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Comparative effects of slowly digestible and resistant starch from rice in high-fat diet-induced obese mice

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Abstract The effects of optimized slowly digestible starch (SDS) and resistant starch (RS) from rice starch on weight gain and lipid metabolism in mice (C57BL/6J mice) fed a high-fat diet were investigated. The optimum conditions for SDS were obtained at the pullulanse concentration (X_1) of 498 μL, storage temperature) (X_2) of 47°C, and A/C cycle (X_3) of 5, and for RS, were determined to be 838 μL (X_1), 62°C (X_2), and a cycle of 3 (X_3) using response surface methodology (RSM). Mice fed SDS and RS for 6 weeks showed both significantly decreased weight gain and fat pad weight (p<0.05). Significant decrease in total lipid, triglyceride, and cholesterol concentrations in serum and liver was observed in both SDS and RS group compared to HFD groups (p<0.05). Although both of intake SDS and RS significantly contributed to beneficial effects, RS groups was more effective than SDS group in all parameters.

Keywords: rice starch, slowly digestible starch, resistant starch, optimization, anti-obesity effect

Introduction

Dietary carbohydrates play a major role in regulating obesity, blood glucose attenuation and insulin response (1). Starch, the dominating dietary carbohydrate in the human diet (2), is classified into three major fractions based on the rate and extent of its digestion: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). RDS is the starch fraction that induces a rapid increase in blood glucose level after ingestion (3). SDS is the starch fraction that is more slowly but completely digested in the small intestine, thereby lowering glycemic response compared to RDS. It was previously reported that foods containing SDS have beneficial physiological activities such as satiety, physical performance, and improved glucose tolerance (4). On the other hand, RS is a fraction that cannot be digested in the small intestine and is fermented in the large intestine to produce short-chain fatty acids (5). As a result, it has been shown to have a variety of beneficial effects such as protection of colon health and a low glycemic index (6).

Rice starch, one of the important dietary sources of carbohydrates, has been related to cooking quality, physicochemical and digestibility. However, the glycemic response of rice starch is known to be relatively high compared to those of other starchy foods and is related to increased risk of obesity, diabetes, and cardiovascular disease (7). Thus, improvement of the nutritional characteristics of rice starch, such as lowering its GI index, is a subject of great importance.

The rate and extent of starch digestion are influenced by intrinsic factors such as the structure of the starch and extrinsic factors such as anti-nutrient components and processing method. It is well known that amylose is less digestible than amylopectin. RS is produced by highly retrograded amylose fraction of starch, the quantity being directly proportional to the amylose content (8). Therefore, to improve the yield of the RS fraction, there have been many attempts to obtain modified starch with a higher percentage of amylose through the use of an enzymatically method (9,10). Some researchers have reported that the yield of RS was increased by treatment with debranching enzymes such as pullulanase, which hydrolyzes α-1, 6 glucosidic bonds of amylopectin (11). However, compared to the current understanding of the RS fraction, little is known about the SDS fraction, although this fraction has various physiological benefits. The purposes of this study were (i) to investigate the optimum conditions for production of rice starch containing SDS and RS by enzyme treatment and (ii) to determine the response of the weight gain effect and lipid profiles in mice and compare the physiological functions of optimized SDS and RS *in vivo*.

Materials and Methods

Materials Rice starch (S-7250), α-amylase (A-3176, Type VI: From porcine pancreas) and porcine pancreatin (P-1750) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Commercial

Table 1. Experimental design and the response of the dependent variables for the yield of slowly digestible starch (SDS) and resistant starch (RS) of rice starch

Test run No	Independent variables					
	Coded variable			Real variables		
	X_1[1)]	X_2	X_3	Pullulanase (NPUN/g dry starch)	Temperature (°C)	Autoclaving cycle
1	-1	-1	-1	446	25	2
2	-1	-1	1	446	25	4
3	-1	1	-1	446	65	2
4	-1	1	1	446	65	4
5	1	-1	-1	1,175	25	2
6	1	-1	1	1,175	25	4
7	1	1	-1	1,175	65	2
8	1	1	1	1,175	65	4
9	0	0	0	810	45	3
10	0	0	0	810	45	3
11	-2	0	0	81	45	3
12	2	0	0	1,540	45	3
13	0	-2	0	810	5	3
14	0	2	0	810	95	3
15	0	0	-2	810	45	1
16	0	0	2	810	45	5

[1)]The X_1, X_2, and X_3 were the concentration of pullulanase (NPUN/g dry starch), storage temperature (°C), and the autoclaving cycle, respectively.

pullulanase (Promozyme® D2) and amyloglucosidase (AMG 300 L, activity 300 AGU/mL) were obtained from Novozymes (Bagsvaerd, Denmark). All other chemicals were analytical grade reagents.

