

GERMINATION BEHAVIOUR, PHYSICO-NUTRITIONAL PROPERTIES, AND DIASTASE ACTIVITY OF BROWN RICE INFLUENCED BY GERMINATION TIME AND TEMPERATURE

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(Received: 19 March 2017; accepted: 4 June 2017)

Germinated brown rice received great attention as healthy ingredient and can be used as an alternative source in the malting and brewing industry. The germination capacity, physico-nutritional properties, sugars, and diastase enzyme activity of brown rice affected by germination times and temperature were determined and compared with control. Soaking in water increased the moisture content of brown rice. Germination rate of brown rice was also increased by higher germination time and temperature and reached maximum after 48 h of germination at 35 °C. However, dry matter loss, grain weight, and density are affected to a lesser extent. Germination significantly ($P < 0.05$) affects the crude protein, fat, fibre, and ash contents. Total carbohydrates content showed linear relationship with germination time and temperature. During germination, hydrolytic enzymes act on starch, reducing its concentration and resulting in higher total and reducing sugars amounts. Increase in germination time and temperature also increased diastase enzyme activity.

Keywords: brown rice, germination, nutritional properties, sugars, diastase enzyme activity

Rice (*Oryza sativa*) known as ‘Queen of cereals’ is a staple food consumed by 50% of the population in the world, particularly in the Asians countries. Brown rice contains 5–7% bran, 2–3% embryo, and the 90–92% endosperm is excellent source of proteins, dietary fibre, vitamins, minerals, organic acids, and health promoting bioactive components, better than milled rice (MOHAN et al., 2010).

In recent times, germinated brown rice (GBR) has been distinguished as one of the novel ingredients and gained a great attention, especially in rice-dependent economies (MOONGNGARM & SAETUNG, 2010). Time and temperature are the main factors affecting germination of brown rice, and both parameters, i.e. germination time and temperature, play important roles in sugar development during the process of germination (SAMAN et al., 2008). Studies reported that the majority of processes take place during the first 48 h of germination, increasing with the increase in temperature (27–37 °C), while the rate of germination drops sharply at lower temperatures (SAMAN et al., 2008). During the process of germination, various biochemical activities produce essential compounds and energy for the formation of the seedling, which drastically changes the physico and biochemical compositions of brown rice. Consequently, the overall nutritional quality improves (HU et al., 2017). Germinated rice grains were not frequently used in breweries as they essentially produce α -amylase during the process. Hence, GBR possesses great potential that could be utilized for malt and as a natural source of hydrolytic enzyme in sugar and brewing industries (AYERNOR & OCLOO, 2007). The objective of the present study was, therefore, to investigate germination behaviour and compositional changes in brown rice related to germination time and temperature.

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1. Materials and methods

Paddy (PR 123) (12.8% m.c) was procured from Punjab Agricultural University, Ludhiana, and husk was removed in a rubber roll Sheller (Stake Rice Mill, Japan) to get brown rice.

1.1. Germination of brown rice

Brown rice was steeped in excess distilled water at 25 °C for 10 h, and then placed in a thin layer on wet muslin cloth on tray and covered with another layer of wet muslin cloth. Grains were allowed to germinate for 12, 24, 36, and 48 h at temperature of 25, 30, and 35 °C, in an incubator (85% relative humidity) (M/s. Narang Scientific Works, New Delhi), followed by drying in tray drier at 45±2 °C, and stored in jars at refrigeration temperature (4–7 °C) for subsequent analysis. Non-germinated brown rice was served as the control.

1.2. Steeping behaviour of brown rice

Percent increase in the moisture content as per standard AACC (2000) was estimated at different soaking time intervals at 25 °C to assess the soaking behaviour of brown rice.

1.3. Germination characteristics of brown rice

Briefly, a selected number (50) of grains were allowed to germinate at variable germination conditions and the numbers of totally germinated grains were counted every 12 h.

