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Development of an all rice malt beer: A gluten free alternative

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ABSTRACT

In the current study three all-rice malt beers were produced in a traditional way from different rice malts each with sufficient endogenous enzyme activity for degradation of the rice components. The use of a simple infusion mashing program with acidification was mandatory as well as for improving hydrolytic enzymes activity as for protein degradation. Following this procedure, a complete saccharification was achieved 1 h after reaching 74 °C as ascertained with the iodine test. Lautering proceeded without difficulty, probably due to the low viscosity of the worts and to the rice husk which was ideal for filtration. Despite their low total nitrogen and FAN (Free-Amino Nitrogen) contents and suboptimal wort sugar compositions, the fermentation for all the samples proceeded regularly. The most important aroma-active substances were determined and compared to a barley malt bottom-fermented beer. Identification of the sensory profiles of the beers was also performed using a trained taste panel.

1. Introduction

Beer is a very popular beverage around the world. Generally the basic ingredient is barley or wheat malt. This can be problematic for individuals who suffer from celiac disease, an intolerance to the gluten proteins found in barley and wheat, and who should follow a gluten-free diet which would also exclude beer (Hager, Taylor, Waters, & Arendt, 2014). This is one reason for which it may be useful to replace the conventional barley or wheat ingredients in beer production with rice, a gluten-free and readily available cereal. Another reason is the possibility to produce beer with alternative raw materials in those countries where barley is not cultivated.

Rice beer production is not without challenges. Meanwhile the malting of barley and wheat produces malts that are suitable for brewing, the malting of rice has not yet yielded an appropriate malt despite several attempts made in recent years (Agu et al., 2012; Ceppi & Brenna, 2010a; Kongkaew, Usansa, & Wanapu, 2012; Zarnkow, Kessler, Burberg, Kreisz, & Back, 2005). The main problem with rice malt is that it fails to complete saccharification during mashing. This means the rice starch does not completely degrade into soluble sugars and low molecular weight starch degradation products in the wort, which is a necessary condition for beer production. As a result, the yield and the rate of lautering decrease and an undesirable haze forms during fermentation and the beer flavor suffers (Narziß & Back, 2012a). One of the reasons for the incomplete saccharification seems the high gelatinization temperature of the rice starch and an insufficiency of starch-degrading enzymes in the rice malt. But also a sufficient protein degradation, especially of the structural protein of the endosperm cell wall, is indispensable for the saccharification of the starch and has to occur prior to or simultaneously with starch modification (Narziß & Back, 2012a). This is more difficult in rice malt than in barley malt probably due to the different protein composition of rice that can be noted also in the absence of gluten. Even in the case of the alternative way to produce beer, by supplementing the unmalted raw material with commercial exogenous enzymes, it is not possible until today to produce a beer-like 100% rice beverage even though this technique is successfully applied to unmalted barley (Steiner, Auer, Becker, & Gastl, 2012), because the rice protein could not be degraded by synthetic enzymes and therefore the protein cannot solubilize in the wort (Narziß & Back, 2012a; Steiner et al., 2012; Zarnkow & Back, 2005). In this case the problem is the low nitrogen content in the wort which is crucial for several reasons. The high molecular weight proteins are important for foam quantity and stability while the low molecular weight nitrogen products e.g. amino acids, measured as FAN, are necessary for yeast nutrition and so for a complete fermentation. Moreover, the formation of

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color and aroma substances by Maillard reactions, which are important for beer quality and flavor, is reduced.

On the other hand the malting process increases proteolytic activity even if the overall low protein content of rice malt and its weak modification during malting and mashing yields less soluble nitrogen in the wort than in barley malt (Ceppi & Brenna, 2010a; Narziβ & Back, 2012a). So the rice beer production is problematic both in the traditional way by using the malted cereal and in a more modern way by adding exogenous enzymes to the unmalted raw material.

The present work will show how to produce a 100% rice malt beer following a traditional method. The prerequisites are a well-modified and saccharifying rice malt and optimized mashing conditions as reported in a recent study (Mayer, Marconi, Regnicoli, Perretti, & Fantozzi, 2014), where three rice malts capable of completely saccharifying through the work of endogenous enzymes were produced for the first time in a laboratory test. Two of the malts were of the same variety, but differed in origin and had different protein contents. To obtain complete saccharification, it was necessary to adapt the pH and temperature settings of the laboratory mashing programs, because rice malt has a different composition of the endosperm cell walls (Shibuya, Nakane, Yasui, Tanaka, & Iwasaki, 1985; Shibuya & Iwasaki, 1978; Shibuya & Misaki, 1978) and a different content and behavior of the amylolytic enzymes than barley malt (Ceppi & Brenna, 2010b; Dziedzoave, Graffham, Westby, & Komlaga, 2010; Iwata, Suzuki, & Aramaki, 2003; Iwata, Suzuki, Takahashi, & Aramaki, 2002; Iwaki & Fuwa, 1981; Nakai et al., 2007; Yamasaki, Nakashima, & Konno, 2008).