Preparation of starch samples Rice starch suspension (10%, w/v, dry basis) in 0.1 M sodium acetate buffer, pH 5.0, was prepared. The suspension was heated in a 250 mL flask at 90°C for 30 min with stirring. Then the sample was placed in a water bath 57.5°C. After equilibrium, the suspension was incubated with different concentrations of pullulanase (81-1,540 NUPN/g dry starch) for 24 h with continuous shaking. After heating at 90°C for 10 min to stop enzyme activity, the resulting suspensions were autoclaved for 1 h which was repeated up to 5 times (1-5 cycles). Afterwards the sample was cooled at various temperatures (5-85°C). The residue was precipitated by adding 95% ethanol and dried at 40°C for 2 days. After drying, the residue was ground and passed through a 100 mesh sieve for later analytical assay. The SDS and RS contents of the rice starch product were determined.

Experimental design and statistical analysis To investigate the effects of reaction parameters on the formation of SDS and RS, an incomplete factorial design with three independent variables at five levels of variation was used. The selected variables were: enzyme concentration (81, 446, 810, 1,175 and 1,540 NUPN/g dry starch), storage temperature (5, 25, 45, 65 and 85°C) and cycle of autoclaving-cooling (1, 2, 3, 4 and 5). The five levels of each variable were coded as −2, −1, 0, +1, and +2 for statistical analysis (Table 1). The design consisted of 16 experimental points that included 23 fractional factorial points, six star points, and two replicates at the center point (Table 1). The dependent variables were the SDS and RS yields.

The relationship between the independent variables and *Y* was modeled using the following nonlinear quadratic equation:

$$Y=\beta_0+\sum_{i=1}^{3}\beta_i X_i+\sum_{i=1}^{3}\beta_{ii}X_i^2+\sum_{i=1}^{2}\sum_{j>i}^{3}\beta_{ij}X_iX_j \quad (1)$$

where, β_0, β_i, β_{ii}, and β_{ij} are the constant, linear, quadratic, and cross-product regression coefficients, respectively. For further elucidation of the relationship, contour plots were generated using the resulting regression models. Statistical Analysis System (SAS) program 9.1 (SAS Institute Inc., Cary, NC, USA) was used for the statistical analysis of the experimental data.

Determinations of starch fraction Enzyme solutions were prepared according to the method of Englyst *et al*. (3) with minor modification. Pancreatin (1 g) was dispersed in water (12 mL) with stirring with for 10 min at room temperature. The enzyme suspension was centrifuged at 1,500× *g* for 10 min and then, the supernatant (10 mL) was mixed with amyloglucosidase (0.2 mL), which was adjusted to 12 mL with distilled water. This enzyme solution was freshly made for each digestibility test. Starch samples (30 mg) were loaded into micro tubes (2 mL), and 0.75 mL of sodium acetate buffer (pH 5.2) and a glass ball were added to each tube, followed by incubation in a shaking incubator at 37°C to equilibrate for 10 min. Then, 0.75 mL of the prepared enzyme solution was added to each tube, and the stroke speed of the shaking incubator was adjusted to 250 rpm. After 20 min for RDS and a further 120 min for SDS, a 2.0 mL tube was boiled for 10 min to stop the reaction and calculate the contents. Then, the content of RS was calculated based on RS=100–RDS–SDS. The hydrolyzed sugar was measured using dinitrosalicylic acid (DNS) method. Maltose concentration was determined using a standard curve of maltose content versus absorbance.

Animals and diets Experiments were performed with 4 week old C57BL/6J male mice obtained by Central Lab oratory Animal Inc. (Seoul, Korea) for 13 weeks. After 7-day acclimatization period on standard rodent chow (Samyang, Seoul, Korea), the animals were stratified by weight and assigned to one of the three diets. The mice were fed a high-fat diet (HFD, 40% beef tallow-modified AIN-76A purified rodent diet #101556 (Dyets Inc., Bethlehem, PA, USA)) for six weeks, respectively. The HFD group was divided into three groups of 10 per group by randomized block design according to diets: (1) the control group fed with high-fat-diet (HFD); (2) the test group fed with HFD+SDS (Rice-SDS); (3) test group added with HFD+RS (Rice-RS). Mice were housed in cages with wire-mesh bottoms in conditioned rooms (23°C; relative humidity 55%). All mice were provided with food and water ad libitum and maintained on the assigned diet for a

