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Influence of Germination Conditions on Starch, Physicochemical Properties, and Microscopic Structure of Rice Flour

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Abstract—**Brown rice (ungerminated rice) flour and germinated rice flours obtained from different germination conditions were studied to investigate changes in total starch, sugars, and physicochemical properties and starch granule structure as affected by germination process. The results revealed that the germination significantly decrease the level of starch but increased the sugar contents after the first day of germination. The peak viscosity, break down, set back, and final viscosity of germinated rice flour was also reduced, whilst gelatinization temperature did not change. The scanning electron microscopy study clearly showed that germination caused important changes in granular structure of starch.**

Keywords- rice, germinated rice, pasting properties, rice starch granule

I. INTRODUCTION

In recent years, germinated seeds have gained a lot of popularity and widely accepted as a functional food because of its nutritious and heath benefits in several aspects. Germination is known to cause important changes in the kernel. Germinated brown rice utilizes the substances in kernel and generates bioactive compounds which provide health benefits whilst the soaking process improves the texture of brown rice and the nutrients in the seed become easier to digest [1].

Starch is the major component of rice grain and its physicochemical properties affects quality of rice in several aspects such as cooking and eating quality, starch digestibility, the extent rate of starch hydrolysis by amylolytic enzymes. Flours prepared from germinated cereal have been documented to have better nutritional values than those of ungerminated flours. However, the chemical compositions of germinated rice seeds depend on a number of factors. The present study purposed to investigate the effect of germination conditions of on the starch and sugar contents, physicochemical properties, and to visualize the structural changes in starch granule. These properties would affect germinated rice product quality, commercial utilization, and might contribute to widen the restricted food uses of rice. Hence, information on some of which could be useful for utilization of germinated rice in specialty foods.

II. MATERIALS AND METHODS

A. Rice samples

Rough rice of *Oryza sativa* L., cultivar RD-6 was purchased from a local rice-milling factory in Mahasarakham province, Thailand. Ungerminated rice (UGR) or brown rice was prepared by removing a husk of the ungerminated rough rice using a laboratory de-husker.

B. *Germinated* rough rice (GRR) preparation

Rough rice (5kg) was soaked in tap water at room temperature for 24, 48, and 72 h and water was changed every 7-8 h. Each soaked rice seed was distributed in plastic baskets covered by cheese cloth and germinated in a germinating cabinet for 24, 48, 72, 96 h (for each steeping time) at 28-30°C and 90-95 % relative humidity. After germination, the germinated seeds were dried at 50°C to approximately 10% of moisture content. The hull, root, and shoot were separated using laboratory de-husker. For the chemical and physicochemical analyses, germinated rice samples were finely ground (80 mesh) to obtain germinated rice flour and used for analyses.

C. Chemical *compositions*

Total starch and reducing sugars were determined according to [2] and [3], respectively. The individual sugars were analyzed by HPLC-RI detector by following the method reported by [4]. The α-amylase activity was assayed using the enzyme assay kits (Megazyme International, Ireland).

D. Pasting *properties*

UGR flour and GRR flour passing through an 80-mesh sieve was analyzed in triplicate for pasting profiles using the Rapid Visco Analyzer (RVA) (Newport Scientific Pvt. Ltd., NSW 2102, Australia) following the AACC method 61-20 [5]. The changes in viscosity during heating, cooking and cooling were recorded; the gelatinization temperature, peak viscosity, breakdown and setback viscosity values were noted from the viscograms.

E. Scanning *electron* microscopy (SEM)

UGR and GRR flour were dispersed as a monolayer on an SEM specimen stub with double-sided conductive tape [6]. After fixation and dehydration, samples were coated with carbon and gold and then examined with a JEOL JSM 5200 scanning electron microscope, operated at an accelerating voltage of 10 kV.

F. Statistical *analysis*

All experiments were conducted in triplicate and the results are expressed as mean ± SD. The statistical examination of the data was performed using the SPSS statistical software version 16.0 (SPSS, INC., Chicago, USA). The means were compared by using the Duncan Multiple Range Test and $p < 0.05$ was applied to establish significant differences.

