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Analysis of the Carbohydrates in an East African Traditional Beverage, *Togwa*

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In sub-Saharan Africa, *togwa* is a widely consumed beverage containing saccharified starch as a basis, being made from cereals and their malt flours. Togwa was prepared from maize flour and finger millet malt in a laboratory, and trace element and amino acid contents in the togwa were analyzed. The major constituents of togwa are carbohydrates, which are starch and starch hydrolysates that seem to be smoothly digested after ingestion. Carbohydrates in field and laboratory togwa were analyzed in detail by two different HPLC systems: normal phase chromatography and a gel permeation chromatography-multi angle laser light scattering system. Togwa was found to contain a very wide range of size distributions of molecules from glucose to starch that have molecular masses in the millions. It was concluded that togwa is an easily digestible and nutritious food.

KEYWORDS: Togwa; traditional beverage; finger millet; α-amylase; carbohydrate; starch saccharification; oligosaccharide

INTRODUCTION

In our previous study, we investigated the traditional nonalcoholic beverage made from cereal and malt, that is, *togwa* in southern Tanzania on the basis of a fieldwork survey, and we prepared it in our laboratory to analyze (*1*, *2*). In southern Tanzania, togwa is made from maize flour and germinated finger millet flour. For the preparation of togwa, the flour of finger millet sprout is added to the warmed maize porridge, and the mixture is left overnight at room temperature. Starch in maize porridge was hydrolyzed and liquefied by α-amylase in finger millet malt. Togwa is opaque and brownish in color due to the solid particles' suspension. The taste of togwa is sweet, occasionally sour, because of lactic acid fermentation. Togwa is usually spoiled by further fermentation for 2−3 days. Materials of togwa are cereals and their malts, and the main component of togwa is carbohydrates (*1*, *2*).

Togwa seems to have been regarded as a lactic acid fermented food rather than as an energy supplemental food such as a weaning or hygienic food (*3*−*8*). However, in southern Tanzania togwa is used as a food for workers in the field. These fields are located on a steep hillside and during the rainy season when the workers' fieldwork is hard and severe; they depend on togwa for an energy source. For example, they drink togwa as a meal during this season while working in the field. In this study, we took notice of togwa as an efficient energy source for workers, because it seems to contain widely distributed starch-degraded products. We then aimed to characterize the molecular component of these starch-degraded products.

Although carbohydrate is the most important energy source among the macronutrients, the role of dietary carbohydrates in human nutrition seems to have been less extensively studied compared to those of protein and fat. One reason may be the methodologies for carbohydrate analysis (*9*). In this study, to analyze a diversity of carbohydrates in togwa, we used the high-performance liquid chromatography (HPLC) technique and enzymatic analysis using glucose oxidase assay. Due to the liquefaction and the saccharification activity of amylase of finger millet malt, starch in maize is degraded, and various kinds of oligosaccharides and dextrin are contained in togwa. Only a few reports have been published about togwa from the aspect of its being an energy-supplying beverage. The objective of this paper is to clarify the specific and latent significance of togwa as a food consumed while working from the view of food chemistry, including the analysis of carbohydrates in togwa.

MATERIALS AND METHODS

Botanical Materials. White maize flour (corn flour white no. 7) was purchased from Sunny Maize Inc. (Shimizu, Japan). Finger millet malt flour was obtained at a rural village in Mbinga, Ruvuma, Tanzania, and kept at −80 °C until use. Finger millet grains were harvested in 1999 and processed (malting, drying, and milling) in the village.

Field Togwa. The field togwa was collected at a rural village in the southern part of Tanzania as described (*2*). The field togwa was kept for 30 h after the addition of finger millet malt to maize porridge. To stop the reaction and curtail the microorganism population, sodium azide was added to the heat-treated field togwa, which was brought back to Japan and lyophilized.

Reagents. Amyloglucosidase (A-7420; from *Aspergillus niger*) and a glucose oxidase assay kit (GAGO-20) were purchased from Sigma (St. Louis, MO). Other chemicals were purchased from Wako Pure

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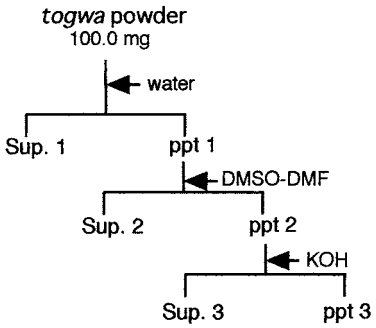

Figure 1. Scheme of fractionation of carbohydrate in togwa. The lyophilized and powdered togwa sample (100.0 mg) was fractionated by solubility in water, organic solvent (DMSO/DMF), and alkaline solvent (2 N KOH).

