Indian black rice: a brewing raw material with novel functionality

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ABSTRACT (250 words current 295)

Indian black rice (*Chakhao Poireiton)* is a pigmented variety, rich in anthocyanins and other phytonutrients. With increasing interest in the use of local raw materials in brewing, it was of interest to develop protocols for malting and brewing with *Chakhao Poireiton* to see in particular whether the antioxidant capacity of anthocyanins could be delivered into finished beer. Protocols for brewing with 100% malted rice were developed and the performance of Indian black rice compared with that of an Italian white rice cultivar suited to brewing. The apparent fermentabilities of rice worts were 69.5% (black) and 67.3% (white), yielding beers of 3.28 and 3.19 % ABV respectively. Black rice worts were FAN deficient (83.5 mg/L relative to 137 mg/L for white rice) and would need nitrogen supplementation to avoid issues with fermentation, e.g. elevated diacetyl. Black rice beer had an orange-red hue as a result of extraction of anthocyanin pigments (2.84 mg/L). The oxidative stability of 100% rice beers was measured using Electron Spin Resonance (ESR) spectroscopy and both samples were found to be unusually stable. Interestingly, when rice beers were blended with a control barley malt derived lager in varying proportions (10, 25, 50%), the oxidative stability was improved, relative to the control lager, particularly so in the case of black rice beer, which contained an antioxidant capacity over and above that of the white rice beer. Future studies are required to determine whether the noted oxidative stability of 100% rice malt beers results in a more flavour stable beer.

KEYWORDS: Indian black rice, 100% rice beer, ESR, beer oxidative stability, beer polyphenols.

INTRODUCTION

India is the largest rice producer in the world. With around 80% of this production utilized for domestic consumption, it is also the largest consumer of rice[1]. The North – Eastern states of India, such as Manipur, are home to a diverse range of traditional aromatic rice landraces[2]. Once such variety, very popular in Manipur is the black rice - *Chakhao Poireiton*, belonging to the species *Oryza sativa* L. *indica*.

Black rice appears black due to the presence anthocyanins, dark purple pigments, which are present in its bran layer. Anthocyanins are antioxidants and the levels accumulated in black rice bran are considered to be one of the highest levels found in foods[3]. Anthocyanins are known for their ability to protect cells from damage due to biotic and abiotic stresses and have also been considered as potential cancer chemo-preventative agents[4]. As a dietary antioxidant, they can also help combat reactive oxygen species, free radicals and help decrease the risk of chronic diseases such as coronary heart disease[5]. Additionally, they are approved for use as a food additive or colouring agent in the EU, Australia and New Zealand with the E number E163 (INS number 163)[6].

In addition to antioxidants, black rice contains high amounts of flavonoid phytonutrients, gamma oryzanol, polyphenols, Vitamin E, dietary fibre and minerals such as iron and copper. It is a better source of plant based protein than normal white rice[7]. It is thus considered to be a premium rice product from a nutritional perspective. There have been very limited studies on black rice *Chakhao Poireiton*. It is a waxy rice and has been reported to be composed of approximately 7% protein, 4% fat, 76% carbohydrate and 2% amylose with a gelatinisation temperature between 75 and 92°C[8]. Figure 1 shows the paddy and different fractions of black rice as it goes through an industrial milling process. However, black rice is usually not polished in order to maintain its bran and the anthocyanin, giving the rice a chewy texture when cooked.

In the brewing industry rice has been used as an adjunct mainly due to its neutral flavour. However, brewing a 100% rice beer is somewhat more challenging. Its starch fails to undergo complete saccharification during mashing. This has been reported to be attributed to the high gelatinization temperature of its starch, insufficient starch-degrading enzymes in malted rice and insufficient degradation of the structural protein of the endosperm cell wall needed prior to or simultaneously with starch modification[9]. There have been only limited studies on the production of rice beer. Usansa et al. reported that enzyme production during malting of rice was dependent on the rice variety and did not correlate with amylose content[10]. There have been reports of successful brewing with 100% rice malt, made possible by optimising the mashing conditions[9], and of the experimental development of speciality rice malts, roasted to enhance flavour and the colour[11]. Amylase activity, needed for starch degradation, depends greatly on the different incubation conditions. For rice, the minimum temperature is 10-12 °C, the optimal temperature is 30-37 °C and the maximum temperature is 40-42 °C inside[12]. Additionally, optimum temperature conditions for malting black rice were reported to be 30 °C[13,14], which is close to room temperature in Asian countries. Ceppi and Brenna, reported development of a gluten-free beer (3.5-4.5% ABV) using Italian rice variety *Loto* and a mashing-in liquor to grist ratio of 1:3.5[15]. Mayer et al, likewise reported an all rice gluten free beer (4.4-4.8% ABV) produced with Italian rice variety *Centauro* and a mashing-in liquor to grist ratio of 1:4[9].

Black rice forms an important part in the Manipuri culture and in ceremonies to prepare various unique dishes. However, the cultivation of *Chakhao* landraces is declining, as it cannot compete with modern high yielding non-coloured varieties[2]. There has been no published work on black rice *Chakhao Poireiton* beer. This research aimed to investigate the use of this Indian black scented rice in the brewing process with a view to improving its commercial value. Finding additional uses of this unique native commodity in the brewing Industry could be of interest to the growing Indian brewing Industry. It could ultimately warrant its agronomical improvement to develop higher yielding varieties and revitalise its cultivation.

MATERIALS AND METHODS

Raw materials

Paddy black rice of the variety *Chakhao poireiton,* was imported from *Imphal*, India. For comparison, paddy white rice of the variety *Centauro* was imported from Pavia, Italy. Both samples of rice were harvested in 2016.

