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ORIGINAL ARTICLE

Red rice conjugated with barley and rhododendron extracts for new variant of beer

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Abstract This study aimed to determine the effect and potential of red rice in conjunction with barley and rhododendron extracts to develop a new variant of beer. In this study red rice, barley, and rhododendron extracts were used in different combinations and the best combination was selected based on quality and sensory characteristics. The results showed that the developed beer was rich in antioxidant activity (47.68 ± 0.96) and contained a good amount of anthocyanin (35.12 ± 0.79), flavonoids (0.119 ± 0.002), and polyphenols (0.410 ± 0.002). The red rice has more dietary significance than that of polished or milled rice, further, the use of rhododendron provides a large number of secondary metabolites such as tannins, saponins, alkaloids, tannins, and flavonoids. Besides, the sensory profile of the developed beer was quite distinct in terms of aroma, taste, and color from other alternatives available in the market.

Keywords Beer · Rhododendron · Red rice · Barley malt · Sensory characteristics

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Introduction

Beer is one of the oldest, low alcoholic, universally consumed beverages produced by the alcoholic fermentation with yeast that leads to hydrolysis of starch into maltose and other sugars, and finally to alcohol (Piazzon et al. 2010). Beer fermentation has 3 phases, initial phase, main fermentation and maturation (Bokulich et al. 2012). The I phase is dominated by Enterobacteria, including *Klebsiella*, *Enterobacter*, *Escherichia*, *Citrobacter*, *Serratia* and *Pectobacterium* and non-*Saccharomyces* yeasts, primarily *Kluyveromyces* in lambic and *Rhodotorula* in coolship ale. When wort pH decreased and ethanol concentration increased flavor. The *Saccharomyces* cells decreased after consumption of all the sugars and gradually replaced by *Brettanomyces* spp. mainly *B. bruxellensis* during maturation (Martens et al. 1992). The main *Saccharomyces* species used for beer preparation are *S. cerevisiae*, *S. kudriavzevii*, *S. pastorianus*, *S. bayanus* and *S. eubayanus* (Capece et al. 2018).

Traditionally, barley *(Hordeum vulgare)* is the main ingredient in beer, followed by hops, and water. Hops incorporation enhances beer taste, reduces pH and has antibacterial activity, however, ineffective against brewer's yeast (Humia et al. 2019). The beer flavour originated from innate chemical volatile compounds of the barley malt, hops and yeast metabolism. The volatile compounds of beer are composed mainly of an aliphatic and aromatic alcohol, esters, organic acids, aldehyde, carbonyl compounds and terpenic substances. Controlled intake of bio compounds present in beer, such as flavonoids and other phenolic compounds dramatically reduce the risk of developing cardiovascular disease, cancer and have antioxidant protective effect (Humia et al. 2019).

Table 1 Grist ratio of red rice and barley malt and with rhododendron extract in worth

Grist treatment	Red rice %	Barley malt %	Water %	Hops % (gm/L)	Rhododendron extract (mL/L)	Mash grist ratio (R:B:W)
1	1	2	9	1	4	1:2:9
2	2	1	9	1	4	2:1:9
3	1	1	6	1	4	1:1:6

R:*B*:*W* rice:barley:water

For the development of beer variants, adjuncts like rice (*Oryza sativa*), other cereals, extracts of aromatic herbs, honey, and others are nicely combined to generate unique flavours, taste, mouth-feel and bitterness. Such addition enhances the quality, shelf life, antioxidant activity, total phenolic and flavonoids content of brewed beer. The chemical composition of rice, especially the high starch content, makes this cereal also perfectly suitable for brewing (Briggs 1998). Mallawarachchi (2016) developed a beer variant with optimized levels of rice (30.75%) and mashing temperature (62 °C). The process of making malt from raw barley followed by the different steps which begin with the cleaning and grading, drying, steeping, germination, kilning, roasting, and end with milling (Asare et al. 2011), whereas in rice, the hydrolysis is carried out by the α-amylase enzyme which increases extract yield and also reduces production costs. Agu (2002) reported that brewing with red rice has a unique flavour and aroma. Red rice is rich in water-soluble vitamins and pigments, often less expensive and rich sources of carbohydrates, energy, thiamine, pantothenic acid and folic acid. Zhang et al. (2017) uses extruded rice as adjunct and reported a higher level of flavouring compounds than traditional beer.