III. Results and Discussion

A. Starch and Reducing sugar content

Before germination, rice grain contained a great amount of starch but only small amount of sugar. The decrease in the starch content of germinated rice was found after germination was taken placed for 2-4 days due to the starch was broken down by amylase and resulting in increasing the concentration of simple sugars. In this study, reducing sugar was found to increase with steeping and germination time (Table 1) due to the hydrolysis of starch. The maximum content was observed at steeping for 2-3 days and germination for 3 to 4 days (9.66-10.06g/100 g flour). A similar observation was reported in germinated brown rice [7].

B. Free sugars

The component of individual free sugars including glucose, maltose, and sucrose is shown in table I. The major of free sugars found in germinated rice were glucose and maltose whereas only small amounts of free sugar were detected in ungerminated rice. The increase in free sugar contents after germination is mostly from the hydrolyzed starch by amylases. Therefore, the higher concentration of sugars was observed in germinated rice that soaked and germinated for the longer times. These results were similar to those reported by [8] in the malts of Indica and Japonica brown rice.

C. Amylase *activity*

The changes in the α-amylase activity of UGR and GRR during germination up to 4 days are presented in Table I. It showed that α- amylase activity was developing in UGR rice and rapidly increased from the second day of germination and slightly decreased at the fourth day. The activity of enzymes depends on temperature, moisture content, and environmental conditions of germination.

D. Pasting properties

Pasting characteristics are one of the most important practical properties of rice flour prepared from germinated rice. The pasting characteristics of the aqueous suspension of both UGR and GRR flours during heating, cooking and cooling of rice samples were recorded in terms of changes in viscosity by RVA are presented in table II. In this study, steeping and germination time significantly affected pasting profiles of germinated rice flour compared to those of the ungerminated rice except pasting temperatures. As the steeping and germination time increased, the values of peak viscosity, breakdown, set back, and final viscosity of GRR decreased. The peak viscosity of GRR was most affected by germination. This was probably due to the degradation of starch by enzyme activity during the germination process. The breakdown viscosity is related to the stiffness of swollen granules whilst the amylopectin is responsible for susceptibility of swollen granules to disintegration when the gelatinized starch slurry is heated and stirred [9]. The heating of the slurry not only causes starch gelatinization but also it activates the enzymes to hydrolyze the starch. As a result of this, the viscosity of the slurry decreases significantly [10]. However, the pasting behaviors are also influenced by the interaction between the chemical components and the crystallinity, size, structure, distribution, and water binding capacity of the starch granules.

E. Physical *properties* of starch

Some of its rheological properties were substantially changed by germination. Ultra-structural changes of the starch, as assessed by scanning electron microscopy, were also monitored during this process. The smooth surface and dense packing of the starch in ungerminated rice seeds (Fig.1 (a)) were outstandingly modified during germination (Fig.1 (b-f)). The figure indicates that starch granules were slightly modified after germination. In the UGR sample, starch granules are characterized by a very smooth surface embedded in a continuous matrix. After 2 to 4 days of germination, visible changes occurred within granules. Starch granules lost their smooth surface, becoming rougher and slightly eroded. The steeping for 3 days and 4 days of germination is illustrated in Fig.1 (f). The surface of the granules was highly affected by germination. The entire granule resembles small irregular fragments held together in such a way that the granule is still in normal shaped. This appearance is similar to that reported by [11] in black eye beans. The starch granule degradation corresponds to the changes in sugar composition mainly increased glucose and maltose.

IV. Conclusions

The ungerminated rice is a poor source of α-amylases but its activity increased sharply after day 2 of germination and decreased thereafter. Germination causes considerable changes in the composition of the starch and sugars, mainly glucose and maltose. The germination affected both pasting properties and starch granule structure.

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REFERENCES

[1] S. Tian, K. Nakamura, and H. Kayahara, "Analysis of phenolic compounds in white rice, brown rice and germinated brown rice," Journal of Agricultural Food Chemistry, vol. 52, Jul. 2004, pp 4808-4813, doi: 10.1021/jf049446f.

