

Production and analysis of non-traditional beer supplemented with sea buckthorn

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Abstract. In recent years, there has been a growing demand for non-traditional beer (craft) with different flavours hence the main aim of this research is to produce beer with sea buckthorns (SBT). Brewing software BeerSmith was used to design the Kölsch beer. After one month of primary fermentation, SBT were sanitised and crashed before adding into the green beer. Carbonation was done using keg with 1.8 bar of carbon dioxide. Physicochemical properties, microbial load and sensory evaluation of Kölsch fruit beer (KFB) were determined. All the physicochemical parameters measured were significantly ($P < 0.05$) influenced by the fermentation time. The pH dropped from 5.8 ± 0.1 to 3.9 ± 0.1 toward the end of the fermentation. There was no microbial growth when KFB was inoculated in the media. °Brix likewise decreases from 13.3 ± 0.43 to 3.86 ± 0.25 . There was a change in the colour of the wort throughout the fermentation from 11.2 ± 0.44 to 32.5 ± 0.56 EBC. A decrease from 1.48 ± 0.02 to 0.86 ± 0.02 mg maltose per 100 mL in the reducing sugar was observed during the entire period of fermentation. A total of 32 volatile compounds were identified. All assessed sensory variables of KFB were significantly different ($P < 0.05$) and preferred by the panellists, however, foaminess and clarity of KFB should have to be improved. KFB showed higher DPPH radical scavenging activity as compared to other types of beer examined due to biologically active substances contributed by SBT.

Key words: sea buckthorns (SBT), Kölsch fruit beer (KFB), sensory analysis, physicochemical and organoleptic properties.

INTRODUCTION

Beer is the world's most established and broadly consumed liquid refreshment and the third most prevalent drink after water and tea (Nelson, 2005). It is an alcoholic beverage obtained by fermentation of wort, which is a mixture of malt, hops and water (Catarino, 2010). According to Leitao et al. (2011), beer is rich in nutrients i.e. carbohydrates, amino acids, minerals, vitamins and cancer preventing phenolic compounds. Hence, moderate drinking would have a positive impact on human health.

Fruit beers have been known around for centuries. Sugar from the fruits have been utilised as a principal constituent in fermentation processes and it was routine for ancient people to mix beer with fruits using the sugar from fruits to help in the maturation process.

According to Mosher (2004), fruit beer is moderately present day creation of the 20th century. Egyptians alluded to the utilisation of dates and pomegranates, however, just a few passing references can be found for this type of beer in interceding years until the 1930's. These beers contain no real natural products, rather a mixture of light beer with a simulated natural product to give a flavour of fruits. Home brewers can do a similar thing by obtaining fruits from shops and adding a suitable amount to their beers. Some brewery industries likewise utilise fruit extract. This functions admirably for natural products like raspberry and apricots that keep up their flavour after ageing. Examples of some commercial fruits beer are New Glarus Belgian Red, Raspberry Tart, Bell's Cherry Stout, Dogfish Head Aprihop, Melbourne Apricot Beer and Strawberry Beer (Strong et al., 2008). Ducruet et al. (2017) added goji berries to amber ale type beer. They brewed at different stage of the production process with the aim of developing beverage with high antioxidant and sensory characteristics. Sour cherry has been also added to mid-young lambic beer (Gert et al., 2014). However, other authors have utilized various fruits in designing their beers (Osta, 2017).

Sea buckthorn (SBT) is a deciduous shrub, generally conveyed everywhere throughout the world, including Russia Federation. It contains various types of nutrients and bioactive substances, such as, vitamins, carotenoids, flavonoids, polyunsaturated unsaturated fats, free amino acid and basic compounds. These compounds change generously among geographical locations, however, their presence is essential for human health. Scientific reviews in the 20th century affirmed therapeutic and wholesome advantages of SBT. Studies are right now experiencing to utilise this natural product in cancer treatment, cardiovascular illness, skin issue, and gastrointestinal ulcer and as liver defensive operator (Mingyu, 1994; Li & Schroeder, 1996; Xing et al., 2002; Zeb, 2004; Ruan, 2007). The fundamental SBT processing products are juice, wine, jam, preserves, compote, and tea (from leaves) (Li & McLoughlin, 2007).

In recent years, there has been a growing demand for beer with fruit flavours. The main purpose of this study is to produce fruit beer using SBT since in present there is no single scientific publication on beer with SBT.