1.4. Dry matter loss

The weights in gram of non-germinated and germinated brown rice (after drying and removing plumule and radicle) were recorded (AYERNOR & OCLOO, 2007). Loss in weight reflects dry matter loss (%).

1.5. Physical properties of brown rice

1.5.1. Thousand-kernel weight. A sensitive electronic balance was used to calculate the thousand-kernel weight of GBR grains.

1.5.2. L/B ratio. The length and breadth of randomly selected un-germinated and germinated brown rice kernels were measured using laboratory scale Vanier calliper.

1.5.3. Bulk density. Ten grams of the samples were placed in a graduated cylinder (25 ml) and packed by gentle tapping of the cylinder on a bench top, ten times. The final volume of the sample was measured and expressed as g ml⁻¹.

1.6. Proximate composition

The proximate composition (protein, fat, fibre, and ash) of the brown rice samples were analysed at 14% moisture using the standard procedures of AACC (2000). Total carbohydrate content was calculated by difference. The energy value (kcal/100 g) of the samples was determined as: (4×protein)+(9×fat)+(4×carbohydrate).

1.7. Total starch

Starch present in the given sample was converted to sugars by acid hydrolysis and the amounts of sugars were estimated by determining the volume of unknown sugar solution required to completely reduce a measured volume of Fehling's solution to red insoluble cuprous oxide (AACC, 2000).

1.8. Total sugars and reducing sugars

Sugars (total and reducing) present in the samples were measured by the amount of ferricyanide reduced by sugar extracts under favourable conditions as per standard AACC (2000) method.

1.9. Diastases enzyme activity

Diastase activity in the flour was determined under standard reaction conditions. The enzyme was extracted with buffer solution at 40 °C, reacted with alkaline ferricyanide, and the amount of reducing sugars thus formed by amyolytic enzyme action was then estimated by the standard AACC (2000) method.

1.10. Statistical analysis

All experimental results were triplicates. Mean values were compared using analysis of the variance (ANOVA) test, and Tukey's posthoc test at $P < 0.05$ was applied in order to establish significant differences between germination conditions (SPSS version 16.0)

2. Results and discussion

2.1. Steeping characteristics of brown rice

The moisture content of brown rice, which had been steeped for different time periods at 25 °C, is shown in Figure 1. Steeping includes the commencement of cell elongation, respiration, embryo's secretory activity, and enzyme activation. Water provided must be enough during steeping to allow good germination, but not at extremely levels, as grains will show a reduction in germination vigour when exposed to an excess of water (SAMAN et al., 2008). Moisture uptake during the early stage of soaking for the first 4 h was very rapid, slowed down subsequently, and then reached a plateau (10 h). After a significant time interval of steeping, moisture content increased 3-fold (11.23 to 31.2%), due to endosperm micropores that took up water by capillary action resulting in rapid hydration (MIAH et al., 2002).