Malting

Malting of the rice varieties was conducted in biological duplicate as paddy rice in an automatic micromalting system (Curio Malting, Milton Keynes, UK). Steeping and germination was conducted at 30 °C in batches of 3.2kg (400 g X 8 cages) for 72.7 hours according to the protocol shown in Table 1. Steep water was renewed for each wet cycle and turning of the malting cages maintained at 1 min in every 10 min throughout the process. Kilning of rice malts used the following cycle: 45°C x 12 h→ 50°C x 12 h → 55°C x 13.5 h→ 70°C x 6 h→ 1 h cooling. Kilned malts were hand deculmed to remove the coleoptile and primary roots. Finished malts were packaged under vacuum into sealed foil-laminated pouches using a vacuum sealer (Audionvac VMS 43).

Brewing of rice beers

Wort preparation

Infusion mashing was conducted in a Braumeister one vessel professional brewing system, (25 L scale; Braumeister, Speidel, Germany) by adapting the process described by Mayer et al[9]. Rice malt (3 kg) was milled using a Bühler Miag Disc mill (Bühler, Braunschweig, Germany) with a gap setting of 0.5mm. The milled grist was added to 22L of brewing water at 45°C. The mixture was supplemented with 3.75g of $CaCl_2.2H_2O$ and mash pH was quickly adjusted to 5.05 using lactic acid. Mashing was conducted by increasing the temperature at a ramp rate of 1°C/min in between the following temperature stands: 30 min at 45°C, 45 min at 65°C, 60 min at 74°C, and 60 min at 78°C giving a total mashing time of 228 min (Figure 2). Iodine tests for starch showed negative towards the end of the 78 °C stand. Lautering was conducted for 30 min with 1 L of sparge water at 80 °C. Mash was subsequently boiled for 1 h with the addition of 5 grams of hops (Zeus T90 pellet hops with specified 15-17% α-acid content) and the wort cooled to 20°C prior to pitching.

Fermentation and maturation

The cooled clarified wort was transferred to a 30 L conical fermenter (FastFerment™, Ontario, Cannada) and pitched with 11 g of Nottingham ale yeast (Danstar; Lallemand Inc., Montreal, Canada). Fermentation temperature was maintained at 20°C for 4 days. Following this the vessel was cooled and stored at 4°C for 6 days to permit the settling out of yeast and precipitate particles. The yeast was cropped from the bottom of the tank. The unfiltered beer was bottled, primed with 3g of dextrose and matured for 21 days at 20°C.

Wort analysis

The following parameters were measured in duplicate:

pH – using hand held pH meter (EzDo 7011). *Specific gravity* – using a hand-held density meter (DMA 35, Anton Paar, Graz, Austria). *Colour* – measured at 430 nm according to EBC 4.7.1 and also using a Hunterlab tristimulus colour measurement system (ColorQuest XE, Hunterlab, Germany). *Free Amino Nitrogen* (mg/L) – using the ninhydrin spectrophotometric method

according to EBC 8.10. *Total polyphenols* (mg/L) – according to the International method (ASBC Beer-35).

Concentrations of individual phenolic compounds were analysed by HPLC according to the method described by Oladokun et al[16]. Briefly, ethyl acetate extracts were evaporated to dryness, reconstituted in methanol and separated using a Waters Alliance 2695 HPLC fitted with a Purospher STAR rp-18 end-capped column (250 x 4.6 mm, 3 µm particle size; Merck Millipore, UK) coupled with a C18 guard cartridge from Phenomenex (UK). Peak areas were extracted at 280 nm and total run time was 65 min. Samples were analysed in triplicate and phenolic acid concentrations were determined from calibration curves generated from external standards run at concentrations of 1, 10, 20, 40 mg/L.

Beer analysis: The following parameters were measured in duplicate as for wort above: pH, specific gravity, colour, Free Amino Nitrogen, total polyphenols, phenolic compounds by HPLC. In addition, alcohol (% ABV) was measured using an Anton Paar Alcolyzer Plus (Anton Paar, Graz, Austria). Foam stability of the beer (30 mm foam collapse time – FCT30) was measured using a Nibem-TPH Foam Stability meter (Haffmans, The Netherlands) according to EBC 9.42.

Measurement of the total anthocyanin content of beers

The total anthocyanin content (TAC) was determined using the pH-differential method [17]. The TAC was calculated using equation (1) and expressed as milligrams of cyanidin 3-glucoside (cyn 3-glu) equivalents/mL of solution:

$$\text{TAC (mg/mL)} = \frac{A \times MW \times DF \times 1000}{\varepsilon} \qquad (1)$$

Where, $A = (A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}$; *MW* is the molecular weight of anthocyanin (449.2 g/mol), *DF* is the dilution factor and ε is molar absorbance of cyn 3-glu (26,900 L/mol/cm).

Measurement of the oxidative stability of rice beers using Electron Spin Resonance (ESR) spectroscopy.

The oxidative stability of beers were assayed using a forcing test at 60 °C during which the time-course of free radical formation was measured using Electron Spin Resonance spectroscopy (Bruker E-scan; Bruker Corporation, MA, USA) with N-tert-Butyl-α-phenylnitrone (PBN) as spin trap[18]. PBN (678 mg) was dissolved first in 500 µL of ethanol (Fisher Scientific) and 500 µL water added. 280uL of PBN solution was added to each beer sample (7 mL). Samples were placed in a 60 °C heating block at 60 second intervals. ESR spectra were recorded with a centre field of 3478 G and sweep width of 17 G. The microwave bridge had a power of 2.31 mW and frequency of 9.77. Receiver gain was 1261, modulation frequency 86 kHz, modulation amplitude 1.1 G, modulation phase 0.85°, time constant 20.48 ms. Scans were aggregated and the peak to peak height of the first derivative of the EPR spectra was recorded as the intensity value at a given time point. Samples were taken at approximately 10 min intervals across the assay time using an autosampler (Bruker Corporation, MA, USA) and the running order was randomised.