MATERIALS AND METHODS

Some of the materials and equipment use in brewing the KFB are presented in Table 1. A brewing software BeerSmith (version 2.3) (USA) was used in designing the Kölch beer.

Table 1. Raw materials and Equipment

| Malt | Hops | Yeast | Equipment | Disinfectants | Fruit |
|------------------------------|---------------------------------------|---|--------------------------|--|------------------|
| Château Pale Ale (4.9 kg) | Hallertauer Hersbrucker (100 g) | Kölsch yeast (Wyeast Labs #2565, UAS) | Mash tun | Ethanol (90%) | Sea buckthorn |
| Château Pilsen 2RS (2.5 kg) | Huel Melon (40 g) | | Mash paddle | Mira acid NV liquid (Mirachem Industries) | |
| Château Wheat Blanc (0.4 kg) | | | Siphon | | |
| | | | Hydrometer Containers | | |

Mashing protocol and wort production

Single infusion method was used in this study. Before starting the brewing, all the equipment was washed and sterilised using Mira acid NV liquid. 7.8 kg of malted grains were measured and milled with roller miller. The milled malt was mixed with 30 L distilled water in mash tun (hydromodule 1:3.8) and heat up to 47 °C for 20 minutes. The temperature was increased to 65 °C after 5 min with a step time of 40 min. Again after the step time, the temperature was once again increased to 78 °C after 10 minutes. Filtration was carried out and the filtrate (wort) then boiled at 100 °C for 90 minutes. 22 L of distilled water was used for sparging process. Mash Paddle was used in stirring the mixture throughout the whole process. Hallertauer Hersbrucker hops were added 60 min before the end of the boiling time. When the boiling time was over the Huel Melon hops was then added. Clean sterilised copper wort chiller was then lower into the boiled wort for cooling. With the aid of sterilised syphon, the cold wort was transferred into fermentation bucket equipped with airlock bubbler.

Pitching and fermentation

Wyeast smack-pact™ sealed with internal nutrient was purchased and activated by shaking package vigorously to release nutrient 3 h before the pitching time. 95% alcohol was use to sanitise the package before opening and pitched the wort in the fermentation bucket. Fermentation was carried out at a temperature of 18 °C for 4 weeks.

Addition of SBT

SBT (Fig. 1) was purchased from the local market in Yekaterinburg, washed with distilled water peracetic acid and sodium hydroxide and was rinsed with distilled water. It was then mashed in a sterilised plastic bowl and added into the new fermentation bucket with the 4 weeks old Kölsch green beer (KGB) for further two weeks fermentation at 18 °C.

Conditioning

Stainless steel keg was used for the carbonation. The keg was initially washed and sterilised with antiseptics (peracetic acid and sodium hydroxide) and dry for several hours before usage. The 6 weeks old KFB was transferred into the keg with the aid of sterilised syphon and pumped 1.8 bar of carbon dioxide (CO₂) from CO₂ tank fitted with an automatic gas pressure regulator that can be set at a predetermined pressure. It was then stored at 4 °C for 1 week.

Physicochemical parameters

°Brix was measured using a refractometer RSG-100ATC. pH was determined using digital pH meter (Hanna, model HI 98127). Titratable acidity (TA) was determined by titrating the sample with 0.1 N NaOH solution with 1% phenolphthalein until slightly pink colouring remains for 30 seconds. Lane-Eynon method was used to quantify the reducing sugars (ASBC, 2007). Colour was determined by measuring the absorbance at 430 nm using spectrophotometer (ParkinElmer, Lambda 35) against distilled water in cuvettes of 10 mm length.

Enumeration of Microorganisms

Using the laminar flow cabinet, a serial dilution of KFB have been performed mixing 1 mL of KFB with 9 mL of sterile peptone physiological saline solution (5 g peptone, 8.5 g NaCl, and 1,000 mL distilled water, pH = 7.0) and homogenised with the help of pipette. Decimal dilutions were plated. Aerobic mesophiles were enumerated by pour plate on nutrient agar (15 g peptone, 3 g yeast extract, 6 g NaCl, 1 g dextrose and 12 g agar, pH = 7.5) incubated at 30 °C for 3 days. Yeasts and molds were numerated by spread plate on yeast mold agar (3 g yeast extract, 3 g malt extract, 5 g peptone, 10 g dextrose, and 1,000 mL distilled water, pH = 4.0) and potato dextrose agar (200 g of sliced unpeeled potatoes boiled in 1 L distilled water, filter through cheesecloth, 20 g dextrose, 20 g agar, pH = 5.6) and incubated at 25 °C for 5 days.

