Test

Reducing power of the wheat beers was determined using a FRAP reagent, in accordance with the method described by Benzie & Strain (1996) and He et al. (2012). The materials prepared for this purpose included a 10 mmol/L TPTZ (2,4,6-tripyridyl-s-triazine; Sigma-Aldrich; Steinheim, Germany) solution, a 20 mmol/L $FeCl_3xH_2O$ (Chempur, Poland) solution, an acetate buffer with pH=3.6 as well as a 40 mmol/L HCl (Chempur, Poland) solution. Subsequently the FRAP reagent was busked by 25 cm^3 of the acetale buffer with 2.5 cm^3 of the TPTZ dissolved in HCl and 2.5 cm^3 of $FeCl_3x6H_2O$. A 6 cm^3 sample of FRAP solution was placed in a test tube with 0.2 cm^3 of the beer and incubated at a temperature of 37°C for 10 minutes; subsequently the absorbance at the wavelength λ=593 nm was examined using Vis V-5000 spectrophotometer (Shanghai Metash Instruments Co. Ltd., China). The blank contained distilled water instead of beer. The results of the FRAP test were expressed as mmol Fe^{2+}/L. All data were obtained in triplicate.

ABTS Test

Antiradical activity of the wheat beers was examined using ABTS radical cation in line with the method developed by Re et al. (1999). A 7 mmol/L ABTS [(2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); Sigma-Aldrich; Steinheim, Germany] solution and a 2.45 mmol/L potassium persulphate solution (Chempur, Poland) were prepared for this purpose. The solutions were combined at 1:0.5 ratio, and stored for 12-16 hours in darkness to enable development of ABTS cation. The $ABTS^+$ solution was added distilled water to achieve absorbance of 0.700±0.02 (at wavelength λ=734 nm). A 3 cm^3 portion of the diluted $ABTS^+$ solution was placed in the test tube with 0.3 cm^3 of the beer, and the after 6 minutes the absorbance value at the wavelength λ=734 nm was determined using Vis V-5000 spectrophotometer (Shanghai Metash Instruments Co. Ltd., China). Results were revision to account for dilution and converted as trolox equivalent (mmol TE/L). All data were obtained in triplicate.

Statistical analysis

To compile the research results, Statistica 13.3 software was used. To interpret the results, one-way analysis of ariance ANOVA (Tukey's test) was applied, with significance level at α=0.05 for physicochemical and antioxidant properties and the wheat beers.

RESULTS AND DISCUSSION

Changes in pH, and contents of extract and alcohol during fermentation of the wheat beers

Alcoholic fermentation carried out by yeast is the most important stage in beer production, because it results in formation of ethyl alcohol as well as compounds (*e.g.* esters and aldehydes) responsible for taste and aroma, ultimately affecting the sensory quality of the product. The changes in extract and alcohol contents and in pH value occurring during the beer fermentation process are illustrated in Figures 2.-5. With each day of the fermentation process there was a drop in apparent extract content and in pH value in the beer, and an increase of alcohol content. The most visible changes in the values of the specific parameters were observed on the second day of the fermentation; the transformations gradually became less pronounced and from the fifth day, on average, they were stabilised at a similar level in each type of wheat beer investigated in the study (Figures 2 – 5). A very rapid decrease in apparent extract content may lead to low carbonation of the beer (saturation may be regulated in the process of bottle conditioning). On the other hand, however, the intensity of changes occurring on the first days of fermentation is associated with content increase of esters and higher alcohols affecting the sensory quality and the flavour of the beer (Ignatov et. al., 2014).

Insufficient changes in the values of the relevant parameters (pH, apparent extract and alcohol content) on the first days of fermentation may reflect poor activity of the beer yeast. A process with such characteristics may lead to production of beer with poor quality or even to complete loss of the wort with yeast added.

Physicochemical characteristics of wheat beers

The highest yield of the fermentation process was obtained for the beer produced from commercially available wheat malt. As for the beers made from the investigated wheat varieties, the highest yield of the production process was identified in the case of EB samples. The value of apparent attenuation ranged from 66.61% in the case of EB to 70.92% in RB samples, and was positively correlated to the ethanol contents identified in the wheat beers (Table 2.).