In the present study, the authors intended to verify if the good results of the laboratory mashing trials can be achieved also under brewhouse conditions and if the quality and quantity of the obtained protein degradation products is sufficient or can affect the course of fermentation or the organoleptic features of the final product. In fact the presence of a balanced amino acid and sugar composition of the wort is necessary for proper fermentation and the formation of fermentation by-products like esters, aldehydes and higher alcohols, which contribute to a beer's flavor and determine its sensory profile.

The main objective of this study was to use the three rice malts that have been previously developed to brew a beverage similar in aroma, taste and mouthfeel to beer. The all-rice malt beers were produced for the first time in a traditional way in a pilot brewery and the final rice beers were evaluated and compared to a barley malt beer. Finally the levels of major aroma-active components of bottom-fermented beers were determined and a sensory analysis was performed to assess the beer-like character of the rice beverages.

2. Materials and methods

2.1. Rice

Three samples of Italian rice were used in this study and all were harvested in 2011. The first sample was Centauro from Sardinia (8.51% protein dry matter), the second sample was Centauro from North Italy (7.62% protein d.m.) and the third sample was Balilla (7.44% protein d.m.). Each rice sample was malted in duplicate as paddy rice in an automatic micromalting system from Custom Laboratory Products (Keith, UK) as has been described in a previous article by the authors (Mayer et al., 2014).

2.2. Brewing

2.2.1. Wort production

The aforementioned malt samples (3 kg) were processed in a 20-L pilot scale brewery (Braumeister, Speidel, Ofterdingen, Germany) in duplicate. The malt was crushed in a two-roller mill (Engl-Maschinen GmbH, Schwebheim, Germany) with a 0.5 mm gap between the rollers. A 1:4 ratio of rice malt grist to brewing water (3 kg of malt to 12 L of water) was used and supplemented with $CaCl_2 \times 2H_2O$ to 85 mg/L of Ca. After mixing the malt grist with water the pH was quickly adjusted with lactic acid to 5.3. The same infusion mashing program was used for the three different rice malt samples.

The optimal pH and temperature conditions for rice malt enzymes have not been described in the literature to our knowledge with the exception of α-glucosidase (55 °C, pH 4.5 (Iwata et al., 2003)), pullulanase (60 °C, pH 5.5 (Iwata et al., 2002) or 55 °C, pH 6.0 (Yamasaki et al., 2008)). Therefore, all temperature rests were empirically developed. Mashing was conducted by increasing the temperature by 1 °C/min and by maintaining the following temperature rests: 30 min at 45 °C, 45 min at 65 °C, 60 min at 74 °C, 10 min at 78 °C. A temperature rest at 55 °C, optimal for α-glucosidase, was avoided because of the already high amount of glucose in the wort (Mayer et al., 2014). The saccharification test with iodine solution was performed every 10 min after the beginning of the temperature rest at 74 °C. All the samples completely saccharified 1 h after reaching 74 °C, before increasing the temperature to 78 °C.

After 10 min of mashing-out, the wort was transferred to the lauter tun for filtration. The lautering time for all samples was approximately 1 h. Sparging was conducted twice with 4 L and once with 2 L of water at 78 °C. The mash was boiled for 60 min. At the beginning of this phase, hop pellets (variety Perle, 7.7 α-acids) were added to achieve 15 International Bitter Units (IBU). The hopping in the final part of wort boiling was avoided in order to minimize the development of flavor compounds from the hop. After boiling and elimination of the hot trub, the wort was cooled to 13 °C. Aeration of the wort was then performed before yeast pitching.

2.2.2. Fermentation

Pitching of the yeast was carried out at 13 °C using 1 g yeast per L of wort. A dry, bottom-fermenting yeast that is commercially-available (Saflager S-23, Fermentis, Marcq-en-Barœul Cedex, France) was introduced after rehydration with sterile water to achieve 10^7 cells/mL in the wort. The use of a bottom-fermenting yeast allowed for better identification of the typical rice malt flavor. The fermentation temperature was maintained at 13 °C for 6 days. Then, after cooling down to 2 °C, the yeast was discharged from the bottom of the tank. The maturation lasted for 10 days. At the end of maturation, the beer was analyzed.

2.3. Chemical analyses

2.3.1. Wort analyses

The following analyses were performed on the worts in duplicate according to the official Analytica-EBC methods (European Brewery Convention, 2007): Extract of Wort (°P), EBC method 8.3; pH of Wort, EBC method 8.17; Color of Wort: Spectrophotometric Method (EBC-U), EBC method 8.5; Viscosity of Wort (mPa*s at 20 °C), EBC method 8.4; Fermentability, Attenuation Limit of Wort – Rapid Method (%), EBC method 8.6.2; Total Nitrogen in Wort: Kjeldahl method (mg/L), EBC method 8.9.1; Free Amino Nitrogen (FAN) in Wort by Spectrophotometry (mg/L), EBC method

8.10.