Volatile compounds analyses by Gas chromatography–mass spectrometry (GC-MS)

Major volatile compounds were identified, by analysing the beer directly with no pre-treatment. GCMS-QP2010 Ultra, Shimadzu, gas chromatograph outfitted with a Mass spectrometer, and a capillary column DB-5 ms (30 m x 0.25 mm, 0.25 µm) covered with 5% diphenyl-95% dimethyl polysiloxane was utilised. Helium was used as the carrier gas at a constant flow rate of 1.5 mL min⁻¹. Without pre-treatment 1 µL of the KFB was injected in the splitless mode (vent time, 60 s) and the Compounds were identified by comparing of mass spectra obtained with Mass Spectral Library of the National Institute of Standards and Technology (NIST).

DPPH radical scavenging activity

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radicals scavenging activity was estimated by their Electron paramagnetic resonance (EPR) (Bruker Elexys E-500, X-band) spectra. The determination of antioxidant activity of beer samples was carried out by the difference in the amount of paramagnetic particles of a stable free radical, measured before and after complete proceeding the chemical reaction between a free radical and an analyte, which is accompanied by a decrease in the intensity of the EPR spectrum of the free radical after adding the analyte by the formula:

$$AOA = \frac{(n_{s1} - n_{s2}) \cdot C_{DPPH}}{n_{s1}}, \quad (1)$$

where *AOA* – the antioxidant activity (meq); *C_{DPPH}* – the DPPH concentration in an initial solution (M); *n_{s1}* – the initial amount of paramagnetic centers of DPPH, units; *n_{s2}* – the amount of paramagnetic centers of DPPH after interaction with the analyte (beers), units (Ivanova et al., 2017).

DPPH (1 mmol) was dissolved in ethanol and without pre-treatment 10 μ l of the samples were pipetted into Eppendorf tube containing 1 mL of DPPH. EPR spectra of every 30 seconds for 15 minutes were recorded during the reaction with an antioxidant using EPR spectrometer Bruker Elexys E-500, X-band.

Organoleptic properties assessment

A total of 15 panellists consisting of unequal numbers of males and females of different ages were randomly selected at the beer festival organised by the Ural craft beer association, Yekaterinburg, Russia. The flavour (aroma), clarity, colour, foaming and saturation with carbon dioxide, mouthfeel, alcohol strength and the overall acceptability of the fruit beer were evaluated according to the Hedonic Rating Scale with the following keys; Liked extremely = 9, like very much = 8, like moderately = 7, like slightly = 6, neither like nor dislike = 5, dislike slightly = 4, dislike moderately = 3, dislike very much = 2, dislike extremely = 1. Regular consumers of beer were selected for recruitment as panellists for sensory evaluation.

Statistical Analysis

Data generated were subjected to analysis of variance (ANOVA) using Origin statistical software (version 8.1) at 5% significance. All measurements were made at least in triplicate. Results were reported as means \pm standard deviations. For sensory analysis a one-way ANOVA and a post-hoc Tukey-Scheffe test were conducted to test the means results obtain at 5% significance.

RESULTS AND DISCUSSION

Reducing sugars content

The reducing sugars content in the wort is vital characteristic in the process of fermentation since yeast transform sugars to liquor, carbon dioxide, and different aggravates that impact the essence of fermented foods and drinks. The pattern in exhaustion of reducing sugars appears in the Fig. 1. There was a general diminishing in reducing sugars content from 1.48 ± 0.01 to 0.88 ± 0.01 mg maltose per 100 mL at the start and at the 4th week of the fermentation, respectively. SBT was added in green beer at the 4th week that the decreasing sugar expanded again in view of the sugars in the fruit itself from 0.94 ± 0.02 till 0.85 ± 0.03 mg maltose per 100 mL at the 5th week and 6th week before the carbonation started. The wellspring of the sugars can likewise influence on fermentation through contrasts in supplements and flavour antecedents. While the most widely recognised hotspot for beer is malted grain, brewers around the globe utilise numerous diverse starches.

The sort of sugars present in wort relies on upon the mashing temperature administrations. Monosaccharides are easier fermentable than polysaccharides and brewers all over the globe utilizes this finding in brewing especially while selecting priming sugars for carbonation. Also, this kind of sugars in wort influences on the flavor of the final beer.