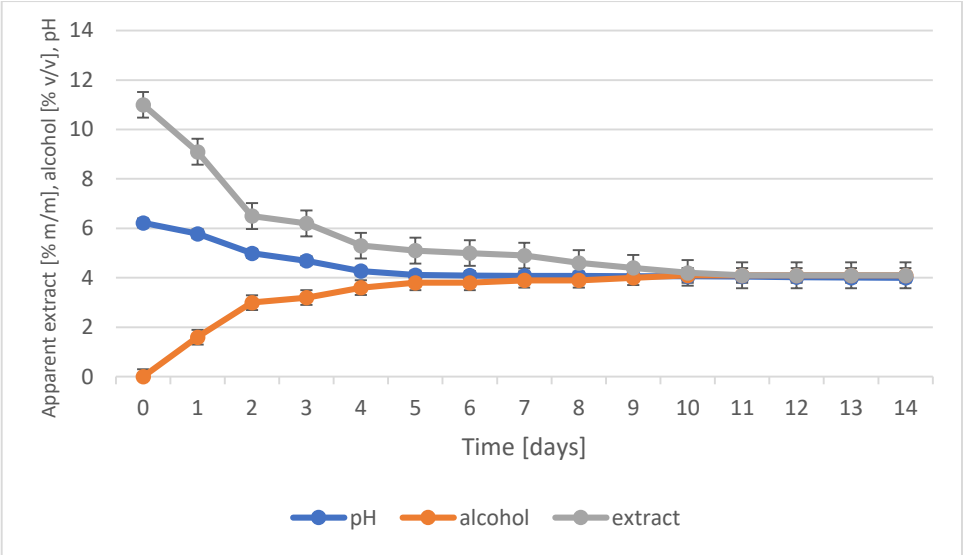

Figure 2. Changes in the contents of apparent extract and content of alcohol, and in pH value during the fermentation process in the case of the wheat beer CB

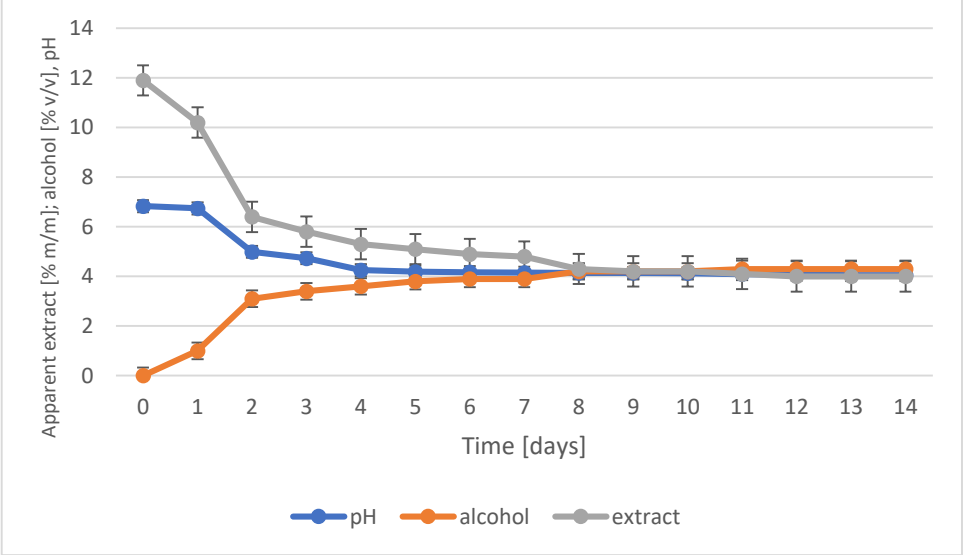

Figure 3. Changes in the contents of apparent extract and content of alcohol, and in pH value during the fermentation process in the case of the wheat beer EB

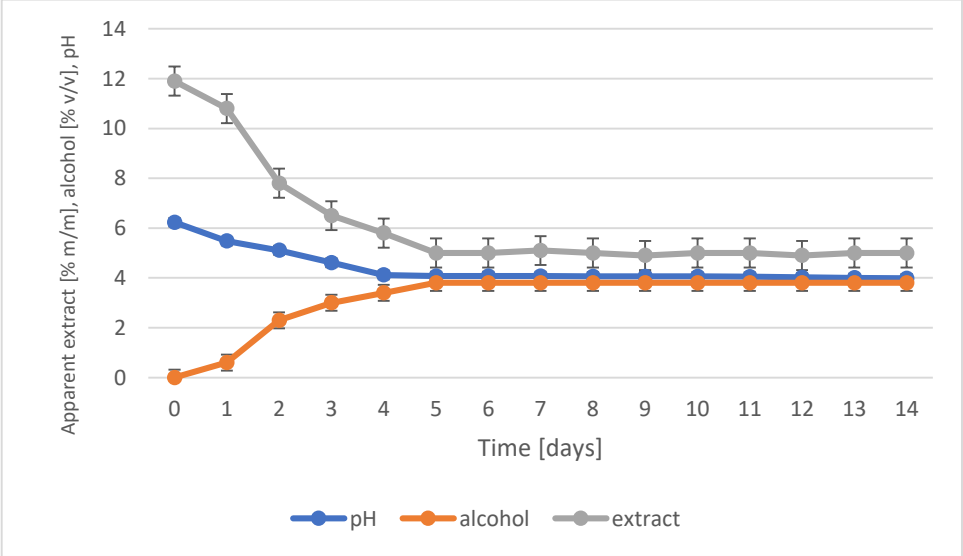

Figure 4. Changes in the contents of apparent extract and content of alcohol, and in pH value during the fermentation process in the case of the wheat beer RB