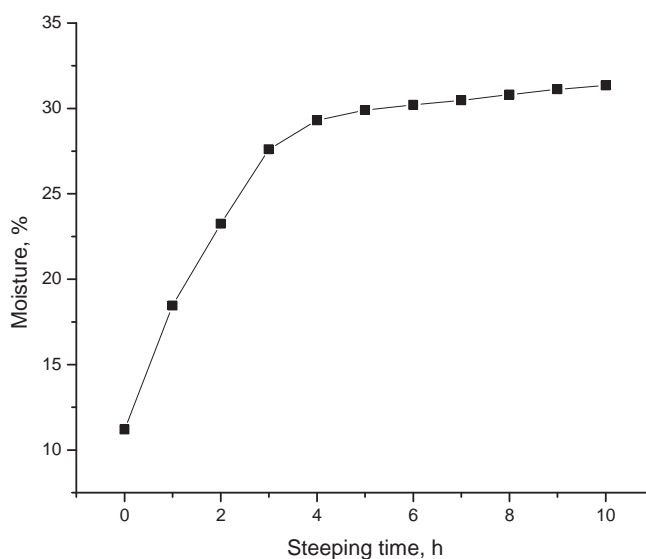


Fig. 1. Steeping characteristics of brown rice

2.2. Germination capacity of brown rice

Germination capacity of brown rice intensified significantly ($P < 0.05$) with increased germination time and temperature (Fig. 2). The mean percent germination capacity of brown rice after 12 h of germination is 68.17, which increased significantly ($P < 0.05$) to 73.38, 81.06, and 87.85% after 24, 36, and 48 h of germination, respectively. The germination capacity of brown rice also increased as the temperature of germination increased. Similar results for increase in germination capacity of brown rice with increase in germination time and temperature were also reported by CACERES and co-workers (2014). Temperature at which grains are germinated is one of the key features that affect germination capacity and it also plays a critical role in sugars development (SAMAN et al., 2008).

2.3. Dry matter loss

Dry matter loss or vegetative loss, the material lost in converting grain into malt, increased significantly with increase in germination time and temperature (Table 1). As the germination duration increased from 12 to 48 h and temperature from 25 to 35 °C, the mean % dry matter loss increased from 0.18 to 2.78% and 0.70 to 1.95%, respectively. Germination of brown rice for 48 h at 25, 30, and 35 °C increased the % dry matter loss to 1.9%, 3.02%, and 4.14%, respectively. The increase in dry matter loss of grains during steeping could be due to leaching out of solids, increased metabolic activity in grains, removal of root and shoot (vegetative growth), and chemical changes occurring during germination (AYERNOR and OCLOO, 2007).

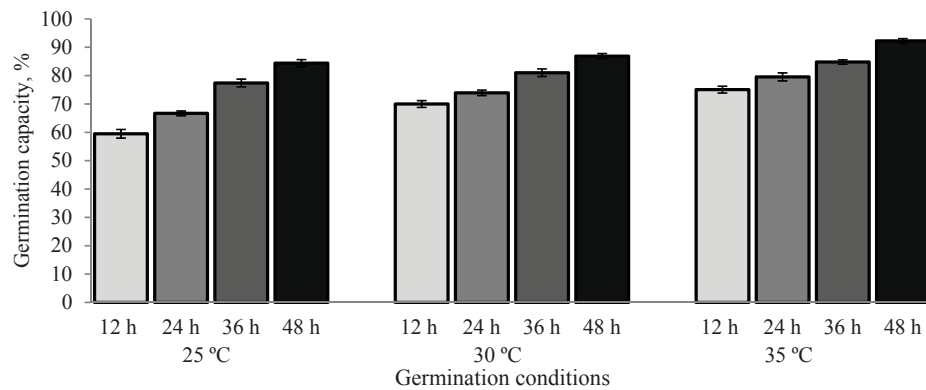


Fig. 2. Germination capacity of brown rice as affected by germination time and temperature

Table 1. Effect of germination conditions on dry matter loss and physical properties of germinated brown rice

