Maltooligosaccharide Composition of Flours, Weaning Foods, and Gruels Prepared from Germinated Rice, Corn, Mungbean, and Cowpea

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(Received August 10, 1989)

Summary Germinated rice, corn, mungbean, cowpea, and weaning foods prepared from their selected combinations were studied for their saccharide profile by TLC, total and soluble sugars content, and amylase activities in the flour and gruel samples. The enzyme system present in the germinated materials resulted in the partial digestion of carbohydrates into maltooligosaccharides, as shown by TLC pattern and soluble sugars determination of all materials germinated for 48, 72, and 96 h. Rice had the highest concentration of amylase, followed by cowpea, corn, and mungbean. The amylolytic activity produced during germination was retained in the flour despite drying and roasting processes involved. The processes of germination and gruel preparation of germinated materials contributed to the digestibility of weaning foods prepared from cereals and legumes.

Key Words maltooligosaccharides, weaning food formulations, germination, amylases, reducing sugars, dextrinogenic property, dietary bulk, amylolytic activity, gruel

The Protein Advisory Group of the United Nations Guideline on Protein-rich Mixtures for Use as Weaning Foods (1) recommended the processing of starchy components with amylases which will reduce the viscosity and water-retention capacity or bulkiness of the mixture, thus allowing the feeding of a more concentrated preparation. Responding to this challenge, four weaning food formulations, namely, germinated rice-mungbean (GRM), germinated rice-cowpea (GRC), germinated corn-mungbean (GCM), and germinated corn-cowpea (GCC) were prepared from a combination of 70% germinated (72-h germination period)
rice/corn and 30% germinated (48 h) mungbean/cowpea (2). The formulations containing from 15 to 20% dry matter in the gruels were found to have 3,000 cps viscosity, which was suitable for infant feeding. Due to the reduced viscosity of the gruel, more solids could be added, thus increasing their nutrient composition (3). During germination, starch is partially broken down to dextrins by enzymes (4). The partially digested starch may be taken advantage of by a child being weaned from a lactose diet to a cereal diet. It was considered to be of interest to investigate the breakdown of starches into maltooligosaccharides through the germination process. The purposes of this study were to show the TLC pattern of sugars formed from starch of germinated rice, corn, mungbean, and cowpea and of their selected combinations into weaning foods; to compare the amount of soluble, reducing, and dispersible sugars to the total sugars in the flour or gruel samples; and to show the amylase activities in the flour and in weaning food samples.

MATERIALS AND METHODS

Germination, flour preparation, and food formulation. Rice, corn, mungbean, and cowpea flours with germination periods of 0, 24, 48, 72, and 96 h and weaning food formulation samples were prepared according to standardized methods (2). Representative samples for analysis were drawn from 1 kg of thoroughly mixed flour of each of the germination treatments. The samples were sealed in thick (0.5 mm) polyethylene bags and were kept in the freezer (-18°C) until use.

Preparation of samples and detection of sugars on TLC. A one gram representative flour sample was added to 10 ml of boiling water, mixed in a vortex mixer for 1 min, and filtered through Whatman No. 2 filter paper. The filtrate was immediately used in the TLC experiments. Six μl of the hot-water extracts was spotted on 20 × 20 cm silica gel TLC plates (Whatman K5F, 250 μm thick) along with 3 μl of standard sugars (40 mg/ml): glucose (G1), maltose (G2), maltotriose (G3), maltotetraose (G4), maltopentaose (G5), maltohexaose (G6), and maltoheptaose (G7). These oligosaccharides were obtained from Nihon Shokuhin Kako, Tokyo.