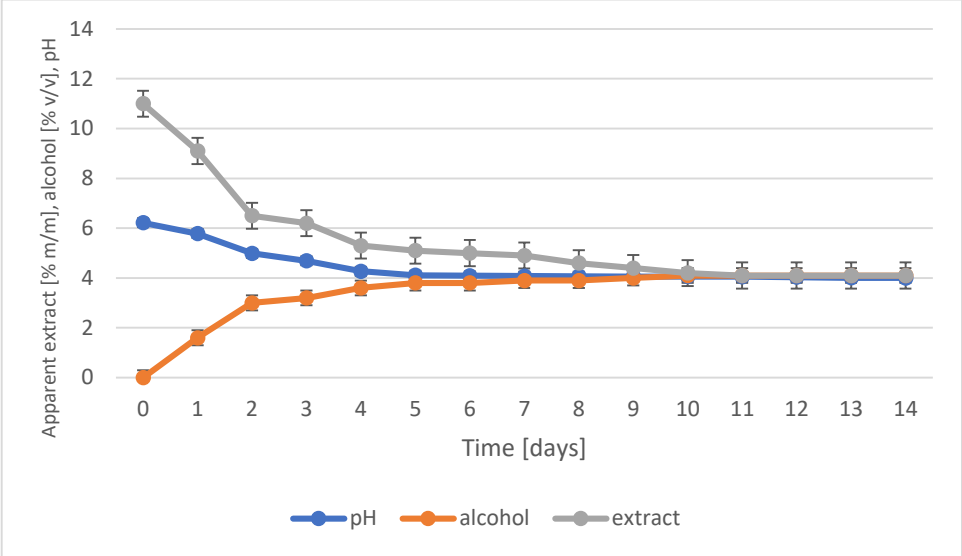

Figure 5. Changes in the contents of apparent extract and content of alcohol, and in pH value during the fermentation process in the case of the wheat beer GB

In a study investigating suitability of Korean wheats for production of beer, Byeon et al. (2021) compared these varieties to an American equivalent which was found with apparent attenuation of 71.4% and ethanol content of 4.87% v/v. Against, Mascia et al. (2014) reported that durum wheat beer, investigated in their study, was found with apparent attenuation of 69.5%, compared to a German and a Czech beer in which apparent attenuation was 79.9% and 69.6%, respectively, and ethanol content amounted to 5.37% v/v and 4.34% v/v, respectively. Wheat beers developed by Depraetere et al. (2004) from mixed feedstock consisting of barley malt (60%) and wheat malt (40%) were found with apparent attenuation in the range of 87.1 – 91.1%. The differences in the value of apparent attenuation in beers produced in the same conditions reflects different contents of nutrients in the worts used in production of RB, as well as CB and GB; these nutrients are used by yeast in the process of fermentation whereby carbon dioxide is released and the available sugars are transformed into ethanol (Byeon et. al., 2021). The content of ethyl alcohol in beer is one of the most important quality determinants because it is responsible for sensory properties of the beer and it affects the economics of production. Furthermore, tax is calculated based on this value (Mascia et. al., 2014).

The wheat beer samples were produced to resemble American Wheat Beer, therefore their characteristics should include moderate bitterness in the range of 15-30 IBU (Oliver 2012). The lowest value of this parameter was identified in CB (below 30 IBU), whereas the Rother beers were found with the contents of bitter substances ranging from 35.8 IBU (RB) to 38.9 IBU (GB; Table 2).

In line with the recipe assumptions, the contents of bitter substances in wheat beers should be at a level of 25 IBU, but due to the slightly reduced efficiency of the production process, the value was significantly higher. Bitterness of beer is significantly associated with the dose of hops and degree of isomerisation of hop α-acids and to a lesser extent with the reaction of proteins with polyphenols contained in the malt Byeon et. al. (2021). The α-acids contained in hop cones significantly contribute to the intensely bitter taste of beer, which is partly neutralised by sugars contained in the beer, resulting in the pleasant taste of the product, and affecting the stability of beer head. The variety and quality of hops, and duration of boiling process affect the content of bitter substances in the beer (Habschied et. al., 2021).

The highest titratable acidity and pH values were identified in EB sample, and all the wheat beers were found with pH exceeding 4.12 (Table 2). Mascia et al. (2014) reported similar values of titratable acidity and pH in the wheat beers investigated, whereas Depraetere et al. (2004) found the wheat beers to have pH of 3.9-4.0. Changes in pH value and acidity in beers may reflect adverse and undesirable transformations in the final product of the fermentation process.

