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Effects of Cereal Powders with Dietary Fibers on Retrogradation Properties of *Jeungpyun*, a Korean Traditional Fermented Rice Cake

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Abstract : This study investigated retrogradation properties of *Jeungpyun* substituted for cereals with dietary fibers of 60% of brown rice, and barley. Quality changes during storage periods of the functional *Jeungpyun*, were studied using α-amylase iodine enzyme digestion methods, X-ray diffraction patterns, and differential scanning calorimetry (DSC). The barley substituted samples showed significantly lower retrogrdation rates than those of control when examined by α-amylase method. The Relative crystallity by X-ray diffraction patterns had typical A type in all samples with appearing big crystallity around its diffraction angle 23°degrees as storage periods were increased. The brown rice and barley *Jeunpyun* made smaller crystallity than that of control, representing slower retrogradation rates. The batter controls had significantly lower ΔH than the other compared samples when measured by DSC, but had significantly higher ΔH after 30 days of storage, implying that the control required more energy for regelatinization after the 30 days of storage.

Keywords : *Jeungpyun*, brown rice, barley, α-amylase, X-ray diffraction, DSC

Introduction

Jeungpyun is a traditional Korean steamed rice cake using fermentation for its preparation. The first step of the preparation is soaking rice and other cereal powders. The soaked rice is sifted and yeast dissolved in sugar solution is added with a little amount of salt and vinegar. Two steps of fermentation are carried out for about 2.5 hr. After the fermentation, the samples are steam cooked and the rice cakes are cooled down to room temperature. Understanding the mechanisms of the process is important in order to obtain suitable criteria for the determination of the unique process and to produce high quality *Jeungpyun* or other rice-based snacks using similar process[1-3]. Several studies have shown that the *Jeungpyun* has viscoelastic and aerated texture like other ordinary cakes[2]. Structure of *Jeungpyun* is unique because it forms a sponge-like texture only with rice flour which does not have gluten. Although the specific mechanism has not been fully determined yet, some studies of *Jeungpyun* are studied in many ways as one of the possibilities of a globalization of Korean food. The main problems of rice based cakes in general are rapid starch retrogradation. In the present study, *Jeungpyun* containing natural dietary fiber materials such as brown rice and barley were developed and the effects of those cereals with dietary powder on the retrogradation of the product were examined to obtain basic data for shelf-life properties of the *Jeungpyun*.

Materials and Methods

Materials

The Japonica type rice and brown rice used in the present study were produced in Kimpo city, Kyunggido. The content of amylose in the rice was 16.9%. Raw barley flour (Taekwang Food Co., Kyungsangdo, Korea), sugar (Cheil Jedang Corporation, Seoul, Korea), activated dry yeast (Ottogi Corporation, Seoul, Korea), brown rice vinegar (Ottogi Corp oration, Seoul, Korea), and

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Table 1. [1]Formulation ratios of *Jeungpyun* with partial substitution of rice flours for brown rice and barley flours

Sample \ Ingredients	Rice Flour	Brown Rice	Barley	Salt	Sugar	Dry Yeast	Water
Control	100	0	0	1	10	1	50
Brown rice	40	60	0	1	10	1	60
Barley	40	0	60	1	10	1	70

[1]rice flour weight basis (%).

salt (Hanju salt, Seoul Korea) were purchased from a local department store. The whole materials were kept at 2°C refrigerator until use.

Preparation of *Jeungpyun*

Preparation of *Jeungpyun* was modified with the standard formula, shown in Table 1. Cereals of plain rice and brown rice were washed, soaked in water for 3 hr. Water was drained, and the soaked cereals were ground with a roller type mill (3HP, Kyung Chang Machinery, Daegu city, North Kyungsangdo, Korea). Due to the unique cohesiveness characteristics of barley, the barley could not be ground and sifted as rice or brown rice after soaking, raw barley flour was used instead. The powders of each cereals were sifted using a 40 mesh sift. The sifted cereals were measured for 500 g each and air-tightly packaged using a vacuum sealer (Ellyon, Kyunggido, Korea). All the prepared samples were kept frozen at –40°C until use. For control, 600 g of rice flour was used and 6 g of dry yeast was dissolved in 30 g (flour weight basis) sugar in 150 g water at 40°C and left to stand at room temperature for 20 min. For next step, 6 g salt, 6 g vinegar, another 30 g sugar, and 150 g water were added and mixed. For 60% cereal substituted samples, 240 g of rice flour and 360 g of each powders were used instead. The first fermentation was carried out in a 35°C incubator (J-IM3 JISI-Co, Sanyo, Tokyo, Japan) for 1.5 hr. The first fermented batter was degassed and the second fermentation was carried out in the same incubator for 1 hr using a container (45 × 45 × 8 cm^3) specially manufactured for this whole experiment. The two step fermented samples were steam cooked for 30 min and allowed to stand for 10 more min at room temperature. The rice cakes were completely cooled down to room temperature for 1 hr before measurements.

