Note

The Effect of Heat-acid Treatment on the Formation of Resistant Starch and the Estimated Glycemic Index in Potatoes

Eri Udagawa,[1, *] Hiroko Matsuda,[1, *, †] Mamiko Tanaka,[1] and Takaaki Shirai[1]

[1]*Laboratory of Salad Science, Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology*

(*4–5–7 Konan, Minato-ku, Tokyo 108–8477, Japan*)

Abstract: Potatoes are generally regarded as high glycemic index (GI) foods. Resistant starch (RS) comprises the starch fraction that is not absorbed in the small intestine, thus controlling the glucose level and improving the intestinal environment. In this study, an analysis of the formation of RS of potato starch samples under different acetic acid-thermal treatment conditions was conducted. Additionally, the relationship between the rates of starch digestion, estimated GI (eGI), and the RS content was evaluated by employing *in vitro* enzymatic models. Compared with control samples, the RS content in the cold-stored samples after acid-boiling was higher, whereas that of samples after heating at 120 °C with acetic acid was decreased. The eGI was negatively correlated with the RS content in potatoes. Cold store after acid-boiling was effective in increasing the RS content. Furthermore, low eGI values may have resulted from higher levels of RS in potatoes.

Key words: resistant starch, estimated glycemic index, potato, starch, cooking, thermal treatment

Potatoes are the third largest food crop worldwide, following rice and wheat, and are valued globally for their ease of cultivation, preparation, and as a readily assimilated source of carbohydrate energy.[1] Starch comprises the majority of the carbohydrate content of potatoes. From a nutritional point of view, starch is generally classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS).[2] RDS is rapidly digested in the small intestine, thereby immediately causing an increase in the blood glucose level after ingestion. SDS is digested slowly but completely, thereby sustaining plasma glucose levels over time.[3)4)5] RS is the portion of starch and/or products of starch hydrolysis that escape digestion in the small intestine, thereby entering the colon for fermentation.[6] During fermentation by colonic microflora, short-chain fatty acids such as acetic, propionic, and butyric acids are formed. Butyrate constitutes a main energy source for the colonocytes and is beneficial to colonic health.[7)8] Additionally, we have provided evidence of cholesterol- and triglyceride-lowering effects of once-daily intake of corn-starch-derived RS in rats.[9]

The glycemic index (GI) is a method of ranking foods according to their postprandial blood glucose response.[10] Food and Agriculture Organization of the United Nations/World Health Organisation (FAO/WHO) Expert Consultation suggested the use of the GI to provide a useful means of facilitating the selection of the most appropriate carbohydrate containing foods for the maintenance of health and treatment of several diseases. Accordingly, foods can be classified into three GI categories: 1) low ($\leq$ 55); 2) medium (55–69), and; 3) high ($\geq$ 70) GI.[11] The consumption of low GI foods could contribute to reduced incidence and prevalence of heart disease, cardiovascular disease, diabetes, obesity, and some forms of cancer.[12)13] Several studies have reported that potatoes generally contain medium to high GI, which has often adversely affected their consumption, but have overlooked the many nutritional and health benefits of potatoes.[11)14] Food products containing RS have lower GI values; therefore, they can be used in controlled glucose release applications.[6]

It is possible to increase the RS content in foods by modifying certain food preparation conditions, including pH, cooking heating temperature and time, and the number of heating and cooling cycles.[6] The RS content of boiled potatoes is already substantial, 4.5 g 100 g^{-1} (starch basis), and could be further increased to 9.8 g 100 g^{-1} (starch basis) by temperature cycling following heat treatment at 6 °C for 24 h, followed by 70 °C for 24 h.[15] In many food systems, organic acids are added to starchy foods, including acetic, ascorbic, citric, and malic acids. These acids are usually added to adjust pH, provide flavor, or to act as natural antimicrobial agents.[16] It was found that heat treatment combined with citric acid hydrolysis increases the RS content of normal maize starch.[17] Potato meal, cold store and the addition of vinegar was found to reduce acute postprandial glycaemia and insulinemia in healthy subjects.[18] However, the effects of acetic acid-thermal treatment on the formation of RS in potato starch is yet to be investigated.

One objective of the present study was to analyze the in-

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fluence of different acetic acid-thermal treatment conditions on the formation of RS and granule structure properties of potato starch samples. The second objective was to evaluate the relationship between the rate of starch digestion, estimated GI (eGI), and the RS content employing *in vitro* enzymatic models.