Enrichment of beer with the extracts of medicinal plants imparts unique sensory characters with an increase in the concentration of bioactive compounds (Ducruet et al. 2017). Plants contain a variety of phenols which hold different biological activities [antioxidant, anti-inflammatory, antiviral and antibacterial] (Sharma et al. 2016). Different researches show that phenols in plants can minimize the risks of diabetes, cancers, osteoporosis, cardiovascular diseases and others. Due to the presence of these lucrative components, researchers suggest the recovery of natural antioxidants from natural sources to utilize these active compounds as functional ingredients in food industries (Galanakis 2018, 2013). The presence of bioactive compounds in natural sources has attracted high interest by consumers due to its high nutritional value. For fluid food, thermal pasteurization or sterilization are the important processes which ensure the food safety, shelf life (destruction of enzymes and microorganisms) and to maintain the natural quality of food (Zinoviadou et al. 2015). The variety of rice with a red aleurone layer is called red rice and its red colour is due to the presence of the pigment proanthocyanidin (Raghuvanshi et al. 2017). Rhododendron (*Rhododendron arboreum*) is rich in bioactive compounds and widely distributed in the Himalayan region with medicinal benefits in various traditional treatments of health conditions like heart disease, diarrhoea, blood dysentery and reduces the oxidation processes in the body by protecting against reactive oxygen species (Chawla et al. 2019). Flowers of rhododendron contain secondary metabolites *i.e.* alkaloids, saponins, tannins, flavonols and others in good concentration and anthocyanins, are the major pigments present in flowers. Recent research revealed that this pigment 'anthocyanin' has the potential to prevent heart diseases and cancer due to the presence of powerful antioxidants (Barba et al. 2015). Vitamin C (ascorbic acid) is a secondary antioxidant which prevents pigment peroxidation and lipid oxidation (Galanakis et al. 2018a, b, c). The extract of flowers can be used as natural food colouring, flavouring agent which is widely utilized in the development of healthcare products and for new product differentiation. Beer, as an absolute natural beverage, holds numerous beneficial effects on the human body such as in preventing the risk of coronary heart disease and to eliminate the risk of pancreatic cancer in the body, if consumed in moderation (De et al. 2016). It is a plentiful source of carbohydrates, amino acids, vitamins, organic acids, fermentation metabolites, phenolic compounds *i.e.* flavanols, flavonoids and others, which contribute flavour, aroma, colour to beer and also enhance the antioxidant activity (Zhao et al. 2010). The addition of antioxidants (tocopherols, ascorbic acid and polyphenols) from natural bioresources sound healthier to the consumers (Galanakis et al., 2018a, b, c and Kovačević et al. 2018).

Beer enriched with extracts of medicinal plants imparts functional concentration of its bioactive compound and new sensory feature. Nowadays the role of adjuncts in brewing has also been reported by many authors (Kumar et al. 2019). Red rice and rhododendron may serve as suitable adjuncts due to their health beneficial properties. Therefore, the present study was designed to develop new technology for preparation of functional beer possibility using adjuncts *i.e.* red rice with extracts of rhododendron in brewing at different ratios to obtain unique flavour, colour, taste, and aroma, with enhanced nutritional and therapeutic properties.

Materials and methods

Raw materials

Raw materials *i.e.* barley and domestic beer sample used as control were procured from the local market of Solan (Himachal Pradesh, India), red rice was procured from Rohru (Shimla, Himachal Pradesh, India), rhododendron (red colour) was harvested from Shimla hills to make extracts, and hops Pellets Cascade 7.8% by DVKSP Impex private limited (Batch number: P91-AIUCAS0028) and yeast culture (*Saccharomyces cerevisiae* Carlsbergensis) was obtained from Mohan Meakin (Solan, HP, India) and maintained at 4–7 °C. The amylase enzyme used was procured from Triton Chemicals (Mysore, India).

Malt production

Barley

The malt was produced by processing of barley by the malting process which began with pre-washing, washing to remove any remaining loose debris and inferior grains and further followed by steeping, germination, and kilning. During steeping, barley grains were immersed in water at 20 ± 1 °C for 6 h. It increased grain volume by 33% and moisture up to 40–47% which facilitates grain germination. Grains were transferred to germination vessels as a slurry for 3 days for the growth of the embryo of the grain, manifested by the rootlets growth and increase in the length of the shoot. After germination kilning was carried out to stop germination and enzymatic activity to stop undesirable flavours and chemical compounds. In kilning green malt was heat-treated for removal of moisture from germinated grain to 4–5% by oven drying at 80 °C for 24 h, the dried grains were then ground into powder (Gomaa2018).

Hydrolysis of red rice (Oryza sativa)

The hydrolysis of red rice started with steeping rice in water for 1 h at 25 °C after that cooking of rice was done for 40 min. at 90 °C and cooled to 70 ± 1 °C to preserve colour pigment in red rice. According to Galanakis et al. (2012), heat treatment (> 60 °C) can destroy the matrix or functional compounds such as anthocyanins and polyphenols. The rice was mashed as starch was gelatinized and then filtered through a muslin cloth and treated with 1% alpha-amylase at 60 °C for 24 h. Alpha-amylase breakdown the starch into sugars and the obtained mixture was rice wort which further mixes with barley powder at the time of mashing (Singh and Ali 2006).