The plates were developed with a solvent system of ethyl acetate–methanol–water, 37:40:23 by volume (5). The developed plates were dipped in glucoamylase (Sumichimu, obtained from Shin-Nihon Kagaku Kogyo, Aichi prefecture) solution, prepared from 125 mg of glucoamylase in 200 ml of cold (5°C) acetone, for 1 min and allowed to air-dry. Formation of glucose was accomplished by incubation in a closed chamber maintained at 40°C for 30 min. The enzymes were inactivated by allowing the plates to stand at 100°C until the plates were thoroughly dried (5 to 10 min). The plates were soaked for 1 min successively in 3 solutions as follows: solution 1 consisted of 1 ml of saturated silver nitrate in 200 ml of cold (5°C) acetone; solution 2 was a mixture of 4 g of sodium hydroxide, 10 ml of water, and 190 ml of ethanol; and solution 3 was 240 g of sodium thiosulfate, 25 g of sodium sulfite, and 10 g of sodium bisulfite dissolved in 1 liter of distilled water. The plates were dried with a hair
blower after each dipping in the solutions; after the third solution, the plates were immersed in water first to eliminate the reagents before drying completely.

Preparation of samples and determination of sugars. a) Total sugars in the flour: One hundred milligram flour samples were placed in screw-capped test tubes and added with 10 ml of 0.1 N HCl. The tubes were placed in a block heater for 100 min at 80°C. The mixture was neutralized with 0.1 N NaOH, centrifuged, and the supernatant was analyzed for total sugar by the anthrone method (6). Results were expressed as mg glucose equivalent per gram sample.

b) Soluble sugar in the flour sample: One gram of each representative flour sample was suspended in 8 ml of 0.1 N HCl, mixed for 1 min (vortex mixer), and allowed to stand for 30 min at ambient temperature to inactivate the enzymes. It was neutralized with 0.1 N NaOH and centrifuged at 3,000 rpm for 10 min. The supernatant was analyzed for soluble and reducing sugars by the anthrone method (6) and Somogyi-Nelson method (7), respectively. Results were reported as above.

c) Soluble sugars in the gruel samples: A one gram representative flour sample was added to 6 ml of distilled water (37°C) and mixed in glass centrifuge tubes for 1 min in a vortex mixer. Gruels were prepared by the method of Mosha and Svanberg (8) to simulate actual gruel preparation, i.e., the mixtures were heated in a boiling water bath for 10 min to reach a cooking temperature of 95°C, then kept at this cooking temperature for 15 min, and cooled to 40°C. The gruels were inactivated with 2 ml of 0.4 N HCl and allowed to stand for 30 min. They were then neutralized with 0.1 N NaOH and centrifuged at 3,000 rpm for 10 min. The supernatant solution was analyzed for soluble and reducing sugar as above.

d) Dispersible sugar in the gruel: One gram sample was prepared into gruel as described in c). The gruel was allowed to stand without moving for 5, 10, 15, and 20 min at ambient temperature; then they were determined for total sugar (6). Samples were carefully taken from the surface of the tube by means of a 1 ml pipette without disturbing the gruel. The tubes were left undisturbed throughout the 20-min sampling period.

Enzyme activity. A one gram representative flour sample from each of the 48-, 72-, and 96-h germination periods for rice, corn, mungbean, cowpea and the four weaning foods (GRM, GRC, GCM, and GCC) was suspended in 10 ml of 0.2% calcium chloride solution (9), mixed in vortex for 1 min, and centrifuged at 3,000 rpm for 10 min. The supernatant solution was immediately tested for enzyme activity using 1% soluble starch in 0.05 M acetate buffer, pH 4.8, as substrate. To tubes containing 0.5 ml of the substrate solution, 0.5 ml of the supernatant solution was added. The samples were incubated at 30°C for 0, 5, 10, 15, and 30 min. In the control (0 min), 1 ml of 1 N HCl was added before the addition of the sample to stop enzyme activity. At the end of each incubation period, 1 ml of 1 N HCl was added to the samples to inactivate the enzymes. Reducing sugar was measured as described above. The amount of glucose equivalent (mg/g) produced from the different samples was plotted against incubation time.

