JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 7701–7707 7701
DOI:10.1021/jf904534q

Experimental Studies To Obtain Rice Malt

Elena L. M. Ceppi and Oreste V. Brenna*

Department of Food Science and Microbiology, Università degli Studi di Milano, via Celoria 2, 20133 Milano, Italy

The production of a rice malt that could be used as an ingredient in gluten-free foodstuffs, especially for brewing purposes, was studied. Different rice varieties were characterized through morphological description and chemical−physical analyses. Each rice variety was germinated in the laboratory in jute bags for different periods. To produce a rice malt with a good diastatic power, steeping and airing conditions, as well as time and temperature during germination, were studied. The endogenous enzymatic activities, which developed during the malting process and which characterize the diastatic power of the obtained rice malts, were also measured. The conditions of the malting process and the drying step were checked and optimized to produce rice malt with the desired color and aroma. Once the most efficient malting procedure had been chosen, the most suitable rice variety underwent the germination and kilning process in a pilot plant. Some saccharification tests were performed in the laboratory to verify the sugar content of worts obtained from the produced rice malts and whether they could be successfully fermented. The results showed that a good rice malt could be obtained, even if it has a lower enzymatic activity than barley malt.

KEYWORDS: Rice; germination; malt; beer; gluten-free; celiac disease

INTRODUCTION

The great majority of baked products are derived from gluten-containing cereals. An increasing number of consumers cannot consume these products, being affected by celiac disease. This genetic disease is a permanent autoimmune enteropathy triggered by the ingestion of gluten-containing cereals. In Italy, celiacs are currently estimated at 400,000 people, with an annual increase of about 10% [official site of the AIC, Associazione Italiana Celiaci: http://www.celiachia.it/celiachia/default.asp (*1*)]. Today there is an increase in the incidence of celiac disease, mainly due to improved diagnostic procedures (*2*). At the moment the only treatment for this disease is the exclusion of some of the most common foods from the diet, such as bread, pasta, biscuits, and pizza. In recent years much research work has been carried out in the area of gluten-free products, and results have often and successfully been transferred to industry, representing a profitable market.

Rice (*Oryza sativa* L.) is one of the most important cereals in the world and is the staple food for more than half of the world's population. Most importantly, in this context, it is a gluten-free cereal. Not only is it the basic cereal for people in Eastern countries, but fermented rice beverages are also traditionally produced there. In Western countries, wine and beer are the most common alcoholic beverages, but beer is a forbidden item for celiacs. Indeed, beer is produced from barley malt, and thus it contains gluten.

A gluten-free malt, like the one obtainable from rice, might well be a resource that could be exploited to allow celiacs to enjoy a beer-like beverage. Other cereals or pseudocereals, such as sorghum or millet (*3*) and buckwheat and quinoa (*4*), can be malted with quite good results, even though none have the same properties as barley.

Malt production is a three-stage process involving steeping (to start embryo growth), germination (to allow enzymatic modification of the endosperm), and kilning (to stop modification and dry the resultant malt). Manipulation of processing time, temperature, and air rests results in malts with different physical, chemical, and biochemical properties (*5*).

Up to now, only a few attempts have been made to malt rough rice, although some preliminary studies on Nigerian rice varieties were reported by Okafor and Iwouno (*6*). In our study, rice was the gluten-free raw material selected because it is similar to barley. It is a typical cereal in northern Italy, where it is widely cultivated (mainly in Piedmont and Lombardy); it has a low lipid content and neutral aroma and taste. Many rice varieties exist and are cultivated following different technologies, although the submersion method is still the most commonly used. The specific aim of this study was to produce a rice malt. Indeed, a malted rice with a fairly good diastatic power and appreciable sensory characteristics could be used by food industries to produce a number of products, such as gluten-free foodstuffs (as well as beer) or baby and dietetic food.

MATERIALS AND METHODS

All chemicals used were of analytical grade, and solutions were prepared using bidistilled water.

Rice Samples. Eight traditional Italian rice varieties were provided by Ente Nazionale Risi (Castello d'Agogna, Pavia, Italy) as rough rice. The samples were harvested in October−November 2005 and 2006 and were stored at 18 °C. The first crop was processed at laboratory scale, whereas the second one was malted in the pilot plant.

Table 1 shows the main characteristics of the rice varieties considered. Rice samples included both waxy (rice F) and nonwaxy varieties

*Corresponding author (telephone +39-02-50319169; fax +39-02-50319190; e-mail Oreste.Brenna@unimi.it).

Published on Web 06/04/2010

Table 1. Main Characteristics of the Studied Rough Rice Varieties[a]

	rice variety							
	A	B	C[b]	D	E	F	G	H
spikelet								
length (mm)	9.81	7.15	8.99	8.63	10.29	7.08	7.46	9.60
width (mm)	2.63	3.32	3.41	3.51	2.73	3.53	3.54	3.98
100 corn weight	2.62	2.60	3.20	3.29	2.84	2.57	2.81	3.84
caryopsis								
length (*l*, mm)	7.61	5.01	6.43	6.14	8.13	4.73	5.27	6.64
width (*w*, mm)	2.25	2.98	2.96	2.29	2.28	2.94	3.11	3.18
ratio *l*/*w*	3.38	1.68	2.17	2.10	3.57	1.61	1.69	2.09
100 corn weight	2.08	2.07	2.80	2.60	2.30	2.16	2.37	3.30
shape	long	rounded	half-tapering	half-tapering	long	rounded	rounded	half-tapering
characteristics								
pearling	absent	absent	absent	absent	absent	present	present	present
amylose content (% dm)	26.47	18.01	19.60	16.70	19.95	0.88	18.53	18.30
hardness (kg/cm^2)	0.91	0.68	0.74	0.63	0.78	0.30	0.65	0.60
stickiness (g·cm)	0.85	3.31	2.80	3.60	2.94	8.05	3.83	3.00

[a] Data are from the Website of Ente Nazionale Risi, www.enterisi.it/ris_schede.jsp; lettering is used for the sake of privacy. [b] Rice C cultivated in traditional way was studied together with the homonymous rough rice obtained by organic farming either with dry-seeded cultural system (C1) or with submersion (C2).