Experimental design

The barley malt, adjunct red rice wort and water were mashed in different grit combinations ratio of 2:1:9, 1:2:9 and 1:1:6, respectively. Water was heated to a temperature of 65 °C and barley malt and hydrolyzed rice wort was added while maintaining the temperature. The temperature was then increased to 75 °C and hops (cascade pellet with 7.8% alfa acid) were added (dry hopping) with continuous stirring and heating for 60 min. The boiled wort was cooled to room temperature and filtered with nylon filter membrane after that filtered wort was inoculated with a starter culture of *S. carlsbergensis* at the rate 5% with rhododendron extract *i.e.* 4 mL/L is added which is selected on the basis of sensory evaluation then the fermentation was carried out for 7–8 days and then the final brewed beer was filtered (Nylon filter membrane) and clarified. The filtered clear liquor was pasteurized at 60 °C for 15 min, packed in glass bottles and stored under refrigerated conditions for further studies.

Analysis of developed beer

Raw material and beer were analyzed for pH, specific gravity, titratable acidity, ascorbic acid, total phenols, ethyl alcohol, beer bitterness, carbohydrates content, protein content, and 2,2-diphenyl-l-picryl hydrazyl (DPPH) radical scavenging assay. The pH of prepared beer was analyzed using DELUXE pH meter (METTLER TOLEDO). Before measurement, the instrument was calibrated with buffer solutions of pH 4, 7 and 9. The specific gravity was measured using a hygrometer. The titratable acidity was estimated by titrating a known aliquot of the sample against N/10 NaOH solution using phenolphthalein as an indicator. The titratable acidity was determined and expressed as per cent citric acid AOAC (1980). The ascorbic acid and total phenols were determined as per literature Thimmaiah (1999). The total and reducing sugars were determined by Lane and Eynon's volumetric method from Ranganna (1986). The total anthocyanin content was determined as per the method of Giusti and Wrolstad (2001). The ethyl alcohol content in the beer samples was determined by the colourimetric method given by Caputi et al. (1968). The bitterness in beer was calculated by Analytica-EBC (2010). The carbohydrate content was estimated using anthrone reagent as per literature Yemm and Willis (1954). The protein content was determined using Lowry's method and the radical scavenging activity of beer was determined using 2,2 diphenyl 2 picrylhydrazyl hydrate (DPPH) method (Brand-Williams et al. 1995).

Sensory analysis

Prepared beer and a market beer were evaluated on nine points hedonic scorecard for organoleptic characteristics like colour, appearance, aroma, acidity, sweetness, body, bitterness, astringency and overall impression of the beer by a panel of judges. Ten trained sensory panellists (Scientists, Shoolini University, Solan, HP, India), judged the developed beer. According to defects, if any, the scores were reduced. For Sample scoring, a mean value of 6 and above were taken as acceptable.

Statistical analysis

The observations of quantitative estimation of physico-chemical parameters were determined in triplicate and presented in mean ± standard deviation (SD). Microsoft Excel, 2016 (Microsoft Corp., Redmond, WA) was used for the calculation of standard deviation (SD). Statistical difference in terms of significant and non-significant values was confirmed by one way and two-way analysis of variance and comparison between means was completed by critical difference value (Kaushik et al. 2018).

Results and discussion

Chemical characteristics of rhododendron (Rhododendron arboreum)

Data pertaining to chemical parameters viz. TSS, pH, acidity, total sugars, reducing, non-reducing sugars and brix/acid ratios of rhododendron flowers are presented in (Table 1) Table 2. The TSS and pH of rhododendron were 9.82°B and 3.06, respectively. The total sugar and reducing sugars were 5.72 and 2.02%, respectively, whereas, titratable acidity (%), ascorbic acid and total phenols were 1.16% citric acid, 29.12 (mg/100 g) and 28.14 (mg/100 mL), respectively. Total anthocyanins content was 35.2 (mg/L). Results obtained from our study were almost similar to the investigation of Solanki et al. (2013) who reported the TSS and acidity of fresh petals as 8.5 and 2.69, respectively. Similar results regarding TSS (6.82°B) and pH (3.02) were reported by Bhatt et al. (2017). Solanki et al. (2013) reported higher values for reducing, non-reducing and total sugar, however, it might be due to that the values presented by them were on a dry basis and our values are on a wet basis. They reported 1.19% acidity, 33.12 mg/100 g ascorbic acid, and 29.67 mg/100 mL anthocyanins content and total phenol content was 34.14 mg/100 mL.