Table 2. Physicochemical analysis of the wheat beers

Parameter	Wheat beer			
	CB	EB	RB	GB
Yield [%]	79,1	69,7	73,2	76,9
Colour [EBC units]	$16^{a}\pm3$	$17^{a}\pm3$	$20^{a}\pm2$	$18^{a}\pm3$
Original extract [°P]	$11.91^{a}\pm0.10$	$11.89^{a}\pm0.06$	$11.90^{a}\pm0.17$	$11.90^{a}\pm0.04$
Apparent extract [% m/m]	$3.53^{bc}\pm0.08$	$3.97^{a}\pm0.07$	$3.46^{c}\pm0.12$	$3.55^{b}\pm0.07$
Real extract [% m/m]	$3.56^{c}\pm0.12$	$4.10^{c}\pm0.06$	$3.72^{b}\pm0.14$	$3.59^{c}\pm0.10$
Apparent attenuation [%]	$70.36^{b}\pm0.05$	$66.61^{d}\pm0.11$	$70.92^{a}\pm0.08$	$70.17^{c}\pm0.08$
Real attenuation [%]	$70.11^{a}\pm0.08$	$65.52^{d}\pm0.10$	$68.74^{c}\pm0.07$	$69.83^{b}\pm0.08$
Content of alcohol [% v/v]	$4.58^{ab}\pm0.04$	$4.37^{c}\pm0.04$	$4.62^{a}\pm0.09$	$4.51^{b}\pm0.07$
pH	$4.12^{b}\pm0.07$	$4.89^{a}\pm0.10$	$4.76^{a}\pm0.11$	$4.24^{b}\pm0.13$
Titratable acidity [0.1M NaOH/100 cm^3]	$2.80^{c}\pm0.12$	$3.34^{a}\pm0.14$	$2.44^{d}\pm0.13$	$2.86^{b}\pm0.12$
Content of bitter substances [IBU]	$29.80^{d}\pm0.45$	$38.0^{b}\pm0.40$	$35.8^{c}\pm0.15$	$38.9^{a}\pm0.55$
Content of carbon dioxide [%]	$0.58^{a}\pm0.20$	$0.56^{ab}\pm0.10$	$0.53^{b}\pm0.20$	$0.58^{a}\pm0.06$
Content of diacetyl [µg/dm^3]	$18^{a}\pm4$	$11^{ab}\pm4$	$15^{ab}\pm4$	$4^{b}\pm2$
Free amino nitrogen (FAN) [mg/dm^3]	$63.45^{d}\pm0.06$	$68.06^{c}\pm0.06$	$78.81^{a}\pm0.09$	$73.15^{b}\pm0.07$
Total polyphenols [mg/dm^3]	$141^{b}\pm9$	$210^{a}\pm8$	$211^{a}\pm8$	$187^{a}\pm10$

Data are expressed as a mean value (n = 3) ± SD; SD – standard deviation.
Mean values within a raw with different letters are significantly different ($p < 0.05$)

The investigated beers were found with very similar contents of carbon dioxide – with the mean value of 0.56% (Table 2). In the study by Mascia et al. (2014) the contents of carbon dioxide in the wheat beers ranged from 0.49% to 0.74%, relative to the type of beer, whereas Depraetere et al. (2004) reported values in the range of 0.57 – 0.60%. Optimum content of carbon dioxide results in carbonation of wheat beer and contributes to the refreshing effect produced by beer; beers with low level of carbonation are not accepted by consumers. Carbon dioxide and proteins are responsible for formation of beer foam because its molecules are enclosed in the colloidal system formed by various types of proteins (Michalski et. al., 2018)

Total polyphenol contents in the wheat beers were statistically varied, ranging between 141 mg/dm^3 (CB) and 211 mg/dm^3 (RB; Table 2). The beer produced from the American wheat cultivar, in the study by Byeon et al. (2021), was found with total polyphenols amounting to 102.8 mg/dm^3, whereas Mascia et al. (2014) reported higher values in the range from 139 to 177 mg/dm^3 in the various types of the wheat beer. Depraetere et al. (2004) reported total polyphenol contents in wheat beers in the range of 125-142 mg/dm^3. Alcoholic fermentation leads to interactions of lipids and proteins with polyphenols contained in the wort subjected to fermentation, which is linked to the possible formation of sediments and haze in the beer (Byeon et. al., 2021). Polyphenol contents and degree of isomerization affect the depth of beer taste (*e.g.*:, polymers of flavanol), and pungency (products of reactions occurring between proteins – proline, and polyphenols). Polyphenols which are located in malt and hops pass into the wort during mashing and boiling process; their concentration depends on malt refinement, as well as duration and temperature of mashing and boiling with hops, when proteins react with polyphenols and sediments may be formed (Habschied et. al., 2021).

The least intense colour was identified in CB sample, whereas the colour-related differences between the beers produced from the investigated wheat cultivars were small but statistically significant, with the highest value identified in RB sample (Table 2). Nevertheless, the colour of all the wheat beers produced in this study was consistent with the norm. The colour of the final product is mainly affected by the type of malt used, as well as the conditions of the mashing and brewing process, when the chemical compounds responsible for taste and aroma (mainly hydroxymethylfurfural and furfural) as well as colour, *e.g.*:, melanoidins (products of Maillard reaction) pass into the beer and give it the appropriate colour and flavour (Byeon et. al., 2021; Michalski et. al., 2018; Mascia et. al., 2014). The high total polyphenol contents in the investigated beers may also have significantly impacted the intensity of hazing and the colour ofthe beverages.

The contents of free amino nitrogen (FAN) in the wheat beers were statistically varied, with higher contents observed in the samples of RB and GB

(Table 2). On average beers contain 100 mg/dm^3 of amino acids. Free amino acids are essential in fermentation mainly for metabolic transformations in yeast, but they also significantly impact quality characteristics of beers, including stability of beer head and sensory properties of beer. During the fermentation and in the final product amino acids are partly transformed into aldehydes. In line with recommendations, contents of free amino acids in the final product should be relatively low, in order to delay beer aging and deterioration of its quality (Kucharczyk et. al., 2018; Gudio et. al., 2007). Higher contents of protein in wheat malt used for beer production correspond to lower polyphenol contents in the final product of fermentation (Mascia et. al., 2014). A similar trend was observed by Byeon et al. (2021) and in the current study with regard to beers made from the investigated wheat cultivars.