RS contents of boiled starches (control, 7.7 ± 0.4 %; acetic acid, 7.7 ± 0.2 %) were significantly lower than those of non-heated starches (control, 73.2 ± 0.9 %; acetic acid, 81.0 ± 0.8 %) because of gelatinization while boiling. Table 1 shows the RS contents of the heat/store-treated starches of the control and acetic acid-treated samples (acetic acid samples). A three-way ANOVA was performed on RS contents [% of total starch: the additive solution (A) × the heat-treatment condition (H) × the store periods (S)]. There were significant differences in RS contents for A ($p < 0.001$), H ($p < 0.001$), and S ($p < 0.05$), and the interaction of $A \times H$ ($p < 0.001$), $H \times S$ ($p < 0.001$), and $A \times H \times S$ ($p < 0.001$), but not for $A \times S$ ($p = 0.09$). As per Tukey's multiple comparison tests, it was suggested that RS values were significantly affected by several conditions ($p < 0.05$). Among the control samples, the cold store did not affect the RS content in boiled samples. However, increased RS contents have previously been found in boiled potato tubers following cold store for 24 and 48 h.[15)19)] According to the previous studies,[20)] starches with a high amylose content gave higher amounts of RS than those with less amylase content. The retrograded amylopectin undergoes melting in the approximate temperature range of 40–70 °C, whereas the melting of retrograded amylose required a higher temperature range of 120–170 °C.[21)] Amylose retrogradation is considered to be a rapid process completed within 48 h, whereas amylopectin retrogradation may continue for weeks.[22)] Additionally, starch granules were observed to swell slightly at 100 °C and to swell remarkably at approximately 120 °C.[23)] These reports indicated that amylose gelatinization was promoted at 120 °C and not at 100 °C. Raatz *et al.*[24)] reported that the increased RS content in cold-stored potato starch compared with that in boiled potato starch was affected by the variety of the potato. In the present study, there were no differences in the RS content between boiled and cold-stored samples after boiling because amylose gelatinization was not greatly promoted in boiling samples. However, RS content was lower in the sample heated at 120 °C than that in the boiled sample. Additionally, this content was higher in the sample that was stored at 6 °C for 24 h or 48 h after heating at 120 °C compared with that in the sample heated at 120 °C. These results indicated that cooling treatment at 6 °C might increase the RS content of cold-stored samples after heating at 120 °C due to the promotion of retrogradation of amylose.

Under acetic acid treatment alone, the RS content in cold-stored samples after boiling increased as compared with that of boiled samples. However, compared with boiled samples, the RS content in acid-treated samples decreased after heating at 120 °C. According to the previous studies, amylose and amylopectin chains in cornstarch heated at 97 °C for 60 min are hydrolyzed by the addition of organic acids, resulting in a reduction of viscosity at a low

Table 1. Resistant starch (RS) content of the potato starches that were heat-treated with/without the addition of acetic acid.

Sample	RS/total starch (%)	
	Control	Acetic acid
Boiled	7.7 ± 0.4[abc]	7.7 ± 0.2[abc]
Boiled and stored (6 °C, 24 h)	7.9 ± 0.5[bc]	8.7 ± 0.4[d]
Boiled and stored (6 °C, 48 h)	6.9 ± 0.9[a]	8.5 ± 0.3[cd]
120 °C	6.3 ± 0.6[e]	2.8 ± 0.2[f]
120 °C and stored (6 °C, 24 h)	7.3 ± 0.5[ab]	2.9 ± 0.1[f]
120 °C and stored (6 °C, 48 h)	7.9 ± 0.6[bcd]	2.7 ± 0.3[f]

Values represent means ± standard deviations (S.D.) ($n = 6$). Means within different letters are significantly different ($p < 0.05$).

pH value.[25)] Ohishi *et al.*[26)] reported that there was no difference in the susceptibility of rice grains boiled at 80 °C to amylase, with or without the addition of acetic acid, whereas hydrolysis of the rice cooked to the last in an electric rice cooker with the addition of acetic acid and amylase was higher than that of rice cooked without acetic acid. Therefore, in the present study, the RS content in acid-treated samples heated at 120 °C decreased because of the promotion of hydrolysis of starch. In the acid-boiled samples, the addition of acetic acid did not affect hydrolysis because of the lack of heating temperature and cooking time. The cooling treatment at 6 °C did not affect the RS content of cold-stored samples after heating 120 °C with acetic acid. Kunlan *et al.*[27)] reported that starch was hydrolyzed to D-glucose and this yield was 77.8 % when heating at 145 °C with HCl. In the present study, the starch in cold stored after heating 120 °C with acetic acid was hydrolyzed to D-glucose, resulting that amylose retrogradation was not promoted.