For instance, ageing of wort, which is high in glucose, produces lagers with higher content of esters than typical groupings of esters (especially ethyl acetic acid derivation and isoamyl acetic acid derivation). On the other hand, wort, which is high in maltose, brings about lower centralizations of these esters. This decreasing trend was also

reported by other investigators (Demuyakor & Ohta, 1993; Villicaña & Saldivar, 2004) in sorghum beer and barley wort.

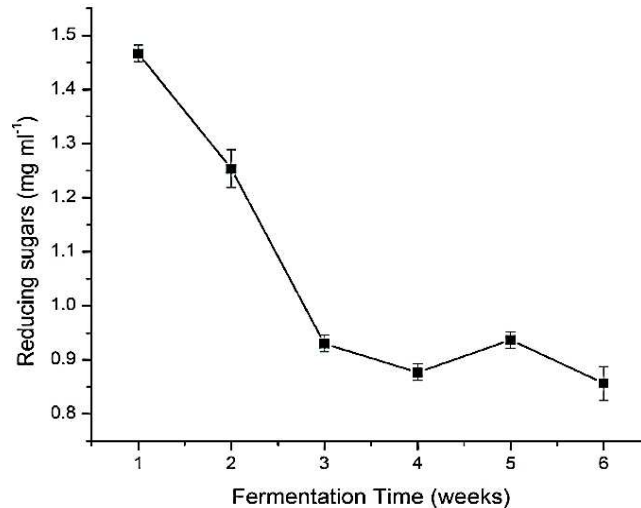


Figure 1. Change in reducing sugars over time.

pH and Titratable acidity

The pH of the wort begins to diminish as the fermentation time advances which differs statistically ($P < 0.05$). A decrease in pH from 5.8 ± 0.1 to 4.6 ± 0.1 at the 4th week was observed in Fig. 2. Additionally, there was no further diminishing in pH after the 4th week when SBT was added in the green beer. pH falls amid ageing accordingly due to utilisation of buffering materials (free amino nitrogen) by yeast and the release of natural acids (Bamforth, 2001). We realized that malt worts are the phenomenal wellspring of nitrogen, minerals, and vitamins. Wort supplies the greater part of the supplements yeast requirement for an appropriate fermentation. As the yeast take up minerals and vitamins from the wort, they begin to produce enzymes important for development and this might likewise be a factor for dropping pH (Zainasheff & White, 2010). The further drop in pH after the 5th week may be assigned to the presence of various phytochemicals (bioactive substances) in SBT which might have excreted and mixed with the green beer.

All parts of the SBT plant are thought to be a decent wellspring of substantial number of bioactive substances like vitamins (A, C, E, K, riboflavin, folic acids), carotenoids (α , β , δ -carotene, lycopene), phytosterols (ergosterol, stigmaterol, lansterol, amyryns), natural acids (malic and oxalic acids), polyunsaturated unsaturated fats and some fundamental amino acids (Beveridge et al., 1999; Yang & Kallio, 2001; Pintea et al., 2005). The hops (Hallertau-Hersbrucker and Huel Melon) used in this study may likewise be one of the elements that contribute in the mode of a change in pH since they contain alpha and beta acids in various amounts. As pH values of the beer diminishes over the normal range from 4.5 to 3.9 there is expanded protection against microbial deterioration, colloidal strength, froth stability (for reasons which were not completely comprehended), diminished flavour solidness, sense of taste, smoothness and drinkability (Melm et al., 1995).

TA is one of the essential parameters for beverages analysis, aside from carbonic acids content. There was a radical increment in the TA from an underlying estimation of $0.65 \pm 0.04\%$ to $2.18 \pm 0.03\%$ of the last month of the fermentation. The dependences of these parameters on fermentation time were shown in Fig. 2. From the general dependable guideline, the lower a pH value, the higher TA. However, not specifically corresponded to this trend, which was mentioned in this study and can be affirmed from Fig. 2. Generally speaking, TA well correlates with the 'acid taste' of the beer. The acidic substances of beer are of incredible hugeness for the protection and tactile attributes (Rajković et al., 2007). TA most likely increases during fermentation processes due to that the substrates in wort are spent and metabolites (lactic, malic, citric, acidic acids) start to aggregate subsequently affecting TA.