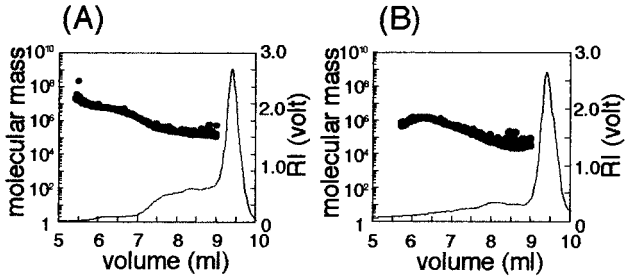

Figure 2. Comparison of the chromatograms between the laboratory togwa (A) and the field togwa (B) by GPC-MALLS. Both togwa samples were lyophilized and powdered. Elution patterns were detected by a refractive index (solid line). Calculated molecular masses are shown in the same figure (extent of dots).

Chemical Industries, Ltd. (Osaka, Japan) and Nacalai Tesque Inc. (Kyoto, Japan).

Preparation of Togwa in the Laboratory. Togwa was prepared in the laboratory according to the method we described before (*2*). Three hundred milliliters of water was added to 53 g of maize flour in a glass vessel reactor. The slurry was continuously stirred at 400 rpm with a turbine and an anchor type propeller and was heated to 90 °C. When the temperature reached 90 °C, the heating was stopped and the slurry was stirred and cooled to 50 °C. Then, 18.6 g of finger millet malt flour was added to the slurry through the inlet hole of the glass vessel reactor. Twenty minutes after the addition of finger millet malt flour, the stirring was stopped. The slurry was then left at room temperature in the reactor. The togwa incubated for 9 h was used as the laboratory togwa for the following analysis (*2*). The laboratory togwa was immediately lyophilized after production and stored in a deaerated plastic bag at 4 °C until analysis.

Analysis of Carbohydrate. Carbohydrate analysis of both the field (Tanzania) togwa and the laboratory togwa was carried out using two types of HPLC, that is, the normal phase column chromatography (NPC) and a gel permeation column chromatography connected with the multiangle laser light scattering photometer system (GPC-MALLS system). The former can analyze the distribution of low molecular saccharides in detail, and the latter can analyze saccharides in a wide range of molecular masses, including high molecular weight polysaccharides.

GPC-MALLS Analysis. Lyophilized togwa powder was dissolved (20 mg/mL) in dimethyl sulfoxide (DMSO)/dimethylformamide (DMF) (DMSO/DMF = 50:50), heated to 80 °C for 15 min, and filtered by a 0.22 μm filter (Ultrafree-MC, Millipore Co., Bedford, MA). Then, the samples were applied to the GPC system (Shodex GPC system-21, Showa Denko Co., Tokyo, Japan) and OH Pack SB-806M HQ (0.8 × 30 cm^2; Showa Denko Co.) at a flow rate of 0.4 mL/min. Measurements were carried out at 40 °C. DMSO/DMF was also used as eluent. Elution from the column was monitored with a MALLS photometer (DAWN DSP, Wyatt Technology, Santa Barbara, CA) and a refractive index (RI) detector (RI-71S, Showa Denko Co.). The data measured by the MALLS photometer and RI detector were processed by the software ASTRA (Wyatt Technology) to calculate the molecular weights of the eluted polymers according to the theory and procedure described before (*10*, *11*).

NPC Analysis. Lyophilized togwa powder was suspended to 4 mg/mL in eluent (acetonitrile/water = 50:50) and filtered with a 0.22 μm filter (Ultrafree-MC, Millipore Co.). The samples were applied to pump units (L-6000, Hitachi, Tokyo, Japan) at a flow rate of 0.5 mL/min. The RI detector (RI-71, Showa Denko Co.) was connected to an Amide-80 column (Tosoh, Tokyo, Japan). NPC was carried out at 40 °C using a column oven (CTO-6A, Shimadzu Co., Kyoto, Japan). Each eluted sugar was identified by the elution time and spiking.