(A, B, C, D, E, G, and H). For some rice varieties, samples obtained with different cultivation techniques and/or by organic farming were analyzed.

Chemical−Physical Analyses. The analyses were mainly conducted according to official Analytica-EBC methods (*7*). All samples were analyzed at least in triplicate. The dimensions of corns were measured using calipers. Thousand corn weight for rough rice varieties and rice malts was evaluated using methods 3.4 and 4.4, the moisture content of the rough rice and of rice malts using methods 3.2 and 4.2 of the Analytica-EBC, and the total nitrogen for paddy rice and malted rice using methods 3.3.1 and 4.3.1. The protein content was calculated by adopting 6.25 as a conversion factor; the color of malts (method 4.7.1) was evaluated using a Beckman DU 650 spectrophotometer (Beckman Coulter SpA, Milano, Italy). The extract of malts by Congress mash determined according to method 4.5.1 of the Analytica-EBC allows the potential of malt for producing wort solubles by a standard mashing program to be determined. Moreover, it is used to establish saccharification rate, odor, speed of filtration, pH of wort, color, and viscosity of wort. The analysis was performed on 50 g of ground malt, weighed in a beaker previously tared with a balance accurate to 0.01 g (Gibertini TM 1600, Italy). Two hundred milliliters of water at 46 °C was added to the sample, and the mixture was maintained at 45 °C for exactly 30 min in a water bath (Haake DC 30, Enco Spinea, Venice, Italy). Then the temperature of the mash was raised at 1 °C per minute for 25 min. When the temperature reached 70 °C, a further 100 mL of water at 70 °C was added; the saccharification rate was measured from this point by transferring a drop of the mash to a spot on a porcelain plate and adding a drop of iodine solution. The test was repeated at 5 min intervals until saccharification was complete (a clear yellow spot was obtained). If this did not occur, the test was stopped after 1 h, so the temperature was maintained at 70 °C for 1 h. Subsequently, the mash was cooled to room temperature in 10−15 min and, after adjustment of the contents of the beaker to 450.0 ± 0.2 g by the addition of water, was filtered through Whatman qualitative filter paper grade 113 (320 mm diameter).

Filtration was stopped when the cakes appeared dry or after 2 h when slow filtration occurred. The speed of filtration was defined as "normal" if it was complete within 1 h; otherwise, it was expressed as "slow". Once the specific gravity had been measured, it was possible to calculate the extract content of malt according to specific formulas. The standard procedure described in method 4.12 of the Analytica-EBC allows the combined activity of α- and β-amylases (diastatic power) of malt under standard reaction conditions to be determined.

Twenty grams of milled pale malt was mixed with 480 mL of cold water in a previously tared beaker and left in a water bath at 40 °C for 1 h ± 2 min. The extraction solution was then cooled to room temperature and subsequently adjusted to 520 g, with a tolerance of ±0.2 g. Samples were filtered using Whatman qualitative filter paper grade 113. The first 200 mL was discarded, and the next 50 mL was used for the analysis.

Five milliliters of the malt extract was added to 100 mL of a 20 g/L starch solution and left in the water bath at 20 °C for 30 min to allow starch hydrolysis. Then enzymes were inactivated by adding 4 mL of a 1 M NaOH solution, and the volume was adjusted. An iodometric determination was performed both on sample and on blank. Unreacted iodine was titrated with 0.1 M thiosulfate solution until the blue color disappeared. Results were expressed according to specific formulas (method 4.12 Analytica-EBC).

Enzymatic kits (Megazyme International Ireland Ltd.) were used to determine α- and β-amylase activities.

Worts obtained by applying the saccharification procedure were analyzed to define color (method 8.5 Analytica-EBC) and reducing sugar content (by using Fehling's reagents). Malts produced in the pilot plant underwent several additional analyses. The germinative energy was determined according to method 3.6.1 of the Analityca-EBC, whereas the Extract Hartong 45 °C analysis was performed as described in method 4.1.4.11 of the MEBAK (*8*). Data concerning soluble nitrogen were obtained by Analytica-EBC method 4.9.1 performed on 20 mL of wort. Worts obtained were also analyzed to determine boiled wort color (method 4.19 of the Analytica-EBC) and viscosity (MEBAK method 4.1.4.4, 1997).

HPLC of Wort Sugar. The HPLC system used consisted of a 600E Multisolvent Delivery System (Waters, Milford, MA), a refractive index detector (1037A, Hewlett-Packard), and a Waters High Performance Carbohydrate column (4.6 × 250 mm) equipped with a Sentry High-Performance Carbohydrate Guard Column (Waters). Isocratic elution was carried out with 75% acetonitrile in water (both LiChrosolv, Merck, Darmstadt, Germany); the flow rate was 1.4 mL/min, and the column was thermostated at 60 °C. Before injection (50 μL), samples were diluted 10 times with water and filtered using Whatman filter paper no. 4. After this passage, the filtrate was further diluted (in a proportion 1:1:3 = sample/water/acetonitrile) and was filtered through a 0.22 μm Millipore GVWP membrane filter. The amounts of glucose and maltose were calculated from the respective calibration curves prepared using standard solutions of glucose and maltose (Sigma-Aldrich). Maltotriose and maltotetraose, to which the only other two peaks emerging could be attributed, were expressed as glucose equivalents. Results were expressed as grams of sugar per liter of sample.

Statistical Analysis. Linear regression of data and analysis of variance were conducted with Statgraphics 5.1 (STCC Inc., Rockville, MD); Fisher's least significant difference (LSD) procedure ($p < 0.05$) was used to discriminate among the means.