Germination temperature	Germination time (h)	Dry matter loss (%)	L/B ratio (mm)	1000-kernel weight (g)	Bulk density (g ml ⁻¹)
Control		–	4.14±0.02 ^A	39.26±0.14 ^a	0.82±0.00 ^A
Soaked 25 °C	10 h	–	4.12±0.07 ^A	39.16±0.48 ^a	0.82±0.01 ^A
	12	0.15±0.00 ^{dH}	4.05±0.02 ^{aA}	38.90±0.05 ^{aB}	0.82±0.01 ^{aA}
	24	0.57±0.01 ^{cG}	4.04±0.02 ^{aB}	38.78±0.08 ^{aB}	0.81±0.01 ^{aAB}
	36	0.89±0.02 ^{bF}	4.00±0.02 ^{aC}	37.43±0.04 ^{bC}	0.79±0.01 ^{aB}
	48	1.19±0.01 ^{aE}	3.99±0.01 ^{aC}	37.02±0.59 ^{bC}	0.78±0.02 ^{aB}
30 °C	12	0.19±0.02 ^{dH}	4.02±0.01 ^{aB}	38.48±0.31 ^{aB}	0.80±0.01 ^{bB}
	24	0.81±0.01 ^{cF}	3.94±0.03 ^{bD}	38.37±0.30 ^{aB}	0.79±0.00 ^{bB}
	36	1.82±0.01 ^{bD}	3.93±0.02 ^{bD}	36.98±0.88 ^{bC}	0.76±0.01 ^{aC}
	48	3.02±0.01 ^{aB}	3.92±0.05 ^{bD}	36.15±0.49 ^{bD}	0.76±0.02 ^{aC}
35 °C	12	0.19±0.21 ^{dH}	4.04±0.05 ^{aB}	37.06±1.10 ^{aC}	0.80±0.00 ^{aB}
	24	1.04±0.02 ^{eEF}	4.02±0.01 ^{aB}	36.76±0.00 ^{abD}	0.78±0.01 ^{bAB}
	36	2.44±0.01 ^{bC}	3.85±0.03 ^{bE}	36.72±0.42 ^{abD}	0.76±0.01 ^{bC}
	48	4.14±0.02 ^{aA}	3.86±0.04 ^{bE}	36.15±0.49 ^{bD}	0.73±0.01 ^{cD}

Values represent mean (n=3) ± standard deviation. Means having different superscripts from A, B, C to J differ significantly (P<0.05) column-wise among different treatments. Means having different superscripts a, b, c, and d show significant (P<0.05) differences during different germination time at each temperature.

2.4. Physical properties of germinated brown rice

2.4.1. L/B ratio. Germination conditions have a non-significant effect on the length/breadth ratio (L/B ratio) of brown rice (Table 1). L/B ratio of non-germinated brown rice was 4.14, which after germination for 48 h at 25, 30, and 35 °C was reduced to 3.99, 3.92, and 3.86, respectively. During soaking and germination, grains absorb water, swell, and subsequently

enlarge, while upon drying, due to substantial loss of moisture, the kernel again shrinks back to smaller size leading to the reduction in L/B ratio.

2.4.2. Thousand-kernel weight. Thousand-kernel weight indicates density and kernel size of the food commodity. Substantial changes in the 1000 grains weight of brown rice grains were observed after germination at different times and temperatures (Table 1). Non-germinated brown rice had a 1000-kernel weight of 39.26 g, which was reduced by 5.70 and 7.92% after 48 h of germination at 25 and 35 °C, respectively. Results in the present study were in accordance with findings of AYERNOR and OCLOO (2007).

2.4.3. Bulk density. Non-germinated brown rice had the highest bulk density (0.82 g ml⁻¹), though germination brought only non-significant decrease in bulk density of grains (Table 1). Bulk density of brown rice germinated under different time and temperature conditions varied from 0.82–0.73 g ml⁻¹. During germination process, grain constituents were lost or utilized as food reserves for energy and growth of the new seedling, which, thus, decreased the bulk density of grain (AYERNOR & OCLOO, 2007). QUADIR and co-workers (2012) also reported 6.06–15.41% decrease in the bulk density for 3 paddy cultivars after germination.