Table 2 Chemical characteristics of rhododendron

Characteristics	Value
TSS (°B)	9.82 ± 0.12
pH	3.06 ± 0.02
Titratable acidity (%)	1.16 ± 0.13
Ascorbic acid (mg/100 g)	29.12 ± 2.03
Total sugar (%)	5.72 ± 0.19
Reducing sugar (%)	2.02 ± 0.11
Total phenols (mg/100 mL)	28.14 ± 0.43
Total anthocyanins (mg/100 mL)	35.2 ± 1.42
Total Proteins (mg/100 mL)	1.39 ± 0.09

Data presented is mean ± SD (n = 3)

Table 3 Chemical characteristics of barley and red rice

Characteristics (mean ± SD)	Barley	Red rice
Moisture (g/100 g)	23.29 ± 0.11^{a}	11.31 ± 0.29^{b}
Ash (g/100 g)	1.29 ± 1.0^{b}	1.49 ± 0.43^{a}
pH	3.16 ± 0.01^{a}	3.18 ± 0.09^{a}
Titratable acidity (%)	0.33 ± 0.44^{a}	0.04 ± 0.07^{b}
Ascorbic acid (mg/100 g)	2.0 ± 0.01^{a}	1.31 ± 0.02^{b}
Protein (%)	5.02 ± 0.05^{a}	0.95 ± 0.06^{b}
Total sugar (%)	8.08 ± 0.57^{a}	3.76 ± 0.19^{b}
Reducing sugar (%)	0.23 ± 0.02^{b}	1.02 ± 0.01^{a}
Total phenols (mg/100 mL)	78 ± 3^{a}	48 ± 2^{b}

Data presented is mean ± SD (n = 3)

$^{a-b}$Means with the different superscript in a row are significantly different ($P < 0.05$) from each other

Table 4 Effect of grist combination on specific gravity of beer

Grist combination	Specific gravity	
	Original gravity	Final gravity
1:1:3	1.035 ± 0.04^{aA}	1.013 ± 0.03^{aB}
2:1:3	1.038 ± 0.06^{aA}	1.010 ± 0.07^{aB}
2:1:3	1.041 ± 0.05^{aA}	1.008 ± 0.06^{aB}

Data presented is mean ± SD (n = 3)

$^{a-b}$Means with the different superscript in a column are significantly different ($P < 0.05$) from each other

$^{A-B}$Means with the different superscript in a row are significantly different ($P < 0.05$) from each other

Chemical characteristics of barley and red rice

The chemical characteristics of barley and red rice are presented in Table 3. Barley showed higher moisture, pH, titratable acidity, ascorbic acid, protein, total sugar and total phenols in comparison to red rice. Red rice showed higher ash content and reducing sugar in comparison to barley. A similar composition was reported for the red rice by Raghuvanshi et al. (2017). The slight variations in the physicochemical parameter might be due to environmental, genetic and biological factors.

Specific gravity

Specific gravity is a widely used method for screening or scrutinizing the wort for beer standardization. The gravity before starting of fermentation is referred to as the original gravity and the measurement after completion of fermentation known as final gravity. This change in the specific gravity is due to yeast fermentation which leads to hydrolysis of starch into maltose and other sugars, and finally to alcohol. Ale beer has original gravity around 1.040, and the final gravity is 1.029 (Preedy et al. 2009). In this study, specific gravity showed a similar trend and also have significant difference among the beer prepared using different grit combination as shown in Table 4 among the various treatments, the original gravity ranged from 1.041 to 1.035 in wort and final gravity ranged from 1.013 to 1.010 at the end of the fermentation.

Effect of different grit ratio on the physicochemical parameter of beer

The data presented in Table 5 shows that there was a significant difference among the beer prepared using different grit combinations with extracts. Among the various treatments, the pH content ranged from 3.61 to 4.02 and highest in the control sample and lowest in grist combination (2), wherein grist combination (1) and (2), pH was 4.02 and 3.87, respectively. Results obtained from our study are almost similar to Cooper et al. (2016) and Pai et al. (2015). In general, the pH ranges from 3.90–4.20 for lager beer in the brewing industry which plays a role in the protein coagulation, hop utilization, effectiveness for enzymes and for making clear beer. Further, the results of pH were in corroboration with the titratable acidity of beer which is also a major factor in brewing industries in terms of chemical stability, colour, odour, and taste. The titratable acidity of beer is affected by the fermentation process, as well as by the quantity and quality of raw material added to the wort, which increases or decreases pH and titratable acidity of the beer. Among the various treatments, the titratable acidity ranged between 0.0973 and 0.1220%. The highest titratable acidity in beer was observed in grist combination (1), whereas, the lower titratable acidity in combination (2), 0.1020 in combination (3) and 0.1113 in the control sample. According to the investigation of Tusekwa et al. (2000), the total acidity of beer was between 0.06 and 0.28%, respectively.