Malting Procedure at Laboratory Scale. The procedure for malting and brewing with some Nigerian rice varieties (*6*) was adapted to suit Italian rice cultivars, following some preliminary experiments. Rough rice was washed several times to remove dirt, sand, straws, and floating grains. The rice was then placed in a vessel fitted with a tap, steeped, and left in the dark (t_0) (Climatic Test Chamber HC 0020, Heraeus Vötsch) at 20 °C for 24 h. During the first day, the water was periodically changed, as required for barley malt. After this period (t_{24}), the water was drained from the grain and air was blown inside the mass for 20 min to remove the carbon dioxide and heat produced by respiration and to supply oxygen to the germinating

grain and then continued for a further 20 min following the addition of water. The same airing procedure (20 min without water and 20 min with water) was repeated after 6 h (t_{30}) at 20 °C.

Rice seeds were transferred into jute bags (40 × 60 cm; the layer deepness varied according to the amount of germinating rice, ranging from 3 to 10 cm), and more water was added before these were left in the Climatic Test Chamber.

Samples were germinated for 3, 5, and 7 days, and the jute bags were wetted at regular intervals according to their needs; intervals were specified in terms of time from the beginning of the germination process, as shown below for samples germinated for 3, 5, and 7 days.

3 days: t_{48}, t_{56}, t_{72}, t_{80}, t_{96}.

5 days: t_{48}, t_{56}, t_{72}, t_{80}, t_{96}, t_{104}, t_{120}, t_{128}, t_{144}/

7 days: t_{48}, t_{56}, t_{72}, t_{80}, t_{96}, t_{104}, t_{120}, t_{128}, t_{144}, t_{152}, t_{168}, t_{176}, t_{192}.

When germination was ended (3 days = t_{102}, 5 days = t_{150}, 7 days = t_{198}), rice "green malt" was placed in aluminum trays, and kilning took place in a heater initially at 50 °C for 5 h and then at 65 °C for 12−18 h depending on the amount of rice.

After kilning, the malt was cooled, seedlings and rootlets were removed, and then the malt was stored for a minimum of 2 weeks to obtain a homogeneous product and vacuum-packed to avoid changes in the moisture content.

Some roasting trials were performed on small amounts of germinated rice to improve color and flavor.

Mashing Procedure at Laboratory Scale. The mashing procedure performed at laboratory scale was essentially based on information given by Okafor and Iwouno (*6*). To produce a "sweet wort", rice malt and water were mixed in a 1:4 ratio; the obtained mash was heated to 50 °C in a HAAKE DC30 water bath and allowed to stand for 30 min. After this first rest, the supernatant was decanted and the starch was heated on a heating plate (GWM, CERAN Schott) to 88 °C (10 min rest) to achieve gelatinization. The supernatant was returned to the cooled and gelatinized starch (expected temperature of 62 °C), and the resulting mash was heated to 67 °C and held at this temperature for 60 min. The pH was tested and adjusted to 5.6 by the addition of a few drops of 85% phosphoric acid. The following 30 min rest was at 71 °C, and then the temperature of the mash was raised and maintained at 73−75 °C for 30 min. At the end of the procedure the mash was filtered using fluted Whatman no. 113 filter paper, and the residue was sparged with water at 78 °C.

Malting Procedure in the Pilot Plant. The best malting conditions defined at laboratory scale were also applied in a German pilot plant (100 kg per batch), located in the Weyermann Malzfabrik (Bamberg, Germany). The pilot plant, called "Unimalt", is a stainless steel cylindrical steep−germination−kilning vessel (SGKV); thus, no transfer is required. It is filled from above through an opening similar to that of cylinder-conical tanks, and it has a false bottom, consisting of slotted metal plates mounted above the true base, so that water can be drained from the grain. The plant is also equipped with a helical turner and a temperature control system, made up of four temperature detectors placed at different levels. Several other parameters, such as relative humidity, fan speed, percentage of recirculated air and its humidity, and percentage of cold air, can be kept under control. On one side the Unimalt is also provided with three openings to collect samples at the top, in the middle, and at the bottom of the vessel.

The following procedure was applied. The paddy rice was placed in the pilot plant and washed several times to remove powder, dirt, and "floating rice". This last was drawn off through a pipe by overflowing. The steeping phase took place at a temperature of 18−20 °C and lasted for 48 h. During this period water was periodically drained from the grain and replaced with fresh, and air was blown into the base of the plant at regular intervals. About 24 h from the beginning of the wet steeping process, water was drained and a dry steeping took place for about 8 h. The second wet steeping lasted for about 15 h, then water was drained again, and the rice was allowed to stand for 1 h.

Once the steeping time had ended, the germination step began. In the first four trials rough rice was germinated at 20 °C for 6 days, and then three more trials were carried out for 7 days. The grain temperature was controlled by adding and draining water and by forcing a stream of attemperated and water-saturated air through the rice mass. Sometimes water was sprayed on the top because the upper portion of rice was often drier than the layers below. At the end of the germination step, the green rice malt was kilned according to the steps reported in **Table 2**.

Table 2. Kilning Conditions Applied to Two Different Sets (W6d and W7d) of Malt Germinated at Pilot Scale for 6 and 7 Days, Respectively

kilning step	6-day germinated malt (W6d)	7-day germinated malt (W7d)
heating	from 45 to 65 °C within 8 h	from 45 to 58 °C within 8 h
pause	4 h, 60 °C	4 h, 58 °C
heating	from 60 to 65 °C within 30 min from 65 to 75 °C within 90 min from 75 to 80 °C within 90 min from 80 to 85 °C within 90 min	from 58 to 63 °C within 4 h
pause	4 h, 85 °C	6 h, 63 °C

Table 3. Diastatic Power of Malts Obtained by Different Rice Varieties Germinated for 7 Days at 20 °C, Reducing Sugar Content, and Expected Alcoholic Content of Sweet Worts[a]

sample	DP (WK units)	reducing sugars (g/100 mL)	expected alcoholic content (% vol)
A	119.7 f	5.54 e	3.32
B	109.4 f	3.93 b	2.36
C	79.2 cde	5.92 f	3.55
C1	68.4 bc	5.05 d	3.03
C2	68.4 bc	5.02 d	3.01
D	71.9 bcd	5.66 ef	3.40
E	51.5 a	3.51 a	2.11
E1	61.6 ab	3.63 a	2.18
F	92.3 e	5.20 d	3.12
G	112.9 f	4.26 c	2.55
H	82.1 de	4.44 c	2.66

[a] Different letters within a column indicate significant differences among rice samples (LSD, $p < 0.05$).

