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Changes in carbohydrates and amylolytic activity during malting of a local variety of rice

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Abstract

A local variety of paddy, "Mottaikaruppan" variety was selected for this study. Both paddy and its dehusked unpolished rice grains were steeped and allowed to germinate. Steep liquor analysis revealed that there was an increase in the release of the soluble materials with steeping time. Germination of dehusked unpolished rice (77.7%) was better than paddy (59.6%) on the fifth day. A change in moisture content during germination was almost the same for both paddy and dehusked unpolished rice after 2½ days. Appreciable drop in starch content (from 89.1 to 84.5g/100g dry matter) was observed from the third day. Increase in reducing sugar and total sugar contents were observed during germination. Increase in malt amylase (40.0 to 367.8Ug^{-1} dry matter) activity was observed from the second to fifth day of germination. Addition of 0.1gL^{-1} gibberellic acid improved the germination of dehusked unpolished rice by 11.7% at 4th day, while addition of Tween-80 (1.0mL/L) with gibberellic acid decreased the germination.

Key words: Rice malt, malting, germination, steeping, rice steep liquor, malt enzymes

Introduction

Food industry mainly involves cereal starch [1]. Modification of starch to sugar syrups of having different DE values has gained important place in food industries. Modifications of starch could be achieved either by using endogenous or exogenous or both enzymes [2]. Using endogenous enzymes is economically beneficial. Several studies were made on malting of rice [3], wheat [4], barley [5], corn [6], oat [4] and millet [4]. Effective utilization of malted rice in food industry needs research and development.

A study was done to find the effects of steeping and germination of a local variety of brown rice ("Mottaikaruppan" variety) on the changes in moisture, starch, total sugar and reducing sugar contents, and activities of endogenous

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amylolytic enzymes. Further, the effects of hormone (gibberellic acid) and surfactant (Tween-80) on malting were also studied to improve the malting of dehusked unpolished rice grains.

"Mottaikaruppan" variety of rice is a traditional variety of paddy cultivated in the Northern region of Sri Lanka. This variety of rice is preferred by the Jaffna inhabitants and its properties have never been studied. Hence, this local variety of rice was selected and the changes in the grains during steeping and germination were studied.

Materials and Methods

Materials

Paddy and dehusked unpolished rice ("Mottaikaruppan" a local land variety to the Northern region of Sri Lanka) were purchased from the local market and used as raw materials. Soluble starch (AR) was from Sigma Chemical Company (USA). All the other chemicals were of analytical grade. All the experiments were performed in triplicates.

Analysis of dehusked unpolished rice

Moisture content of dehusked unpolished rice grains was determined [7]. The dehusked unpolished raw rice was powdered using a domestic grinder at 30^{o}C and the flour was analyzed for starch by hydrolyzing [7] and reducing sugar content was determined using dinitrosalicylic acid method [8], protein by Kjeldhal method [7], fat [7], fibre [7] and ash [7] contents with respect to its dry weight. Raw rice supernatant was prepared by mixing rice flour (1.0g) with distilled water (10.0mL) at 30^{o}C was analyzed for reducing sugar content (by dinitrosalicylic acid method) [8].

Determination of rice malt amylolytic activity

Rice malt enzymes were extracted by mixing 1.0g of soaked/malted rice powder samples with 10gL^{-1} NaCl (10.0mL) for 15min at 30^{o}C and centrifuging (MSE bench centrifuge) at 3000rpm for 5min. The supernatant was analysed for amylolytic activity [9].

One unit of rice amylolytic activity (U) is the amount of enzyme, which releases 1.0µmole of glucose from 40gL^{-1} soluble starch in 1min at pH 5.0 and 60^{o}C. The activity of malt amylolytic activity is presented as Ug^{-1} dry malt powder.