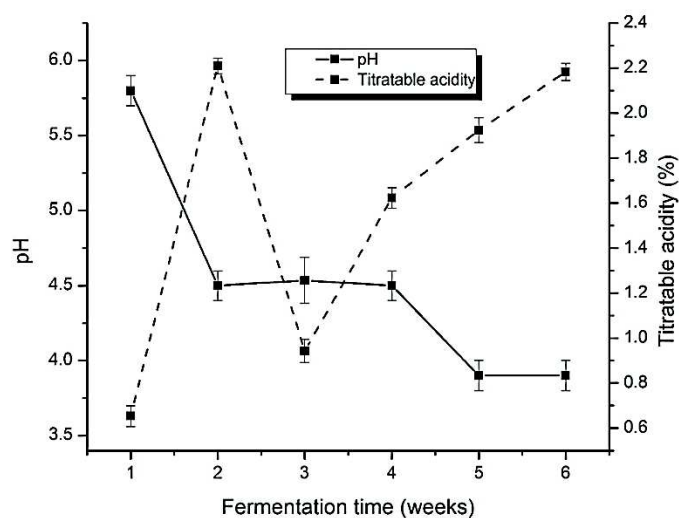


Figure 2. Change in pH and titratable acidity over the fermentation period.

Volatile compounds

Yeast can create 500 distinctive flavour and aroma substances (Zainasheff & White, 2010). Yeast metabolites, which impact beer flavour, consist of natural acids, medium chain-length (8–10 carbon particles) aliphatic alcohols, sweet-smelling alcohols, esters, carbonyls and different sulfur-containing compounds (Boulton & Quain, 2001).

A total of 32 volatile compounds were identified by gas chromatography in the beer during maturation and presented in Table 2. There were 10 acids, 6 alcohols and 16 esters. After adding SBT berries to the beer on the stage of its maturation, more volatile compounds were identified due to the fact that SBT plant itself contained a considerable number of compounds with therapeutic effect. These compounds gave an impact in flavour, pH, TA and mouthfeel. For instance, the acidic acids, such as propanoic and isobutyric acids as well as others contributed fundamentally in pH of both the green and final beer.

Mostly, volatile acids can contribute with vinegary, mushy, and fatty smells and, in addition, provides with sharpness, stringency and rancidity (Pinho et al., 2006). In the present study 10 of such acids were identified.

Esters significantly impact the character of beer. They contribute in the fruity fragrances and flavours of beer. According to Meilgaard (1975), even the ‘cleanest-tasting’ lagers contain esters in quantities up to 50. With no esters a beer would appear to be very insipid. The esters of ethyl acetic, ethyl caproate, and isoamyl acetic acids in beer appear due to a specific yeast strain activity and ageing conditions (Zainasheff & White, 2010).

Table 2. Volatile compounds found Kölch fruit beer

| | |
|--------------------------------|---------------------------------|
| 2-methyl-3-Buten-2-ol | Hexanoic acid |
| 3-methylButanal | Ethanol |
| 3-methyl-1-Butanol | Fusel oil |
| Propanoic acid | Ethyl esters |
| Isobutyric acid | Octanoic acid |
| Butyl isobutyrate | Ethyl butanoate |
| Butanoic acid | Ethyl caprylate |
| 1-methylbutyl ester | 2-Methylbutyl isobutyrate |
| 3,7-dimethyl-1,6-Octadien-3-ol | Isovaleric acid |
| Acetaldehyde | Ethyl isovalerate |
| Ethyl aldehyde | Isoamyl acetate |
| Ethyl Acetate | Bicyclo[4.2.0]octa-1,3,5-triene |
| Acetic acid | Pentanoic acid |
| Ethyl ethanoate | Isopentyl pentanoate |
| Isoamyl alcohol | 3-Methylbutanoic acid |
| 2-methylButyraldehyde | Caprylic acid, Ethyl octanoate |

Volatile natural compounds are typical constituents of many matured nourishments and drinks and are classified as esters, alcohols, aldehydes, acids, terpenes, ketones, sulphur mixes, amines, phenols etc. (Cortacero-Ramírez et al., 2003; Lui et al., 2005). The greater part of these organic constituents do not influence on beer, whereas there are various pathways to generate numerous different compounds which spill out of the cell and affect beer flavour. Volatile compounds are seen as fundamental beer constituents in brewery business as they affect the nature of beer and upgrade purchaser acknowledgement (Erny et al., 2009; Lui et al., 2005). Similar volatile compounds were identified by Shale et al. (2013). Some of these compounds had positive impact on fragrance (i.e. esters), whereas others affect negatively (i.e. fusel oil).