After kilning, the malt was cooled to 35 °C by decreasing the temperature of the circulating air. Then seedlings and rootlets were removed by rubbing seeds against a sieve, and the malt was packed into waterproof bags used for barley malt and analyzed.

RESULTS AND DISCUSSION

Preliminary Studies on Eight Rice Varieties. To select the most suitable varieties for the malting process, in the beginning, rice samples were germinated for 7 days only. **Table 3** shows the diastatic power (DP) of malts, reducing sugar content, and expected alcoholic content of sweet worts obtained at laboratory scale. According to the DP values, rice malts A, B, and G can be identified as the best samples from an enzymatic point of view, as their amylolytic activity exceeds 100 Windisch−Kolbach (WK) units. Instead, rice malts obtained by germinating samples E, E1, C1, and C2 had a low amylolytic activity, whereas the other samples showed mean levels of DP ranging between 71.9 and 92.3 WK units. These values are lower than those obtained from barley malt, which are usually higher than 200 WK (*9*).

Some saccharification tests allowed a sufficient amount of sweet wort to be obtained within a reasonable time (< 60 min), thus the reducing sugar content of sweet worts could be determined. Filtrates were found to contain variable amounts of reducing sugars according to the Fehling method. In particular, data ranged between 3.51 (sample E) and 5.92 g/100 mL (sample C) and a first grading showed that the worts obtained from malts A, C, C1, C2, D, and F were the best ones, with values ≥ 5.00 g/100 mL of reducing sugars. The expected alcohol content was quite low and in some cases < 3.00% vol, but it must be taken into consideration that, during this first step, no boiling was performed, so sweet wort was not concentrated, as usually happens during beer production.

Once the approximate amount of reducing sugar was known, worts were analyzed by HPLC to determine the wort sugar

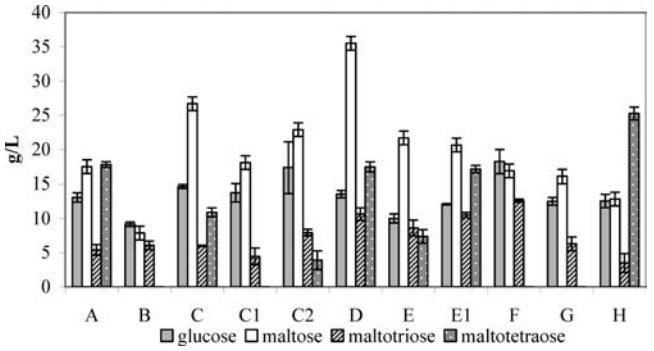

Figure 1. Glucose, maltose, maltotriose, and maltotetraose contents of sweet worts obtained from different rice varieties germinated for 7 days.

composition, to better quantify and qualify the types of sugars. As shown in **Figure 1**, the main sugar was maltose in almost all of the samples except for worts obtained from malts B, F, and H, in which it was lower than or approximately equal to the glucose content.

Glucose, maltose, and maltotriose were detected in all worts; on the contrary, maltotetraose was absent in samples B, C1, F, and G.

Generally the amount of maltotriose ranged between 3.5 and 6.5 g/L (as glucose equivalents), but in worts C2, D, E, E1, and F values of about 8.0−13.0 g/L were reached. Results obtained by HPLC were considered together with those concerning reducing sugar content and diastatic power in order to select some rice varieties for a subsequent study.

The samples chosen were rough rices A, C, D, F, and G. Sample A was selected for its good amylolytic activity and consequently its positive behavior during mashing. Its reducing sugar content was quite high, and it had a balanced wort sugar composition. In fact, maltotriose and maltotetraose, which are essential for enhancing the body of the beer, were present in fairly good amounts (5.38 and 17.78 g/L, respectively).

With regard to malt C, the DP was not so high, but the reducing sugar content (the highest among all of the rices analyzed) and the amount of glucose and maltose in the wort indicated that it might be a promising sample. Indeed, these sugars could be fermented by yeast in a future fermentation step, whereas trimer and tetramer, which were present in satisfying amounts, would improve the mouthfeel of the beer.

Rice D, with a very good reducing sugar content, was also chosen for the carbohydrate composition revealed by HPLC testing. It was characterized by the highest amount of maltose (35.5 g/L) and by a good level of glucose. Moreover, the amounts of maltotriose and maltotetraose were sufficient to ensure a fairly good body in the final product.

Sample F, the only waxy variety tested, proved to be a malt with a good diastatic power and produced worts rich in sugars. Even if the chromatogram indicated that worts produced using malt F were lacking in the tetramer, the collected filtrates were characterized by substantial glucose, maltose, and maltotriose contents.

The last selected rice was sample G. Its DP was the determining factor in its choice; indeed, its amylolytic activity was higher than those of other malts. With regard to the sugar quality and quantity of worts produced from malt G, fairly good results were observed.

Characterization of the Five Selected Rough Rice Varieties before and after Malting. Thousand corn weight and total nitrogen and protein contents of the five paddy rices were determined to better characterize the samples (**Table 4**). The data regarding the 1000 corn weight identified rices C and D, the half-tapering ones, as the heaviest varieties. The total nitrogen content in samples A, C, D, and F was slightly higher than 1% and ranged between 1.17 and 1.21% (dm), whereas results for the protein content were about 7.31−7.63% (dm). Rough rice G was noticeable for its low nitrogen content and consequently for the smaller protein content [values reported in the literature vary widely (*10*)].