Steeping and malting of paddy and dehusked unpolished rice

Paddy and dehusked unpolished rice grains (500g) were steeped in distilled water (1.0L) containing 0.1gL^{-1} $Na_2S_2O_5$.The rice steeped-liquor collected at different time intervals was analysed for soluble materials such as total sugar

[7], reducing sugar [8] and endogenous malt amylolytic activity [9].

At 12h, the steeped liquor of paddy and rice were drained off and the paddy and dehusked unpolished rice grains were allowed to germinate in a moistened bag wetted with 0.15gL^{-1} $Na_2S_2O_5$ and kept in dark at 30^oC for 6 days. Soaked & malted paddy and rice samples were collected at different time intervals and malting was arrested by drying the soaked/germinated grains at 35^oC for 2 days. Moisture content [7] and percentage of germination were determined.

Preparation and analysis of soaked & malted rice powder and soaked/ malted rice extract supernatant

Soaked and malted rice samples were dried at 35^oC for 2 days were ground to fine powder in a domestic grinder, where the temperature did not exceed 35^oC. Soaked and malted rice powders were analyzed for starch content, with respect to their dry weights.

Soaked/ malted rice powder sample (1.0g) was mixed with distilled water (10.0mL) at 30^oC for 15min and the suspension was centrifuged at 3000rpm for 5min and the supernatant was analyzed for total sugar [7], reducing sugar [8], and endogenous malt amylolytic activity [9].

Effect of Gibberellic acid and Tween-80 on the germination of dehusked unpolished rice grains

Dehusked unpolished rice grains (500g) were steeped in distilled water (1.0L) containing 0.1gL^{-1} $Na_2S_2O_5$ and different concentrations of gibberellic acid (00, 0.01, 0.1 and 1.0gL^{-1}) or different concentrations of gibberellic acid (00, 0.01, 0.1 and 1.0gL^{-1}) and Tween-80 (1.0mL/L) at 30^oC for 12 hours. The procedure was followed as described in Section 2.4, by spraying aqueous solutions of $Na_2S_2O_5$ (0.15gL^{-1}) containing the respective concentrations of gibberellic acid or different concentrations of gibberellic acid(00, 0.01, 0.1 and 1.0gL^{-1}) and Tween-80 (1.0mL/L).

Results

Effect of steeping process on soluble material content of dehusked unpolished rice and rice steep liquor

Increase in malt amylase activity with decrease in reducing sugar and total sugar content of soaked rice extract supernatant during steeping period predicts that sugars formed by the action of amylolytic activity may have leached out of the rice grain into the rice steeped liquor as well as utilized for the germination process (Figure 1). This was supported by the analysis of rice-steeped liquor for reducing sugar, total sugar and amylolytic activity. Increase in reducing sugar, total sugar contents and malt amylolytic activity with steeping time was observed in rice steeped liquor analysis (Table 1).

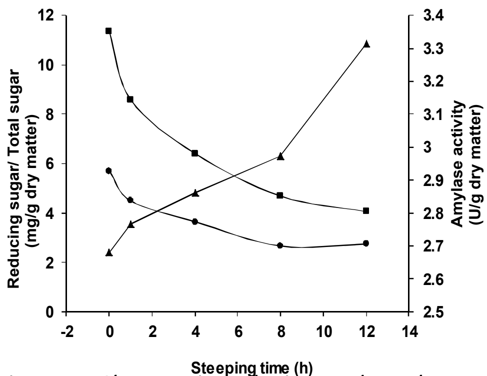

Figure 1: Changes in reducing and total sugar contents and amylase activity in the soaked rice supernatant with different steeping time (0, 1, 4, 8 and 12h) at 30°C. (■), Total sugar; (●), Reducing sugar and (▲), Amylase activity.

Comparison of the germination of dehusked unpolished rice and paddy

Germination of dehusked unpolished rice commenced on the second day while the paddy on third day. The results revealed that 77.7% of dehusked unpolished rice and 59.6% of paddy had germinated on the fifth day.