°Brix

The °Brix gives the information of sugars and substances content in the wort. A general decrease in their quantities was observed throughout ageing due to their utilisation by fermenting yeast (Fig. 3) which differs statistically ($P < 0.05$). A decrease from 13.3 ± 0.43 to 3.8 ± 0.25 °Brix can be caused by consumption of sugars that was mentioned in early work by Avicor et al. (2015).

There was no supplementary decrease in °Brix observed after addition of SBT presumably because no discharge of sugars from the organic product (SBT) in the green beer. Sugar utilisation started quickly after pitching and the most elevated rate of sugar consumption may happen after the cell biomass has achieved maximal density. From Fig. 3 °Brix decreased drastically from 13.3 ± 0.43 to 3.9 ± 0.25 during 3 weeks of the ageing time. The catabolism of sugars gives yeast energy and carbon skeletons for

anabolic pathways. This basic activity results in an extensive decline of the solutes in the wort (Briggs et al., 2004). Embden-Myerhof-Parnas cycle is the real sugar catabolic pathway in yeast, functions under both oxygen and anaerobic conditions and is the cause by which around 70% of exogenous sugars are absorbed (Briggs et al., 2004).

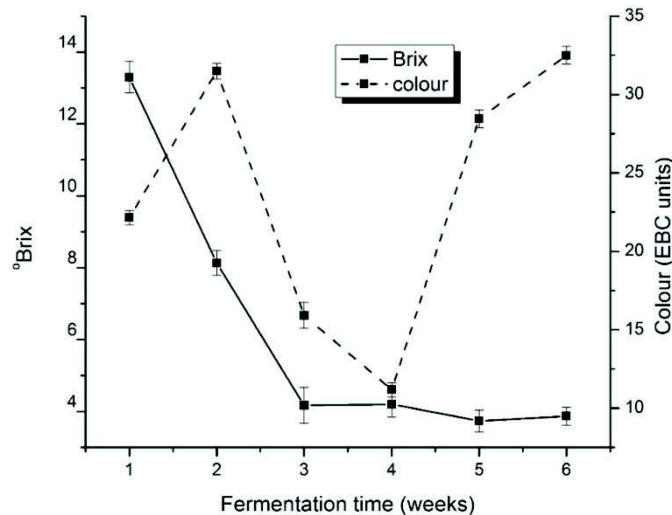


Figure 3. °Brix and colour of wort vs. fermentation time.

Colour

We observed an increase in colour measured in EBC units after the 1st week of maturation which are statistically different ($P < 0.05$). This fact can be explained by extraordinary fermentation and massive foam production accompanying with evolving yeast metabolites. However, in the next two weeks there was a decrease in colour due the consumption of the specific solutes in the wort resulting in the less intensive fermentation accompanying with lower foam production. The addition of SBT was found to increase the colour of the Kölch green beer. It should be noticed that SBT contains fermentable sugars and other substances that subsequently increase in gravity of the green beer at the 4th week. However, colour continues to develop until the 6th week (Fig. 3).

In food and beverage products, the consumers regularly survey the nature of products by their colour and appearance. The colour and appearance of products are essential characteristics of their quality (Lawless & Heymann, 1998; Cao, 2013). They affect the impression of different qualities, i.e. smell and flavour (DuBose et al., 1980).

Most consumers expect to taste clear beer; they may presume low quality and reject beer that is not clear (Cao, 2013). Producing clear beer is a fundamental prerequisite of all brewers. The outcomes were in concurrence with the early report by Cao (2013).

Microbial analysis of fruit beer

No microbial growth was observed on the plated Petri dishes inoculated with KFB on different media.

Strict hygienic conditions were observed in the whole brewing processes by utilizing a sanitised equipment. Processes such as mashing, wort boiling, filtration,

aseptic bottling, conditioning and cold storage in the brewing decrease the potential for microbial contamination.

Different parameters are vital for the microbial stability of beer and include pH, concentration of hops bitterness, content of ethanol, CO₂, SO₂, natural acids, acetaldehyde and different metabolites as well as supplements and storage temperature (Jespersen & Jakobsen, 1996). In our study pH and ethanol content of final beer were found to be 4 and 5.2%, respectively. Natural acids such as octanoic, caprylic acid and hexanoic acids as well as hops (Hallertauer Hersbrucker, Huel Melon) of KFB may be the factors which inhibit microbial growth in the plated dishes.