Table 4. Thousand Corn Weight and Total Nitrogen and Protein Contents of the Five Selected Rough Rice Samples[a]

rough rice	1000 corn weight (g)	total N (% dm)	protein content (% dm)
A	27.1 b	1.18 b	7.37
C	32.4 d	1.17 b	7.31
D	31.9 c	1.21 b	7.56
F	26.7 b	1.18 b	7.37
G	26.0 a	0.90 a	5.63

[a] Different letters within a column indicate significant differences among rice samples (LSD, $p < 0.05$).

It is evident that rough rice has lower nitrogen and protein contents than barley, which has about 10.5−12.0% dm (*11*, *12*). Because nitrogen and protein are strictly related to foam and haze formation in beer, it was decided to check their contents in raw material to predict what would happen with malting and brewing.

Each sample was then germinated for 3, 5, and 7 days to better observe the trend of the examined variables. The resultant malts underwent the same analyses as for the eight varieties, as well as some other determinations such as extract EBC and color of wort, which are more indicative for brewing purposes. The main results are reported in **Table 5**. It can be noted that the 1000 corn weight decreased slightly in all samples with germination time because of the greater growth of rootlets and shoots, which are removed after the kilning process.

Moisture content was also determined; this parameter was a little higher than usual for barley malt [≤5%, (*13*)13], and values increased slightly in the course of germination time. **Table 5** also shows data concerning EBC extract and related practical information that contributed to the final selection of malts suitable for beer production. To this end, both speed of filtration and saccharification rates were taken into account.

The main results indicate that the speed of filtration was normal for all samples except for malts C, D, F, and G after 3 days of germination and for malt G at the fifth day. On the contrary, malt A was characterized by very good filterability and dry spent grains already at the first germination stage. The saccharification rate was also monitored, and the only malt characterized by complete saccharification after 60 min was sample F, which was germinated for 7 days.

In general, malts obtained from the five rough rice samples show similar data regarding extract, except for malt A, which is characterized by an extract percentage of dry matter much lower than other samples, with a maximum average of 34.98% in the dry matter in correspondence to the seventh day. Rice A germinated in an inhomogeneous way, and faulty sampling could be the origin of this poor result.

As reported in the literature, extract values for barley malt are defined as good if >82% and as poor if <79% (*9*); therefore, on the basis of these considerations, 7-day malts obtained from rough rices D, F, C, and G showed improved values, close to good levels.

Applying the procedure to determine the EBC extract, a small amount of filtrate was taken to assess the malt color. This analysis was not performed on samples obtained from malt F because of the high viscosity of the filtrate, which could not be filtered through a Millipore Millex HA 0.45 μm membrane filter, as required by the official method. Results are expressed as EBC units derived from absorbance at 430 nm, and reference data concerning a pilsner malt (2.5−4.5 EBC) were taken into account. In light of these data, rice malts were paler than pilsner malt, and

Table 5. Thousand Corn Weight, Moisture, and Extract of Rice Varieties Germinated for 3, 5, and 7 Days and Reducing Sugar and Expected Alcoholic Contents after Fermentation of Worts Obtained from Malts[a]

malt	germination time (days)	1000 corn weight (g)	moisture (%)	extract (% dm)	speed of filtration	saccharification	color of malt (EBC)	reducing sugar content (g/100 mL)	expected alcoholic content (% vol)
A	3	24.4	5.26 a	15.58 a	slow	>60 min	1.40 a	1.09 a	0.65
	5	23.8	5.32 b	19.16 b	normal	>60 min	1.33 a	3.10 b	1.86
	7	23.3	5.42 c	34.98 c	normal	>60 min	1.65 b	6.06 c	3.64
C	3	30.4 b	5.77	57.49 a	slow	>60 min	1.58 a	1.97 a	1.18
	5	28.3 ab	5.74	71.31 b	normal	>60 min	1.55 a	5.15 b	3.09
	7	26.5 a	5.79	74.38 c	normal	>60 min	2.42 b	8.05 c	4.83
D	3	29.4 b	5.45 a	60.2 a	slow	>60 min	1.73 a	1.50 a	0.90
	5	26.7 a	5.61 b	70.2 b	normal	>60 min	1.74 a	5.60 b	3.36
	7	26.1 a	5.69 b	75.81 c	normal	>60 min	2.18 b	8.31 c	4.98
F	3	24.4 b	5.58 a	nd	slow	>60 min	nd	1.47 a	0.88
	5	23.2 ab	5.64 a	74.19	normal	>60 min	nd	5.36 b	3.22
	7	22.0 a	5.72 b	75.83	normal	>60 min	nd	6.13 c	3.68
G	3	24.1	5.38 a	nd	slow	>60 min	nd	nd	nd
	5	23.5	5.54 b	68.53 a	slow	>60 min	1.70 a	3.71 a	2.22
	7	23.1	5.61 b	72.15 b	normal	>60 min	1.88 b	6.96 b	4.18

[a] For each malt sample, different letters within a column indicate significant differences related to germination time (LSD, $p < 0.05$).

only samples C and D showed values closer to the reference minimum, with 2.42 and 2.18 EBC units, respectively. There was almost no difference in color between samples obtained after 3 and 5 germination days, whereas the color increased in 7 day malts, surely because of the larger amounts of sugars that can take part in Maillard reactions.

After the above-mentioned analyses, all samples underwent the same mashing procedure as that used in the laboratory and were analyzed to detect the amount of reducing sugars (**Table 5**). As expected, the values increased with the extension of the germination period, and malts germinated for 7 days were observed to have a higher sugar content than those reported under Preliminary Studies on Eight Rice Varieties. Malts germinated for 3 days gave worts with low amounts of sugar, in the 1.09−1.97 g/100 mL range. It proved to be difficult to use samples at the fifth day of germination for brewing purposes, whereas 7-day malts gave good results. Among these last samples, the best ones were produced from rough rices C and D (8.05 and 8.31 g/100 mL, respectively), although the others were characterized by a high reducing sugar content.