Hydrolysis of starch into maltose and other sugars by the action of yeast leads finally to alcohol and naturally liberated carbon dioxide. The alcohol content was significantly different among all the treatments which might be due to the differences in the composition of other nutrients

Table 5 Effect of different grit ratio on physicochemical parameter of beer

Characteristics (mean ± SD)	Control	1:1:3	2:1:3	2:1:3
pH	4.12 ± 0.06^{b}	3.47 ± 0.08^{a}	3.61 ± 0.07^{ab}	3.87 ± 0.10^{b}
Titratable acidity (%)	0.0913 ± 0.001^{a}	0.1220 ± 0.002^{b}	0.1020 ± 0.002^{a}	0.0973 ± 0.001^{a}
Alcohol (%)	5.63 ± 0.44^{b}	4.17 ± 0.36^{a}	3.85 ± 0.52^{a}	4.02 ± 0.41^{a}
Total phenols (mg/mL)	0.313 ± 0.008^{a}	0.410 ± 0.002^{c}	0.377 ± 0.016^{b}	0.340 ± 0.002^{a}
Protein content (mg/mL)	4.12 ± 0.04^{c}	3.90 ± 0.021^{c}	2.87 ± 0.05^{a}	3.35 ± 0.002^{b}
Carbohydrate content (mg/mL)	40.87 ± 0.702^{d}	37.3 ± 0.42^{c}	32.87 ± 0.702^{a}	35.2 ± 0.42^{b}
Reducing sugars (mg/mL)	1.992 ± 0.012^{b}	1.116 ± 0.003^{a}	1.113 ± 0.021^{a}	1.108 ± 0.001^{a}
Flavonoids (mg/mL)	0.097 ± 0.001^{a}	0.112 ± 0.001^{b}	0.119 ± 0.002^{b}	0.115 ± 0.003^{b}
Total anthocyanins (mg/L)	8.18 ± 0.64^{a}	25.34 ± 0.71^{b}	35.12 ± 0.79^{d}	29.11 ± 0.53^{c}
IBU-bitterness unit	19.03 ± 0.39^{c}	16.2 ± 1.26^{b}	13.9 ± 0.97^{a}	14.7 ± 0.06^{a}
DPPH (scavenging activity) %	27.11 ± 1.47^{a}	46.47 ± 1.52^{b}	47.68 ± 0.96^{b}	45.12 ± 1.06^{b}

Data presented in table is mean of three values ± SD

$^{a-d}$Means with the different superscript in a row are significantly different ($P < 0.05$) from each other in wort and conditions during fermentation. The alcohol content in various treatments ranged from 3.85 to 4.17 and 5.63% in the control sample. Among all the treatments the highest alcohol content observed in grist combination (1) was 4.17% and the lowest 3.85% in the grist combination (2), whereas, in the grist combination (3) alcohol content was about 4.02% in it. Results obtained in the present study are similar to as reported by Han et al. (2016); Cooper et al. (2016) and Pai et al. (2015).

Polyphenolic components in beer were mainly derived from barley malt, red rice, rhododendron extracts and hop which influence the colour, taste, bitterness of final ferment beer and these polyphenolic components claimed to be a good source of antioxidants as investigated by Leitao et al. (2012). While the red rice and extracts of rhododendrons are a good source of phenolic compounds as it contains alkaloids, flavonoids, glycosides, saponins, tannins, steroids, and phlorotannins, whereas, the red rice is also a good source of polyphenolic components which 143.38 mg GAE/100 g as investigated by Raghuvanshi et al. (2017). In our study, the highest phenolic content was observed in grist combination (1) which is 0.410 mg/mL and lowest in grist combination (3) 0.340 mg/mL whereas in control sample the polyphenolic components was less from all the treatments *i.e.* 0.313 mg/mL which is similar to the study of Pai et al. (2015). Ng and Mocek (1973) investigated that the phenolic compounds in beer are ranging from 150 to 350 mg/L, whereas, in grist combination (2) concentration of phenolic compounds is 0.377 mg/mL.

According to the research of Devolli et al. (2018), the protein in beer is a mixture of the heterogeneous molecules which plays an important role in the mouthfeel, stability, and quality of the beer. Protein content in various treatments ranged from 2.87 to 3.90 mg/mL, and 4.12 mg/mL in the control sample, whereas, the highest protein content among all treatments observed in grist combination (1) and the lowest in the grist combination (2), whereas, grist combination (3) showed 3.35 mg/mL protein content. Result obtained from our study is similar to the study of Pai et al. (2015).

Kunze et al. (2011) investigated that malted barley contains 70–85% total carbohydrates and the majority of them are glucose, maltose, dextrin, arabinoxylans, and maltotriose. These fermentable sugar are further utilized by yeast to convert sugar into alcohol and also contribute to the sweetness and body of the beer. In our study, the amount carbohydrates in grist combination (1) was 37.3 mg/mL, whereas, lowest 32.87 mg/mL was determined in grist combination (2) and 35.2 mg/mL in grist combination (3), whereas, in the control, sample have highest carbohydrate content among all the treatments (40.87 mg/mL). Reducing sugar are those type of sugars that have aldehyde or ketone group which help to the molecules of sugars to behave like reducing agent (Kunz et al. 2011) because yeast utilize only low molecular weight sugar *i.e.* glucose, sucrose, and maltose, whereas, in case of oligosaccharide they don't have free aldehyde or ketone group to ferment (Pai et al. 2015). In our study, the reducing sugar content in beer ranges from 1.116 to 1.108 and 1.992 mg/mL in the control sample. The highest content of reducing sugar in our treatments was 1.116 mg/mL in grist combination (1), whereas, in grist combination (2) it was 1.113 mg/mL and lowest 1.108 mg/mL in grist combination (3).