Table 2. Composition of experimental diets (unit: g/kg diets)

Ingredient	Experimental groups[1)]		
	HFD	SDS	RS
Casein	200	200	200
DL-methionine	3	3	3
Corn starch	150	-	-
Sucrose	150	150	150
Rice-SDS	-	150	-
Rice-RS	-	-	150
Beef tallow	400	400	400
Cellulose	50	50	50
Mineral mixture	35	35	35
Vitamin mixture	10	10	10
Choline bitartrate	2	2	2
Total calories (kcal/kg)	5,542	5,542	5,542
Total (g/kg)	1000	1000	1000

[1)]HFD, High fat diet: 40% beef tallow modified AIN-76A purified rodent diet #101556 (Dyets Inc.)

six-week period. Food intakes were measured twice weekly, and body weights were recorded once a week. The compositions of control and experimental diets are shown in Table 2. The care and treatment of mice were approved by the Hanyang University Lab Animal Care Committee, which were in accordance with the Korean Guide for the Care and Use of Laboratory Animals.

Serum collection and various organ preparations Food was removed for 24 h at the end of the experimental term, and the mice were anesthetized with dry ice and dissected. Blood was collected from the heart, was placed at room temperature for 2 h and then centrifuged at 1,500× *g* for 30 min at 4°C to obtain serum. The adipose tissue (perirenal and epididymal), and liver were excised from each mice and the wet weights were measured. Serum and organs were then snap-frozen in liquid nitrogen and stored at −70°C for further analysis.

Lipid profile Serum lipid profiles were analyzed in a blood chemistry analyzer (Olympus AU 400; Olympus, Tokyo, Japan). For liver analysis, total lipids were homogenized in chloroform: methanol (2:1, v/v) by using a modified method of Folch *et al*. (12). After homogenization, liver samples were measured with enzymatic colorimetric procedures for triglyceride and total cholesterol using a commercial kit (Asan Pharmaceutical Co., Seoul, Korea).

Statistical analysis Statistical analysis was performed by Statistical Package for the Social Sciences (SPSS, Version 12.0, SPSS Inc., Chicago, IL, USA). The results were expressed as mean with standard deviation (SD) and significant differences were determined by one-way analyses of variance (ANOVA) followed by Duncan's multiple comparison tests.

Table 3. Regression equation coefficients for yield of slowly digestible starch (SDS) and resistant starch (RS) of rice starch

Factors	SDS[1)] (%)	RS (%)
Constant	25.733999**	29.1316**
Pullulanase	1.104068 (NS)	1.426609**
Storage temperature	0.954912 (NS)	2.271825**
A/C cycles	1.849906*	1.157653*
(Pullulanase)^2	-2.555536**	-0.606567 (NS)
(Storage temperature)^2	-2.91351**	-1.740151**
(A/C cycles)^2	-0.258477 (NS)	-1.382652**
Pullulanase x Storage temperature	0.297682 (NS)	0.925396 (NS)
Pullulanase x A/C cycles	-0.358604 (NS)	-0.745149 (NS)
Storage temperature x A/C cycles	0.019258 (NS)	-0.844586 (NS)
R^2	0.8749	0.9515
F	4.66	13.07
Probability of *F*	0.0373	0.0027

*, ** significant at $p<0.1$, $p<0.01$, respectively.
[1)]SDS, slowly digestible starch; RS, resistant starch

Results and Discussion

Optimization for reaction conditions of SDS and RS The regression coefficients calculated for the yields of SDS and RS by response surface regression (RSREG) are shown in Table 3. As shown in Table 3, both regression coefficients of model showed high value of R^2 [R^2= 0.87 for SDS and 0.95 for RS], respectively. The polynomial regression equations using Eq. 2 and 3 are given by:

$$\%SDS=25.73^{**}+1.84X_3^{*}-2.55X_1^{2**}-2.91X_2^{2**} \quad (2)$$

$$\%RS=29.13^{**}+1.42X_1^{**}+2.27X_2^{**}+1.15X_3^{*}-1.74X_2^{2**}-1.38X_3^{2**} \quad (3)$$