Figure 2 shows the amounts of glucose, maltose, maltotriose, and maltotetraose determined by HPLC. All rice malts showed a general increase in the amount of sugars up until the seventh day. This determination was performed to ascertain the sugar composition of worts and to determine which were the most balanced, again in accordance with the main aim of this study. As mentioned above, each 3-day malt was characterized by insufficient modification. After 5 days of germination, the sugar content increased and both maltotriose and maltotetraose were detected, except in samples A 5d and G 5d, in which the tetramer was absent. In addition to having fairly good amounts of the sugars responsible for the body in beer (trimer and tetramer), samples germinated for 7 days had a much higher maltose content with respect to glucose. This may be explained by the fact that β-amylase activity, which splits starch into disaccharides, predominates at this stage. We can observe that, in sample A 3d, only glucose was detected, whereas in the other 3-day tests maltose was already present. No sweet wort was collected from malt H.

Sample C already seemed to be the best at the first germination stage; after 5 days, its wort was characterized by all four sugars in satisfactory amounts, whereas, at the seventh day, maltose and

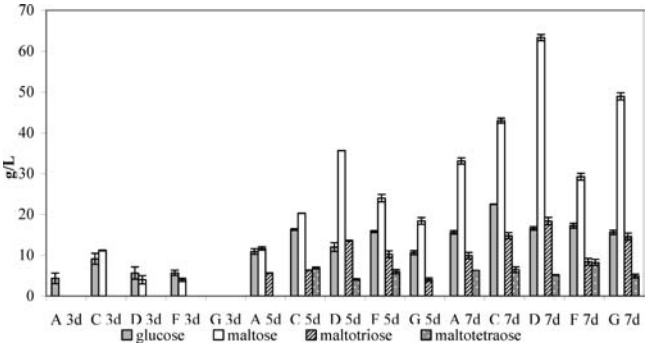

Figure 2. Glucose, maltose, maltotriose, and maltotetraose contents of worts obtained from malted rices A, C, D, F, and G after 3, 5, and 7 days of germination.

maltotriose amounts doubled with respect to the values at the previous stage. Worts obtained from malt D had a trend similar to those from malt C.

To better explain the sugar content and composition of sweet worts, the data on diastatic power and α- and β-amylase activities are discussed below.

As shown in **Table 6**, the DP of the malted rice showed increasing values until the seventh day, ranging between 39.7 and 225.1 WK units. Malts A and C had similar trends and values at all three germination stages, with a good increase after the second phase, whereas sample D differentiated itself especially between the fifth and seventh days of germination. On the contrary, malts F and G showed a completely different and leveled behavior. Indeed, even if after 3 days of germination the amylolytic activity of these two samples was about double that of the others, following this, the value for amylolytic activity remained almost unchanged until the seventh day.

Malts obtained at laboratory scale had a low DP compared to values for barley malt [> 215 WK units (*9*)] except for samples A and C germinated for 7 days, which had enzymatic activities of 220.3 and 225.1 WK units, respectively.

After this first analysis, the activities of both α- and β-amylases were determined using enzymatic kits (**Table 6**). Regarding

Table 6. Enzymatic Activities of Malts A, C, D, F, and G at Different Germination Times[a]

malt	germination time (days)	DP (WK units)	α-amylase activity	β-amylase activity
A	3	39.7 a	6.34 a	52.03 a
	5	101.2 b	12.45 b	55.6 a
	7	220.3 c	16.66	88.14 b
C	3	47.2 a	4.73 a	51.19 a
	5	96.1 b	20.04 b	71.82 b
	7	225.1 c	37.02 c	113.02 c
D	3	54.3 a	1.91 a	46.42 a
	5	94.2 b	8.75 b	70.04 b
	7	145.1 c	21.56 c	139.12 c
F	3	90.6 a	3.08 a	56.10 a
	5	116.0 b	17.5 b	69.09 b
	7	120.0 b	17.76 b	90.10 c
G	3	94.0 a	0.6 a	53.94 a
	5	94.2 a	2.59 b	59.79 a
	7	119.5 b	12.03 c	93.91 b

[a] For each malt sample, different letters within a column indicate significant differences related to germination time (LSD, $p < 0.05$).

α-amylase activity, it can be noted that even if malted rice samples were characterized by similar initial values, they then followed different trends. The best results were recorded for malt C after 5 and 7 days, whereas, for malted rice F, the α-amylase activity increased until the fifth day and then remained constant until the seventh day. Sample G showed the lowest activity which, however, increased about 5 times, from 2.59 to 12.03 CU/g of malt, after 5 and 7 days, respectively. This explains the behavior of malt G during saccharification tests, for which improvements in liquefaction and filtration were observed with samples germinated for 7 days. In general, it can be said that after 3 days of germination, the α-amylase activity is very scanty in all samples, whereas during the last stage of germination all malts reach medium to high levels of activity.

Results regarding β-amylase activity (**Table 6**) indicate similar trends and values for all malts. There were fairly good levels of activity in samples germinated for 3 days, and this may be because β-amylase was already present in ungerminated rice, as it is in barley (*14*), and only needed to be activated during germination, when new β-amylase was also synthesized. The higher β-amylase activity connected with the seventh germination day was responsible for the improvement noted during saccharification tests with respect to trials performed with 5 day samples. All samples showed a general increase in both α- and β-amylase activities up to the seventh day. In view of the results obtained, it was decided that germination for 7 days at 20 °C was needed. Moreover, it was possible to identify two varieties of rough rice samples, which appeared to be the most suitable for malting and consequent brewing purposes. These were samples C and D. Despite these considerations, experimentation on rice D could not continue, as it was no longer cultivated in amounts sufficient to satisfy the demand for brewing use. For this reason further tests were performed only on rice C, and larger amounts (approximately 15 kg instead of 5−7 kg) were germinated for 7 days at 20 °C and analyzed. The results obtained were quite similar to those for the small laboratory batches, so it was decided to test the malting procedure in a pilot plant.