Analysis of bulk fermentation volatiles in rice beers by Head Space Gas chromatography (GC-HS-FID)

Volatile analysis was conducted using a modified version of EBC Method 9.39. Beer samples were chilled to 4°C and sonicated for about 10 seconds. Degassed beer sample (10 mL) was transferred to a headspace vial, 50 µl of internal standard (10,000 ppm 1-butanol) added, followed by 3.5g of sodium chloride and the vial was quickly sealed tight using a crimper.

Analysis was conducted by HS-GC-FID (SCION 456-GC, Bruker Corporation, MA, USA) fitted with ZB-Wax column (60 m × 0.25 mm i.d., 0.50 µm film thickness), under a constant 15 psi pressure with helium as carrier gas. Injection volume was 500 µL at a split ratio of 1:20. Run time was 36.25 min with an additional 20 min agitation time. The GC oven was programmed for an initial 85°C for 10 min, 110°C for 13 min (ramp @ 25°C/min), 200°C for 13.25 min (ramp @ 8°C/min). The temperatures of the injection port and FID detector were 150°C and 250°C, respectively.

Vicinal diketone (VDK) analysis by Head Space Gas Chromatography (GC-HS-ECD)

VDK analysis was conducted using a method based on EBC 9.42.2 which can detect and quantify diacetyl (0-0.25 ppm) and 2,3-pentanedione (0-0.25 ppm) using 2,3-hexanedione as an internal standard. Samples were chilled to 4°C, degassed in a cooled shaking incubator at 175 rpm for 5 min and filtered through a 0.45 µm syringe filter. Beer samples (5 mL) were transferred to individual headspace vials, 50 µl of internal standard (5 ppm) added, followed by 3.5g of ammonium sulphate and the vial was quickly sealed tight using a crimper. Analysis was conducted by HS-GC-ECD using a SCION 456-GC (Bruker Corporation, MA, USA) fitted with a Restek Rtx-5MS (30 m × 0.25 mm id × 0.25 µm df) column, under a constant 50 psi pressure and with helium as carrier gas. Injection volume was 500 µL at a split ratio of 1:5. Run cycle time was 12 min with an additional 20 min agitation time. The GC oven profile started with an initial hold at 30°C for 2 min followed with a linear ramp to a final temperature of 120°C which was held for 2 min (ramp at 70°C/min). The temperatures of the injection port and ECD detector were 110°C and 150°C, respectively.

Elemental analysis of rice beers using Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Beer samples were degassed by sonication (5 min). All samples were diluted (1:10) with nitric acid (2%) by pipetting the sample (1 mL) and nitric acid (2%, 9 mL) into a 10 mL plastic sample tube. Sample tubes were capped and inverted three times. Diluted samples were stored at 2˚C pending elemental analysis.

A multi-element analysis of diluted beer sample was undertaken by ICP-MS (Thermo-Fisher Scientific X-SeriesII) with a 'hexapole collision cell' (7% hydrogen in helium) to remove polyatomic interferences. Samples were introduced from an autosampler (Cetac ASX-520 with 4 x 60-place sample racks) through a concentric glass venturi nebuliser (Thermo-Fisher Scientific; 1 mL/min). Internal standards were introduced to the sample stream via a 'T'-piece and included Sc (100 ng/mL), Rh (20 ng/mL) and Ir (10 ng/mL) in 2% nitric acid. External multi-element calibration standards (Claritas-PPT grade CLMS-2, Certiprep/Fisher) included Fe, Cu and Mn in the range 0-100 µg/L. Sample processing was undertaken using Plasmalab software

(version 2.5.4; Thermo-Fisher Scientific) set to employ separate calibration blocks and internal cross-calibration where required.

RESULTS AND DISCUSSION

Paddy rice samples were germinated at 30°C, kilned at 70°C and hand deculmed. Both varieties of rice germinated at a similar rate and after two days distinct coleoptiles were visible (Figure 3). Total malting time was reduced from the 8 days [19] used in a prior study, to just 3 days. This short malting time was achieved at a higher germination temperature of 30°C, and would most likely result in lower malting losses (in roots and coleoptile). Under similar conditions: steeping for 24 hours and germinating at 30°C, six white Thai rice cultivars have been reported to result it average malting losses of 10%, 20% and 40% for a total malting time of 4, 5 and 6 days respectively[10].

Rice malts were mashed using the schedule shown in Figure 2. Lautering proceeded without difficulty, probably due to the low gravity of the worts (ca. 9°P) and to the rice husk forming an efficient filter bed. For both the rice varieties, fermentation of the wort resulted in a similar decrease in pH, and specific gravity (Figure 4). This suggests that the two varieties of rice produced worts of similar quality which had comparable fermentabilities (69.5 and 67.3% for black and white rice worts respectively; Table 2) generating final ethanol contents in the region of 3.2% ABV.

The rice grist (3 kg) yielded 1.29 ±0.02 and 1.19 ±0.06 kg of oven dried spent grains for black and white rice malts respectively. This equated to an extract rate of approximately 60% by weight for each malt. Rice husk comprises ~20% of paddy rice[20]. Although, this is higher than for traditional brewing feedstocks such as barley (10%[21]), the husk has been reported to assist in an efficient lautering process[19]. However, rice husk is tougher than barley husk and of the two rice varieties used, black rice has a tougher husk than the white rice husk. This could create challenges in scaling up this process, especially when pumping the mash. Careful milling would be required in order to balance husk preservation (to aid lautering) against potential impacts on the physical properties of the mash.