The wort yield (%) at the end of wort production was calculated as:

$$\text{Yield } \% = \frac{E \, x \, V \, x \, F}{G} \times 100$$

E: Extract content (1 g/0.1 L)
V: wort volume (L)
G: grain bill (g)
F: correction factor

2.3.2. Beer analyses

The beer samples were characterized for several quality attributes using the following Analytica-EBC methods (European Brewery Convention, 2007): Original Extract of Beer (°P), EBC method 9.4; Alcohol in Beer by Distillation (% v/v), EBC method 9.2.1; pH of Beer (method 9.35); Color of Beer: Spectrophotometric Method (EBC-U), EBC method 9.6; Total Nitrogen in Beer: Kjeldahl Method (mg/L), EBC method 9.9.1; Free Amino Nitrogen (FAN) in Beer by Spectrophotometry (mg/L), EBC method 9.10; Foam Stability of Beer using the NIBEM-T Meter 30 s (sec), EBC method 9.42; Viscosity of Beer: Glass Capillary Viscometer (mPa*s at 20 °C), EBC method 9.38. The apparent degree of fermentation was determined by following the Mebak method 2.8.4 (Mitteleuropäische Brautechnische Analysenkommission, 2013).

The contents of various amino acids (aspartic acid, glutamic acid, serine, threonine, arginine, histidine, methionine, valine, leucine, isoleucine, lysine, glycine, alanine, tyrosine, and phenylalanine) were determined in rice malt wort and beer by high performance liquid chromatography (HPLC) by quantifying the fluorescence of the orthophtaldialdehyde (OPA)/mercaptoethanol derivatives as reported by Marconi et al., 2013.

The composition of sugars in the worts and beers from rice malt were determined by HPLC coupled with evaporative light scattering detector (ELSD) as reported by Floridi, Miniati, Montanari & Fantozzi, 2001.

The free dimethyl sulphide (DMS) concentration in beer was determined according to the method from Stafisso, Marconi, Perretti & Fantozzi, 2011.

Aldehydes, alcohols and esters in beer were quantified according to slightly modified methods proposed by Vesely, Lusk, Basarova, Seabrooks & Ryder, 2003, as shown by De Francesco, Turchetti, Sileoni, Marconi & Perretti, 2015, based on solid-phase microextraction with on-fiber derivatization. An Agilent Technologies 6850 gas chromatograph equipped with an Agilent Technologies Mass Spectrometer 5975C (Santa Clara, CA) coupled with a Maestro Autosamples Gerstel Multi Purpose Sampler (Baltimore, MD) was used. The gas chromatograph-mass spectrometer was equipped with a glass direct inlet liner (1.5 mm inner diameter and 140 µl volume) and a DB-5MS capillary column of 60 m × 0.32 mm × 1 µm (J&W Scientific, Folsom, CA) consisting of cross linked 5% phenyl methyl siloxane. A 65-µm poly(-dimethylsiloxane)/divinyl benzene (PDMS/DVB) fiber coating (Supelco, Bellefonte, PA) was used.

2.4. Statistical analyses

Statistical analyses were performed using SigmaPlot Software (version 12.0; Systat Software, Inc., San Jose, CA). Different matrices were compared by one-way repeated measures analysis of variance (ANOVA), and the results were further analyzed using the Holm–Sidak test and the Tukey test.

2.5. Sensory analysis

A sensory evaluation of the beers was carried out by a trained, 18-member tasting panel (9 women and 9 men, 24–65 years old), following the EBC method 13.10 (Sensory Analysis: description analysis (IM) (European Brewery Convention, 2007)). The scores ranged from 0 to 9. A score of 0 meant that the attribute was absent whereas a score of 9 indicated that the attribute was extremely strong. The twelve attributes assessed for the taste were: fruity/estery, alcoholic/solvent, cooked vegetable, cereal, malty, oxidized/aged, bitter, sweet, sour, astringent, body and linger, while the seven attributes assessed for the aroma were: fruity/estery, alcoholic/solvent, cooked vegetable, cereal, malty, oxidized/aged and sweet. Blind-tasting was conducted on all the experimental samples. The results were depicted in a spider plot and the values were expressed as the mean of the two technological replicates.

3. Results and discussion

In the current study three 100% rice malt beers were produced in duplicate using a traditional method. The aim was to control the behavior of the rice malt in the brewing process achieving complete saccharification in the mashing step, an unproblematic wort filtration and a regular fermentation. An additional goal was the identification of typical flavor characteristics of the rice malt beers.