Flavonoids are the polyphenolic components that have beneficial health effects in terms of antioxidant activity. The major source of flavonoids in beer is major barley, hops, and rhododendron. Total flavonoid content in rhododendron was 1276.5 mg/100 mL as investigated by Kumar et al. (2019), whereas, in red rice, the total flavonoid content was 120.0 mg R.E./100 g of flavonoid as studied by Raghuvanshi et al. (2017). In our treatments, the beer made with a high concentration of red rice showed maximum flavonoids content *i.e.* 0.119 mg/mL in combination (2) and lowest in grist combination (1) 0.112 mg/mL and 0.115 mg/mL in grist combination (3) wherein the control sample flavonoids content found less than all of the treatments *i.e.* 0.097 mg/mL. Brewed beer also analyzed for the total anthocyanin content in beer which was about 25.34 mg/L in grist combination (1), 35.12 mg/L in grist combination (2), and 29.11 mg/L in combination (3), wherein, in control sample the total anthocyanin content found lowest *i.e.* 8.18 mg/L among all the treatments. This pigment, anthocyanin in beer was majorly due to rhododendron and red rice. The ripening and ageing period can affect anthocyanin co-pigments (hydroxycinnamic acids) which plays a dominant role in colour stabilisation. Anthocyanins, catechins, and other flavonoids contribute to the astringency and also scavenge the free radicals which act as anti-inflammatory, anticarcinogenic, antiatherogenic, antimicrobial and other (Galanakis et al. 2015).

Hops contribute bitterness, flavour, aroma and also have an antibacterial effect in beer which is originated from alfa-acid. In commercial beer, the bitterness ranges from 10 to 50 BU wherein in our treatments there was no significant difference in the level of bitterness in grist combination (1), (2) and (3) was 16.2, 13.9 and 14.7 BU. The control sample was the most bitter with 19.03 BU.

Brewed beer was also analyzed for antioxidant activity DPPH (scavenging activity) which depends upon the type of raw material and method of fermentation. There is a correlation between phenolic content and antioxidant activity. The total antioxidant concentration of extracts is based on the determination of total phenols and particular fractions. Phenolic compounds can donate hydrogen atoms, radical scavenging and also contribute to the redox

Table 6 Effect of red rice in conjunction with barley and rhododendron extracts on sensory score

Treatments	Maximum score	Control sample	Grist combination (1)	Grist combination (2)	Grist combination (3)
Appearance	(2)	1.2 ± 0.03	1.4 ± 0.02	1.8 ± 0.04	1.6 ± 0.03
Colour	(4)	2.1 ± 0.25	2.9 ± 0.17	3.8 ± 0.21	3.1 ± 0.15
Aroma	(4)	2.3 ± 0.11	2.8 ± 0.09	3.4 ± 0.13	3.2 ± 0.14
Acidity	(2)	1.7 ± 0.02	1.4 ± 0.03	1.2 ± 0.02	1.3 ± 0.04
Sweetness	(1)	0.6 ± 0.01	0.5 ± 0.01	0.8 ± 0.2	0.7 ± 0.02
Body	(2)	1.8 ± 0.4	1.7 ± 0.3	1.8 ± 0.2	1.8 ± 0.4
Flavour	(2)	1.2 ± 0.07	1.4 ± 0.05	1.8 ± 0.06	1.6 ± 0.05
Bitterness	(2)	1.6 ± 0.1	1.4 ± 0.1	1.3 ± 0.2	1.2 ± 0.2
Astringency	(1)	0.8 ± 0.03	0.7 ± 0.05	0.5 ± 0.04	0.6 ± 0.05
Overall acceptability	(20)	13.3 ± 1.03^{a}	14.2 ± 0.93^{ab}	16.4 ± 0.84^{c}	15.1 ± 1.12^{b}

Scale, according to (Amerine et al. 1965)

Data presented in table is mean of ten values ± SD

$^{a-b}$Means with the different superscript in a row are significantly different ($P < 0.05$) from each other

reactions which lead to either antagonistic and synergistic effect on the overall antioxidant capacity (Galanakis et al., 2018a, b, c). There are some phenolic compounds that help to increase antioxidant activity which improves the stability and increases shelf life by the formation of saturated and unsaturated aldehydes by oxidation (Zhao et al. 2010). Among all treatments, grist combination (2) made with high ratio red rice showed highest scavenging activity *i.e.* 47.68%, wherein the grist combination (1) and (3) scavenging activity was 46.47 and 45.12%, respectively. Scavenging activity of the control sample was lowest among all the treatments *i.e.* 27.11%. Results obtained are almost similar to Pai et al. (2015).