The value for the SDS yield was most significantly affected by the negative quadratic coefficient of pullulanase concentration (X_1) and storage temperature (X_2). Contrast to the results of SDS yield, the RS yield was most significantly affected by the positive linear regression coefficients of pullulanase concentration (X_1) and storage temperature (X_2) as well as the negative quadratic coefficient of storage temperature (X_2) and A/C cycle (X_3) (Table 3). The SDS yield increased from 11.53 to 26.57% as a function of different reaction condition (Fig. 1A). SDS yield was increased with increasing pullulanase concentration and storage temperature until it reached maximum value at intermediate level after the SDS yield began to decrease. Also, A/C cycle had a positive effect the SDS yield. Similar to the SDS yield, the yield of RS was in the range from 16.45 to 31.10% as shown in Fig. 1B, which showed that the increasing pullulanase concentration, storage temperature, and A/C cycles caused an increase of RS yield. The optimal conditions to obtain for SDS and RS yields by canonical analysis were determined as follows: X_1 (pullulanase concentration)=

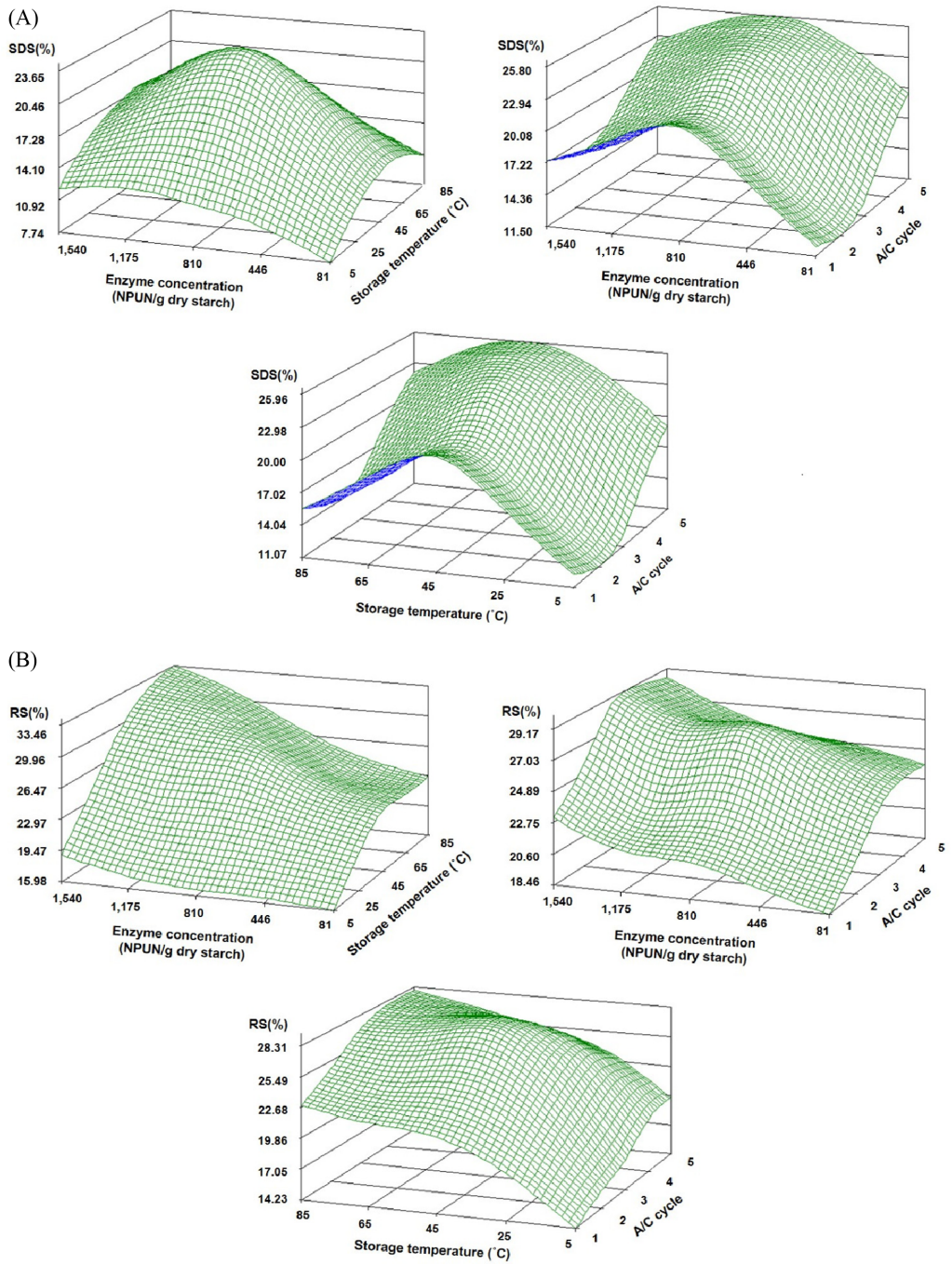

Fig. 1. Response surface plots for the effect of enzyme concentration (μL), storage temperature (°C), and A/C cycles on the yield of SDS (A) and RS (B). The z axis is the response variables SDS (%), and the x axis and y axis are the independent variables.