α-Amylase iodine enzyme digestion method

The degree of retrogradation of *Jeungpyun* was observed by α-amylase iodine enzyme digestion method[4]. The 12 m*l* of samples were placed in test tubes labled as A, B, and C. 2.5 m*l* of 0.1N acetate buffer (pH 5.6) was added to the tubes A and B and 1ml of 1N NaOH was added in test tube C. The prepared test tubes were stand at 30°C for 5 hr for swelling and gelatinization of starch. The tubes were neutralized with 2N acetic acid (pH 7.0) and the same sample amounts were calibrated with 0.1N acetate buffer. Unactivated α-amylase (1 m*l*) was added to the tube A and 1 m*l* of activated α-amylase(3.7 unit/m*l*) was added to each tube B and C. They were reacted in 37°C shaking water bath for 10 min. and the enzyme reactions were suspended with 2.5 m*l* of 4N NaOH in ice bath. They were neutralized with 4N HCl up to pH 7.0. The 1 m*l* of the neutralized samples were added to 10 m*l* distilled water and 100 μ*l* of 1% I_2-10% KI solution and they were reacted for 20 more min. Optical density was measured at 623 nm and retrogradation rate was calculated[4].

X-ray diffraction

The degree of retrogradation and relative crystallinity were measured by X-ray diffraction using X-ray diffractometer (Rigaku Geigerflex G/max II-A, To Kyo, Japan). A piece of *Jeungpyun* (10 × 5 × 3 cm^3) was wrapped in a piece of wrap and kept at cool temperature (4°C). The samples were stored for 0, 1, 2, 3, 7 and 30 days. After the storage, each sample was freeze dried, ground, and sifted using a 100mesh sift to obtain powder form samples. Table 2 shows the measurement conditions for the X-ray diffractometer. Relative crystallinity was calculated by the rate of retrogradation from the degree of X-ray diffraction

Table 2. Conditions of X-ray diffractometer

Target	: CU-Kα
Filter	: Ni
Voltage and current	: 35 KV, 20 mA
Scanning speed	: 10/min
2θ Scanning region	: 4-400

according to the method of Komiya and others[5].

Differential Scanning Calorimetry (DSC)

For DSC (6100, SEIKO INS, Chiba, Japan) measurements, three stages of samples were collected. For the first step, 20 g of each sample was taken immediately after the two fermentation steps. After the steam cooking for gelatinization, 20 g of fresh *Jeungpyun* sample was taken at room temperature. Finally, 20 g of *Jeungpyun* stored for 30 days were taken. These samples were freeze-dried quickly at –40°C, freeze-dried again for 15 hr. They were ground, and sifted using a 100 mesh sift to obtain powder. They were dried in a 70°C vacuum oven for 3 hr and left in a desiccator until use. The powder samples (1.8 ± 0.1 mg) were placed directly into a hermetic aluminum pan and distilled water (7.2 ± 0.1 mg) was placed into the pan using a micropipet. The sample was sealed and left in room temperature for one hour. The samples were heated with the reference of Al_2O_3 (aluminum oxide, 10 mg), from 0°C to 150°C at an increment of 10°C/min to measure onset temperature (To), peak temperature (Tp), and conclusion temperature (Tc) of gelatinization[6,7]. Relative degree of retrogradation in each sample was expressed in percent (%) as ratios of enthalpy before and after gelatinization.

Statistical analysis

All testing was replicated 3 times. Statistical analysis was done using SAS package[8]. For the determination of difference between samples, Duncan's multiple range test was used with significance level at $P<0.05$.