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RESULTS

Preliminary detection of the digestion products of germinated rice, corn, weaning food formulations (Fig. 1, top photo), mungbean and cowpea (Fig. 1, bottom photo) revealed formation of maltooligosaccharides in the germinated materials. The sugars were more visibly clear at 48, 72, and 96 h of germination. Presence of glucose and maltooligosaccharide series was shown in all materials studied. Cereals and the weaning food formulations showed a better separation of the sugars on TLC, compared to the legume samples. In the latter, the presence of other constituents like peptides and/or minerals may have overlapped with, or disturbed the chromatography of the sugars.

Results in Table 1 showed that the total sugars in the flour samples generally decreased after a four-day (96 h) germination period in all the materials studied. The soluble sugar increased in both the flour and gruel samples. In rice flour, the average chain length of the sugars was 2, as shown in the ratio of soluble: reducing sugar in Table 1. TLC also showed that G1 and G2 were the major components of rice germinated at 48, 72, and 96 h. A notable increase in the soluble sugar occurred during gruel preparation, amounting to 23, 48, and 51% of the total sugars, for the 48-, 72-, and 96-h samples, respectively. Production of sugars corresponding to about G6 was formed in the gruel samples of the 96-h sample and TLC also showed, in addition to G1 and G2, the prominence of G6 and G7. In corn, polymers of 2 to 3 chain length (Table 1) were shown to be formed in both the flour and gruel samples. At the germination period of 72 h, the starch degradation products in corn, as soluble sugars, were 16 and 28% of the total sugars for the flour and gruel samples, respectively. At 96 h, the soluble sugars increased to 20 and 33% in the flour and gruel, respectively.

In the legumes, decrease in the soluble sugars for the flour samples was noted in mungbean germinated for 72 h and in cowpea germinated for 24 h, but these soluble sugars increased during the preparation of gruel. The soluble sugars in mungbean had an average of 4 to 7 chain length (Table 1) in the flour and gruel samples. Figure 1 (bottom photo) also showed the prominence of the higher maltooligosaccharides. In the flour samples, the soluble sugars ranged from 21 to 38% of the total sugars at 48, 72, and 96 h of germination. During gruel preparation, the amount of soluble sugars increased to 56% of the total sugars. In cowpea, the major sugars formed at 48, 72, and 96 h of germination were sugars with 4 chain length for both flour and gruel samples. Cowpeas germinated for 96 h, though, showed the formation of all the maltooligosaccharides, as shown by TLC. The conditions during gruel preparation contributed to the production of the higher sugars with about 5 to 6 chain length at 96 h of germination. In the cowpea flour, the soluble sugars were 20 to 27% of the total sugars, but in the gruel, a range of 48 to 60% was found at 48, 72, and 96 h of germination.

The soluble sugars in Table 1 were those that were obtained in the supernatant, i.e., after centrifugation. In actual conditions, however, the gruel administered to a