Sensory evaluation

The sensory analysis of red rice beer in conjunction with barley and rhododendron extracts at different grit ratio (Table 6) shows that there was a notable difference in the various quality parameters. Among all the treatments, the beer brewed with the high ratio of red rice with extracts of rhododendron was higher ranking because of better colour, flavour, aroma, sweetness, appearance and overall acceptability except acidity and bitterness.

Conclusion

The research confirms that there is a possibility of brewing beer with red rice in combination with barley malt and rhododendron extracts to obtain a new variant of beer in the market which has a positive effect on the quality of beer in terms of antioxidant activity, better anthocyanins content and another physicochemical character. Beer prepared with a higher ratio of red rice with barley malt and extracts has better colour, aroma, and sensory acceptability. The use of hydrolyzed red rice as an adjunct with barley malt to provide enough nutrients for yeast to convert sugar into alcohol and provide another physicochemical character while the use of extracts of rhododendron was to provide unique colour, flavour, and aroma as compared to other available options in the market. Therefore, this method of brewing is an alternative to the most commonly used methods and adjuncts to prepared beer with the use of good quality of adjuncts *i.e.* red rice and extracts of rhododendron which are never used and change the regular methods used in brewing to obtain unique one.

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References

Agu RC (2002) A comparison of maize, sorghum, and barley as brewing adjuncts. J Inst Brew 108(1):19–22

Analytica-EBC (2010) Verlag Hans Carl, Nürnberg

AOAC (1980) Official Methods of Analysis. Hortwitz W (ed) 13th ed., Association of Official Analytical Chemists, Washington, DC, p 1015

Asare EK, Jaiswal S, Maley J, Båga M, Sammynaiken R, Rossnagel BG, Chibbar RN (2011) Barley grain constituents, starch composition, and structure affect starch in vitro enzymatic hydrolysis. J Agric Food Chem 59(9):4743–4754

Barba FJ, Galanakis CM, Esteve MJ, Frigola A, Vorobiev E (2015) Potential use of pulsed electric technologies and ultrasounds to improve the recovery of high-added value compounds from blackberries. J Food Eng 167:38–44

Bhatt M, Abrol GS, Kumar S, Nautiyal BP (2017) Preparation and evaluation of functionally enriched squash from rhododendron

(*Rhododendron arboreum* Sm.) flowers. Int J Food Ferment Technol 7(1):191–196

Bokulich NA, Bamforth CW, Mills DA (2012) Brewhouse-resident microbiota are responsible for multi-stage fermentation of American coolship ale. PLoS ONE 7:e35507

Brand-Williams W, Cuvelier ME, Berset CLWT (1995) Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol 28(1):25–30

Briggs DE (1998) Grain and Pulses. In: Briggs DE (ed) Malts and Malting. 1st ed. Blackie Academic and Professional, 2–6 Boundary Row, London, pp. 35–78. ISBN:0 412 29800 7

Capece A, Romaniello R, Siesto G, Romano P (2018) Conventional and non-conventional yeasts in beer production. Ferment 4(38):1–11

Caputi A, Ueda M, Brown T (1968) Spectrophotometric determination of ethanol in wine. Am J Enol Vitic 19(3):160–165

Cooper CM, Evans DE, Yousif A, Metz N, Koutoulis A (2016) Comparison of the impact on the performance of small-scale mashing with different proportions of unmalted barley, Ondea Pro®, malt and rice. J Inst Brew 122(2):218–227

Devolli A, Dara F, Stafasani M, Shahinasi E, Kodra M (2018) The influence of protein content on beer quality and colloidal stability. Int J Innov Approaches Agric Res 2(4):391–407

Ducruet J, Rébénaque P, Diserens S, Kosińska-Cagnazzo A, Héritier I, Andlauer W (2017) Amber ale beer enriched with goji berries–the effect on bioactive compound content and sensorial properties. Food Chem 226:109–118

Galanakis CM (2012) Recovery of high added-value components from food wastes: conventional, emerging technologies and commercialized applications. Trends Food Sci Technol 26(2):68–87

Galanakis CM (2013) Emerging technologies for the production of nutraceuticals from agricultural by-products: a viewpoint of opportunities and challenges. Food Bioprod Process 91(4):575–579

Galanakis CM, Kotanidis A, Dianellou M, Gekas V (2015) Phenolic content and antioxidant capacity of Cypriot wines. Czech J Food Sci 33(2):126–136

Galanakis CM (2018) Phenols recovered from olive mill wastewater as additives in meat products. Trends Food Sci Technol 79:98–105

Galanakis CM, Tsatalas P, Galanakis IM (2018) Phenols from olive mill wastewater and other natural antioxidants as UV filters in sunscreens. Environ Technol Innov 9:160–168

Galanakis CM, Tsatalas P, Charalambous Z, Galanakis IM (2018) Control of microbial growth in bakery products fortified with polyphenols recovered from olive mill wastewater. Environ Technol Innov 10:1–15