498 μL; X_2 (storage temperature)=47°C; X_3 (A/C cycle) = 5 for SDS and X_1 (pullulanase concentration)=838 μL; X_2 (storage temperature)= 62°C; X_3 (A/C cycle)=3 for RS. For the application of selected independent variables, 29.13% and 32.64% for SDS and RS yield were expected, respectively, which was close to actual experimental observations (SDS=26.94%, RS=30.11%).

Characteristics of SDS- and RS-rice starch

***Weight gain, food intake, food efficiency and fat pads weight*:** There was no significant difference in initial body weight between the different groups (p>0.05) (Table 4). However, the addition of the SDS and RS to the high-fat diet (HFD) had significant (p<0.05) effects on the mice final body weight which was significantly (p<0.05)

Table 4. Weight gain, food intake, tissue and fat pads weight of C57BL/6J mice fed high-fat diet after 6 weeks of treatment with experimental diets

	HFD[1)]	Rice-SDS	Rice-RS
Initial weight[2)], *g*	29.77±0.69[ns5)]	29.08±1.37	28.92±1.67
Final weight, *g*	38.64±1.79[a6)]	35.88±1.50[b]	34.86±0.47[b]
Weight gain[3)], *%*	29.76±5.07[a]	23.71±10.40[ab]	20.78±5.95[b]
Food intake, *g/day*	94.40±0.28[a]	90.05±4.73[b]	97.63±11.36[a]
Food efficiency[4)], *g gain/g food*	0.094±0.000[a]	0.076±0.003[b]	0.062±0.013[b]
Fat pad weight			
Perirenal fat, *g*	0.29±0.08[a]	0.23±0.06[b]	0.20±0.04[b]
Epididymis, *g*	2.06±0.35[a]	1.99±0.32[ab]	1.91±0.30[ab]

[1)]HFD, high-fat diet; Rice-SDS, rice-slowly digestible starch; Rice-RS, rice-resistant starch
[2)]Initial weight: mean body weight at the beginning of feeding the experimental diets
[3)]Weight gain (%)=[(Final weight (g)−initial weight (g))/initial weight (g)]x100
[4)]Food efficiency=mean body weight gain (g)/mean food intake (g)
[5)]ns, not significant
[6)]a-bValues with different subscripts within the same row are significantly different among samples at α=0.05 level by Duncan's multiple range test.

Table 5. Lipidic parameters in serum and liver of C57BL/6J mice fed high-fat diet after 6 weeks of treatment with experimental diets

	HFD[1)]	Rice-SDS	Rice-RS
Serum			
Total lipid, *mg/dL*	337.81±8.19[a2)]	317.13±11.82[a]	269.39±9.37[b]
Triglyceride, *mg/dL*	98.89±4.63[a]	78.99±6.76[b]	68.79±4.10[b]
Total cholesterol, *mg/dL*	165.74±4.07[a]	155.38±4.43[a]	136.27±5.26[b]
HDL cholesterol, *mg/dL*	102.00±1.63[ns3)]	97.20±2.25	86.40±2.74
LDL cholesterol, *mg/dL*	63.74±2.55[a]	58.18±2.25[a]	49.87±2.68[b]
Liver			
Total lipid, *mg/g liver*	114.13±7.29[a]	80.62±10.05[b]	55.96±6.89[c]
Triglyceride, *mg/g liver*	30.76±2.20[ns]	28.14±2.37	25.56±1.35
Total cholesterol, *mg/g liver*	1.07±0.43[a]	0.83±0.51[ab]	0.44±0.28[b]