Compared with control samples, the RS content in cold-stored samples after acid-boiling was higher. This result indicates that cold store after acid-boiling was effective in increasing the RS content. In the present study, acetic acid was added to the starch before thermal treatment. Majzoobi *et al.*[28)] reported that the addition of acetic acid after gelatinization affected the physicochemical properties of wheat starch. Hence, the RS content may vary according to the timing of the addition of acetic acid.

The surface of starch granules was investigated using SEM (Figs. 1–2). The non-heated starch granules were elliptically shaped with a smooth surface (Fig. 1). The size of these starch granules ranged from 15 to 30 μm. However, the heated starch samples lost their individually and formed agglomerates with rough surfaces, possibly due to the destruction of the granular structure of the starch during heating (Fig. 2). Lee *et al.*[29)] also reported that potato starch exhibited aggregation of granules and a distorted granular shape after 40 % moisture heat treatment.

There were no differences in the structures between the boiled control and acetic acid samples (Fig. 2). However, the acid-treated starch samples heated at 120 °C displayed pores on the surface (Fig. 2). Majzoobi *et al.*[16)] showed that pregelatinized starch with acetic acid was altered to a more uneven structure, with the effect increasing with an increasing concentration of acetic acid. Additionally, it is reported
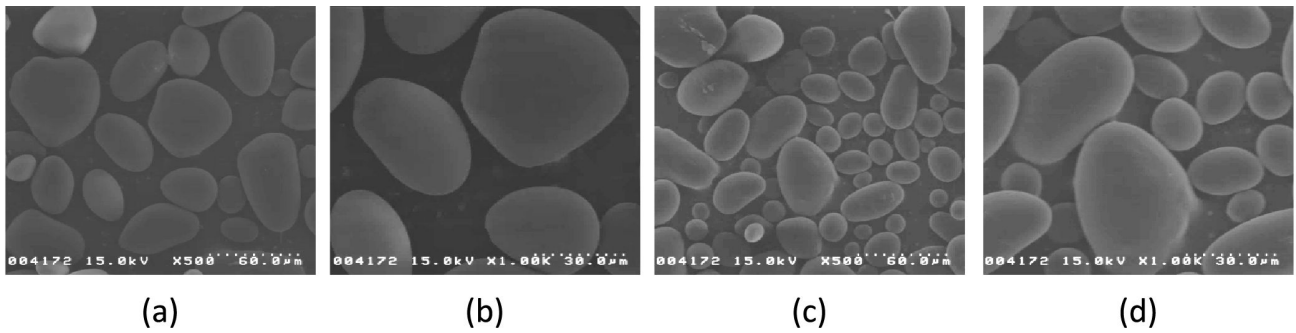
Fig. 1. Scanning electron microscope (SEM) photographs of starch granules of potato that were not heated with/without addition of acetic acid.