The average Free Amino Nitrogen (FAN) contents of rice worts were 83.5 and 137 mg/l for black and white rice respectively (Table 2). Protein contents of the two rice varieties have been previously reported to be 6.6-7.7% for *Chakhao Poireiton*[8,22] and 7.6-8.8% for *Centauro*[19]. In addition to having a higher FAN content, the white rice FAN was more completely assimilated by the yeast, with 89% consumption across fermentation compared to 66% of FAN utilisation for the black rice beer (Table 2). However, this apparently did not impact on alcohol production. The FAN content of all rice malt wort (12°P) was previously reported to range between 160-179 mg/L [9]. It can be concluded that, despite the relatively low wort FAN level in the present experiments, there was sufficient yeast growth to ferment the wort and produce alcohol. It is likely that any brewing process based on 100% black rice malt would require nitrogen-supplementation for optimal yeast health and fermentation progression, particularly when brewing at higher gravities where higher wort FAN levels are required[23].

Rice beers poured with a generous, coarse white foam and were visually somewhat hazy (Figure 5), with the white rice beer being substantially more turbid. The reasons for the different turbidities in finished beer is not clear based on present data, however, one possibility is that the elevated polyphenol content of black rice aided the precipitation of haze active protein from the black rice beer either in the brewhouse or through

fermentation/maturation. It is likewise possible that the difference results from different protein or starch solubilisation during processing of the 2 different varieties. The most striking difference between the two beers was in terms of colour (Figure 5, Table 2). Black rice beer had a pink-orange hue (elevated a* colour co-ordinate; Table 2) whereas the white rice beer had a more conventional golden lager hue. Usually in brewing the wort/beer colour increases at higher pH values as the extent of colour pick-up via the Maillard reaction is greater at a higher wort pH values. However, with black rice wort and beer the colour is primarily imparted by anthocyanins, whereby pH directly impacts the colours of the pigments – tending towards red hues at the low beer pH values resulting for black rice beer (pH 3.6). It can also be noted that the buffering capacity of both rice beers was lower than is encountered for barley malt brewed beers, where a final pH of around 4.0-4.2 is typical. This could relate to the very low residual FAN content in both rice beers.

Phenolic content of rice beers

Anthocyanins were only detected in the black rice wort and beer (Table 2). The anthocyanin content of de-husked powdered *Chakhao Poireiton* has been reported to be around 740 mg/kg[4]. Taking the percentage of husk in paddy rice to be approximately 20%[20] suggests an actual anthocyanin content in the region of ~600mg/kg. Anthocyanin content of black rice wort was 4.9±1.7 mg/l (Table 2) and was further reduced during fermentation to 2.8±1.1 mg/l (Table 2). The apparently low transfer rate of anthocyanins into wort is most likely due to their heat labile nature. Much of the anthocyanin is believed to have degraded during the mashing process at temperatures up to 78 °C. Cyanidin-3-glucoside is one of the major anthocyanins present in *Poireitin*[4]. The cyanidin-3-glucoside content of black rice (*Oryza sativa* L. *japonica* var. SBR) has been reported to decrease by up to 80% during cooking[24](e.g. for 20 min in a pressure cooker). *Chakhao Poireiton* has been reported to have anthocyanins which are more heat stable compared to other black rice varieties[22]. Hence, use of other black rice varieties under similar processing conditions could result in almost total loss of anthocyanins.

A decrease in TPC was observed between wort and finished rice beers (Table 3). This could reflect losses through adsorption to yeast cells or through chill haze formation and removal. For both the wort and beer samples, brewing with black rice resulted in a 4-fold greater TPC than with white rice (Table 3). In lager beers the typical TPC is in the range of 150-340mg/l[25]. HPLC analysis indicated that both rice beers contained a broad range of phenolic compounds Table 3). It was notable that protocatechuic acid was only detected in black rice worts. This compound could be associated with the degradation of cyanidin-3-glucoside (or related anthocyanins) as has previously been reported during cooking of black rice (*Oryza sativa* L. *japonica* var. SBR)[24]. Tyrosol and indole-3-acetic acid were only detected in rice beers and not in the respective worts, indicating that they were formed or imparted to the beer through fermentation. There was a corresponding increase in the sum of individual phenolic acid contents after fermentation: 12.06 to 25.6mg/l (black rice wort to beer) and 2.62 to 30.25 mg/l (white rice wort to beer). Tyrosol is an antioxidant and the Ehrlich pathway degradation product of the amino acid tyrosine. Hence the greater amounts noted in white rice beers (Table 3) likely corresponds with the noted higher wort FAN content (Table 2).

Flavour properties of rice beers

Fermentation volatiles were analysed by gas chromatography using a headspace injection technique (Table 4). Concentrations of volatile esters such as ethyl acetate, ethyl hexanoate and isoamyl acetate, in the rice beers were comparable with, but towards the lower end of the ranges typically reported for barley malt beers (Table 4). VDK analysis indicated that the black rice beer contained diacetyl (0.34 mg/L; Table 4) in excess of its flavour threshold (0.1-0.15 mg/L). This would be regarded as a flavour defect in conventional lager beers and was most likely caused here by the noted low FAN values in black rice wort (Table 2). VDKs are released into the fermenting wort and are subsequently assimilated by yeast towards the end of fermentation[26]. Diacetyl is formed as an off-shoot of the pathway for valine synthesis in yeast and the higher values observed in black rice worts and beer (Figure 7 and Table 4) reflect: i) increased activity through the valine synthesis pathway and ii) slower uptake and assimilation of diacetyl by the reduced cell mass of yeast resulting from the low wort FAN content (although this did not materially impact on fermentation progression relative to the white rice beer fermentations). At low concentrations diacetyl provides a butterscotch-like aroma whereas pentanedione is detected as honey-like[27]. Of the two main VDK in beer, diacetyl is generally present in concentrations that are approximately 10 times higher than those for 2,3-pentanedione[28]. The latter was also true of the beers in this study.

Beer foam is one of the important visual attributes by which consumers judge beer quality[29]. This characteristic is influenced by both the raw materials used and the brewing process. Black rice beers (257±23.8sec) had a significantly higher Nibem foam stability (FCT30) than white rice beers (209±15.7sec) in this study.