[2] J. Holm, I. Bjorck, A. Drews, and N. G. Asp, A rapid method for the analysis of starch. Starch, vol. 38, 1986, pp. 224–226, doi: 10.1002/star.19860380704.

[3] M. Somogyi, "Notes on sugar determination," Journal of Biological Chemistry, vol. 195 Jan 1952, pp. 19-23.

[4] P. Ruperez, "Soybean oligosaccharide potential as new ingredients in functional food," Nutricion Hospitalaria, vol. 21, Jan, 2006, pp.92-96.

[5] American Association of Cereal Chemist (AACC). 2000. Method 22-08, 61-20. In: Approved methods of the American Association of Cereal Chemist, Inc. 10th ed. St. Paul, Minn.: AACC.

[6] M. Langton, and A.M. Hermansson, "Microstructural changes in wheat starch dispersion during heating and cooling," Food Microstructure, vol. 8, 1989, pp. 29–39, doi: 10.1111/j.1745-4603.1996.tb00085.x.

[7] S. H. Oh, J. R. Soh, and Y. S. Cha, "Germinated brown rice extract shows a nutraceutical effect in the recovery of chronic alcohol-related symptoms," Journal of Medicinal Food, vol. 6, 2003, pp. 115-121.

[8] B.H. Mohan, N.G. Malleshi, T. Koseki, "Physico-chemical characteristics and non-starch polysaccharide contents of Indica and Japonica brown rice and their malts" LWT - Food Science and Technology, vol. 43, 2010, pp. 784–791, doi:10.1016/j.lwt.2010.01.002

[9] J. Reka and S. Andras, "Pasting behavior of amylose, amylopectin and their mixtures as determined by RVA curves and first derivatives," Starch, vol. 60, 2008, pp. 70-78,

[10] doi: 10.1002/star.200700634.

[11] D. J. Manners, "Some aspects of the structure of starch.," Cereal Foods World, vol. 30, 1985, pp. 461467.

[12] H.C. Silva and B.S. Luh, "Changes in oligosaccharides and starch granules in germinating beans," Canadian Institute Food Science and Technology Journal, vol. 12, 1979, 103-107.

TABLE I. STARCH, SUGAR CONTENTS, AND ENZYME ACTIVITY DURING GERMINATION OF RICE SEEDS

Germination conditions	Total starch	Reducing sugar	Glucose	Maltose	Sucrose	alpha-amylase (U/g flour)
Control	83.91±0.62^{a}	0.92±0.03^{f}	0.09±0.00^{g}	0.64±0.02^{h}	0.05±0.00^{f}	1.42±0.46^{g}
S1G1	82.88±1.08^{a}	3.21±0.04^{e}	0.22±0.02^{f}	1.04±0.06^{g}	0.24 ±0.07^{e}	21.30±0.72^{f}
S1G2	83.39±1.04^{a}	4.23±0.39^{d}	0.28±0.03^{e}	1.62±0.02^{f}	0.61± 0.04^{c}	45.36±1.05^{e}
S1G3	80.16±1.18^{b}	4.41±0.59^{d}	1.03±0.06^{b}	1.96±0.04^{e}	0.53± 0.05^{d}	54.59±0.86^{d}
S1G4	78.69±1.71^{bc}	8.51±0.46^{b}	1.09±0.06^{b}	2.98±0.45^{b}	0.98±0.08^{ab}	64.46±1.46^{c}
S2G1	79.22±0.66^{bc}	7.95±0.94^{b}	0.39±0.02^{d}	1.84±0.16^{d}	0.71 ±0.09^{c}	61.90±1.25^{c}
S2G2	79.07±1.28^{bc}	8.02±0.35^{b}	1.26±0.12^{ab}	2.83±0.02^{c}	0.92± 0.02^{b}	86.97±2.92^{a}
S2G3	78.39±3.75^{bc}	7.05±0.73^{c}	1.24±0.06^{ab}	2.65±0.08^{d}	1.01± 0.14^{a}	84.82±0.86^{a}
S2G4	75.96±1.85^{cd}	9.82±0.39^{a}	1.33±0.14^{a}	3.45±0.31^{a}	1.16± 0.18^{a}	81.58±1.79^{b}
S3G1	76.09±0.94^{d}	7.20±0.91^{c}	0.48±0.08^{c}	1.88±0.15^{e}	0.50±0.04^{d}	56.36±0.66^{d}
S3G2	76.19±1.18^{d}	7.88±0.41^{b}	1.18±0.04^{b}	2.97±0.07^{b}	0.66±0.01^{c}	83.87±1.66^{ab}
S3G3	75.25±0.47^{d}	10.06±0.49^{a}	1.27±0.07^{a}	3.03±0.12^{ab}	1.09 ±0.06^{a}	82.55±1.39^{b}
S3G4	74.24±0.48^{d}	9.66±0.27^{a}	1.36±0.11^{a}	3.27±0.23^{ab}	1.08± 0.16^{a}	80.55±1.93^{b}