(a) control (500×), (b) control (1,000×), (c) acetic acid (500×), and (d) acetic acid (1,000 ×).
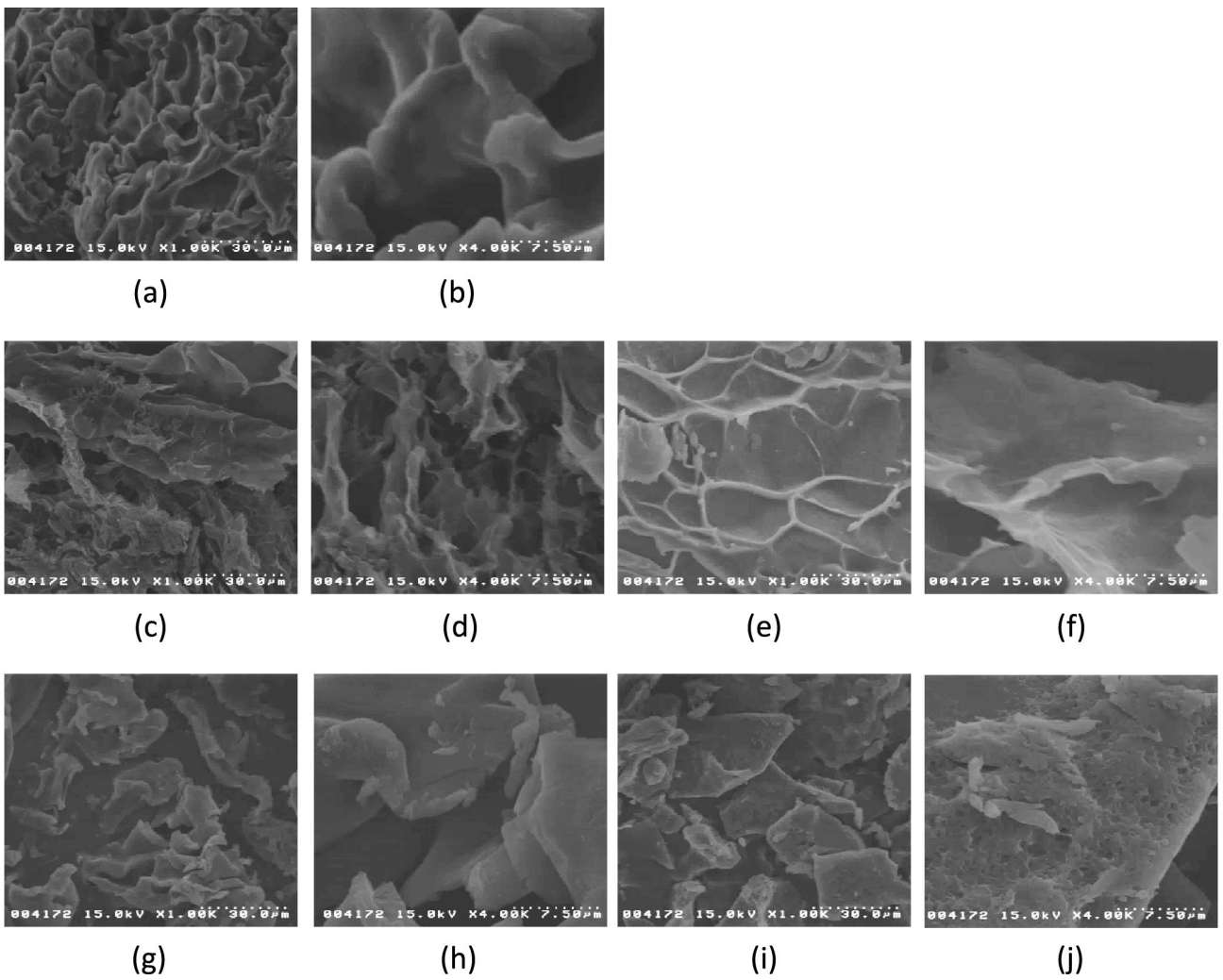
Fig. 2. Scanning electron microscope (SEM) photographs of starch granules of potato that were heat-treated with/without addition of acetic acid.

Boiled (1,000×) (a) and (4,000×) (b); boiled and stored (6 °C, 24 h) (1,000×) (c) and (4,000×) (d); boiled and stored (6 °C, 24 h) + acetic acid (1,000×) (e) and (4,000×) (f); 120 °C (1,000 ×) (g) and (4,000 ×) (h); and 120 °C + acetic acid (1,000 ×) (i) and (4,000 ×) (j).

that structural features such as surface pores and channels increase the effective surface area, thereby facilitating the rapid diffusion of amylases to substrates in maize starch.[30] In the present study, the RS contents in samples heated at 120 °C with acetic acid were decreased. Hence, these pores were caused by hydrolysis of amylose and amylopectin.

The hydrolysis curves of white rice and potato samples are shown in Fig. 3. These curves can be used to predict the change of the postprandial elevation of blood glucose levels. Using these hydrolysis curves, the degree of hydrolysis, hydrolysis index (HI), and eGI were calculated and are shown in Table 2. In the current study, white rice was used as a reference food as it is a staple food in Asia. The hydrolysis of potato samples was lower than that of white rice. Compared with the control boiled sample, the rate of

Table 2. Degree of hydrolysis, *in vitro* hydrolysis index (HI), and estimated glycemic index values (eGIs) obtained from HI in the potato starches that were heat-treated with/without addition of acetic acid ($n = 2$).