Complete saccharification, ascertained with the iodine test, was achieved due to the developed, optimized mashing program. Indeed, the starting temperature of 45 °C for 30 min with correction of the pH to 5.3 improved protein degradation which was a prerequisite for complete saccharification of the starch, as previously reported (Mayer et al., 2014). The lower pH-value might also enhance the activity of the most hydrolytic enzymes for starch and cell wall degradation (Meussdoerffer & Zarnkow, 2009). Only the optimum pH of α-amylase is slightly higher, between 5.6 and 5.8, so the activity of this enzyme could be therefore negatively influenced. However, this fact could be compensated by the activity of limit dextrinase which is increased by a lower pH-value (5.4–5.5 (Stenholm & Home, 1999)). Limit dextrinase content in rice malt is more than 10-fold higher than in barley malt, so this enzyme acquires great importance in the mashing process of a rice malt. The adequate temperature rests at 65 °C for limit dextrinase and β-amylase and 74 °C for α-amylase lead to complete saccharification.

In this study lautering happened without any difficulty, in contrast to the long filtration times of the laboratory worts (Mayer et al., 2014), executed at 20 °C. This may have been due to the higher filtration temperature in the plant (78 °C), as well as the low viscosity of the worts (Table 1) and the use of the rice husks which formed an ideal filter bed. Rice wort yields at the end of the wort production ranged from 64 to 66%. The fermentability values of the rice malt worts of the North Italian and Sardinian Centauro varieties were 71.5 and 73.0% respectively. This is below the value of a pale barley malt wort, which is in the range of 78–85% (Narziß & Back, 2012b). This level of fermentability was observed only in the Balilla rice malt wort (79%), probably caused by its higher β-amylase content (Mayer et al., 2014).

The very clear color of the rice malt worts (Table 1) and, as a consequence, the very clear color of the final beers (Table 2) are probably due to the low kilning temperature (70 °C) of the rice malts (Mayer et al., 2014) and the low soluble nitrogen content in the worts (Table 1). Indeed, less Maillard products and their precursors were produced, which are responsible for a great part of the wort color. Also the low pH of the cast wort (wort after boiling prior to fermentation), about 5.2 for Centauro and 5.4 for Balilla (Table 1), may have decreased the wort color (Narziß & Back, 2012b). The wort color of the variety Balilla was slightly darker than Centauro,

Table 1
Rice malt worts quality attributes.

	North Italian Centauro	Sardinian Centauro	Balilla
	Mean±sd	Mean±sd	Mean±sd
Extract (°P)	12.65 ± 0.25[a]	12.78 ± 0.01[a]	12.71 ± 0.50[a]
pH	5.20 ± 0.01[a]	5.21 ± 0.03[a]	5.41 ± 0.09[a]
Color (EBC-Unit)	5.4 ± 0.1[a]	5.5 ± 0.1[a]	6.9 ± 0.1[b]
Viscosity (mPa s at 12 °P)	1.59 ± 0.01[a]	1.61 ± 0.01[a]	1.68 ± 0.01[b]
Fermentability (%)	71.5 ± 2.1[a]	73.0 ± 0.1[a]	79.0 ± 0.1[b]
Soluble nitrogen (mg/L at 12 °P)	688 ± 9[a]	718 ± 13[a]	675 ± 17[a]
FAN 12 °P	179 ± 5[a]	160 ± 3[a]	174 ± 6[a]
Yield (%)	64 ± 1[a]	66 ± 2[a]	64 ± 2[a]

n = 2 technological repetitions. Values in the same row followed by a different letter are statistically different ($p \leq 0.05$). sd = standard deviation.

Table 2
Rice malt beers quality attributes.

	North Italian Centauro	Sardinian Centauro	Balilla
	Mean±sd	Mean±sd	Mean±sd
Original extract (% P)	12.45 ± 0.07[a]	12.45 ± 0.07[a]	12.50 ± 0.57[a]
Alcohol (%v/v)	4.59 ± 0.18[a]	4.77 ± 0.03[a]	5.12 ± 0.30[a]
Apparent degree of fermentation (%)	68.5 ± 2.8[a]	71.4 ± 0.1[ab]	76.2 ± 0.2[b]
pH	4.24 ± 0.03[a]	4.21 ± 0.01[a]	4.24 ± 0.01[a]
Color (EBC-Unit)	4.4 ± 0.1[a]	4.3 ± 0.4[a]	5.0 ± 0.8[a]
Total nitrogen (at 12 °P) (mg/L)	462 ± 14[a]	465 ± 6[a]	415 ± 39[a]
FAN (at 12 °P) (mg/L)	75 ± 10[a]	79 ± 1[a]	67 ± 6[a]
Viscosity (at 12 °P)	1.47 ± 0.01[a]	1.45 ± 0.01[a]	1.53 ± 0.06[a]
Nibem (sec)	169 ± 21[a]	166 ± 21[a]	157 ± 4[a]

n = 2 technological repetitions. Values in the same row followed by a different letter are statistically different ($p \leq 0.05$). sd = standard deviation.

but the final beer color values were not statistically different (Table 2).