Malting in the Pilot Plant. Table 7 shows results for some of the best malts obtained by applying the slightly modified malting procedure in the German pilot plant.

Moisture and protein content and germination energy were determined in rough rice (crop 2006) before being malted in the pilot plant. The moisture content was 12.5%, whereas a lower protein content than that usually found in barley samples [7.27% as compared with about 11.5% in the dry matter (*12*)] was found. The germination energy, calculated as the percentage of grains that can be expected to germinate when malted normally, was equal to 83 and 87% after 3 and 5 days, respectively, enough to try a pilot malting, even if not an optimal value.

It was decided to test a procedure more similar to that used to produce barley malt with a higher maximum kilning temperature (85 °C), but still keeping 20 °C as the steeping and germination temperature. A longer dry steeping and a shorter germination step (6 days) were suggested by German technicians to allow water outside to penetrate into the corn and to reduce the growth of rootlets and acrospires, with a consequent saving of starchy substrate. A similar 6-day germination had already been tested at laboratory scale with a 20% loss in the enzymatic activity in comparison with a 7-day malt; however, the suggestion was followed as it could be a good chance to try it in the 100 kg plant, where all variables could be kept under better control.

Data shown in **Table 7** refer to first malts obtained with a 6-day germination. The final moisture content (4.0−4.9%) was lower than that obtained at laboratory scale, due to a stronger kilning program, as shown in **Table 2**, which may, however, have had negative consequences on enzymatic activity. Concerning EBC extract determination, the method developed for barley malts has been applied to rice malt, even if rice starch has a higher gelatinization temperature than that of barley. The lack of gelatinization may explain why lower extract values, ranging between 61.7 and 68.0%, are usually obtained with rice. The same extract determination was subsequently calculated on dry matter according to the moisture content of the samples.

The Hartong index helps to evaluate if malts are undermodified (< 35%) or overmodified (> 40%). Normal values for barley malts range between 35 and 40% (*9*); thus, these rice malts are undermodified (**Table 7**). The required saccharification time is > 60 min, whereas a pH value of about 6.3 is a little bit higher than that required during mashing. Figures for wort color were quite low (1.8−2.0 EBC units) even if, after boiling, the color increased to acceptable levels, ranging between 4.2 and 6.2 EBC units. Viscosity, mainly due to β-glucans, was calculated on Congress (EBC) wort, giving higher values than those recommended (< 1.60 mPa·s) and confirmed that insufficient modification occurred in rice malts.

The protein content in malts ranged between 7.0 and 7.6% in the dry matter, as compared with the 10% found in pilsner malt (*13*). The total soluble nitrogen was determined, as it would contribute to the "body" and mouthfeel of the beer and as it is also strictly related to beer foam (or "head") stability. Instead, the "Kolbach index", that is, the soluble protein/total protein ratio, was determined as for EBC congress wort, as a measure of the protein modification in malting.

According to the reference data for barley malt (*9*), it can be said that these rice malts have a low soluble protein percentage (average value = 1.2%) and a low Kolbach index value (15.1−18.6% instead of 35−45%), which means that they were poorly modified during germination. Some problems that may in fact arise with undermodified malts include low extract recovery and slow wort separation; moreover, the worts obtained may be cloudy, the hot break may form poorly, and the wort will ferment slowly, with a low yield.

After these first pilot malting trials, it was decided to extend germination for 7 days and to reduce the dry steeping time again. Comparison of the results concerning 7-day malts (**Table 7**) with those obtained for 6-day malts shows that the moisture content

Table 7. Characteristics of Malts Germinated for 6 and 7 Days in the German Pilot Plant, W6d and W7d

	malt						
parameter[a]	W6d-1	W6d-2	W6d-3	W6d-4	W7d-1	W7d-2	W7d-3
moisture content (%)	4.0 ± 0.1	4.7 ± 0.1	4.9 ± 0.2	4.9 ± 0.1	5.1 ± 0.1	5.2 ± 0.1	5.0 ± 0.1
extract							
EBC extract (%)	61.7 ± 1.6	66.4 ± 1.9	66.4 ± 1.8	68.0 ± 1.3	68.7 ± 1.4	69.7 ± 1,7	69.7 ± 1.5
(% dm)	64.3 ± 1.2	69.7 ± 2.3	69.8 ± 2.0	71.5 ± 1.9	72.4 ± 1.8	73.5 ± 1.9	73.4 ± 1.8
extract Hartong 45 °C (H_{45}) (% dm)	15.3 ± 0.2	17.0 ± 0.9	15.3 ± 0.4	15.7 ± 0.4	18.5 ± 0.4	18.1 ± 0.6	22.1 ± 0.3
color							
wort color (EBC)	1.9 ± 0.1	2.0 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.8 ± 0.1
boiled wort color (EBC)	4.2 ± 0.1	4.7 ± 0.1	4.4 ± 0.2	6.2 ± 0.1	4.8 ± 0.1	4.8 ± 0.1	6.4 ± 0.1
pH, viscosity, and saccharification time							
wort pH	6.32 ± 0.09	6.30 ± 0.18	6.32 ± 0.15	6.33 ± 0.21	6.14 ± 0.13	6.13 ± 0.15	6.10 ± 0.09
saccharification time (min)	>60	>60	>60	>60	>60	>60	>60
viscosity cal 8.6% (mPa·s)	1.94 ± 0.04	1.53 ± 0.05	1.59 ± 0.07	1.57 ± 0.05	1.54 ± 0.06	1.55 ± 0.06	1.55 ± 0.05
viscosity cal 12.0% (mPa·s)	2.60 ± 0.10	1.82 ± 0.05	1.93 ± 0.03	1.90 ± 0.04	1.85 ± 0.04	1.87 ± 0.05	1.87 ± 0.05
nitrogen/protein determination							
protein content (% dm)	7.3 ± 0.2	7.0 ± 0.3	7.6 ± 0.1	7.5 ± 0.1	7.1 ± 0.1	7.0 ± 0.2	7.2 ± 0.01
TSN (mg/L)	193 ± 4	207 ± 6	184 ± 5	199 ± 6	255 ± 7	260 ± 7	296 ± 8
soluble protein (% dm)	1.2 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.9 ± 0.1
Kolbach index (% dm)	16.4 ± 0.4	18.6 ± 0.5	15.1 ± 0.6	16.6 ± 0.6	22.4 ± 0.8	23.2 ± 0.5	25.7 ± 0.6

