Indigestible Fractions of Starch Hydrolysates and Their Determination Method

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Several types of starch hydrolysates that have dextrose equivalents of 12–16 were compared in terms of structural analysis, *in vitro* digestibility via the Prosky method, and digestibility as estimated by measuring the glycaemic index in humans, with their correlations noted. Results showed that digestion of starch hydrolysates decreased with the initial moisture content during their preparation. Structural analyses of samples suggested that the decrease in digestion was due to glycosidic linkage conversion. Conceivably, this glycosidic linkage conversion translates to condensation, a reverse reaction to hydrolysis. Furthermore, it was also shown that digestibility of maltodextrins clearly differed between enzymatic hydrolysis and acidic hydrolysis, and that the molecular weights of indigestible fractions differed according to the hydrolysis method. *In vitro* digestibility of starch hydrolysates was also compared with their digestibility in humans by measuring glycaemic index. The portions determined indigestible from the *in vitro* test coincided with findings in humans. Similar results were obtained from the *in vitro* and *in vivo* tests. The *in vitro* test method used in this study may provide a simple and reliable approach to evaluate the digestibility of starch hydrolysates in humans.

Starch hydrolysates can be classified into pyrodextrin (subsequently referred to as dextrin), maltodextrin and/or corn syrup, *etc.*, depending on its production process or extent of hydrolysis. These hydrolysates are not used as sweeteners like glucose and fructose but as binders and carriers. In recent years, such hydrolysates have been used as energy sources in infant milk powder, sports drinks, enteral and tube feeding preparations. The growing popularity of these starch hydrolysates is mainly due to their excellent digestibility and moderate osmotic pressure that is derived from their average molecular weights.

Information concerning the digestibility of starch hydrolysates from past literature^{1,2)} is only available for dextrins; *viz.*, studies related to the changes in digestibility by amylases accompanying the structural changes of starch by dextrinization (thermal hydrolysis). For maltodextrins and corn

syrups, available information is minimal, esp. organized information on their digestibility or absorbability in human. Therefore, the present study was undertaken to obtain information on the digestibility of starch hydrolysates. Briefly, enzyme and acid hydrolysates of starch were prepared under various conditions with their *in vitro* digestibility through continuous hydrolysis mediated by α -amylase and glucoamylase in addition to structural determination. Furthermore, digestibility in humans was estimated by tolerance test and compared to the *in vitro* digestibility test.

MATERIALS AND METHODS

Materials. Cornstarch containing 13% moisture and 0.05% nitrogen was obtained commercially (Nihon Shokuhin Kako, Co., Ltd., Tokyo, Japan). Maltodextrin (DE 11) was obtained as Pinedex #2 (Matsutani Chemical, Co., Ltd., Itami, Japan), and first grade reagents (Wako Pure

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Table 1. Conditions of preparation.

Sample		A	B	C	D	E	F	G	H
Hydrolysis by		Enzyme	Hydrochloric acid						
State of cornstarch		Slurry			Powder				Separated of Sample G
Condition of Preparation	Moisture (%) at hydrolysis	75	95	75	50	25	10	5	
	Conc. of HCl (ppm) at hydrolysis	—	—	—	500	750	750	750	
	pH at hydrolysis		1.8	1.8	—	—	—	—	
	Conc. of α -amylase (%) at hydrolysis	0.4	—	—	—	—	—	—	
	Temperature °C	85	125	130	130	127	129	130	
	Reaction time (min)	100	10	20	20	30	40	60	

Chemical Industry Co., Ltd, Tokyo, Japan) were employed.

Sample preparation. Eight samples (Table 1) were prepared by the following methods.

Enzyme hydrolysis. Cornstarch (50 g) was added to 125 mL of water, and the solution was adjusted to pH 6.0 by the addition of 3% sodium hydroxide (NaOH). α -amylase (0.2 g, Kleistase; Daiwa Kasei Co., Ltd, Japan) was added to the solution for hydrolysis, which was stirred to form a paste, being kept at 85°C for 100 min in a boiling water bath. The enzyme was inactivated by adjusting the solution to pH 4 by adding dilute hydrochloric acid (HCl). After neutralization, the acid hydrolysate solution was bleached with activated carbon, filtered through diatomaceous earth, and desalted with ion-exchange resin (100 mL of a 1:1 mixture of Amberlite 200C [H-type] and Amberlite IRA-68 [OH-type]; Organo Co., Ltd., Tokyo, Japan; packed in a 2-cm diameter column). The solution was then concentrated and freeze-dried to powder (Sample A).