Concerning nitrogen levels, the total nitrogen contents of the worts (675–718 mg/L, Table 1) were satisfactory in relation to the crude protein content, but low compared to a barley malt wort (950–1.100 mg/L, (Narziβ & Back, 2012b)). This could negatively affect the beer foam as confirmed by the low Nibem values (Table 2) of the beers. The FAN content in the rice malt worts ranged from 160 to 179 mg/L (Table 1). Generally, for a barley malt wort of 12 °P the FAN content should not be less than 150 mg/L (Meussdoerffer & Zarnkow, 2009) for a proper fermentation, but 200–250 mg/L are recommended since a low amount of total FAN promote overproduction of fermentation by-products like higher alcohols. In fact, higher alcohol concentrations above 100 mg/L can damage the flavor and quality of bottom-fermented beers (Kunze, 2004). Indeed, Table 3 shows that higher alcohols are present in greater amounts in the rice malt beers, probably due to the reduced concentration of the amino acids and a relatively high pitching temperature of 13 °C.

However, their concentration was inside the range described by the literature for a barley malt bottom-fermented beer (Mitteleuropäische Brautechnische Analysenkommission, 2013) and only the 2-Methyl-1-propanol was slightly higher.

The amino acid profile of the Centauro wort samples (Table 4, amino acid profile of the Balilla wort sample not available) shows that key free amino acids were present in adequate quantities, albeit less than in a barley malt wort. The exceptions were leucine and methionine, which were very scarce, and arginine, which was present in a relatively higher amount.

Successful fermentation also depends on the wort sugar composition. Table 5 shows the different sugar profiles of the rice malt worts of Centauro samples compared to a barley malt wort (sugar profile of the Balilla wort sample not available). In the barley malt wort the most abundant fermentable sugar is maltose, however in the Centauro rice malt worts maltose is in the same range of glucose and there were higher levels of maltotetraose, maltopentaose and maltohexaose. The same result was previously obtained in the laboratory wort for all three malts (Mayer et al., 2014). This is an indication of the different amylolytic enzymes working in the rice malts. Moreover, the sum of the fermentation onset stage sugars glucose, fructose and sucrose were 49% and 42% of the total content of the fermentable sugars for the North Italian Centauro and the Sardinian Centauro varieties, respectively. According to Annemüller, Manger & Lietz, 2011, this value should not exceed 25%, because maltose and maltotriose utilization could be delayed causing a slow primary fermentation.

Overall, despite the low total nitrogen and FAN contents and the suboptimal wort sugar composition, the fermentation for all the three samples proceeded regularly (Fig. 1). The limit attenuation was nearly achieved after 5 days of primary fermentation at 13 °C. The quality attributes of the three rice malt beers are reported in Table 2. The pH values dropped down to 4.21–4.24 probably due to the pH-correction during mashing. The alcohol contents of the rice malt beers were in an acceptable range, while the total nitrogen and FAN contents were low and this could be a reason for the poorly developed body (Fig. 2).

Table 4 shows the strong decreases of the single amino acids from wort to beer. The amino acid present in the highest amount in all three beers was arginine, as in the worts. Amino acids are non-volatile, taste-active compounds. Arginine, for example, has a bitter taste. Their concentrations in beer are very low and below the threshold limits, but like all the single taste and aroma compounds they can have interactions with other taste qualities. Through such synergistic and masking effects many compounds can result in different flavor impressions which characterize a beer (Schönberger, 2004). For this reason the most important volatile aroma-active compounds were determined in the beers and compared to a barley malt bottom-fermented beer (Table 3). These compounds, such as higher alcohols, originate from the raw material or are formed during the brewing process. The aroma-active

Table 3
Volatile compounds in rice malt beers.