Control = ungerminated rice
S1G1, S1G2, S1G3, and S1G4 refer to the steeping time for one day and germination for 1, 2, 3, and 4 day, respectively.
S2G1, S2G2, S2G3, and S2G4 stand for the steeping time for 2 day and germination for 1, 2, 3, and 4 day, respectively.
S3G1, S3G2, S3G3, and S3G4 refer to the steeping time for 3 day and germination for 1, 2, 3, and 4 day, respectively.
[a] Means within columns followed by the same letter are not significant different at $p < 0.05$

TABLE II. PASTING PROPERTIES (RVU) OF UNGERMINATED AND GERMINATED RICE FLOUR

Germination condition	Pasting temperature (°C)	Peak viscosity	Break down	Final viscosity	Set back
Control*	68.90±2.81	89.08 ± 2.70[a]	64.08 ± 3.41[a]	101.46 ± 2.53[a]	66.45 ± 2.66[a]
S1G1	68.81±1.56	83.91 ± 1.18[b]	14.00 ± 0.82[bc]	29.45 ± 0.88 [b]	9.54 ±1.23 [b]
S1G2	66.44±1.13	18.87 ± 1.35[d]	10.96 ± 1.11[d]	13.25 ± 2.23[d]	5.33 ± 0.09 [c]
S1G3	66.78±1.01	1.04 ± 0.65[g]	1.87 ± 0.06 [e]	0.50 ± 0.59[g]	0.33 ± 0.02f
S1G4	67.02±1.32	1.37 ± 0.06[g]	2.500 ± 0.11[e]	0.58 ± 0.47[g]	0.54 ± 0.03[e]
S2G1	67.22 ±1.76	21.00 ± 0.46[c]	15.33 ± 0.23[b]	24.41 ± 0.12[c]	9.75 ± 0.35 [b]
S2G2	66.31±1.18	7.25 ± 0.46[e]	13.12 ± 0.41[bc]	5.29 ± 0.53[f]	0.58 ± 0.07[e]
S2G3	67.22±1.81	5.41 ± 0.12[ef]	11.75 ± 0.35[cd]	5.46 ± 0.29[f]	1.37 ± 0.17[d]
S2G4	66.78±1.42	5.37 ± 0.06[ef]	12.87 ± 0.41[bc]	6.29 ± 0.17[e]	1.20 ± 0.17[d]
S3G1	67.14± 0.80	6.29 ± 0.05[e]	14.21 ± 0.05[bc]	6.42 ± 0.35[e]	1.50 ± 0.35[d]
S3G2	67.25±0.94	6.33 ± 0.12[e]	1.37 ± 0.06[e]	5.91 ± 0.23[ef]	5.95 ± 0.17[c]
S3G3	66.60±1.03	2.71 ± 0.29[fg]	2.21 ± 0.65[e]	0.79 ± 0.65[h]	0.29 ± 0.09[f]
S3G4	66.78±1.90	2.62 ± 0.17[fg]	1.16 ± 0.12[e]	2.00 ± 0.11[g]	0.51 ± 0.02[e]