Results and Discussion

α-Amylase iodine enzyme digestion method

Table 3 shows the results of measuring the degrees of retrogradation using the α-amylase iodine enzyme digestion method. With the increase in storage time, the degree of retrogradation increased in each sample. By 24 hr of storage, no significant difference was seen in the control sample and brown rice sample, but a significant difference was seen between control and barley substituted samples. The delay in retrogradation was highest in barley samples with 15.30%, followed by brown rice substituted sample(17.10%) and barley substituted sample(20.03%) by 48 hr of storage. The similar trend was shown in the 72 hr stored sample groups with higher percentage ($P<0.05$) representing the cereals fortified with dietary fibers have positive effects in extending the shelf-life of *Jeungpyun.*

Table 3. Retrogradation degree of *Jeungpyun* using α-Amylase substituted for flours of brown rice and barley during storage at 4°C

Hr	Control	Brown Rice	Barley
24	$^{2)C}10.27^{a1)}$	$^{C}9.90^{a}$	$^{C}8.24^{b}$
48	$^{B}20.03^{a}$	$^{B}17.10^{b}$	$^{B}15.30^{c}$
72	$^{A}34.37^{a}$	$^{A}30.40^{b}$	$^{A}24.33^{c}$

Same letters are not significantly different each other ($p<0.05$).
1)mean values of retrogradation degree for storage time at each treatment (row).
2)mean values of retrogradation degree for treatment at each storage time (column).

X-ray diffraction

Figs. 1-3 show the patterns of X-ray diffraction for degree of retrogradation and relative crystallinity in *Jeungpyun* prepared with substituting rice flour for brown rice and barley flours with storage time. The crystal peaks were seen starting around 15.5 degree to 23.0 degree, showing the typical Type A crystalline pattern. The peak height around 22-23 degree of the newly formed diffraction angle was used as the index of relative crystallinity of starch. With the increase in storage time, X-ray diffraction became sharper, suggesting that retrogradation was progressing. Kainuma and others[9] and Wu and Sarko[10] explained this result with the rearrangement of chains remaining after hydrolysis and strong bonding between molecules in the non-crystal area of starch. Table 4 shows the results of retrogradation

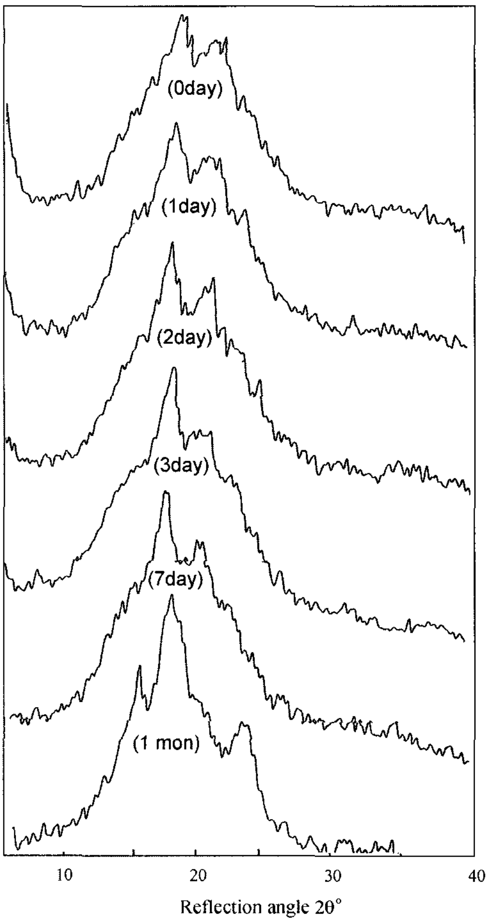

Fig. 1. X-ray diffraction patterns of control Jeungpyun during storage at 4°C.

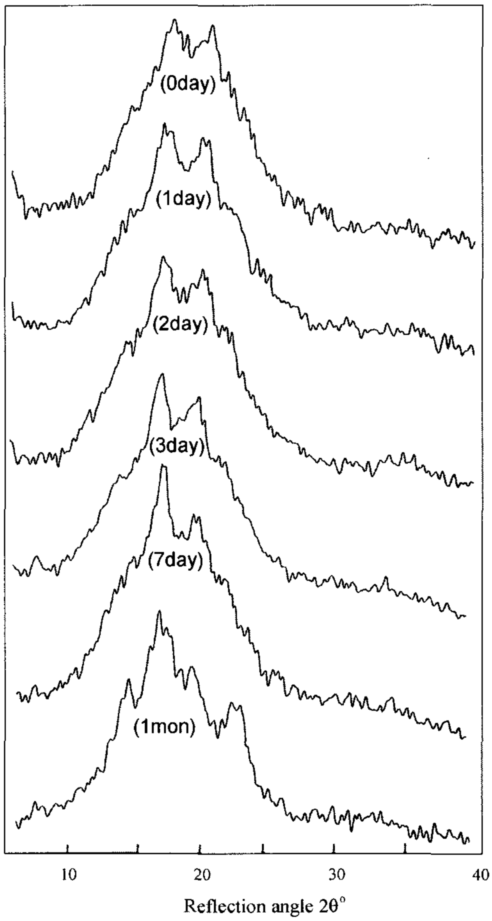

Fig. 2. X-ray diffraction patterns of Jeungpyun substituted for barly flour during storage at 4°C.