2.5. Proximate composition of germinated brown rice

The nutritional composition of brown rice non-germinated and germinated under varying time and temperature conditions in this study are shown in Table 2. Germination had inverse effect on the protein content of the grain. With extended time and increased temperature of germination, the protein content of brown rice decreased significantly ($P < 0.05$) (Table 2). After 48 h of germination at 25, 30, and 35 °C, protein content of brown rice was reduced by 5.20, 5.82, and 6.35%, respectively. KIM and co-workers (2012) also reported that germination caused decrease in protein contents in different rice cultivars, due to loss of nitrogenous compounds during soaking and increased protease activity during germination. In contrast, studies of MOONGNARM and SAETUNG (2010), however, reported increase in the protein content of germinated rice, due to net synthesis of enzyme proteins. Fat content was observed to be noticeably higher in non-germinated brown rice (2.45%) but it decreased significantly ($P < 0.05$) with increase in germination time and temperature by 1.37% to 2.41% (Table 2). The decrease in fat content at different germination conditions ranged from 21.22 to 44.08%, and it decreased as a result of the increased lipolytic enzymes activities, which hydrolyse fat components into fatty acids and glycerol during germination (CHINMA et al., 2009). Similar results of reduction of fat content were also reported by MOHAN and co-workers (2010). Non-germinated brown rice flour had the highest ash content (1.35%), while brown rice germinated at 35 °C for 48 h had the lowest ash content (1.08%). There was significant ($P < 0.05$) decrease in ash content (4.4 to 46.5%) in germinated brown rice as the germination progressed in time, which was due to loss of minerals as outgrowth of root and shoots were removed after drying. MOONGNARM and SAETUNG (2010) also reported decrease in ash content during germination. Crude fibre content of brown rice was significantly ($P < 0.05$) influenced by germination time and temperature (Table 2). Non-germinated brown rice had crude fibre content of 1.18%, which after 48 h of germination at 25, 30, and 35 °C, decreased significantly by 4.13, 5.08, and 8.47%, respectively, due to degradation of cell wall of outer bran layer. In contrast, OHTSUBO and co-workers (2005), however, found that crude fibre content in germinated brown rice increased. Studies of ONGOL and co-workers (2013) are in accordance with the

Table 2. Effect of germination conditions on changes in proximate composition of germinated brown rice

Germination temperature	Germination time (h)	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Crude ash (%)	CHO	Energy (kcal)
Control	10h	7.85±0.04 ^A	2.45±0.04 ^A	1.18±0.02 ^A	1.41±0.02 ^{AB}	73.12±0.04 ^J	345.95±0.32 ^A
	Soaked 25 °C	7.78±0.02 ^A	2.44±0.03 ^A	1.17±0.00 ^{AB}	1.42±0.03 ^A	73.19±0.05 ^J	345.85±0.08 ^A
	12	7.62±0.04 ^{Ba}	2.41±0.04 ^{Aa}	1.17±0.01 ^{aAB}	1.41±0.03 ^{aAB}	73.39±0.06 ^{dI}	345.78±0.27 ^{aA}
	24	7.20±0.05 ^{Cb}	2.22±0.02 ^{bB}	1.16±0.01 ^{abAB}	1.39±0.01 ^{aAB}	74.03±0.06 ^{eH}	344.92±0.18 ^{bB}
	36	6.85±0.05 ^{Dc}	2.10±0.06 ^{bBC}	1.14±0.00 ^{bBCD}	1.37±0.02 ^{abBC}	74.54±0.03 ^{gG}	344.44±0.30 ^{bBCD}
25 °C	48	6.35±0.05 ^{Fd}	1.93±0.07 ^{cDE}	1.13±0.00 ^{cCD}	1.34±0.02 ^{bCD}	75.26±0.13 ^{eE}	343.77±0.33 ^{eD}
	12	7.12±0.04 ^{Ca}	2.14±0.01 ^{aBC}	1.15±0.02 ^{aABC}	1.36±0.01 ^{ABC}	74.23±0.04 ^{dH}	344.62±0.15 ^{aBCD}
	24	6.67±0.04 ^{Eb}	2.03±0.08 ^{abCD}	1.13±0.02 ^{aCDE}	1.30±0.03 ^{bDE}	74.87±0.06 ^{fF}	344.45±0.36 ^{aBCD}
	36	6.12±0.04 ^{Gc}	1.92±0.05 ^{bCDE}	1.12±0.00 ^{aDE}	1.28±0.03 ^{bEF}	75.56±0.09 ^{bD}	343.99±0.23 ^{aCD}
	48	5.82±0.06 ^{Hd}	1.87±0.07 ^{cE}	1.12±0.00 ^{aDE}	1.16±0.02 ^{eH}	76.01±0.11 ^{aC}	344.15±0.42 ^{aBCD}
30 °C	12	6.89±0.04 ^{Da}	2.05±0.02 ^{aCD}	1.14±0.00 ^{aBCD}	1.24±0.02 ^{aFG}	74.68±0.04 ^{dFG}	344.72±0.11 ^{aBC}
	24	6.34±0.07 ^{Fb}	1.85±0.06 ^{bE}	1.12±0.00 ^{bDE}	1.23±0.02 ^{aG}	75.46±0.08 ^{eDE}	343.83±0.30 ^{bD}
	36	5.71±0.04 ^{Hc}	1.77±0.04 ^{bE}	1.10±0.01 ^{cF}	1.18±0.01 ^{bH}	76.25±0.06 ^{bB}	343.77±0.07 ^{bD}
	48	5.20±0.06 ^{I d}	1.37±0.09 ^{cF}	1.08±0.01 ^{dF}	1.08±0.05 ^{eI}	77.26±0.03 ^{aA}	342.19±0.47 ^{eE}