The primary contaminants originate from the raw materials and brewery equipment, whereas the secondary contaminants are acquainted with the beer before packaging and kegging (Moretti, 2013). In the research (Sarlin et al., 2005) there has been reported that usually grains can be contaminated with *Fusarium graminearum* and *F. moniliforme* resulting in gushing (spontaneous ejection of beer from its container) of beer. The barley used in this study was acquired from credible producers in Germany who are guarantees of its microbial safety.

The use of clean water in brewery cannot be underestimated. The fundamental concern is the presence of spoilage organism in water used for brewing, for instance, dilution of beer after high gravity fermenting or from vessels rinsed with contaminated water (Hill, 2009). Filtered water was used in the entire brewing process and it assists to avoid introducing contaminated water into wort/beer. However, boiling also helps to eliminate contamination.

Hops are known for their antibiotic properties. As known, the majority of gram positive microorganisms are inhibited by hops, despite the fact that gram negative organisms are unaffected (Hill, 2009). The usage of two sorts of hops in brewing KFB provided higher antibiotic properties to inhibit microbial contamination.

Sensory analysis

The final KFB brewed within 6 weeks was evaluated for consumer acceptability using 15 panellists. The average values of the analysis were used to plot a radar chart presented in Fig. 4, which consists of the sequence of equi-angular spokes, representing 7 variables. All the variables assessed were significantly different ($P < 0.05$).

Esters and alcohols identified in the present study were found to contribute to aroma (8.60 ± 0.82) with a higher average score 9. It is obvious for an aroma variable. The hops in the present study were used for a dual purpose as flavour and bitterness contributors. Nevertheless, SBT in its turn contain some natural aroma substances as well. The responses from the panellists with respect to mouthfeel were also impressive and can be confirmed by score 8 for this variable in the chart. The mouthfeel (8.40 ± 0.63) is important to the aroma when it comes to beer products as they correlate. The filtration processes during the wort production affect the clarity of KFB, because poor filtration results in non transparency of beer. KFB obtained after the 4th week was transparent enough, but it was altered after the mashed SBT was added providing with score 7 for this variable in the chart.

The average score for foaminess (6.86 ± 2.20) was good, although not excellent like for other variables, which indicate that 1.8 bar CO₂ utilised for the carbonation was not sufficient enough to replace the lost CO₂ in the KFB. CO₂ content needs to be increased up to 2 bar to achieve excellent foaminess.

The amount of alcohol in KFB is assessed by alcoholic strength. The score 8 of alcohol strength (8.40 ± 0.73) on the chart indicates the acceptance of the alcohol percentage (5.2%) by the panellists. 13 out of 15 testers assessed alcohol strength of the beer brewed as extremely similar to calculated one by BeerSmith (version 6.0).

The colour (7.92 ± 0.79) of the final beer is very important for both a brewer and consumers. Pleasant colour will attract more consumers and the brewer will earn more profit in return. SBT contributed to colour of KFB and panellist generally liked it. The score 8 on the radar chart supports this point (Fig. 4). 4 panellists marked score 9 (liked extremely) while 6 assessors gave score 8 (like very much), 7 – (like moderately) for this variable.

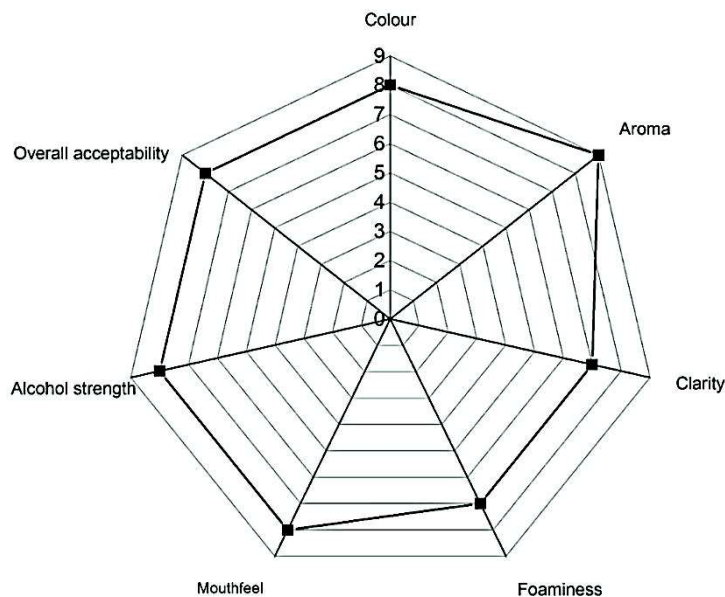


Figure 4. Average hedonic scores of sensory analysis of KFB brewed within 6 weeks.