The lowest content of diacetyl was found in GB sample, however the relevant value identified in all the wheat beers was consistent with the norm (Table 2). The accepted concentration in beers is <25 μg·dm^{-3}, except for some types of beer in which higher contents are desirable, *e.g.*:, in Czech Pils. Diacetyl (butane-2,3-dione), which is a side product of alcoholic fermentation, affects the flavour of beer. To reduce the contents of diacetyl in beer it is necessary to appropriately select the yeast strain, to maintain activity of the yeast as long as possible, especially during beer maturation phase, and to increase the temperature periodically (Michalski et. al., 2018; Kucharski & Tuszyński, 2017).

Antioxidant potential of the wheat beers

Beer is known to contain antioxidants, such as polyphenols, vitamins, melanoidins and bitter acids (Bogdan & Kordialik-Bogacka, 2016; Ditrych et. al., 2015). Beers with a higher DPPH radical scavenging capacity prezent better stability of taste, even with a longer storage period, which is related to the reduced production of trans-2-nonenal or other aldehydes (He et. al., 2012). Antiradical activity of beer, determined using DPPH method, was in the range between 1.16 and 1.21 mmol TE/L, except for EB sample in which the activity was much higher, and amounted to 1.60 mmol TE/L (Table 3). Reducing power of the wheat beers was more varied – the highest value was found in RB sample (2.55 mmol Fe^{2+}/L), slightly lower in EB, and the remaining wheat beers presented significantly lower reducing power (Table 3). A study by Ditrych et al. (2015) investigated top-fermented wheat beer and reported 0.5 mmol TE/L antiradical activity, measured with DPPH test, and 0.7-0.8 mmol Fe^{2+}/L reducing power. Furthermore, this research team observed higher antioxidant activity in non-stabilized beer.

The value of the potential is related to the wheat/barley cultivar selected for malt production and to the process applied in beer production. Ditrych et al. (2015) also observed that higher polyphenol contents in beers positively affect the antioxidant activity of beer measured using DPPH and FRAP methods.

A study by He et al. (2012) showed that reducing power of unpasteurized wheat beers (FRAP) stored at 20°C was in the range of 1.71-1.48 mmol Fe^{2+}/L and the values tended to decrease with longer duration of storage (storage days 0-18). Increased formation of radicals in stored beer is related to both storage duration and the technological processes applied, e.g., beer pasteurization delays the loss of oxidative stability, but longer duration of the process negatively affects both polyphenol contents and antioxidant potential of the beer (Ditrych et. al., 2015; Hoff et. al., 2013; Cao et. al., 2011). Antiradical potential of the wheat beers was also assessed in terms of reducing capacity related to ABTS radical cation; the relevant value was in the range between 2.18 mmol TE/L in RB and 2.94 mmol TE/L in EB sample (Table 3). A study by Szwajgier (2009), focusing on antioxidant activity of barley beers (lagers), assessed antiradical activity of phenolic acids using the DPPH radical and ABTS radical cation and the findings showed the mean values of 2.28 mmol TE/L and 4.58 mmol TE/L, respectively. The method with the use of DPPH is selective and is used to assess hydrophobic compounds contained in food products, whereas the method using ABTS may be applied to assess both hydrophobic and hydrophilic antioxidants contained in the samples subjected to the relevant analysis.

Sensory analysis of wheat beers

Sensory characteristics of wheat beers are determinants of the specific beer style, and they contribute to attractiveness of the product and acceptance for the given beer among consumers. Sensory characteristics of beer to the highest extent is related to the taste and aroma of the product and to a lesser degree, to the colour of beer and quality of the beer head, the latter properties also being rather important. The wheat beers investigated in this study were slightly hazy and iridescent which is characteristic for wheat beers, contributing to the colour obtained in the beer (16-20 EBC, Table 2).

Table 3. Antioxidant potential of the wheat beers produced

Parameter	Wheat beer			
	CB	EB	RB	GB
Antiradical activity of beer – DPPH method [mmol TE/L]	$1.16^{b}\pm0.06$	$1.60^{a}\pm0.11$	$1.17^{b}\pm0.07$	$1.21^{b}\pm0.05$
Reducing power of beer – FRAP method [mmol Fe^{2+}/L]	$1.48^{d}\pm0.08$	$2.34^{b}\pm0.06$	$2.55^{a}\pm0.07$	$1.83^{c}\pm0.07$
Antiradical activity of beer – ABTS method [mmol TE/L]	$2.21^{b}\pm0.06$	$2.94^{a}\pm0.05$	$2.18^{bc}\pm0.05$	$2.06^{c}\pm0.05$

Data are expressed as a mean value (n = 3) ± SD; SD – standard deviation.
Mean values within a raw with different letters are significantly different ($p < 0.05$)