Although detailed sensory characterization of the 100% rice beers was beyond the scope of the present study, the beers were tasted by experienced brewers within our team. VDK character was picked up, particularly on the black rice beers. As discussed above this could readily be addressed by nitrogen supplementation of the wort prior to fermentation. Furthermore, rice beers had their own individual characteristics, being slightly sour (due to the lower beer pH) and with an aroma note present which was reminiscent of cooked rice pudding.

Oxidative stability of rice beers

Lag time values determined using the ESR forced ageing method indicate the endogenous anti-oxidative potential of a beer and are directly related to its oxidative stability. Furthermore, the T150 value (signal after 150 min of the assay with PBN spin trap) is commonly cited as a comparative index of the extent of radical formation after a fixed time of forcing. It was immediately notable that both rice beers were unusually stable in terms of their ESR forced ageing responses (Figures 6A and 6B). The traditional inflection in signal intensity associated with exhaustion of the antioxidant capacity was very hard to discern for 100% rice beers as the ESR signals generated were relatively flat with only a gradual increase in signal intensity over 150 min at 60°C. The ESR traces observed for black and white rice beers were very similar to one another both in the freshly fermented and matured beers. A typical ESR lag-time curve for a barley malt-derived commercial lager beer, which was run under the same conditions as a part of the same experiment, is plotted (orange curve) on Figures 6A and 6B by way of comparison. The question arises – were the 100% rice beers highly oxidatively stable because they contained a relatively powerful array of antioxidants, or

because they lacked pro-oxidant species which are normally present in barley malt beers? We decided to investigate this further by performing ESR forcing tests on samples generated by blending proportions of each rice beer (25, 50%) into the commercial lager beer (Figure 6C, black rice; Figure 6D, white rice). Interestingly, the blending of black rice and commercial lager beers resulted in the expected reductions in T150 values, in proportions that approximately corresponded with the blend ratio and the T150's of the individual samples (Figure 6C). At 50:50 the commercial lager/ black rice mix had a T150 value (48,600) that was around 53% of that of the commercial lager beer (90,400). However, with the white rice beer, incorporation at 25% made no difference to the measured T150 relative to the commercial lager beer and even in a 50:50 blend ratio the T150 value (72,900) was as much as 81% of that in the commercial lager alone. Based on these results it is speculated that 100% rice beers lack significant pro-oxidant species which are present in malt derived lager beers and also that they contain antioxidant species which can enhance the antioxidant capacity of beers. Furthermore, it can be concluded that the black rice beers contained species which improved the oxidative stability of the commercial lager beer when the two were mixed in blends and that this trend was more evident when blending in the black rice beer as opposed to the white rice beer (comparing Figures 6 C & D).

Elemental analysis of rice beers using Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Thirty-one metallic elements, including almost all essential and toxic metals such as lead, cadmium, mercury, arsenic, silver, and thallium, were quantified in both of the beers by ICP-MS (Table 5). For comparative purposes a 'control lager' beer brewed from 100% barley malt was submitted for analysis alongside the rice beers to highlight major differences in elemental composition relative to the primary grist materials used.

Rice beers were relatively rich in magnesium and contained less potassium than the control lager. When considering the oxidative stability of beers there is much focus on the concentrations of transition metals such as iron, copper and manganese, which can catalyse the formation of pro-oxidant radical oxygen species[18]. In view of the noted oxidative stability of 100% rice beers it is interesting to note that they contained very low levels of iron and copper relative to the control lager (Table 5). However, the converse was true of manganese which was present at mg/L quantities, more than 10-fold higher than in the control lager (177.8 µg/L). Apparently this did not damage the oxidative stability of the 100% rice beers, perhaps due to the form in which manganese is present when brewing with 100% rice. This may favour the extraction of manganese through the brewing process, since reports elsewhere in the scientific literature of typical manganese contents of the raw materials themselves do not suggest such a high discrepancy as was noted here in the finished beers (raw rice 21 mg/kg dry weight, raw wheat 31 mg/kg and raw barley 29 mg/kg[30,31]).

Arsenic concentrations in rice beers (14.86 µg/l and 27.9 µg/l for black and white rice beer respectively) are of particular interest due to concerns about arsenic contents in rice. These results will reflect differences in the mineral contents due to the soil of the cultivation areas (India vs Italy) and also the cultivars (black vs white). Arsenic content of Italian beers has been reported to be 3-24 µg/l[32], Polish beer ranged from 2-13 µg/l[33] and for beers bought in New York ranged from 0.33 to 21.32 µg/l[34]. Arsenic contents of 100% rice beers have not previously been reported.

Conclusions

We have demonstrated the feasibility of using the Indian black rice *Chakhao Poireiton* to make beer in a process using 100% malted rice. This could be of interest as an alternative raw material for the brewing industry in the fastest growing economy in the world, namely India. Black rice beers contained a much higher total polyphenol content (TPC) than white rice beers and were pinkish-orange in colour due to the presence of anthocyanin pigments at low pH. The FAN content of black rice malt worts was low and supplementation with additional nitrogen sources would doubtless be required for commercial brewing. Interestingly, ESR measurements indicated that beers made from 100% malted rice were unusually oxidatively stable and in future studies it would be interesting to see if this confers enhanced flavour stability through shelf-life. When blended with a barley malt-derived lager beer in various proportions the black rice beer in particular improved the oxidative stability of the resulting blend on a proportionate basis. It is proposed that the black rice beer both lacked pro-oxidant species which are present in barley malt-derived beers and also contains additional anti-oxidant species (e.g. anthocyanins). Further studies are required to develop understanding of the impacts of adjunct and alternative raw materials usage on beer oxidative stability and to establish whether the noted oxidative stability of 100% rice beers would actually confer a particularly flavour stable product.

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Notes

The authors declare no competing financial interest.

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Table 1. Steeping and germination conditions used for malting of rice samples in the study.