Sample preparation by acid hydrolysis and dextrinization. Each specific amount of HCl was added to the cornstarch (for starch in a powder state, 5% HCl was sprayed over the powder), and the initial moisture content was adjusted to the required conditions described in Table 1 (samples $\leq$ 10% moisture content were vacuum-dried at 70°C). A 100-mL anti-pressure bottle (5 cm in diameter) was filled with 50 g of sample and sealed. Samples were hydrolyzed to DE 12–16 by autoclaving at 125–130°C under the conditions described in Table 1, which were obtained in a preliminary study to designate starch hydrolysates of *ca.* DE

15 by changing the reaction times. These hydrolysates were dissolved in distilled water to prepare a solution of 5 to 10% concentration, neutralized with 3% NaOH, desalted with ion-exchange resin, concentrated, and freeze-dried to powder (Samples B, D and E). Sample C for digestibility test and the tolerance test and Sample G for fractionation and other studies were prepared in the same way and conditions.

Separation of the indigestible fraction. The amylase-resistant fraction of Sample G was prepared as follows. Briefly, *ca.* 2 kg of Sample G was dissolved in water to obtain a solution of 20% concentration. The solution was treated with α -amylase (Termamyl 60L; Novo Industry Co., Ltd., Tokyo, Japan) at 0.2% to solid. Hydrolysis was performed by keeping the solution at pH 6.0 for 60 min. The solution was then adjusted to pH 4.5, hydrolyzed by adding 0.1% glucoamylase (Glucosyme NL; Amano Pharmaceutical Co., Ltd., Nagoya, Japan) kept for 24 h, and then desalted with ion-exchange resin. After the sample was concentrated to 50%, glucose (*ca.* 45%, generated by hy-

Fig. 1. The method for determining the digestibility.

drololysis of digestible fraction) was removed from this solution (*ca.* 4 L) by passage through a column packed with 10 L of XFS-43279 (a Na-type strongly acidic cation ion-exchange resin; Dow Chemical Co., Ltd., Tokyo, Japan) at 60°C and SV (specific velocity) 0.3 before further elution with distilled water. The remaining portion was purified again by ion-exchange resin, concentrated and subsequently dried (sample H).

Direct reducing sugar determination. Direct reducing sugar was determined by the Willstätter-Schudel method.³⁾ In this method 0.04 N NaOH solution, 0.04 N I₂ solution, 0.04 N sodium thiosulfate solution and 5% HCl were used as reagents.

Method for linkage analysis. The linkages of starch hydrolysates were determined by methylation analysis previously described by Hakomori *et al.*⁴⁾ Gas chromatographic analysis was done using GC-14A and the detector FID (both from Shimadzu, Kyoto, Japan) under the following conditions. Column: DB-1 fused silica capillary (60 mm × 0.255 mm ID, 1.0 m film; J & W Scientific, Tokyo, Japan); column temperature: 50°C for 1 min, then 280°C for 10 min and left to stand for 10 min; injection temperature: 300°C; eluent: helium.

In vitro digestibility test. The digestibility of starch hydrolysates was determined by measurement of the digestible content via the method shown in Fig. 1. This procedure was based on the Prosky method,⁵⁾ an assay for dietary fiber determination. Briefly, the sample was hydrolyzed through mediation of two types of amylases (Termamyl 120 L; Novo Industry Co., Ltd., Tokyo, Japan/A-9913; Sigma Co., USA) and protease (P-5380; Sigma Co., St. Louis, USA) before dilution. Glucose produced by the hydrolysis of the digestible fraction was determined by the glucose-oxidase method^{6,7)} (Determiner Kit GLE; Kyowa-Medic Co., Ltd., Tokyo, Japan). The amount of glucose thus determined was multiplied by the starch index 0.9 to obtain the digestible portion (%) of the original sample.

$$\text{Digestibility (\%)} = \frac{\text{Glucose (mg)} \times 0.9}{\text{Sample (mg)}} \times 100$$

Molecular weight distribution. After the digestible test solution was desalted with the ion-exchange resin, the molecular weight distribution

was determined by high performance liquid chromatography (HPLC) analysis using 665A-12 pump and L-3300RI monitor differential refractometer (all from Hitachi, Co., Ltd., Tokyo, Japan) under the following conditions: column, tandem Shodex Ionpack S-801, S-802, S-805 and S-806 (8 mm × 50 cm) (Showadenko, Co., Ltd., Tokyo, Japan); column temperature, 60°C; mobile phase, water; flow rate, 1.0 mL/min. Before determining test samples, glucose, maltose and Shodex standard P-82 were determined as standards.