degree of stored *Jeungpyun* using X-ray diffraction. Relative crystallinity (Table 5) was calculated by dividing the results into crystal and non-crystal areas around the diffraction angle of 23.0 degree. Relative retrpgradation degree of *Jeungpyun* prepared by substituting for brown rice and barley one month after storage according to X-ray diffraction was about 2 times higher in the control sample compared with the fresh sample, 1.9 times higher in the brown rice substituted sample, and 1.8 times higher in barley substituted sample. The results represent that the retrogradation was delayed in the substituted sample groups. Relative crystallinity by one day after storage was higher in the control sample and brown rice substituted sample compared with barley substituted sample. Starting two days after storage, it was higher in the control sample, followed by brown rice substituted sample and barley substituted sample. The results indicate a significant decrease of retrogradation in the barley substitutied samples ($P<0.05$). The finding that the retrogradation was delayed most significantly in barley substituted sample was because barley substituted sample contains the dietary fiber β-glucan so that dietary fiber extract would interfere with the gelatinization during the process of rearrangement of normal starch molecules after the gelatinization of starch and bond with some amylose and amylopectin to prevent starch molecules to form hydrogen bonding[11].

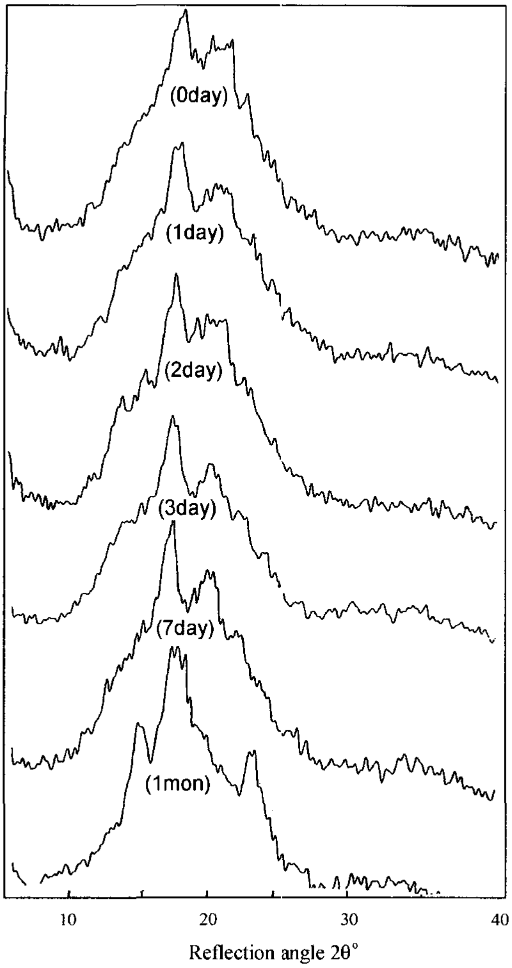

Fig. 3. X-ray diffraction patterns of Jeungpyun substituted for brown rice flour during storage at 4°C.

Differential Scanning Calorimetry (DSC)

Table 6 shows the results of measuring the changes in Jeungpyun prepared by substituting rice flour for cereals with dietary fibers during storage using DSC. No thermal change was seen in the fresh sample obtained from *Jeungpyun* one hour after steaming since no retrogradation occurred. Peaks appeared between 40-60°C in the sample stored for one month under cool condition due to the dissociation of hydrogen bonds formed at the time

Table 4. Ratio of retrogradation in *Jeungpyun* replaced with flours of brown rice and barley by X-ray diffraction during storage at 4°C

Days	0	1	2	3	7	30
control	100^{f}	$^{A2)}$108.3$^{e1)}$	^{A}117.9^{d}	^{A}131.7^{c}	^{A}151.0^{b}	^{A}201.4^{a}
Brown rice	100^{f}	^{A}107.8^{e}	^{B}114.4^{d}	^{B}127.3^{c}	^{B}145.1^{b}	^{B}188.9^{a}
barley	100^{f}	^{B}106.5^{e}	^{C}111.9^{d}	^{C}121.2^{c}	^{C}139.1^{b}	^{C}180.8^{a}

Same letters are not significantly different each other ($p<0.05$).
1)mean values of Ratio of retrogradation for storage period at each treatment (row).
2)mean values of Ratio of retrogradation for treatment of each days (column).