Higher Alcohols	North Italy Centauro	Sardinia Centauro	Balilla	Literature value of barley malt bottom-fermented beer
	Mean±sd	Mean±sd	Mean±sd	
1-Propanol (mg/L)	17.6 ± 0.8[a]	16.2 ± 1.9[a]	17.6± 2.4[a]	5–20*
2-Methyl-1-propanol (mg/L)	36.1 ± 5.0[a]	34.1 ± 4.4[a]	37.4 ± 3.3[a]	5–20*
3-Methyl-1-butanol (mg/L)	54.1 ± 5.8[a]	60.1 ± 8.9[a]	58.8 ± 5.9[a]	30–70*
2-Methyl-1-butanol (mg/L)	26.4 ± 2.3[a]	28.8 ± 3.6[a]	27.8 ± 2.4[a]	8–30*
2-Phenylethanol (mg/L)	30.4 ± 4.2[a]	23.0 ± 1.9[a]	26.5± 2.1[a]	8–40*
2-Furanmethanol (mg/L)	0.62 ± 0.08[a]	0.48 ± 0.04[a]	n.d.	–
Esters				
Ethyl acetate (mg/L)	13.8 ± 0.5[a]	12.0 ± 0.1[a]	9.9 ± 2.4[a]	10–40*
Ethyl butanoate (mg/L)	0.07 ± 0.01[a]	0.05 ± 0.02[a]	0.06 ± 0.01[a]	0.05–0.15*
3-Methylbut-1-yl ethanoate (iso-amyl acetate) (mg/L)	0.62 ± 0.01[a]	0.33 ± 0.16[ab]	0.15 ± 0.03[b]	0.5–3*
Ethyl hexanoate (mg/L)	0.08 ± 0.01[a]	0.10 ± 0.01[a]	0.09 ± 0.01[a]	0.05–0.3*
Ethyl octanoate (mg/L)	0.23 ± 0.02[a]	0.31 ± 0.01[a]	0.23 ± 0.02[a]	0.1–0.5*
Aldehydes				
Ethanal (mg/L)	19.1 ± 0.5[a]	30.3 ± 9.3[ab]	41.9 ± 10.4[b]	2–10*
2-Methyl-1-butanal (μg/L)	12 ± 1[a]	8 ± 1[a]	11 ± 2[a]	60**
3-Methyl-1-butanal (μg/L)	46 ± 6[a]	24 ± 1[b]	36 ± 7[ab]	20**
Hexanal (μg/L)	19 ± 4[a]	20 ± 9[a]	22 ± 1[a]	4.5**
Furfural (μg/L)	42 ± 6[a]	51 ± 3[ab]	67 ± 1[b]	40**
Methional (μg/L)	25 ± 6[a]	16 ± 3[a]	15 ± 5[a]	–
Phenylacetaldehyde (μg/L)	43 ± 7[a]	46 ± 3[a]	58 ± 23[a]	45**
Trans-2-nonenal (μg/L)	n.d.	n.d.	n.d.	–
Sulfur compounds				
Dimethylsulfide (μg/L)	73 ± 4[a]	70 ± 20[a]	66 ± 15[a]	<100**

n = 2 technological repetitions, 4 analytical repetitions. sd = standard deviation; n.d. = not detectable; RT = retention time in minutes.
Values in the same row followed by a different letter are statistically different ($p \leq 0.05$).
* Mitteleuropäische Brautechnische Analysenkommission, 2013.
** Kunze, 2004.

Table 4
Amino acid profiles of rice malt worts and rice malt beers (mg/L).

	Rice malt worts		Literature value of barley malt wort*	Rice malt beers		
	North Italian Centauro	Sardinian Centauro		North Italian Centauro	Sardinian Centauro	Balilla
	Mean±sd	Mean±sd		Mean±sd	Mean±sd	Mean±sd
Aspartic acid	59 ± 13[a]	55 ± 12[a]	*40–100*	3.1 ± 0.2[AB]	4.3 ± 1.1[A]	1.8 ± 0.6[B]
Glutamic acid	68 ± 9[a]	64 ± 8[a]	*35–130*	0.9 ± 0.3[A]	n.d.	1.3 ± 0.6[A]
Serine	73 ± 16[a]	68 ± 15[a]	*40–140*	4.7 ± 0.2[A]	4.3 ± 0.9[A]	1.8 ± 0.5[B]
Histidine	97 ± 13[a]	91 ± 13[a]	*20–120*	25 ± 1[A]	23 ± 1[A]	21 ± 3[A]
Arginine	188 ± 28[a]	177 ± 26[a]	*60–200*	60 ± 15[A]	48 ± 15[AB]	27 ± 9[B]
Glycine	34 ± 2[a]	32 ± 2[a]	*20–60*	31 ± 2[A]	26 ± 1[A]	18 ± 6[B]
Threonine	51 ± 8[a]	48 ± 8[a]	*40–110*	19 ± 2[AB]	24 ± 1[A]	13 ± 4[B]
Alanine	65 ± 13[a]	61 ± 12[a]	*60–200*	24 ± 10[A]	18 ± 1[A]	11 ± 5[A]
Tyrosine	113 ± 25[a]	106 ± 23[a]	*60–200*	20 ± 7[A]	20 ± 5[A]	9 ± 3[B]
Methionine	7 ± 4[a]	7 ± 4[a]	*20–70*	9 ± 1[A]	6 ± 1[B]	6 ± 1[B]
Valine	117 ± 25[a]	110 ± 24[a]	*80–210*	8 ± 2[A]	7 ± 1[A]	3 ± 2[B]
Phenylalanine	93 ± 20[a]	87 ± 19[a]	*60–220*	7 ± 1[A]	8 ± 2[A]	4 ± 2[A]
Leucine	45 ± 11[a]	42 ± 10[a]	*100–300*	4 ± 1[A]	5 ± 2[A]	3 ± 2[A]
Isoleucine	102 ± 27[a]	96 ± 25[a]	*50–150*	10 ± 1[AB]	12 ± 5[A]	5 ± 2[B]
Lysine	111 ± 26[a]	104 ± 24[a]	*60–200*	8 ± 1[A]	8 ± 3[A]	4 ± 1[B]

n = 2 technological repetitions, 4 analytical repetitions; sd = standard deviation. n.d. = not detectable.
Values in the same row followed by a different letter are statistically different ($p \leq 0.05$), lowercase letters for wort samples and capital letters for beer samples.
* Mitteleuropäische Brautechnische Analysenkommission, 2011.

esters, other fermentation by-products that are highly desired in beer, were present in small amounts, even if their concentration was inside the range described by the literature for a barley malt bottom-fermented beer (Mitteleuropäische Brautechnische Analysenkommission, 2013) and only the iso-amyl acetate was slightly lower.