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Fig. 1. Thin-layer chromatograms of hot-water extracts of germinated rice, corn, weaning foods (top photo), mungbean and cowpea (bottom photo). Formulation: 70 cereal: 30 legume. Germination period: 72 h, cereal; 48 h, legume. Abbreviations: GRM, germinated rice-mungbean; GRC, germinated rice-cowpea; GCM, germinated corn-mungbean; GCC, germinated corn-cowpea.
| Sample  | Germination period (h) | Total sugar<sup>b</sup> | Soluble sugars | Gruel |
|---------|-----------------------|-------------------------|----------------|-------|
|         |                       |                        | Reducing sugar<sup>b</sup> | Soluble sugar<sup>b</sup> | Reducing sugar | Soluble sugar |
| Rice    | 0                     | 805                     | 24             | 50    | 24   | 66  |
|         | 24                    | 791                     | 25             | 48    | 26   | 56  |
|         | 48                    | 791                     | 36             | 48    | 37   | 187 |
|         | 72<sup>c</sup>        | 791                     | 32             | 64    | 63   | 382 |
|         | 96                    | 791                     | 33             | 75    | 80   | 400 |
| Corn    | 0                     | 763                     | 32             | 48    | 37   | 70  |
|         | 24                    | 763                     | 32             | 56    | 36   | 64  |
|         | 48                    | 763                     | 40             | 67    | 42   | 94  |
|         | 72<sup>c</sup>        | 763                     | 56             | 123   | 85   | 216 |
|         | 96                    | 750                     | 60             | 155   | 108  | 248 |
| Mungbean | 0                    | 625                     | 35             | 128   | 35   | 130 |
|         | 24                    | 611                     | 36             | 211   | 38   | 287 |
|         | 48<sup>c</sup>        | 562                     | 40             | 216   | 49   | 316 |
|         | 72                    | 569                     | 56             | 120   | 60   | 320 |
|         | 96                    | 562                     | 57             | 104   | 65   | 315 |
| Cowpea  | 0                     | 659                     | 35             | 160   | 35   | 163 |
|         | 24                    | 645                     | 36             | 144   | 37   | 188 |
|         | 48<sup>c</sup>        | 631                     | 48             | 144   | 54   | 308 |
|         | 72                    | 631                     | 56             | 141   | 58   | 380 |
|         | 96                    | 631                     | 57             | 131   | 65   | 315 |
| GRM     |                      | 763                     | 34             | 123   | 57   | 392 |
| GRC     |                      | 756                     | 40             | 91    | 54   | 407 |
| GCM     |                      | 777                     | 40             | 171   | 54   | 464 |
| GCC     |                      | 756                     | 32             | 139   | 54   | 306 |

<sup>a</sup> Average of two determinations, no significant difference between values at $p<0.05$.

<sup>b</sup> Reducing sugar and total/soluble sugars were determined by Somogyi-Nelson and anthrone method, respectively, and the values were expressed as glucose equivalent (mg/g sample).

<sup>c</sup> These germination products were made into gruels of GRM, GRC, GCM, and GCC. Abbreviations: GRM, germinated rice-mungbean; GRC, germinated rice-cowpea; GCM, germinated corn-mungbean; GCC, germinated corn-cowpea.
Table 2. Dispersible sugar\textsuperscript{a} in the gruels prepared from germinated cereals and legumes.\textsuperscript{b}

| Sample     | Germination period (h) | Time of standing after gruel preparation |
|------------|------------------------|----------------------------------------|
|            |                        | 5 min | 10 min | 15 min | 20 min |
| Rice       | 72                     | 710   | 735    | 764    | 769    |
| Corn       | 72                     | 725   | 738    | 748    | 751    |
| Cowpea     | 48                     | 591   | 605    | 612    | 623    |
| Mungbean   | 48                     | solid-not determined                     |

\textsuperscript{a} Sugars were analyzed by the anthrone method and expressed as mg glucose equivalent/g sample. \textsuperscript{b} Average of three determinations, no significant differences among samples at $p < 0.05$.

The child is not centrifuged, so it contains all the dispersible sugars. The values in Table 2 represent the amount of sugars taken at the surface of the container when the gruel was allowed to stand without disturbing the tubes, at different times of sampling (5, 10, 15, and 20 min). The dispersible sugars in the gruel of the 72-h rice sample were 89, 92, 96, and 97\% of the total sugar at 5, 10, 15, and 20 min sampling, respectively. In corn germinated for 72 h, the dispersible sugars in the gruel ranged from 95 to 98\% over the 20-min standing period. For the gruel prepared from cowpea germinated for 48 h, the dispersible sugars were 93 to 98\% of the total sugars, throughout the 20-min sampling period. Mungbean gruels prepared from 48-h germination showed a firm, gelatinized structure, thus making it impossible to take samples.

Figure 2 shows the activity of the enzymes generated at the 48, 72, and 96 h of germination in the four materials and in the weaning food formulations prepared from their selected combinations. Figure 2A and 2B show that the enzymes produced in rice had the highest amylolytic reaction to soluble starch substrate, compared to the other materials. When the 72-h germinated rice was used in the weaning foods, at a level of 70\%, there was a higher concentration of the enzymes in the rice formulations, GRM and GRC, as compared to the corn formulations, GCM and GCC (Fig. 2D).