Cycle	Duration (h)	Temp. (°C)
Steep Cycle 1 (wet)	6.00	30
Steep transfer time (14 min per wet cycle)	0.23	30
Steep Cycle 1 (dry)	3.00	30
Steep Cycle 2 (wet)	6.00	30
Steep transfer time (14 min per wet cycle)	0.23	30
Steep Cycle 2 (dry)	3.00	30
Steep Cycle 3 (wet)	6.00	30
Steep transfer time (14 min per wet cycle)	0.23	30
Germination Cycle 1	24.00	30
Germination Cycle 2	24.00	30
Total time	72.69	

Table 2. **Black and white rice wort and finished beer analytical parameters**.

	pH	Specific gravity	% ABV	Apparent Fermentabilty (%)	FAN (mg/mL)	Anthocyanins (mg/L)	EBC colour	Hunter Lab colour		
								L*	a*	b*
Black rice wort	5.40 ±0.20	1.0367 ±0.0001	-	69.5	83.5 ±13.4	4.93 ±1.8	13.58 ±1.2	29.98 ±1.3	11.67 ±1.1	14.71 ±0.09
White rice wort	5.36 ±0.06	1.03705 ±0.0007	-	67.3	137 ±18.4	0	5.3 ±0.5	43.94 ±0.6	-1.18 ±0.03	9.50 ± 0.1
Black rice beer	3.65 ± 0.06	1.0112 ±0.004	3.28 ±0.07	-	28 ±2.8	2.84 ±0.8	8.26 ±2.2	35.68 ±1.9	6.37 ±1.8	12.99 ±1.0
White rice beer	3.80 ±0.08	1.0121 ±0	3.19 ±0.13	-	15 ±0	0	3.41 ±1.2	44.75 ±0.7	-1.22 ±0.2	7.49 ±1.1

Values are average ± SD of two biological replicates. L*: Light vs. dark where a low number (0-50) indicates dark and a high number (51-100) indicates light; a*: Red vs. green where a positive number indicates red and a negative number indicates green; b*: Yellow vs. blue where a positive number indicates yellow and a negative number indicates blue.

Table 3. Phenolic compound concentrations (mg/L) in rice worts and beers (by HPLC and ASBC Beer-35)

	TPC	HMF	Protocatechuic acid	Tyrosol	4-HBA	Caffeic acid	Vanillic acid	Homo-vanillic acid	4-Hydroxy benzaldehyde	p-coumaric acid	Ferulic acid	Indole-3-acetic acid
						(mg/L)						
Black wort	200.7 ±26.4	0.28 ±0.0	6.09 ±0.03	0	0	0.04 ±0.0	3.37 ±0.0	0.12 ±0.02	0.08 ±0.02	0.62 ±0.02	1.46 ±0.02	0
Black beer	177.12 ±0.0	0.22 ±0.0	7.43 ±0.01	10.64 ±0.03	0	0.03 ±0.0	3.06 ±0.01	0.17 ±0.02	0.04 ±0.01	0.50 ±0.0	1.37 ±0.0	2.14 ±0.01
White wort	58.63 ±6.4	0.22 ±0.01	0	0	0	0	0.29 ±0.01	0.15 ±0.0	0.08 ±0.01	0.82 ±0.0	1.06 ±0.03	0
White beer	43.77 ±0.4	0.18 ±0.02	0	24.68 ±0.05	0.33 ±0.02	0	0.52 ±0.01	0.25 ±0.03	0.31 ±0.02	0.99 ±0.01	1.23 ±0.03	2.09 ±0.03

Values are average ± SD of two biological replicates.

TPC: Total phenolic content; HMF: Hydroxymethylfurfural; HBA: 4-Hydroxybenzoic Acid.

Table 4. Fermentation volatile concentrations in rice beers.

	Black rice beer (mg/L)	**White rice beer** (mg/L)	**Literature value for Barley malt bottom-fermented beer[9]** (mg/L)
VDK			
Butanedione	0.34 ±0.113	0.055 ±0.021	
Pentanedione	0.03 ± 0.014	0.005 ±0.007	
Esters			
Ethyl acetate	7.0 ±0.14	11.5 ±3.0	10-40
Isobutyl acetate	0.125 ±0.007	0.105 ±0.007	
Ethyl butyrate	0.06 ± 0.014	0.06 ±0	0.05-0.15
Isoamyl acetate	0.675 ±0.078	0.465 ±0.021	0.5-3
Ethyl hexanoate	0.025 ±0.035	ND	0.05-0.3
Ethyl octanoate	0.07 ±0.042	0.05 ±0.014	0.1-0.5
Higher alcohols			
n-propanol	13.8 ±0.56	11.9 ±1.4	5-20
isobutanol	62.5 ±4.1	52.5 ±1.9	5-20
Isoamyl alcohol	72.1 ±5.9	83.0 ±16.0	30-70

Data are the mean of two biological replicates ± SD; ND = not detected.

Table 5. **Elemental composition of beers brewed from 100% malted black or white rice cultivars as compared with a control lager beer brewed from 100% barley malt.**

mg/L	B	Na	Mg	P	S	K	Ca	Ti
Black	0.180	11.58	99.68	596.95	20.69	270.59	40.36	0.009
White	0.163	12.20	94.52	470.62	24.46	221.88	32.49	0.007
Control	0.155	10.05	55.79	466.61	68.44	601.08	22.71	0.007

µg/L	Li	Be	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn
Black	2.41	0.040	11.69	0.023	1.12	3421.6	5.71	0.083	4.767	18.72	16.24
White	2.73	0.048	9.44	0.078	1.80	1858.6	14.27	0.096	7.41	35.57	8.98
Control	2.03	0.078	14.41	0.581	8.23	177.8	99.70	0.120	5.14	209.99	25.61

µg/L	As	Se	Rb	Sr	Mo	Ag	Cd	Cs	Ba	Tl	Pb	U
Black	14.87	1.89	2153.39	119.99	0.41	0.003	0.019	0.627	51.85	0.009	0.718	0.003
White	27.96	0.71	190.35	97.88	13.52	0.003	0.072	0.115	21.51	0.008	1.190	0.007
Control	0.50	0.56	135.49	78.78	2.77	0.002	0.027	0.164	14.04	0.031	3.154	0.005

Values are the average of 8 replicate determinations.