Concerning aldehydes, their concentrations in the rice malt beers were in the range of a barley malt beer (Kunze, 2004). They play an important role in flavor stability, and they are produced by oxidation of the corresponding alcohols or are derived from fatty acids and lipids present in the malt during the various stages along the malting and brewing process (Baert, De Clippeleer, Hughes, De Cooman, & Aerts, 2012). The DMS content was also low.

Table 5 shows the sugar profile of the rice malt beers. The data confirmed the complete fermentation of the worts because nearly all fermentable sugars were consumed by the yeast.

Finally, a sensory analysis was conducted on the rice malt beers. All three beers were characterized by having a pale yellow color and a white, coarse foam which rapidly collapsed. Flavor attributes were depicted on spider plots (Fig. 2). All attributes are in a low range and confirm the relatively flat character of the beer. No particular off-flavor was revealed even though ethanal exceeded the perception threshold limit of 10 mg/L (Kunze, 2004). All panelists recognized a vanillin taste in the three beers, which is a descriptor

Table 5
Sugar profiles of rice malt worts and rice malt beers (g/L).

	Rice malt worts		Literature value of barley malt wort*	Rice malt beers		
	North Italian Centauro	Sardinian Centauro		North Italy Centauro	Sardinia Centauro	Balilla
	Mean±sd	Mean±sd		Mean±sd	Mean±sd	Mean±sd
Fructose	1.0 ± 0.1[a]	1.1 ± 0.2[a]	*1.9*	n.d.	n.d.	n.d.
Glucose	33.8 ± 4.0[a]	36.9 ± 0.9[a]	*8.5*	n.d.	n.d.	0.10±0.05
Sucrose	1.1 ± 0.1[a]	1.6 ± 0.2[b]	*3–5*	n.d.	n.d.	n.d.
Maltose	22.6 ± 0.3[a]	38.3 ± 0.8[b]	*54–64*	0.3±0.1[A]	0.4±0.1[A]	0.5±0.1[A]
Maltotriose	15.5 ± 0.5[a]	15.8 ± 0.3[a]	*11–13*	1.4±0.4[A]	2.5±0.6[A]	3.1±0.5[A]
Maltotetraose	12.3 ± 0.2[a]	12.3 ± 0.8[a]	–	15.6±0.1[A]	14.8±1.3[A]	12.5±0.7[A]
Maltopentaose	5.9 ± 1.6[a]	4.3 ± 0.2[a]	–	6.0±0.2[A]	4.9±1.1[A]	3.8±0.2[A]
Maltohexaose	13.7 ± 4.2[a]	12.7 ± 1.0[a]	–	20.0±0.9[C]	7.4±0.5[B]	3.8±0.5[A]
Maltoheptaose	n.d.	0.5 ± 0.1	–	0.9±0.3[A]	0.8±0.1[A]	0.5±0.2[A]

n = 2 technological repetitions, 4 analytical repetitions; sd = standard deviation. n.d. = not detectable.
Values in the same row followed by a different letter are statistically different ($p \leq 0.05$), lowercase letters for wort samples and capital letters for beer samples.
* Mitteleuropäische Brautechnische Analysenkommission, 2013.

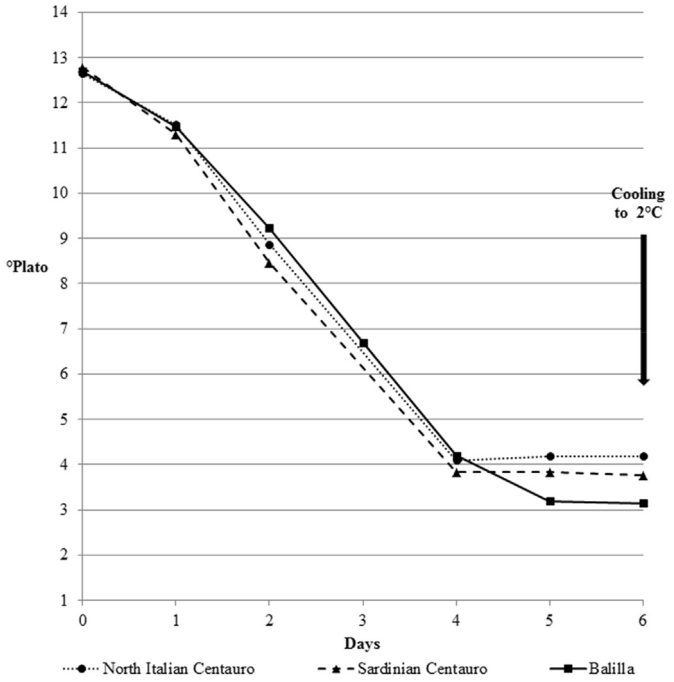

Fig. 1. Course of fermentation. n = 2 technological repetitions.