The overall acceptability (8.33 ± 0.72) are utilised for various goals in a product research, such as product, product optimisation, new product development, appraisal of market potential. Overall acceptability rating of KFB was between 9 (like extremely) and 8 (like very much), with the exception of one score 7 (like moderately). The acceptability was impressive judging from the panellists' scores given on the heptagon radar chart (Fig. 4). There was no statistically significant difference ($P > 0.05$) between variable means except for foaming and aroma ($P < 0.05$).

Antioxidant activity

Antioxidant activity of any substance is defined as its ability to capture free radicals. It is a vital guard of living systems, contrary to oxidative stress (Valko et al., 2006; Oliveira et al., 2009). Diseases like cancer, diabetes, cardiovascular disease, Alzheimer's disease, and other neurodegenerative disorders are caused by oxidative stress (Halliwell, 1994; Giacco & Brownlee, 2010). Numerous antioxidant compounds have anti-inflammatory, antiatherosclerotic, antiproliferative, antimutagenic, anticarcinogenic, antibacterial, or antiviral activities to a more noteworthy or lesser

degree (Liu et al., 2002; Ratnam et al., 2006). Fruit juices, beverages and hot drinks contain high amounts of antioxidants, like polyphenols, vitamin C, vitamin E, Maillard reaction products, β -carotene, and lycopene (Ramadan-Hassanien, 2008).

Beer is considered as one of the main sources of antioxidants to consumers. The amount and composition of the antioxidants vary not only on the qualities of the materials, also on the brewing processes. For example, grains and malt are the principal source of antioxidant compounds in beer and hops contribution is lower (Jurková et al., 2012).

Antioxidant activity of KFB in the comparison with two other industrial alcoholic beverages (Nectar from Bosnia and Baltica from St Petersburg, Russia) was determined using EPR spectroscopy of free radicals.

The DPPH radical scavenging activity of the alcoholic beverages is shown in Fig. 5. KFB sample showed higher DPPH radical scavenging activity of $1.53 \times 10^{-4} \text{ mol} \times \text{equ}$ ($R^2 = 0.93$) than Nectar beer $1.16 \times 10^{-4} \text{ mol} \times \text{equ}$ ($R^2 = 0.69$) and the least Baltica beer $9.85 \times 10^{-5} \text{ mol} \times \text{equ}$ ($R^2 = 0.96$). The higher antioxidant activity of KFB is thought to be caused by incorporating SBT, which possesses its own antioxidant activity due to presence of essential substances mentioned above.

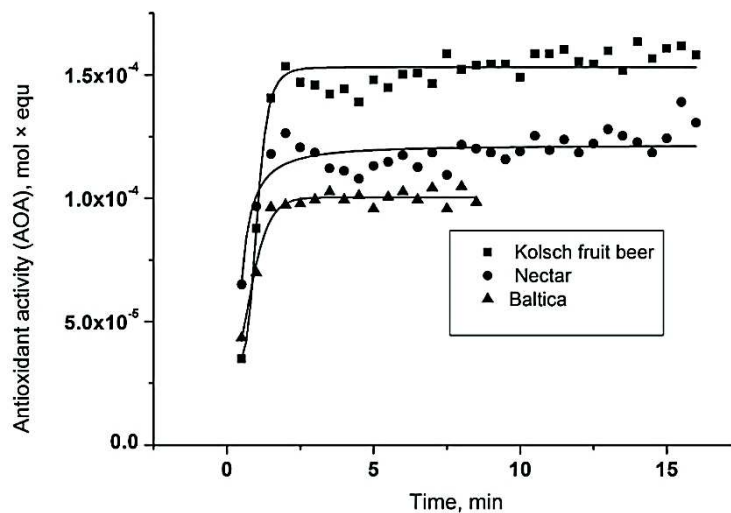


Figure 5. DPPH radical scavenging activity of different beers vs. time.

CONCLUSION

The present study provided information on the use of SBT in producing fruit beer. Fermentation time had an influence on the pH, reducing sugars, °Brix, colour and TA. There was a decrease in reducing sugar content and °Brix as the fermentation proceeded until when the SBT has added that it increase and decreased again. A total of 32 volatile compounds were identified, 10 acids, 6 alcohols and 16 esters. KFB showed higher DPPH antiradical activities than two other alcoholic beverages, which were used for comparison. Panellists generally had a higher preference for the KFB because of the taste and flavour contributed by SBT.

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