Changes in starch, total sugar & reducing sugar contents and amylolytic activity of dehusked unpolished rice during germination

Starch, total sugar and reducing sugar contents of soaked rice and malted rice-extract supernatants prepared from rice, soaked and germinated in different periods were estimated (Figure 2). The starch content began to fall as germination commenced. Steep decrease in starch content was observed between third (72h) and fourth (96h) days of germination. Total sugar content increased from 18h of germination. Reducing sugar also showed similar variation like total sugar. Linearity in the increase of total and reducing sugar levels were observed from 30 to 120h.

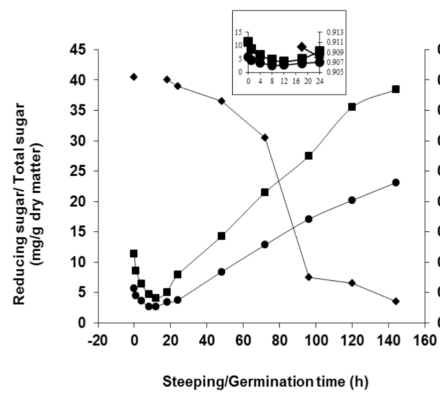

Figure 2: Changes in starch, reducing and total sugar contents of soaked and malted rice prepared from dehusked unpolished rice grains ("Mottaikaruppan") germinated for different periods. (♦), Starch; (■), Total sugar and (●), Reducing sugar.

Raw (non - germinated) rice grains showed amylolytic activity of 2.68Ug^{-1} dry matter, which increased during steeping and germination. Increase in malt amylolytic activity during steeping period is insignificant compared to the increase in the activity during germination period. Appreciable increase in malt amylolytic activity was observed from the second day (48h, 40.0Ug^{-1} dry matter) of germination and steep increase in enzyme activity was observed up to fifth day (120h, 367.8Ug^{-1} dry matter) of germination (Figure 3). Due to microbial contamination, this experiment was not continued after 6 days and thereby we could not observe the changes in the amylase activity during the later stages of germination.

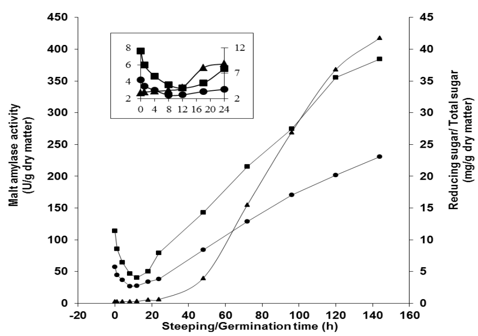

Figure 3: *Relationship between malt amylase activity and total and reducing sugar contents in soaked/malted rice supernatant, produced by the degradation of starch in soaked rice/malted rice with germination time. (■), Total sugar; (●), Reducing sugar and (▲), Amylase activity.*

Effects of gibberellic acid and gibberellic acid & Tween-80 (surfactant) on rice malting

The effect of gibberellic acid on malting of dehusked unpolished rice was studied by adding different concentrations of gibberellic acid (00 to 1.0gL^{-1}) to the steeping water as well as to the spraying water during germination/malting. When the concentration of gibberellic acid was increased from 00 to 0.1gL^{-1} there was about 11.7% improvement in malting on the fourth day of germination. Further increase in gibberellic acid concentration to 1.0gL-1 decreased the malting by 4.2%. So, it was decided to use0.1gL^{-1} gibberellic acid for malting of rice and for steeping and spraying.

To improve malting, it was decided to supplement the steeping water containing $Na_2S_2O_5$ and different concentrations of gibberellic acid with the surfactant, Tween-80. When Tween-80 (1.0mL/L) was incorporated in addition to different concentrations of ibberellic acid, significant decrease in ıe malting percentage was observed /ith respect to germination period.

iscussion

ontents of "Mottaikaruppan" rice are comparable to the results reported by Houston and Kohler [10] for brown rice, where the variety was not specified.