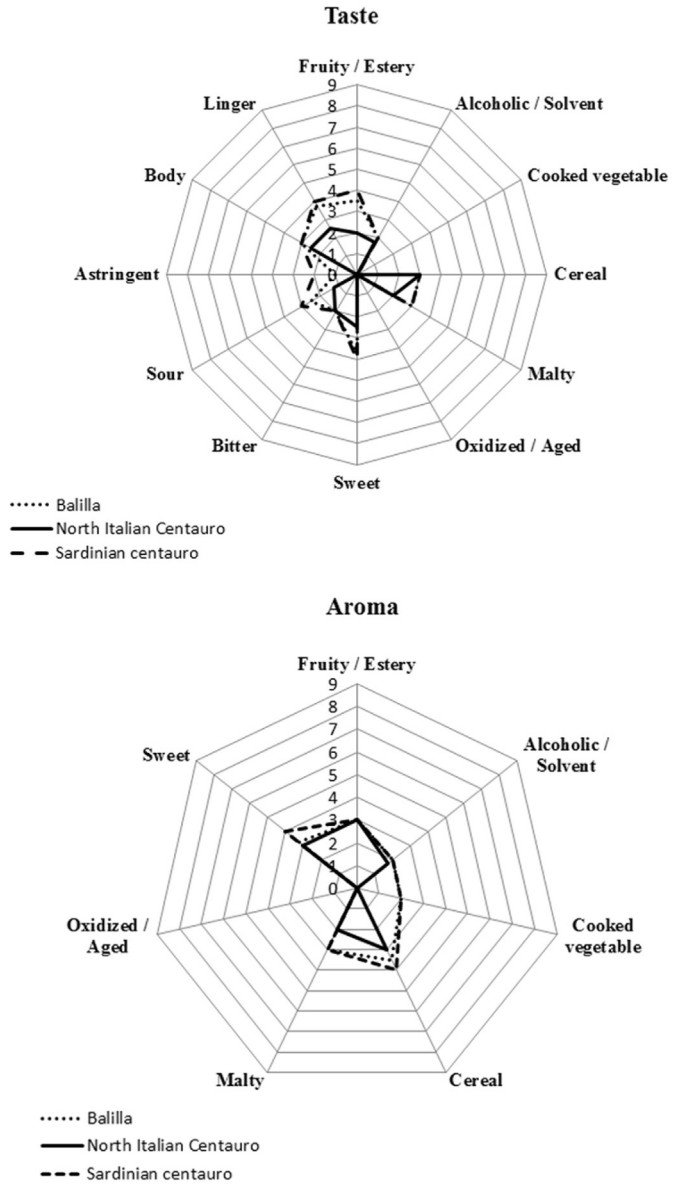

Fig. 2. Spider Plot of the taste and aroma characteristics of the rice malt beers. Legend. 10-point scale with 1-point increments (0 = none, 9 = extremely strong).

for a malty flavor, which could be typical for a rice beer. In Balilla rice beer the panelists specified a “rice” taste as descriptor for the cereal attribute.

4. Conclusions

In conclusion, the main objective of the current study, to produce for the first time an alcoholic beverage from an all-rice malt that is similar to a barley malt beer was achieved. The obtained beer samples showed a content of volatile compounds comparable with a barley malt bottom-fermented beer. The sensory profile of the rice malt beer was similar to a barley malt beer in aroma, taste and mouthfeel, but certainly more flat. Moreover, the color was very pale and the foam rapidly collapsed. These sensorial features of the rice malt beer can be surely improved in the future. The tests were performed on a pilot plant in a traditional way applying an optimized acidic mashing program, often used in barley malt brewing, but mandatory for rice malt brewing. In this way the main obstacles, such as incomplete saccharification of the rice starch and the

usually low content of protein degradation products in the rice malt wort, which have prevented up to today the production of a 100% rice malt beer, have been overcome. The use of a suitable saccharifying and well modified rice malt was decisive. Lautering happened without problems, probably due to the low viscosity of the worts and to the rice husk which was ideal for filtration. The subsequent fermentation took place regularly because the necessary protein degradation products for yeast nutrition were sufficient. Sensory analysis was performed on the final product with encouraging results. No off-flavour was revealed and the beer like character of the beverage was confirmed, representing a good alternative for the diet of individuals who suffer from celiac disease. In the future, the sensory profile may be improved by higher kilning temperatures of the malt or by means of a different yeast strain, hop variety or proportion of ingredients.

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