Sugar tolerance test and glycaemic index. After 12 h of fasting, 5 healthy adult males (aged 25 to 55 yrs) were orally administered 50 g of each test sample in a 30% solution. Blood was collected from the vein at 0, 30, 60, 90 and 120 min after sample ingestion to determine the blood glucose levels by the glucomutarotase GOD method, and the insulin levels were monitored by the enzyme immunoassay method. The glycaemic index (GI) was determined by the area-under-the-curve (AUC) of blood glucose and calculated by the following equation.⁸⁾

$$\text{GI (\%)} = \frac{\text{AUC of Sample}}{\text{AUC of Glucose}} \times 100$$

Five test samples (glucose, maltodextrin, Sample C, G and H) were administered randomly to each subject (one test sample/week/subject). All results were expressed as the mean ± SE, and differences were assessed by the Student's *t*-test for statistical significance.

RESULTS AND DISCUSSION

Relationship between the preparation method of starch hydrolysates and their digestibility.

The effect on digestibility of samples prepared under various conditions in relation to the initial moisture content on hydrolysis revealed that digestibility attenuated with decreases in the moisture content of the samples on hydrolysis (Table 2). Although those samples had almost the same DE values (12–16), 1→4 linkage (straight chains) gradually decreased, while terminal (non-reducing ends) and branching residues (1→4, 1→6 linkage, 1→4, 1→2 linkage) gradually increased along increases in 1→6 linkage and 1→3 linkage.

Table 2. Glucosidic linkages and digestibility.

Sample		A	B	C	D	E	F	G	H
<i>In vitro</i> digestibility (%)		98.0	90.3	87.1	86.0	80.3	61.6	38.0	2.0
Direct reducing sugar		12.0	13.2	14.4	12.2	14.2	16	12.5	12.5
Glucosidic linkage (%)	terminal	13.1	13.8	17.7	18.0	14.8	21.4	25.1	29.3
	1→4	82	78.2	73.7	74.6	76.1	64	40.6	32.5
	1→6	—	—	0.9	0.6	1	3.5	10.9	11.3
	1→4, 1→6	4.1	4.7	4.1	3.8	3.6	4.4	6.8	10.7
	1→3	—	0.9	0.9	0.5	2.2	3.2	6.9	7.3
	1→4, 1→3	0.8	1.1	1	1.4	1	1.1	1	1.9
	1→4, 1→2	—	1.1	0.9	1.1	1.3	1.7	2.4	2.6
	others	—	—	0.8	—	—	0.7	6.3	4.5

These results show that digestibility of starch hydrolysates decreases with the changes in glucosidic linkages, especially through branching. When acid hydrolysates were compared with the enzyme hydrolysate, acid hydrolysates had less digestibility due to the various linkages other than 1→4 and 1→6.

The patterns of molecular weight distributions of an enzyme hydrolysate [Sample A], 6 types of acid hydrolysates [Samples B, C, D, E, F and G] and the indigestible fraction of an acid hydrolysate obtained through the *in vitro* digestibility test [Sample H] are shown in Fig. 2. In those figures, the glucose was produced by hydrolysis of the digestible fraction by amyloglucosidase, and the components other than glucose comprised the indigestible fraction, which was not hydrolyzed by amylases. Furthermore, the molecular weight distributions of the indigestible fraction of the acid hydrolysates [Samples B, C, D, E, F and G] clearly differed from those of the enzyme hydrolysate [Sample A]. The enzyme hydrolysate indicated a trace of indigestible fraction of low molecular weight and the acid hydrolysates' indigestible fraction was distributed in the molecular weight range of 10^2 to 10^4 . Among the acid hydrolysates, although the content of the indigestible fraction is inconsistent, the pattern of the molecular weight distribution of indigestible fraction does not differ appreciably from hydrolysate to hydrolysate. In this study, an indigestible fraction was invariably present in all acid hydrolysates of starch, regardless of the conditions of hydrolysis. This indigestibility of starch hydrolysates is pre-

sumably due to the formation of glucosidic linkages (e.g. 1→2, 1→3), which are not hydrolyzed by amylases or human digestive enzymes and are not commonly present in starch. The structural changes in the manufacturing process of dextrin could be attributed to reverse synthesis, which concurs with hydrolysis,²⁾ transfer intermediated by 1→6 anhydro-D-glucose, which is produced by intramolecular dehydration of reducing ends,^{2,9)} or direct conversion of linkages between hydrolyzed molecules.^{1,2)} It is not surprising that both the syntheses in reverse reaction and hydrolysis occurred even in a high moisture content on hydrolysis of, for example, maltodextrin. In other words, hydrolysis of starch is invariably a reverse reaction accompanied with condensation. This could be proven from the fact that when glucose being obtained by acidic or enzymatic hydrolysis, gluco-oligosaccharides such as isomaltose, gentiobiose and panose are also produced.¹⁰⁾ In the present study, a chemical equilibrium between hydrolysis and condensation was achieved in each condition, and we thus assume that starch hydrolysates having different digestibility were produced.