However, significant difference in protein content was observed. This variation could be due to either the degree of polishing in addition to varietal differences and the environmental conditions [11] prevailing in the region. Although environmental factors strongly influence the protein content, there is evidence that it is heritable [12].

Steeping process helps the water to enter the grains and moisture softens the seed coat and enables the embryo to come out easily. It also facilitates the germination by producing endo-enzymes to act on the macromolecules present in the embryo and endosperm. The results indicated that steeping of rice has stimulated the production of amylolytic activity. The amylase would have attacked the starch beads and helped in the release of soluble starch and reducing sugars. This is evidenced by the reducing and total sugar contents of rice steeped liquor and dehusked rice extract supernatant (Table 1). Thus steeping the rice for longer time could have led to the changes in the starch bead and hence led to the changes in the properties of the flour prepared.

Generally the paddy is used for germination [3]. Preparation of malted rice powder becomes difficult after the germination process because the dehusking process is not feasible or difficult. Therefore to find the possibilities of germination of dehusked rice, paddy and dehusked unpolished rice of "Mottaikaruppan" variety were treated similarly and their germination properties were compared. Higher and faster rate of germination of dehusked unpolished rice compared to paddy could be due to the removal of husk, which has aided the diffusion of water into and the respiration of rice grain than paddy. This is illustrated by the difference in the moisture contents of rice and paddy during germination. In this local variety of paddy and rice, initial moisture content was about 15.5% (dry basis). During germination, increase in moisture content was observed both in paddy and dehusked unpolished rice. However, the entry of water into the rice was more than that into paddy during the early stages of germination. Husk would have acted as the barrier for the entry of water into paddy. During later stages of germination, there was no significant difference in moisture content of paddy and rice (i.e., after 2½ days the moisture content was same in paddy and dehuked rice). Thus in addition to moisture some other factors are also contributing to the germination process. It was expected that the milling of paddy to dehusk and to prepare unpolished rice could lead to a decrease in germination percentage by removing some part of the embryo. However, the observations indicated that milling to just remove the husk was rather useful than giving an adverse effect. One explanation for this positive effect of milling could be the heat exposure of the dehusked rice during milling and the heat could have helped the rice grains to overcome the dormancy. This needs further investigations.

Only 50.0% of the corn grains germinated under the specified laboratory conditions during malting on fourth day [13]. The decreased germination percentage of dehusked rice could be because of the disturbance in the native structure by milling while the corn grains used were not milled or polished [13].

From these results it was concluded that dehusked unpolished rice of “Mottaikaruppan” variety possesses high rate of germination property than that of paddy, inspite of its susceptibility for microbial contamination. So, it was decided to study the effect of germination on the macromolecules of dehusked unpolished rice. Lineback and Ponpipom [4] observed that amylolytic activity in malted wheat, oat and pearl millet increased during germination and tended to become constant during the later stages of germination i.e., after 8 to 14 days. The highest □-amylase activity was exhibited in malt flour of finger millet germinated at 15^{o}C for 9 days and at 20^{o}C for 6 days, while the highest β-amylase activity was displayed in the malt flour germinated for 5 days at 30^{o}C [14]. Further, pearl millet has active α-amylase while oat has less active α-amylase. Even though, α-amylase activity was increased in all the cereals during germination, wheat starch granules were not severely attacked by α-amylase [4]. The mode of action of the endogenous enzymes on starch for instance is different in barley and wheat [15]. This was revealed by observing the morphological changes of the starch granules during germination [15].

The linearity between malt amylase activity and increase in total and reducing sugar contents with decrease in starch content was observed up to 120h (Figures 2 and 3). After 120h, the increase of total and reducing sugar levels were not linear even though the amylase activity was increased. This could be because of the increased utilization of the sugars compared with their rate of production. These results indicated that endo-enzymes produced during the germination process have enhanced the hydrolysis of starch and production of sugars. The amount of rice amylase present in the non-germinated rice grains increased gradually during steeping and germination processes and act on the starch macromolecules to produce sugars, which were utilized for the germination of rice grains.