Sample	% hydrolyzed	HI	eGI	
			Rice = 100	Glucose = 100
Boiled	25	77	78	63
120 °C	31	90	90	72
Boiled + acetic acid	28	85	86	69
Boiled and stored (6 °C, 24 h) + acetic acid	25	72	74	59
120 °C + acetic acid	29	90	90	72
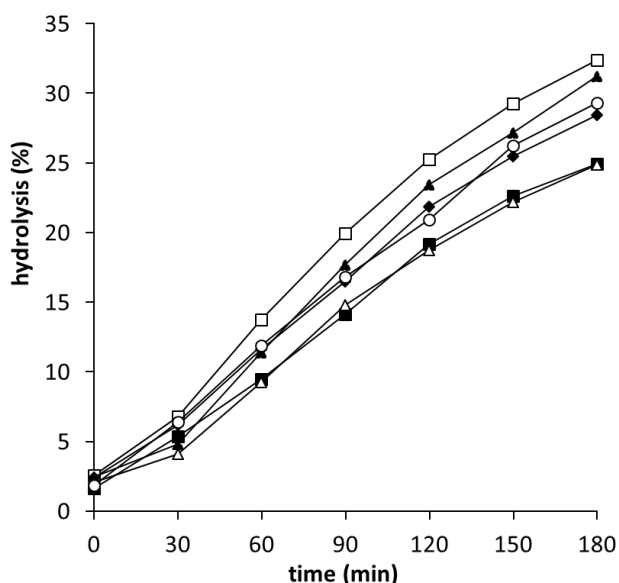
Fig. 3. *In vitro* digestible starch hydrolysis kinetic curves of boiled rice and heat-treated potato starches with/without addition of acetic acid.

□, boiled rice; ■, boiled; ▲, 120 °C; ◆, boiled + acetic acid; △, boiled and stored (6 °C, 24 h) + acetic acid; ○, 120 °C + acetic acid.

hydrolysis per 1 h of the control sample heated at 120 °C was 1.2-fold, that of the acid-boiled sample and acid-treated starch samples heated at 120 °C was 1.17-fold, and that of the cold-stored sample after heating at 120 °C with acetic acid was 0.98-fold. The eGI values of all potato samples were calculated according to the reference white rice (eGI = 100) as 74–90. In previous studies, glucose was commonly used as a reference food for calculating eGI. The values obtained in the present research were multiplied by 0.8 to obtain eGI values, as suggested by Sugiyama *et al.*[31] According to this calculation, the eGI of all potato samples ranged from 59 to 72. In previous studies, three varieties of boiled Australian potatoes produced a GI ranging from 87 to 101,[32] whereas the GI of boiled English new potatoes and boiled or baked Canadian potatoes showed intermediate values ranging from 59 to 70.[10)33] Different processing methods, starch characteristics are known to influence the eGI of potatoes and potato products.[34)35] In the present study, although the eGI values of boiled and cold-stored samples after acid-boiling were lower than that of the other samples, whereas the eGI values of samples heated at 120 °C were higher, the difference was not statistically significant. The eGI was negatively correlated with the RS content ($r = -0.652$, $p < 0.05$), indicating that the low eGI

values may have resulted from their higher levels of RS. These results agree with those of previous studies; Pinhero *et al.*[36] reported that a significant and strong positive correlation was observed between eGI and RDS, whereas a significant and strong negative correlation was observed between eGI and RS in potato.

In the present study, the effect of different acetic acid-thermal treatment conditions on the formation of RS and the relationship between the RS content and eGI in potato starch was analyzed. After acid-boiling, the RS content was significantly increased with time spent in cold store. On the other hand, after 120 °C treatment, the RS contents in acid-treated samples were decreased and pores were evident on the surfaces of starch granules. These results suggested that the acid-heating temperatures influence RS formation in potato. In addition, there was a negative correlation between eGI and RS, indicating that cold store after acid-boiling of potato starch was effective in lowing eGI. Therefore, potato RS, cold stored after boiling with acetic acid might benefit human health. The present study may provide a scientific basis for the production and use of RS.