Galanakis CM, Tsatalas P, Charalambous Z, Galanakis IM (2018) Polyphenols recovered from olive mill wastewater as natural preservatives in extra virgin olive oils and refined olive kernel oils. Environ Technol Innov 10:62–70

Giusti MM, Wrolstad RE (2001) Characterization and measurement of anthocyanins by UV-visible spectroscopy. Curr protoc Food anal Chem 1:F1–2

Gomaa AM (2018) Application of Enzymes in Brewing. J Nutri Food Sci Forecast 1(1):1002

Han H, Kim J, Choi E, Ahn H, Kim WJ (2016) Characteristics of beer produced from Korean six-row barley with the addition of adjuncts. J Inst Brew 122(3):500–507

Humia BV, Santos KS, Barbosa AM, Sawata M, Mendonça MDC, Padilha FF (2019) Beer molecules and its sensory and biological properties: a review. Molecules 24(1568):1–19

Kaushik R, Chawla P, Kumar N, Janghu S, Lohan A (2018) Effect of pre-milling treatments on wheat gluten extraction and noodle quality. Food Sci Technol Int 24(7):627–636

Kovačević DB, Barba FJ, Granato D, Galanakis CM, Herceg Z, Dragović-Uzelac V, Putnik P (2018) Pressurized hot water extraction (PHWE) for the green recovery of bioactive compounds and steviol glycosides from *Stevia rebaudiana* Bertoni leaves. Food Chem 254:150–157

Chawla P, Kumar N, Kaushik R, Dhull SB (2019) Synthesis, characterization and cellular mineral absorption of gum arabic stabilized nanoemulsion of *Rhododendron arboreum* flower extract. J Food Sci Technol 56(12):5194–5203

Kumar D, Hager AS, Sun A, Debyser W, Javier Guagliano B, Singh V (2019) Improving fermentation rate during use of corn grits in beverage alcohol production. Beverages 5(1):5

Kunz T, Lee EJ, Schiwek V, Seewald T, Methner FJ (2011) Glucose–a reducing sugar? Reducing properties of sugars in beverages and food. Brew Sci 64:61–67

Leitao C, Marchioni E, Bergaentzlé M, Zhao M, Didierjean L, Miesch L, Ennahar S (2012) Fate of polyphenols and antioxidant activity of barley throughout malting and brewing. J Cereal Sci 55(3):318–322

Mallawarachchi S, Bandara LRLM, Dilshan SKDHS, Gunawardena S (2016) Optimization of mashing process in beer production using rice as an adjunct. Moratuwa Engineering Research Conference. https://doi.org/10.1109/MERCon.2016.7480155

Martens H, Dawoud E, Verachtert H (1992) Synthesis of aroma compounds by wort enterobacteria during the first stage of lambic fermentation. J Inst Brew 98:421–425

Ng E, Mocek M (1973) A method for estimating total polyphenols in beer. J Inst Brew 79(2):165–169

Pai TV, Sawant SY, Ghatak AA, Chaturvedi PA, Gupte AM, Desai NS (2015) Characterization of Indian beers: chemical composition and antioxidant potential. J Food Sci Technol 52(3):1414–1423

Piazzon A, Forte M, Nardini M (2010) Characterization of phenolics content and antioxidant activity of different beer types. J Agric Food Chem 58(19):10677–10683

Raghuvanshi RS, Dutta A, Tewari G, Suri S (2017) Qualitative characteristics of red rice and white rice procured from local market of Uttarakhand: a comparative study. J Rice Res 10:49–53

Ranganna S (1986) Handbook of analysis and quality control for fruit and vegetable products. Tata McGraw-Hill Education, New York

Sharma S, Kaushik R, Sharma P, Sharma R, Thapa A, Indumathi KP (2016) Antimicrobial activity of herbs against *Yersinia enterocolitica*. Ann Univ Dunarea de Jos of Galati Food Technol 40(2):119–134

Singh V, Ali SZ (2006) In vitro hydrolysis of starches by CC-amylase in comparison to that by acid. Am J Food Technol 1(1):43–51

Solanki SN, Huria AK, Chopra CS (2013) Physico-chemical characteristics of buransh (*Rhododendron arboreum*)-a nutritious and edible flower. J Hill Agric 4(1):50–52

Thimmaiah SK (1999) Standard Methods of Biochemical Analysis, 1st edn. Kalyani, New Delhi

Tusekwa TCEM, Laswai HS, Towo EE, A. B. (2000) Traditional alcoholic beverages of Tanzania: production, quality and changes in quality attributes during storage. Int J Food Sci Nutr 51(2):135–143

Yemm EW, Willis A (1954) The estimation of carbohydrates in plant extracts by anthrone. Biochem J 57(3):508–514

Zhang D, He Y, Ma C, Li H (2017) Improvement of beer flavor with extruded rice as adjunct. J Inst Brew 123:259–267

Zhao H, Chen W, Lu J, Zhao M (2010) Phenolic profiles and antioxidant activities of commercial beers. Food Chem 119(3):1150–1158