Gibberellic acid is a growth-promoting hormone. This was added to overcome dormancy of seeds, accelerate malting process [16], increase respiration rate of the embryo [17] and stimulate the rate of hydrolytic enzymes production [4]. The dose of gibberellic acid is critical because at certain concentrations malt may be over modified, its colour may be affected and the sugar/ dextrin and amino acid/ protein ratios may be increased to higher levels [16]. Hence, it is necessary to select the optimum gibberellic acid concentration for malting of rice. The results revealed that after a certain concentration of gibberellic acid, it would show inhibitory or adverse effects on malting. Gibberellic acid improved the malting of barley [5]. However, prolonged exposure affected malting [16]. Then, the effect of surfactant together with gibberellic acid (hormone) was studied to improve the malting of dehusked unpolished rice grains. Penetration of gibberellic acid is accelerated by lightly brushing or abrasing the grain i.e., by creating small faults in the pericarp or testa [16]. In this study, a surfactant was used as an alternative to abrase the grains. This observation is contradictory to the results reported by Arasaratnam et al. [14] for the malting of corn, where the addition of Tween-80 (1.0mL/L) increased the percentage of malting. The possible explanation could be given based on the thickness of the seed coat. Since the seed coat of corn is thicker than rice grains, the concentration of the Tween-80 applied for the malting of corn might be sufficient to enhance the malting percentage. The same concentration showed negative effect in the malting of rice grains, since its seed coat is thinner compared to corn. If less concentration of Tween-80 had been used, sometimes a positive effect would have been observed. Since these additives are not recommended in food industry, further investigations were not carried out.

Conclusion

Modification of cereal starch has gained important place in food industry and nutritive value of cereals could also be improved by malting of cereals. This study was carried out on the locally available “Mottaikaruppan” variety of rice. Steeping helped the water to enter the grains to initiate germination and 40.0% of moisture was sufficient to support growth and biochemical alterations in the grain during malting. Germination of dehusked unpolished rice was better than paddy. Starch content decreased while total protein content was more or less unchanged during germination. Soluble materials such as reducing sugar, total sugar and activities of malt carbohydrases increased during malting. Increases in the extractable soluble materials during malting, indicated that malted rice (dehusked unpolished “Mottaikaruppan” variety) could be used for the preparation of malt-derived foods.

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Table 1: *Comparison of soaked rice supernatant and rice steeped liquor obtained by soaking the dehusked unpolished "Motaikaruppan" rice grains in distilled water containing 0.1gL^{-1} $Na_2S_2O_5$ for different periods at 30^{o}C. Soaked rice grains separated from the rice-steeped liquor were spread on an absorbent tissue for 15min and homogenized in a domestic grinder to obtain soaked rice flour.*

	Soaked rice supernatant			Rice steeped liquor		
Steeping time [h]	**Reducing sugar [mg/g DM]**	**Total sugar [mg/g DM]**	**Amylolytic activity [U/g DM]**	**Reducing sugar [mg/g DM]**	**Total sugar [mg/g DM]**	**Amylolytic activity [U/g DM]**
0	5.714	11.375	2.679	-	-	-
1	4.492	8.585	2.766	0.678	1.284	0.570
4	3.647	6.413	2.861	2.000	3.436	0.885
8	2.661	4.683	2.974	2.933	4.307	0.885
12	2.738	4.058	3.316	4.328	5.108	0.894

* Soaked rice flour was mixed with distilled water (1.0g/10.0mL), left at 30^{o}C for 15min with occasional shaking and centrifuged at speed 3000rpm for 5min to obtain soaked rice supernatant to estimate reducing sugar, total sugar and soluble protein contents.

* Soaked rice flour was mixed with 10gL^{-1}NaCl (1.0g/10.0mL) for the extraction of amylolytic activity in soaked rice supernatant.

DM – Dry matter.