Beer head is one of the most important attributes affecting attractiveness of the beer for consumers and impacting their decision to get a given beverage again. American-style wheat beer should be characterised by ample and long-lasting frothy foam (Oliver, 2012). In the own research the foam of the beer from commercially available wheat malt (CB) was found with the best quality indicators. As regards the beers made from the investigated wheat cultivars, RB samples were found with ample white beer head and GB samples stood out for the good stability and structure of the beer head (Figure 6).
Quality and stability of beer head is affected by proteins contained in the malt and passing into the wort during the mashing. Glutenin and gliadin (gluten proteins in wheat) during the malting process are transformed into high-molecular mass proteins soluble in water. These impact the foaming properties and hazing of beer, the effect particularly resulting from their interaction with polyphenols (Yin et. al., 2017; Xie et. al., 2014). The contents of proteins in the wort are stable during the boiling process and the final stage of fermentation, and the largest decrease in protein contents is observed during the initial phase of the fermentation. Addition of wheat malt in beer production positively affects stability of beer head, owing to greater content of soluble proteins and high molecular weight proteins, the latter being responsible for stability of beer foam. Proteins from barley malt are responsible for the structure of foam particles (Depraetere et. al., 2004). Much attention has also been paid to protein LTP1 responsible for transfer of lipids, as well as protein Z, which significantly affect formation and stability of beer foam (Hu et. al., 2019; Wu et. al., 2015; Evans et. al., 1999). A study by Wu et al. (2015) showed that the fraction of proteins with a mass of 2.1-7.6 kDa is mainly responsible for beer foam, whereas the quality of beer is influenced by the fraction of proteins with a mass of 13.2 – 100.0 kDa.
The sensory analysis of the wheat beers showed that CB samples were characterised by the most balanced flavour profile, the important attributes being crispness producing a refreshing effect in the mouth, as well as sweetness, resulting from the lower content of bitter substances (29.8 IBU, Table 2; Figure 7). The GB and RB samples were intensely bitter and produced a sense of fullness of taste (Figure 7).

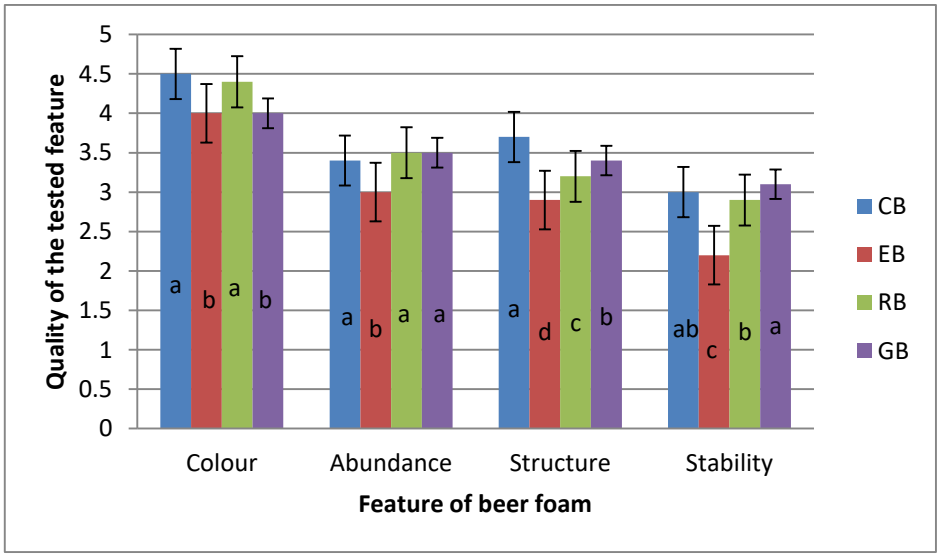

Figure 6. Quality characteristics of the foam in the wheat beers

In all the beers it was possibile to notice grainy and malty accents characteristic for the style of American Wheat Beer, particularly in the GB samples (malty hints). A malty taste of beer is induced by such compounds as maltol and furaneol (Faltenmaier et. al., 2014). The sensory profile of the wheat beers was varied, which was confirmed with physicochemical analyses. The yeast strain used affects the taste of the resulting wheat beer (particularly its phenolic flavour) to a greater extent than the temperature applied during the fermentation process. The chemical compounds significantly affecting the taste of beer are formed resulting from interactions of carbonyl compounds, esters, sulphur compounds, alcohols, phenolic compounds and organic acids (Faltenmaier et. al., 2014). Off-flavours of wheat beers may be associated with inadequate pH maintained in the wort or beer, and with changes in the temperature, yeast strain failing to match the type of beer produced, or the use of chlorinated water which results in formation of chlorophenols in the produced beer (Habschied et. al., 2021).

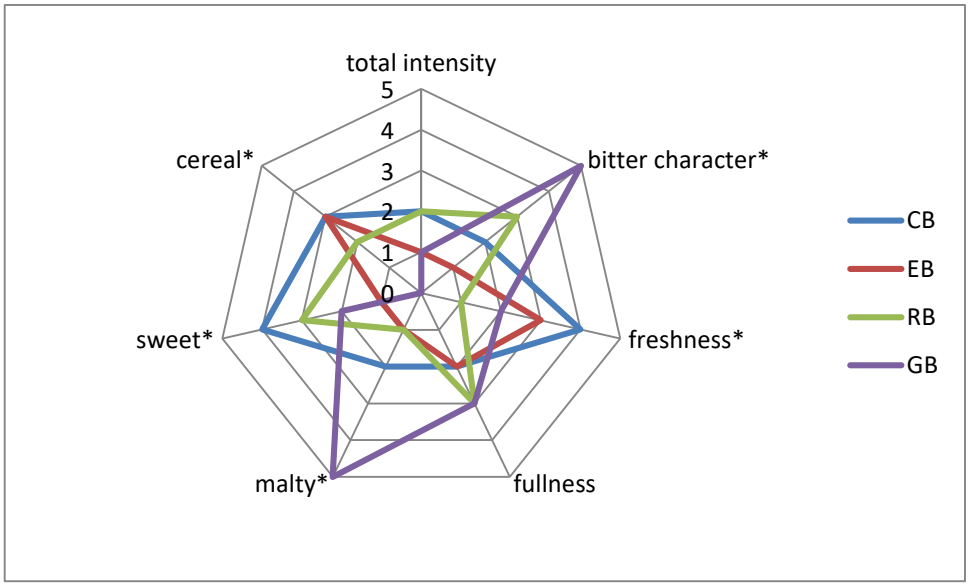

Figure 7. Sensory profile of the wheat beers (* marks the attributes which were statistically different at $p \leq 0.05$)