DISCUSSION

The ratio of amylose to amyllopectins is different in every material, so the amount of glucose, and how fast it is produced during hydrolysis or starch breakdown, also differ. In this study, germination of the seeds caused the breakdown of the starch into maltooligosaccharides by the action of amylolytic enzymes generated during the germination process.

The decrease in the total sugars of all the materials studied could be due to
increased α-amylase activities during germination. In the flour samples, the increase in the soluble sugars could be attributed to the increased rate of mobilization of soluble carbohydrates in the endosperm of cereals during germination. Hsu et al. (10) cited that soluble carbohydrates are an important energy source during the early stages of germination. In the case of the legumes, the decrease may have been caused by the more rapid utilization of the sugars in respiration than the rate at which they are formed by the degradation of reserve carbohydrates. Aman (11) also found that the total and soluble sugars decreased during the germination of mungbean. During gruel preparation, the soluble sugars were increased in all the materials. Aside from the effect of germination, it was found in this study that there

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was also an additive effect of the process of gruel preparation, in increasing the solubility of sugars. The conditions during gruel preparation, such as addition of water, heating, and stirring favored amylolytic activity, thus making possible the continuous production of maltooligosaccharides. Mosha and Svanberg (8) found that the amylases in germinated cereals are active at over 85°C.

Increase in the reducing sugars of all the germinated materials implies that higher polysaccharides, like starch, have undergone sequence of enzymatic degradation.

The amount of soluble sugars obtained with centrifugation may indicate the partial digestibility of the weaning foods prepared from the germinated materials. However, the amount of dispersible sugars (Table 2) available to the child, i.e., without centrifugation, therefore include the soluble sugars, dextrins, and other partial degradation products of starch, which were stabilized in the starch gel during cooking.

The results in Fig. 2 showed that rice had the highest concentration of amylases, followed by cowpea, corn, and mungbean. Although rice had the least amount of soluble sugars in the flour sample (Table 1) compared to the other materials, its total enzyme activity (Fig. 2) was high. This may be due to the localization of the enzymes at the germ area, or due to the nature of the substrate, i.e., raw starch in Table 1 as compared to soluble starch in Fig. 2, or may be due to the rigid structure of the rice starch and so the enzymes produced were not involved in the reaction with starch. Murata et al. (12) found that maltose series oligosaccharides showed a gradual increase from 6 to 12 days of germination at 30°C, and that the activity of α-amylase is very low at the initial stages, up to 4 days, and then increases abruptly in this period. Nevertheless, the amylolytic enzymes produced in rice also increased the production of reducing sugars in GRM and GRC (Fig. 2D) at 70% addition of germinated rice in the weaning foods. Palmiano and Juliano (13) found that the major enzyme for starch degradation in germinating rice was α-amylase. Literature data (14,15) also support that germinated rice had more than one amylase; in fact, three independent amylases were present—a starch liquefying, a dextrinizing, and a saccharifying enzyme.

The enzymes generated during the germination process were shown to have been retained in the flour regardless of heating treatments during drying and roasting. This remaining enzyme activity continued to produce oligosaccharides during the gruel preparation. Changes in the starch composition, shown by the pattern of maltooligosaccharides in TLC and amylase activities of flours and gruels, have a favorable impact on the digestibility, as well as on the viscosity reduction, of the weaning foods prepared from germinated cereals and legumes.

Further study to quantify the kinds of sugars present in the germinated cereals, and legumes and in weaning foods by the HPLC method is recommended.

The authors are grateful to the Japan Society for the Promotion of Science Ronpaku Program for the chance to do part of the research at the Department of Nutrition and Food
Science, Ochanomizu University, Tokyo, Japan, and at the Food and Nutrition Research Institute, Department of Science and Technology, Manila, Philippines.

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