[a] Values are from triplicate analyses of each different malt.

was a little higher (5.0−5.2%), but in an acceptable range for storage. This may be due to the milder kilning regimen applied to these samples; in fact, the highest temperature reached was 63 °C, instead of 85 °C as in the first tests.

The lower temperature is probably also related to the greater extract values, perhaps deriving from a higher enzymatic activity, which was better preserved during the kilning process. The Hartong index also improved (18−22%), whereas color was more or less the same, except for sample W7d-3, which was characterized by a value of 2.8 EBC units.

The pH values remained quite a lot higher than those required for mashing; moreover, no saccharification was obtained within 60 min following the EBC Congress mash method. Total soluble nitrogen increased (255−296 mg/L) as well as the Kolbach index, which showed a maximum of 25.7% on dry matter in malt W7d-3.

Even if lower than those obtained for batches produced at laboratory scale, the amount of fermentable sugar is enough to allow a good fermentation and consequently the production of a beer-like beverage with a satisfying alcoholic degree.

The possibility of obtaining a gluten-free malt from rough rice was demonstrated both at laboratory scale and in a pilot plant. The best malting conditions were found to be germination for 7 days at 20 °C.

The 7-day malts are undermodified when compared to reference data for barley malts, but the small improvement obtained in their properties in the pilot plant could positively affect some characteristics of the final product.

However, in view of the results obtained, some problems will surely arise; in particular, the viscosity of rice worts will definitely negatively affect the lautering process.

ACKNOWLEDGMENT

We acknowledge the precious contribution of the Wayermann Malting Co. staff, S. Weyermann and T. Kraus-Weyermann in particular, for their kind hospitality to E.L.M.C., and thank A. Richter for his valuable support. We highly appreciate the help of V. Lavelli in statistical analysis.

LITERATURE CITED

(1) Associazione Italiana Celiaci, http://www.celiachia.it/celiachia/default.asp.

(2) Bonamico, M.; Ferri, M.; Nenna, R.; Verrienti, A.; Di Mario, U.; Tiberti, C. Tissue transglutaminase autoantibody detection in human saliva: a powerful method for celiac disease screening. *J. Pediatr.* **2004**, *144*, 632–636.

(3) Nout, M. J. R.; Davies, B. J. Malting characteristics of finger millet, sorghum and barley. *J. Inst. Brew.* **1982**, *88*, 157–163.

(4) Schoenlechner, R.; Siebenhandl, S.; Berghofer, E. Pseudocereals. In *Gluten-free Cereal Products and Beverages*, 1st ed.; Arendt, K., Dal Bello, F., Eds.; Food Science and Technology, International Series; Academic Press: San Diego, CA, 2008; pp 149−176.

(5) Briggs, D. E. *Malts and Malting*; Blackie Academic and Professional, Thompson Science: London, U.K., 1998.

(6) Okafor, N.; Iwouno, J. Malting and brewing qualities of some Nigerian rice (*Oryza sativa* L.) varieties and some thoughts on the assessment of malts from tropical cereals. *World J. Microbiol. Biotechnol.* **1990**, *6*, 187–194.

(7) *European Brewery Convention, Analytica − EBC*, Verlag Hans Carl, Getarnke-Fachverlag: Nurnberg, Germany, 1998.

(8) *MEBAK - Methodensammlung der Mitteleuropäische Brautechnische Analysenkommision*; Selbstverlag der MEBAK: Freising-Weihenstephan, Germany, 1997.

(9) Davies, N. Malt and malt products. In *Brewing: New Technologies*; Bamforth, C. W., Ed.; CRC Press: Boca Raton, FL, Woodhead Publishing: Cambridge, U.K., 2006; pp 68−101.

(10) Juliano, B. O. Polysaccharides, proteins, and lipids of rice. In *Rice: Chemistry and Technology*, 2nd ed.; Juliano, B. O., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1985.

(11) Zangrando, T.; Marconi, M. Le materie prime, il processo di produzione e la qualità − le materie prime. In *Il libro della Birra − Guida completa sul mondo della birra: la storia, gli ingredienti, la produzione, la degustazione, il servizio*; Edizioni Calderini de Il Sole 24 ORE Edagricole s.r.l.: Bologna, Italy, 2002; pp 15−24.

(12) Kunze, W. Beer production (fermentation, maturation and filtration) − beer stabilization. In *Technology Brewing and Malting*, 3rd international ed.; VLB: Berlin, Germany, 2004; pp 487−508.

(13) Briggs, D. E.; Boulton, C. A.; Brookes, P. A.; Stevens, R. Malts, adjuncts and supplementary enzymes. In *Brewing: Science and Practice*; Woodhead Publishing: Cambridge, U.K., 2004; pp 11−51.

(14) Sopanen, T.; Laurière, C. Release and activity of bound β-amylase in a germinating barley grain. *Plant Physiol.* **1989**, *89*, 244–249.

Received for review December 23, 2009. Revised manuscript received May 21, 2010. Accepted May 25, 2010.