It should be noted that Samples B and C (Table 1) are so-called maltodextrins, which were hydrolyzed in the slurry and then converted to soluble substances,¹¹⁾ while Samples D, E, F and G are dextrins, which were hydrolyzed in the powder form.¹²⁾ As mentioned above, the molecular structure of dextrin changed and the digestibility of dextrin decreased according to the moisture content of starch on hydrolysis. In particular, when the moisture content of starch was below 25%, the

Fig. 2. The HPLC elution profiles of each starch hydrolysate after *in vitro* digestible test.

The peak of 10^3 shows the digestible part and the other peak shows the indigestible part.

decrease in digestibility was significant. When Samples B and C (maltodextrins) were compared

with Samples D and E (dextrins) in terms of molecular structure and digestibility, there were no

clear differences seen between maltodextrins and dextrans. These results show proof that the reverse reactions are not only applied to dextrin but also to maltodextrin. Hitherto, it was thought that maltodextrin is perfectly digestible; however, the present results reveal that the digestibility of maltodextrin depends on its preparation method and preparation conditions.

Maltodextrin and dextrin should not be classified by digestibility, but by preparation method. From our findings, Sample H may be a new type of maltodextrin, which is prepared by fractionating a part of the maltodextrin produced from hydrolyzing dextrin with amylases.

Relationship between in vitro digestibility of starch hydrolysates and digestion in humans.

In order to estimate the digestibility of the starch hydrolysates in humans, blood glucose and insulin levels were compared by ingestion in 5 healthy volunteers (Fig. 3). For Samples C, blood glucose levels at 60 min after ingestion were lower

than those of standard glucose and standard maltodextrin. For Sample G, blood glucose levels at 30 and 60 min and insulin levels at 30, 60 and 90 min were significantly lower compared to those of glucose. For Sample H, the indigestible fraction by fractionation, showed very little change; therefore the results suggest that the sample was resistant to digestion and absorption.

The *in vivo* digestion in humans was estimated by measuring the glycaemic index (GI), and compared with the results in the *in vitro* study (Table 3). According to Jenkins, the degree of digestibility and absorbability of foods is expressed as GI, which is the ratio of AUC of blood glucose by ingesting the food compared to that by ingesting glucose (100 is assigned to GI of glucose).⁸⁾ The GI values obtained from the *in vivo* test were

Table 3. GI (digestion *in vivo*) and digestibility *in vitro*.

Sample No.	Glucose	Maltodextrin	C	G	H
GI	100	94	84	47	1
Digestibility <i>in vitro</i>	100	98	86	44	2

Fig. 3. Changes in plasma glucose (left) and plasma insulin (right) levels after the administration of 50 g, glucose (○), maltodextrin (□), Sample C (■), Sample G (●) and Sample H (▲) in five healthy subjects.

Values are shown as means: * $p < 0.05$ vs. glucose; ** $p < 0.01$ vs. glucose.

correlated with the results in the *in vitro* digestion test, suggesting that the indigestible fraction in the *in vitro* test was not digested or absorbed in the upper gastrointestinal tract of humans and was therefore passed intact into the colon.

Furthermore, this *in vitro* test was found to provide a useful and convenient means to: (i) estimate the digestibility and absorbability of starch hydrolysates in humans and (ii) determine the content of indigestible fraction in starch hydrolysates.

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澱粉部分分解物中の難消化性成分とその評価方法

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直接還元糖値 12-16 を有する数種の澱粉分解物の調製を行い、それらの構造分析、食物繊維の定量法であるプロスキー法に準じた *in vitro* による消化性試験、およびヒトによる Glycaemic Index の測定を指標にした消化試験を実施し、それぞれの相関性について検討した。この結果、酸分解の試料については分解時の初期水分が減少するに従い消化性が減少した。これは、用いた試料の構造分析の結果、グリコシド結合の変換によることが示唆された。また、このグルコシド結合の変換は加水分解と逆反応である縮合反応(転移反応)によるものと推定された。その他、酵素分解のマルトデキストリンと酸分解のそれとの間には明らかな消化性の違いが存在し、難消化性画分の分子量にも差が認められた。次に、*in vitro* 試験による消化性とヒトでの消化吸収性を比較した。消化性試験における難消化性の画分は、ヒトにおいても難消化吸収性の画分であることが確認された。また、二つの試験によって得られた測定値は非常に近似していた。このことより、今回、用いた *in vitro* 試験法はヒトの消化吸収性を測定するための簡便法になることが示唆された。