Figure 1. Different fractions of black paddy rice (*Chakhao Poireiton*) as it undergoes industrial milling.

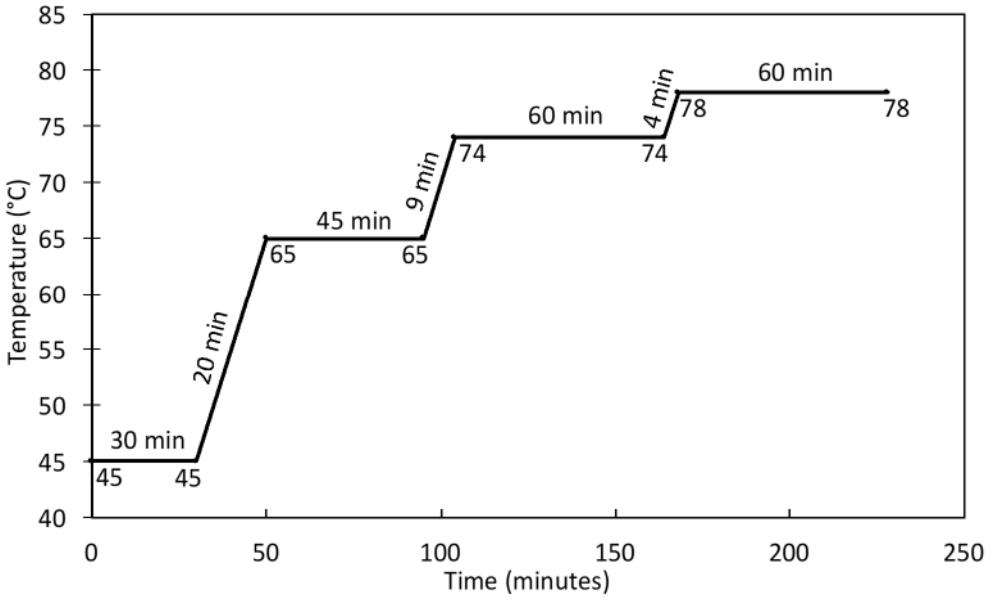

Figure 2. Time-temperature mash schedule used for brewing beers with 100% malted rice

Figure 3. Germinated paddy black (*left*) and white rice (*right*)

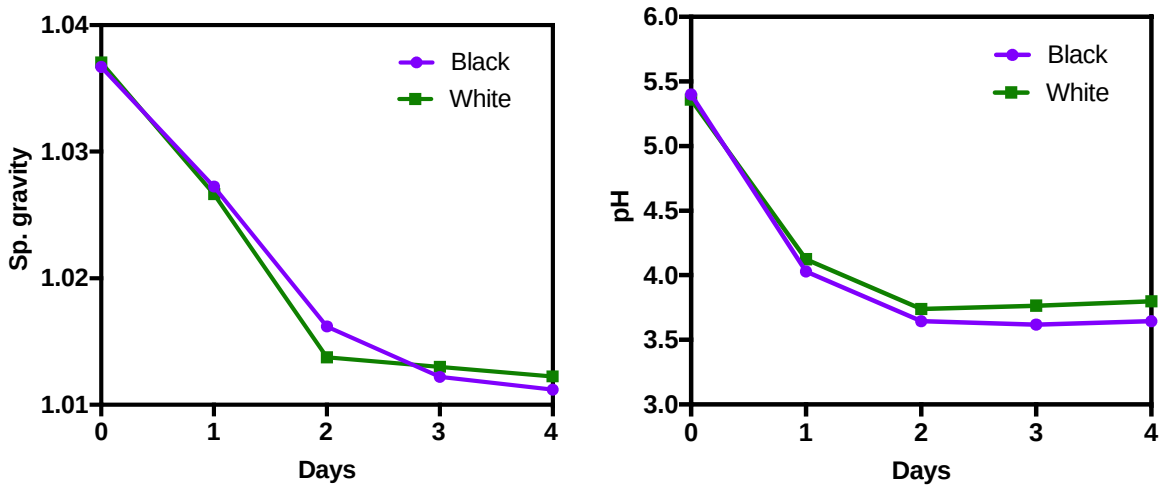

Figure 4. Changes in specific gravity and pH through fermentation of black and white rice worts

Black rice beer White rice beer

Figure 5. Black and white rice beers photographed after 21 days of bottle conditioning.