Control = ungerminated rice
S1G1, S1G2, S1G3, and S1G4 refer to the steeping time for one day and germination for 1, 2, 3, and 4 day, respectively.
S2G1, S2G2, S2G3, and S2G4 stand for the steeping time for 2 day and germination for 1, 2, 3, and 4 day, respectively.
S3G1, S3G2, S3G3, and S3G4 refer to the steeping time for 3 day and germination for 1, 2, 3, and 4 day, respectively.
[a] Means within columns followed by the same letter are not significant different at $p< 0.05$

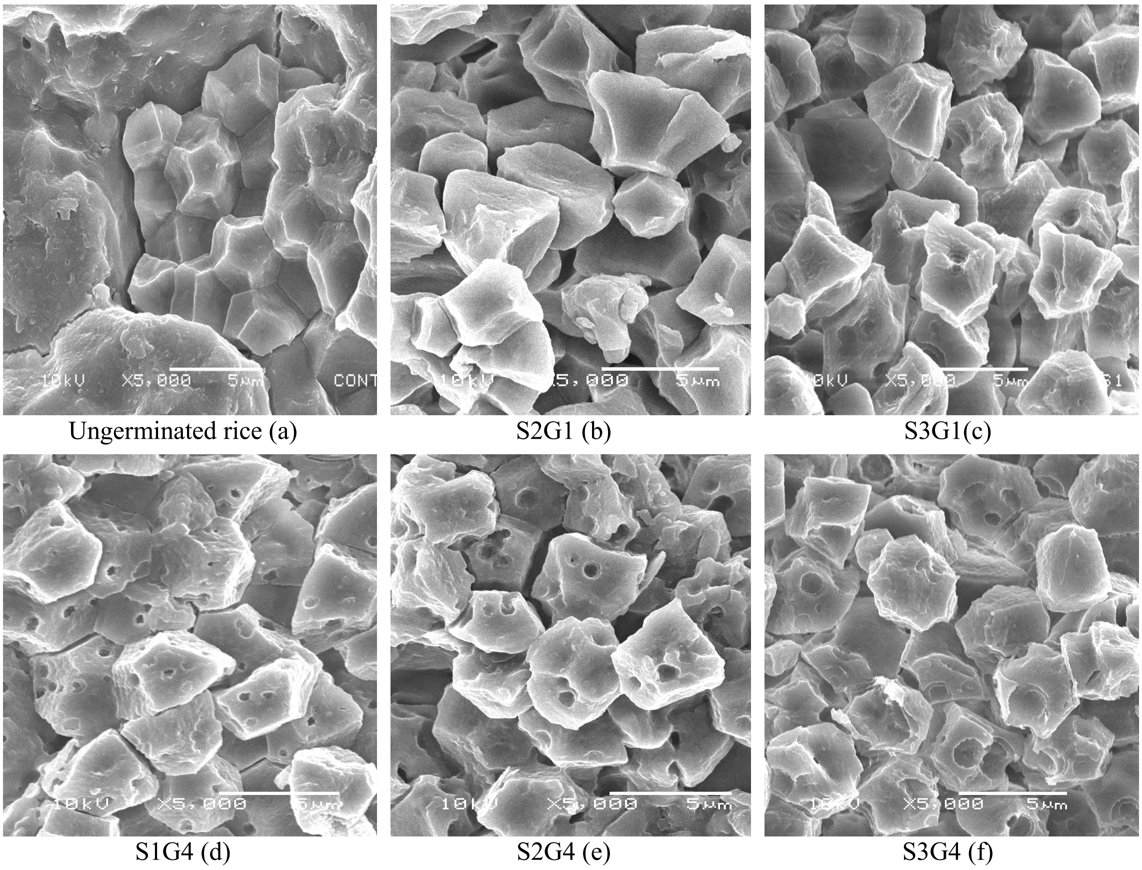

Figure 1. Ultra-structural changes of the starch, as assessed by scanning electron microscopyS2G1 (a) and S3G1 (b) refer to the steeping time for two and three days and germination for one day, respectively.S1G4 (c), S2G4 (d), and S3G4 (e) refer to the steeping time for one, two, and three days and germination for four days, respectively.