CONCLUSIONS

Selection of appropriate wheat cultivar for production of malt and beer is one of the crucial operations which make it possible to obtain fine quality product and to ensure optimum economics of production. Based on the assessments performed in the study it was shown that beer produced from commercially available wheat malt (CB) presented the best physicochemical and sensory parameters (as regards the characteristics of beer head and beer flavour). The results related to the specific wheat cultivars investigated in this study showed high quality properties in GB sample (*e.g.*: yield – 76,9%; real attenuation – 69,83% or content of diacetyl – 4 μg/dm^3), and slightly lower in RB sample (*e.g.*: content of alcohol – 4,62% v/v or real extract – 3,72% m/m). A similar relationship was observed in the sensory assessment. The findings related to EB sample showed relatively low effectiveness of the production process and final beer fermentation, and at the same time high contents of polyphenols (210 mg/dm^3) and good antioxidant activity. As for the investigated wheat cultivars, wheat malts produced from *Gimantis* and *Rockefeller* cultivars can effectively be used as a raw material in beer production; on the other hand, malt from *Elixer* cultivar, found with higher antioxidant contents, may be used as an additive to be applied in a small quantity in the mashing process in order to enhance the beer in terms of its potential health benefits.

FUNDING
The project is financed by the program of the Minister of Science and Higher Education named "Regional Initiative of Excellence" in the years 2019–2022, project number 026/RID/2018/19, the amount of financing PLN 9 542 500.00.

REFERENCES

1. Adadi, P., Kovaleva, E., Glukhareva, T., Shatunova, S.A. & Petrov, A.S. (2017). Production and analysis of non-traditional beer supplemented with sea buckthorn. *Agron. Res*. 15, 1831–1845. https://doi.org/10.15159/AR.17.060.
2. Belcar, J., Matłok, N. & Gorzelany, J. (2020). Technological assessment of winter cultivar of common wheat (*Triticum aestivum* L.) and winter barley (*Hordeum vulgare* L.) for pale malt production. *Acta Univ. Cib. Series E: Food Technology*. 1(24), 89-98. https://doi.org/10.2478/aucft-2020-0024.
3. Benzie, O. F. F. & Strain, J. J. (1996). The ferric Reducing Ability of Plasma (FRAP) as a Measure of „Antioxidant Power": The FRAP Assay. *Analytical Biochemistry*. 239, 70-76. DOI: 10.1006/abio.1996.0292
4. Bogdan, P., Kordialik-Bogacka, E. (2016). Antioxidant activity of beers produced with the addition of unmalted quinoa and amaranth. *FOOD. Science. Technology. Quality.* 3 (106), 118-126.
5. Byeon, Y. S., Lim, S.-T., Kim, H.-J., Kwak, H. S. & Kim, S. S. (2021). Quality Characteristics of Wheat Malts with Different Country of Origin and Their Effect on Beer Brewing. *J. Food Quality*. ID:2146620.
6. Cao, L., Zhou, G., Guo, P. & Li, Y. (2011). Influence of pasteurising intensity on beer flavour stability. *J. Inst. Brew*. 117, 587–592. https://doi.org/10.1002/j.2050-0416.2011.tb00508.x
7. Depraetere, S., Delvaux, F., Coghe, S. & Delvaux, F. R. (2004). Wheat Variety and Barley Malt Properties: Influence on Haze Intensity and Foam Stability of Wheat Beer. *J. Inst. Brew*. 110(3), 200-206. https://doi.org/10.1002/j.2050-0416.2004.tb00203.x
8. Ditrych, M., Kordialik-Bogacka, E. & Czyżowska, A. (2015). Antiradical and Reducing Potential of Commercial Beer. *Czech J. Food Sci.* 33(3), 261-266. doi: 10.17221/658/2014-CJFS
9. European Brewery Convention. *Analytica EBC*. (2004). Hans Carl Getränke-Fachverlag, Nürnberg.
10. Evans, D. E., Sheehan, M. C. & Stewart, D.C. (1999). The Impact of Malt Derived Proteins on Beer Foam Quality. Part II: The Influence of Malt Foam – positive Proteins and Non-starch Polysaccharides on Beer Foam Quality. *J. Inst. Brew.* 105, 2, 171-178.
11. Faltermaier A., Waters D., Becker T., Arendt E. & Gastl M. (2014). Common wheat (*Triticum aestivum* L.) and its use as a brewing cereal – a review. *J. Inst. Brew.* 120, 1-15. http://doi.org/10.1002/jib.107.
12. FAO. (2019). *FAOSTAT Statistical Database*; Food and Agriculture Organization of the United Nations (FAO). Available online: http://www.fao.org/faostat/en/#data/QC (accessed on 19 September 2021).
13. Fogarasi, A.-L., Kun, S., Tankó, G., Stefanovits-Bányai, É., & Hegyesné-Vecseri, B. (2015). A comparative assessment of antioxidant properites, total phenolic content of einkorn, wheat, barley and their malts. *Food Chem.* 167, 1-6. DOI: 10.1016/j.food-chem.2014.06.084
14. Gudio, L., A., Curto, P., Bovin N., Benismail, C., Goncalves, A. & Barro, S. (2007). Predicting the organoleptic stability of beer from chemical data using multivariate analysis. *E. Food Res. and Techn.* 226, 57-62.
15. Habschied, K., Košir, I. N., Krstanović, V., Kumrić, G. & Mastanjević, K. (2021). Beer Polyphenols – Bitterness, Astrigency, and Off-Flavors. *Beverages*. 7, 38. https://doi.org/10.3390/beverages7020038
16. He, G., Du, J., Zhang, K., Wei, G. & Wang, W. (2012). Antioxidant capability and potableness of fresh cloudy wheat beer stored at different temperatures. *J. Inst. Brew*. 118, 386-392. DOI 10.1002/jib.5
17. Hoff, S., Lund, M.N., Petersen, M. A., Frank, W., & Andersen, M.L. (2013). Storage stability of pasteurized non-filtered beer. *J. Inst. Brew.* 119, 172–181. DOI:10.1002/jib.85
18. Hu, X., Jin, Y., & Du, J. (2019). Differences in protein content and foaming properties of cloudy beers based on wheat malt content. *J. Inst. Brew.* 125, 235-241. https://doi.org/10.1002/jib.550.
19. Ignatov, I., Kaneva, M., Valchev, M., & Vasileva, N. (2014). Research on the fermentation process in wheat beer. *PROCEEDINGS OF THE INTERNATIONAL CONFERENCE „AGRI-FOOD SCIENCES, PROCESSES AND TECHNOLOGIES"*. AGRI FOOD. May 14-15, 2014. Sibiu, Romania.
20. Kucharczyk, K., Tuszyński, T., & Żyła, K. (2018). Effect of yeast dose on the content of amino acids (FAN) in beer produced in multi-tank technology. *Adv. in Food Proces. Tech.* 2; 20-24.
21. Kucharczyk, K. & Tuszyński, T. 2017. Presence of diacetyl and 2,3-pentaodione in beer. *FOOD. Science. Technology. Quality*. 24,4(113), 17-26. DOI: 10.15193/zntj/2017/113/207