Values represent mean (n=3)± standard deviation. Means having different superscripts from A, B, C to J differ significantly (P<0.05) column-wise among different treatments. Means having different superscripts a, b, c, and d show significant (P<0.05) differences during germination time at each temperature. CHO: carbohydrates; kcal: kilocalories

present study. Carbohydrate content of brown rice samples germinated at varying conditions ranged between 73.18% and 77.20% (Table 2). Carbohydrate content increased significantly ($P<0.05$) in brown rice with proliferation through germination. Non-germinated brown rice was observed to have the highest energy value (364.18 kcal) and brown rice germinated at 35 °C for 48 h had the lowest value (341.93 kcal).

2.6. Total starch

Data on the starch content of brown rice as influenced by germination conditions are presented in Table 3. Non-germinated brown rice had 82.82% starch, which decreased significantly ($P<0.05$) as germination progressed at higher time and temperature values. When brown rice germinated at 25, 30, and 35 °C for 48 h, starch content decreased by 5.28, 8.80, and 12.47%, respectively, due to the increased activity of amylases during germination, which hydrolyse starch to yield 6-carbon sugars (AYERNOR & OCLOO, 2007). Similar results for reduction in starch content of germinated rice and brown rice were also reported by CHUNG and co-workers (2012).

2.7. Total sugars and reducing sugars

Total sugars and reducing sugars contents of brown rice as affected by germination time and temperature are presented in Table 3. Increase in germination time from 12 to 48 h and temperature from 25 to 35 °C, significantly ($P<0.05$) increased the accumulation of sugars in brown rice. Total sugars (% glucose) and reducing sugars (% glucose) contents of non-germinated brown rice were 1.53 and 0.45%, respectively. Mean total sugars content after 12 h was 2.17%, which increased by 31.80 and 111.98% after 24 and 48 h of germination, respectively. An increase of 64.44 and 186.67% in reducing sugars contents was observed after 24 and 48 h of germination, respectively. Similar trend was also observed when germination temperature was increased. The pattern of increase and changes in reducing sugars content observed in the present study was similar to those reported in earlier studies by MOONGNARM and SAETUNG (2010). The increase in total and reducing sugars and non-reducing sugars contents after germination is mostly at the expense of hydrolysis of starch presumably by the initial action of α -amylase enzyme on the molecules of starch (MOHAN et al., 2010).