EXPERIMENTAL

Potato and rice starches were obtained from Wako Pure Chemical Industries (Osaka, Japan) and J-Oil Mills (Tokyo, Japan), respectively. Rice starches were only used in the measurement of eGI values.

Potato starch (5 g) was mixed with 20 mL of 1 M acetic acid (starch:acetic acid = 1:4) and the mixtures were packaged under vacuum in pouches. They were boiled or pressure-cooked in an autoclave at 120 °C for 20 min. As a control, distilled water instead of acetic acid was added. After cooling in ice-water for 10 min, the samples were stored in a refrigerator (6 °C) for different periods (0, 24, or 48 h). Finally, the samples were freeze-dried using a condenser with the temperature set at approximately −40 °C and ground.

The RS content was measured using the RS assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) by following the manufacturer's instructions with modifications. The potato samples were incubated in a shaking water bath with pancreatic α-amylase and amyloglucosidase for 16 h at 37 °C. During this time, non-RS was dissolved and hydrolyzed to D-Glucose by the combined action of the two enzymes. Each sample was suspended in an equal volume of 99.5 % ethanol and centrifuged at 1,500 × G for 10 min, following which RS was recovered as a pellet. Each

pellet was washed twice in 50 % industrial methylated spirits and 50 % ethanol, followed by centrifugation. The supernatant was completely removed. The supernatant was completely removed. RS in the pellet was dissolved in 2 M potassium hydroxide solution by vigorously stirring in ice-water over a magnetic stirrer for 30 min. To completely hydrolyze the remaining starch, 1.2 M acetate buffer (pH 3.8), amyloglucosidase, and α-amylase were added, and the samples were incubated at 50 °C for 30 min. The samples were then centrifuged at 1,500 × G for 10 min, and the amount of D-Glucose in the supernatant was measured with glucose oxidase/peroxidase reagent (GOPOD). Non-RS was determined by pooling the supernatant, washing it, adjusting the volume to 100 mL with 0.1 M acetate buffer (pH 4.5), and measuring the content of D-Glucose with GOPOD.

The surface structure of starch granules in potato samples was observed using a scanning electron microscope (S-4000, Hitachi High-Technologies, Tokyo, Japan). For achieving conductive samples, the starch samples were mounted on a metal stub and sputtered with copper. SEM images were captured at an accelerating voltage of 15 kV. Each sample was captured at magnifications of 500 ×, 1,000 ×, and 4,000 ×, respectively.

The eGI was measured using the method of Granfeldt *et al.*[37] with modifications. The samples (0.5 g) were incubated in a shaking water bath with 0.5 mL of 1.5×10^5 U/ mL pepsin (Wako Pure Chemical Industries) for 30 min at 37 °C. After incubation with pepsin, the pH was adjusted to 6.8 using sodium hydroxide solution, after which 0.5 mL of 110 U/ mL α-amylase (Wako Pure Chemical Industries) was added. The samples were adjusted to a volume of 15 mL with 0.05 M phosphate buffer (pH 6.8), transferred to dialysis tubing, and incubated at 37 °C for 3 h with stirring in a beaker with 0.05 M phosphate buffer (pH 6.8). Aliquots (1 mL) of the dialysate were removed every 30 min. Ten microliter of 300 U/mL amyloglucosidase (Megazyme International Ireland Ltd.) was added and the samples were incubated at 50 °C for 20 min. D-Glucose in the sample was measured with GOPOD. The hydrolysis index (HI) was obtained based on the relationship between the areas under the hydrolysis curves (AUCs) for potato products and for rice. The eGI was calculated using the equation: eGI = $6.272 + 0.912 \times$ HI.[15]

Values are expressed as means ± standard deviation (SD) of the mean. Statistical analyses were performed by a three-way ANOVA for *A* that is distilled water and acetic acid, *H* that is boiling and 120 °C, *S* that is store for 0, 24, and 48 h, and the interaction of $A \times H$, $A \times S$, $H \times S$, and $A \times H \times S$. Tukey's multiple comparison tests were used to compare the value between any two of the 12 sample groups (6 heat/store condition × 2 kinds of additive solution) for RS contents of potato starch. Correlation coefficients for the relationships between eGI and RS contents in each condition were calculated by the least-squares method. A difference of $p < 0.05$ was considered to be statistically significant. Calculations of the areas under the curve (AUC) for calculating the HI value were based on the trapezoid rule.

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