22. Kunze, W. (2010). Technology Brewing and Malting. 4th updated edition. *VLB Berlin*, Germany, ISBN 978-3-921690-64-2.
23. Mascia, I., Fadda, C., Dostálek, P., Olšovská, J., & Del Caro, A. (2014). Preliminary characterization of an Italian craft durum wheat beer. *J. Inst. Brew.* 120, 495-499. DOI 10.1002/jib.176.
24. Michalski, J., Sąsiadek, W., & Owsiany, K. (2018). Characteristics of selected chemical compounds responsible for the organoleptic characteristics of beer. *Scientific works of the Univ. of Economics in Wrocław*. 542, 106-121.
25. Oliver, G. (2012). A mesa do mestre-cervejeiro: descobrindo os prazeres das cervejas e das comidas verdadeiras. *Editora Senac São Paulo*, ISBN: 9788539601745.
26. PN-A-79093-6:2000. Beer - Research methods - Determination of carbon dioxide content. (2000). *Polish Committee for Standardization.*
27. Re, R., Pellegrini, N., Proteogente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidants activity applying and improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine*. 26, 9/10; 1231-1237. DOI: 10.1016/s0891-5849(98)00315-3
28. Szwajgier, D. (2009). Content of Individual Phenolic Acids in Worts and Beers and their Possible Contribution to the Antiradical Activity of Beer. *J. Inst. Brew.* 115(3), 243-252. DOI:10.1002/j.2050-0416.2009.tb00376.x
29. Verstrepen, K. G. Derdelinck, J., Dufour, J. Winderick, J. Thevelein, I. Pretorius, F. & Delvaux F. T. (2003). Flavor-active esters: Adding fruitiness to beer. *J. of Bioscience and Bioengineering*. 96, 2, 110–118.
30. Wu, X., Du, J., Zhang, K., Ju, Y., & Jin, Y. (2015). Changes in protein molecular weight during cloudy wheat beer brewing. *J. Inst. Brew*.121, 137-144. DOI 10.1002/jib.198
31. Xie, L., Jin, Y., Du, J., & Zhang, K. (2014). Water – soluble protein molecular weight distribution and effects on wheat malt quality during malting. *J. Inst. Brew.* 120, 399-403. DOI 10.1002/jib.182.
32. Yin, H., Deng, Y., He, Y., Dong, J., Lu, J., & Chang, Z. (2017). A preliminary study of the quality attributes of a cloudy wheat beer treated by flash pasteurization. *J. Inst. Brew.* 123, 366-372. DOI 10.1002/jib.429.