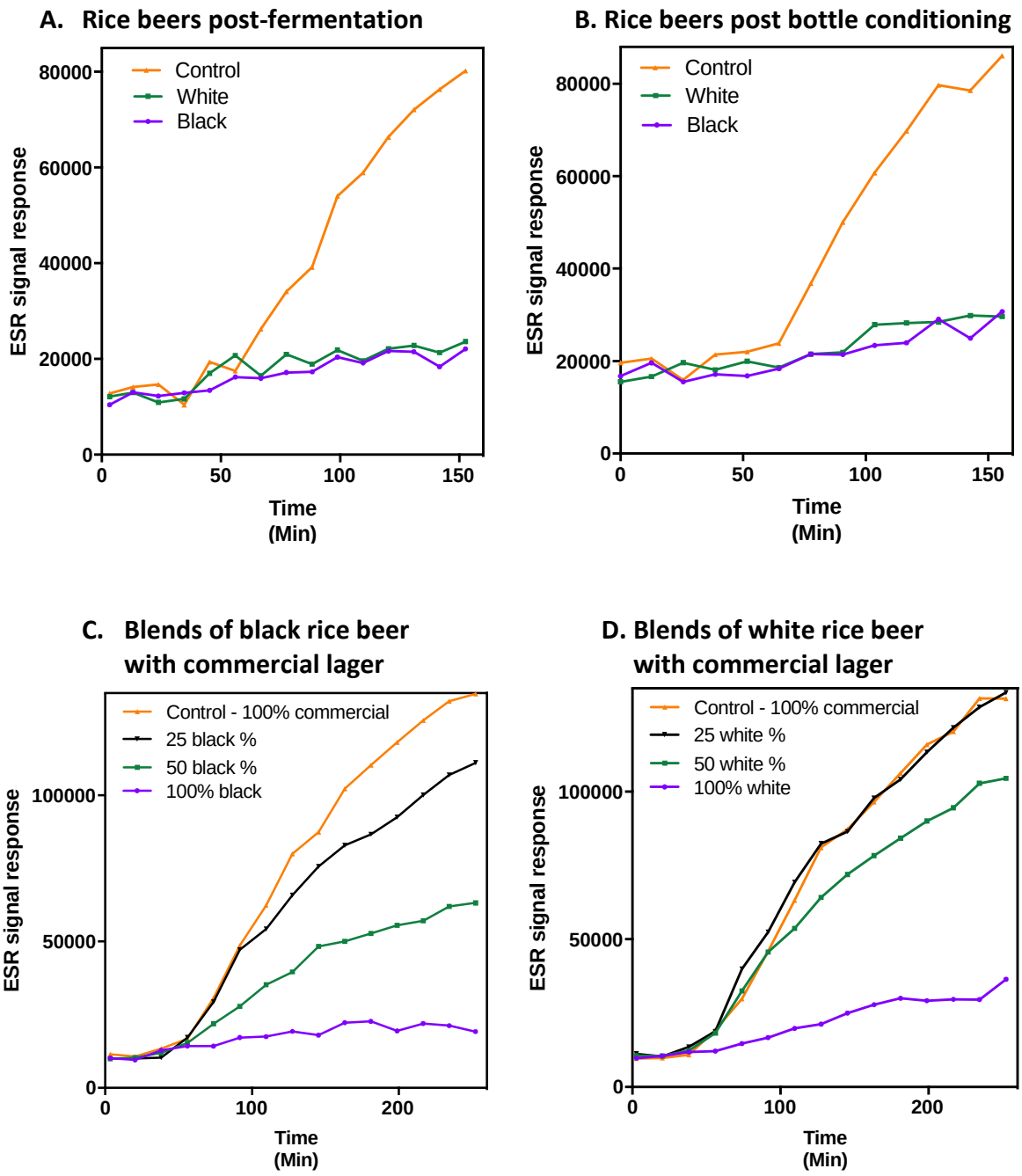

Figure 6. Oxidative stability of rice beers, and blends of rice beers with a commercial lager assessed using ESR spectroscopy

Control - commercial lager beer brewed using 100% barley malt; **A** - ESR response of back and white rice beers after fermentation; **B** - ESR response of back and white rice beer after 21 days of bottling (mature); **C,D** – ESR response of mature black and white rice beer respectively, mixed at different proportions with the control lager beer; Data are average of two biological replicates.

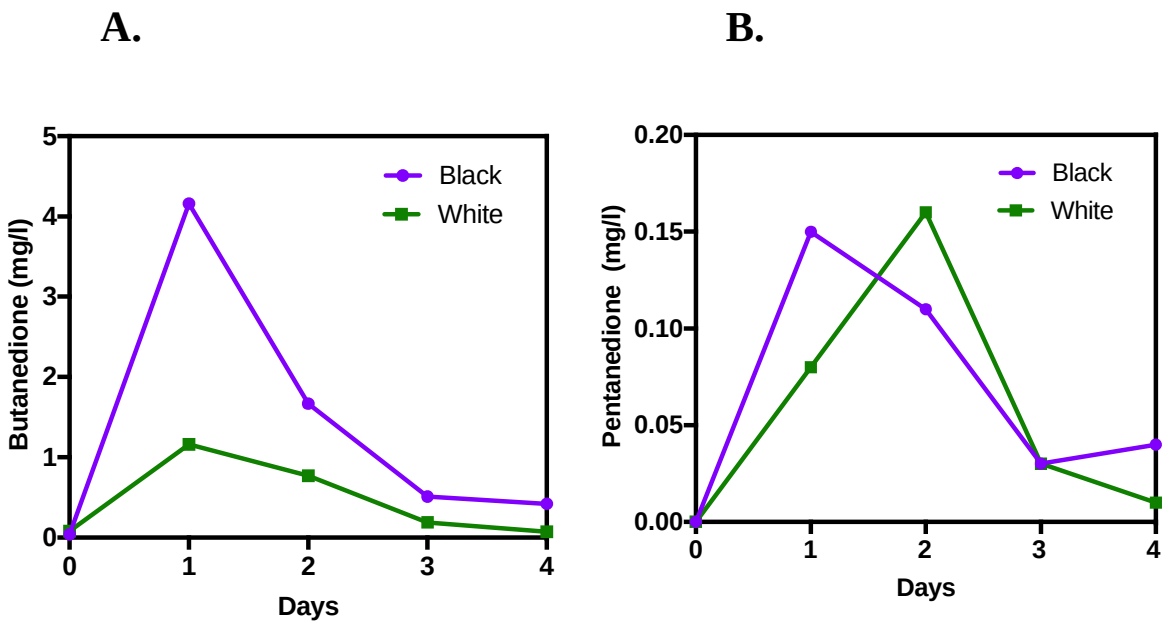

Figure 7. Example VDK profiles through fermentation of malted black and white rice worts. A. butanedione and B. pentanedione.