[1)]HFD, high-fat diet; Rice-SDS, rice-slowly digestible starch; Rice-RS, rice-resistant starch
[2)]a-cValues with different subscripts within the same row are significantly different among samples at α=0.05 level by Duncan's multiple range test.
[3)]ns, not significant

reduced compared to the HFD group. However, there were no significant differences between SDS and RS treatment groups. After 6 weeks, the weight gain of HFD group (29.76%) was higher than SDS (23.71%) and RS (23.71%) group. Furthermore, SDS- and RS-group reduced the wright gain for HFD-fed mice by 20.33 and 30.17%, respectively (data no shown). There are many studies reported that RS intake decreased body weight (13,14). These beneficial effect might be due to that RS decrease energy absorption, thus giving rise to a decrease in epididymal fat pads (15). But, in other studies with diabetic or obese mice, RS did not affect the final body weight or weight loss. A study by Kim *et al*. (16), where the diet containing resistant starch from corn and rice was supplied to normal and diabetic rats, did not observe a significant effect on body weight loss. Jeong *et al*. (17) also reported that rats fed resistant starch had slightly lower weight gain, but this was not significant. Also, supplementation of SDS in high fat-diet significantly decreased the body weight gain, which was explained by a substantial amount of slowly digestible fraction. It is well known that SDS continuously supplies the blood glucose during digestion at a slow rate, which might responsible for weight loss (18). The food intake in the RS group was increased compared with HFD group, while SDS group was significantly lower than in those fed HFD and RS diet (p<0.05). Food efficiency was significantly higher in HFD group than SDS and RS groups (p<0.05). Perirenal and epididymal fat pads were significantly decreased with SDS and RS compared to the high-fat control group (p<0.05). Particularly, both of fat pads in RS groups were lower than that of SDS, but there were not significantly different between SDS and RS.

Lipidic parameters in serum and liver As it can be shown in Table 5, total lipid values in serum were significantly lower in RS groups (269.39 mg/dL) than in HFD-fed groups (337.81 mg/dL) (p<0.05). However, no significance effect of SDS group (317.13 mg/dL) on total lipid was found. Mice fed SDS (78.99 mg/dL) and RS (68.79 mg/dL) diets had significantly (p<0.05) lower serum triglycerides than the HFD group (98.89 mg/dL). The addition of RS component was efficient in reducing serum cholesterol (Total-, HDL-, and LDL-cholesterol) (p<0.05). In contrast to the cholesterol parameters in serum in SDS groups was also somewhat lower than HFD group, however, insignificantly (p>0.05). The serum cholesterol-lowering effect of the diet containing RS is in agreement with other studies. It has been shown that RS replaced with digestible starch in the diet can lower serum cholesterol concentrations in normal or hypercholesterolemic rats (19-21). Some researchers have reported several possible mechanism for the hypoglycemic or hypolipidemic effects of RS. Behall *et al*. (22) reported that RS can lower the level of plasma cholesterol, which might be a consequence of hepatic metabolism regulation. Also, Choi *et al*. (23) suggested that hypo-cholesterolemic effect of RS may a result of increased fecal bile acid and sterol excretion or the synthesis of fermentation products attributed to the inhibition of cholesterols synthesis in the livers.

Table 5 shows results of the effects of SDS and RS on liver total lipid, triglycerides, and total cholesterol concentrations in mice fed a high-fat diet. Total liver lipid values were significantly (p<0.05) lower in mice fed the SDS (80.62 mg/g) and the RS diet (55.96 mg/g) than in those fed the high-fat control diet (114.13 mg/g). Liver triglyceride in the high-fat control diet was 30.76 mg/g, and there were no significant differences between mice fed the SDS (28.14 mg/g), or RS diets (25.56 mg/g). Significant differences (p<0.05) in liver total

cholesterol were observed between the mice fed high-fat control (1.07 mg/g) and those fed RS (0.44 mg/g). Similar results were reported by Kim *et al*. (16) who found lowered liver total lipid and cholesterol in rats at high level of intake of RS form rice. It was may be due to the interference of intestinal cholesterol and bile acid absorption (24).

In present study, the SDS and RS yield from rice starch depends on reaction conditions. The optimal conditions for SDS fraction were obtained at the (pullulanse concentration, X_1) of 498 μL, (storage temperature, X_2) of 47°C, and (A/C cycle, X_3) of 5, and for RS fraction, were determined to be 838 μL (X_1), 62°C (X_2), and cycle of 3 (X_3). The pullulanase concentration and storage temperature are important factor for the SDS and RS than A/C cycle. Weight gain and hypolipidemic effects in serum and liver of SDS and RS prepared under the optimized conditions were determined in mice fed high-fat diet. In addition to, the results also showed that the RS fraction had a better hyperlipidemic and anti-obesity effect than SDS. This study showed that combined reaction condition of enzyme concentration, storage temperature and A/C cycle in rice starch positively influences on the SDS and RS fraction as well as its physiological properties.

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