Table 5. Relative crystallity of *Jeungpyun* replaced with flours of brown rice and barley by X-ray diffraction during storage at 4°C

Days	0	1	2	3	7	30
Control	0.145$^{1)}$	0.157	0.171	0.191	0.219	0.292
Brown rice	0.153	0.165	0.175	0.195	0.222	0.289
Barley	0.151	0.161	0.169	0.183	0.210	0.273

Same letters are not significantly different each other ($p<0.05$).
1)Relative crystallity was estimated from the area of crystalline peaks in the diffraction degree.

Table 6. DSC Characteristics of *Jeungpyun* replaced with flours of 60% brown rice and barley comparing fresh batter and one month storage at 4°C

Sample	*Jeungpyun* batter				*Jeungpyun* after steaming				Re.H/In.H
	TO	Tp	Tc	ΔH	To	Tp	Tc	ΔH	*100(%)
Control	57.6^{a}	63.9^{b}	72.9^{b}	7.5^{bc}	36.9^{a}	43.3^{a}	50.7^{a}	9.3^{a}	123.9^{a}
Brown rice	64.1^{a}	71.1^{a}	79.7^{a}	7.9^{bc}	37.7^{a}	43.4^{a}	50.7^{a}	6.7^{bc}	84.9^{b}
Barley	57.1^{a}	61.1^{b}	71.9^{b}	8.6^{a}	37.8^{a}	43.5^{a}	50.9^{a}	6.0^{c}	70.2^{b}

Means of three replications ; same letter in a column are not significantly different each other ($p<0.05$).

of starch retrogradation[12]. The enthalpies were calculated from the samples before gelatinization and results of the gelatinization temperature. Relative degree of retrogradation was calculated from DSC thermograms from one-month-old Jeungpyun samples. Onset temperature (To), peak temperature (Tp), and closing temperature (Tc) before gelatinization showed similar or higher degrees in the brown rice substituted samples compared with the control sample or barley substituted samples. The temperatures were not significantly different in the sample stored for one month between control and substituted sample groups. Wada and others[13] reported that the onset temperature is low and enthalpy of gelatinization is also small if the crystallinity of starch particle is small. Hydrogen bonding forms during storage so that starch molecules experience partial rearrangements of their structure. Thus, more retrogradation occurs if the enthalpy (ΔH) needed to regelatinize the rearrangement is larger. In the samples before gelatinization, ΔH of control was 7.50 mJ/mg, 7.87 mJ/mg in the brown rice substituted sample, and 8.60 mJ/mg in barley substituted sample showing no significant difference present in enthalpy between the control and brown rice substituted samples, but enthalpy was significantly higher in the barley substituted sample ($P<0.05$). In the samples stored for one month, ΔH was 9.30 mJ/mg in the control, 6.68 mJ/mg in brown rice substituted sample, and 6.03 mJ/mg in the barley substituted sample. Thus, enthalpy was higher in the control sample compared with the substituted sample groups ($P<0.05$). The results suggest that retrogradation progressed more in the control sample since the energy needed for regelatinization after stored in cool place for 30 days was more in the control sample compared with the substituted samples. The relative retrogradation obtained by dividing the enthalpy of each sample at the time of regelatinization by the gelatinization enthalpy was significantly higher in the control sample compared with the brown rice and barley substituted samples. This result was also shown in the result of X-ray relative crystallinity.

According to the above findings on the storagability of *Jeungpyun* prepared by substituting rice flour for brown rice flour and barley flour, a significant delay in retrogradation was seen in brown rice and barley substituted samples compared with the control sample. Thus, *Jeungpyun* substituted large amount of rice for cereals with dietary fiber has possibility extending the shelf-life of those kind of prodects. Those products are expected to receive attentions of health-concerned consumer response for its positive functionality of the dietary fiber such as prevention of cancer and retardation of aging.

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