2.8. Diastase activity

Diastase activity provides a measurement of the saccharification potential of malted grains. Germination time and temperature have significant ($P<0.05$) effect on the diastase enzyme activity of brown rice (Table 3). Non-germinated brown rice has diastase activity of 96.64 mg maltose/10 g, which increased significantly ($P<0.05$) as germination time and temperature increased (Table 3). The mean diastase activity of brown rice after 12 h of germination was 456 mg maltose/10 g, which after 48 h of germination increased to 757 mg maltose/10 g. When germination was done at 25 °C, the mean diastase activity was 543 mg maltose/10 g, which increased to 796 mg maltose/10 g at 35 °C. AYERNOR and OCLOO (2007) also reported that diastase activity increased significantly as germination time increased.

Table 3. Effect of germination conditions on contents of sugars and starch and diastase activity of germinated brown rice

Germination temperature	Germination time (h)	Total sugars (% glucose)	Reducing sugars (% glucose)	Starch (%)	Diastase activity (mg maltose/10 g)
Control		1.53±0.09 ^J	0.45±0.09 ^I	82.82±1.28 ^A	96.6±0.0 ^I
Soaked 25 °C	10 h	1.64±0.00 ^{IJ}	0.49±0.00 ^{HI}	82.62±1.82 ^A	185.1±0.0 ^{HI}
25 °C	12	1.97±0.10 ^{dHI}	0.61±0.00 ^{cGHI}	81.14±0.11 ^{aAB}	285.9±0.0 ^{cG}
	24	2.26±0.08 ^{cGH}	0.74±0.00 ^{bcFGH}	80.46±0.16 ^{bAB}	516.1±16.7 ^{bD}
	36	2.64±0.00 ^{bF}	0.85±0.00 ^{bEFG}	79.54±0.16 ^{cABC}	703.9±0.0 ^{aC}
	48	3.46±0.02 ^{aE}	1.29±0.10 ^{aD}	78.45±0.08 ^{dBCD}	666.31±16.3 ^{aC}
30 °C	12	1.96±0.10 ^{dHI}	0.73±0.00 ^{cFGH}	80.85±0.23 ^{aAB}	369.3±16.4 ^{dF}
	24	2.51±0.09 ^{cFG}	1.05±0.09 ^{bcDE}	78.01±1.51 ^{abBCD}	444.5±16.6 ^{eE}
	36	3.14±0.19 ^{bE}	1.29±0.10 ^{bD}	76.77±0.25 ^{bCDE}	693.4±16.4 ^{bC}
	48	4.35±0.08 ^{aC}	1.79±0.08 ^{aBC}	75.53±0.25 ^{bDEF}	787.7±16.1 ^{aB}
35 °C	12	2.58±0.08 ^{dFG}	0.97±0.00 ^{dEF}	78.17±0.84 ^{aBCD}	712.7±13.1 ^{cC}
	24	3.81±0.09 ^{cD}	1.57±0.09 ^{cC}	76.02±0.44 ^{abDE}	796.3±16.3 ^{bB}
	36	4.75±0.00 ^{bB}	1.99±0.00 ^{bB}	74.01±0.82 ^{bEF}	818.0±16.3 ^{abAB}
	48	5.98±0.00 ^{aA}	2.66±0.09 ^{aA}	72.49±1.34 ^{bF}	857.1±0.0 ^{aA}

Values represent mean ± standard deviation. Means having different superscripts from A, B, C to J differ significantly ($P < 0.05$) column-wise among different treatments. Means having different superscripts a, b, c, and d show significant ($P < 0.05$) differences during different germination time at each temperature.

3. Conclusions

From the present study it can be concluded that longer steeping time increased the moisture content of brown rice. Germination capacity and dry matter loss of brown rice increased as germination time and temperature increased, while L/B ratio and bulk density decreased. Increase in germination time and temperature had inverse effect on the nutritional composition of germinated brown rice. Production of sugars increased during germination with reduction in starch content, and maximum sugar content was observed after 48 h of germination at 35 °C. Enzymatic activity also increased and reached its peak after 48 h of germination at a temperature of 35 °C.

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