Distribution of Carbohydrate in Togwa. *Fractionation and Determination of Glucose.* We fractionated togwa by solubility to evaluate the distribution of carbohydrate as shown in **Figure 1**. The togwa used for this experiment was reproduced in our laboratory. Lyophilized togwa powder (100.0 mg) was suspended in 10 mL of water. The slurry was centrifuged at 3000*g* for 30 min and the supernatant (sup 1) was obtained. The residue (ppt 1) was suspended in 10 mL of water and centrifuged again at 3000*g* for 30 min. It was then dried by centrievaporation using a centrifugal vaporizer (CVE-100D, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) connected to a freeze-dryer (FD-5N, Tokyo Rikakikai Co., Ltd.) equipped with a vacuum pump. Afterward, the sample was dissolved in 5 mL of DMSO/DMF solvent, heated at 80 °C for 15 min, and centrifuged at 3000*g* for 30 min. The residue (ppt 2) was washed twice to remove DMSO with 95% (v/v) ethanol and dried using centrievaporation. Dried ppt 2 was dissolved in 3 mL of 2 N KOH and left at room temperature for 30 min. After centrifugation at 3000*g* for 10 min, the supernatant (sup 3) was obtained and the precipitate (ppt 3) was collected and dried. Sup 3 (300 μL) was suspended in 1.2 mL of water and brought to pH 4.5 with 650 μL of 2 M CH_3COOH. An aliquot of the solution (215 μL) was diluted with 860 μL of the 0.1 M sodium acetate buffer (pH 4.5). To this diluted sample was added 50 μL of a 5 mg/mL amyloglucosidase solution to a total volume of 1125 μL. The reaction mixture was kept at 60 °C for 90 min. The digested sample was diluted, and the glucose concentration of the sample was determined. The other supernatants (sup 1 and sup 2) were diluted and hydrolyzed with amyloglucosidase (A-7420) at pH 4.5 and 60 °C for 90 min. Measurements were done in triplicate and are expressed in milligrams ± SD. The amount of carbohydrate dissolved in each supernatant solution was calculated as a glucose equivalent.

Total Carbohydrate in Togwa. To measure total carbohydrate in togwa, lyophilized togwa powder (100.0 mg) was dissolved in 10 mL of 2 N KOH and left at room temperature for 30 min. An aliquot (300 μL) was diluted with 1.5 mL of water and brought to a pH of 4.5 with 650 μL of 2 M CH_3COOH. The solution was diluted 20-fold with sodium acetate buffer (pH 4.5). To the diluted solution (1075 μL) was added 50 μL of amyloglucosidase solution (5 mg/mL), and the mixture was hydrolyzed at a pH of 4.5 at 60 °C for 90 min. The concentration of glucose was determined using the glucose oxidase assay method. Measurements were done in triplicate, and the amounts were expressed in milligrams ± SD.

Total Carbohydrate in Each Fraction through GPC-MALLS. A GPC-MALLS system was also used to clarify the distribution of carbohydrate in togwa. A lyophilized and powdered togwa sample was directly suspended in DMSO/DMF (10 mg/mL). Subsequently, these samples were applied to the GPC-MALLS system as described before. To evaluate the amount of total carbohydrate in each fraction, the eluted sample was collected from the flow channel between the LS detector and the RI detector, and the amount of carbohydrate in each 0.4 mL fraction was measured by using the phenol−sulfuric acid method (*12*).

RESULTS AND DISCUSSION

Comparisons between the Laboratory Togwa and the Field Togwa Using HPLC. Figures 2 and **3** show comparisons between laboratory togwa and field togwa using two different HPLC systems. **Figure 2** shows an elution pattern of molecules of the laboratory togwa (**Figure 2A**) and the field togwa (**Figure 2B**) by GPC-MALLS. Solid lines were RI of the eluate, and

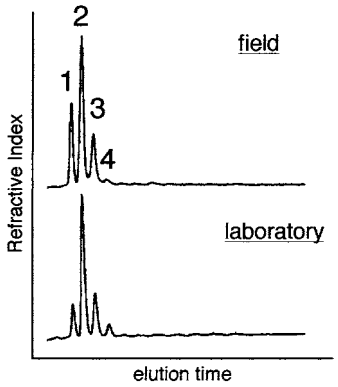

Figure 3. Comparison of chromatograms between the field and laboratory togwas by normal phase column chromatography using an Amide-80 column. The laboratory togwa was collected 9 h after the finger millet malt flour had been added, but the field togwa was collected 30 h afterward. Peaks: 1, glucose; 2, maltose; 3, maltotriose; 4, maltotetraose.

the molecular sizes for each eluate are shown in the same figure. The fraction between 7.5 and 9.0 mL of the field togwa contains less carbohydrate than that of the laboratory togwa. This may be due to the difference in incubation time to prepare togwa. Nevertheless, as a result of degradation of the starch by α-amylase in finger millet malt, both of the togwa samples contained many kinds of starch-degraded molecules with a wide range of molecular sizes. In **Figure 3**, distinct peaks corresponding to those for glucose, maltose, maltotriose, and maltotetraose are observed. Peaks of oligosaccharide (degree of polymerization, DP, = 5−10) were traces on NPC, and therefore the polymerization degree of the glucose unit of the saccharides in the water soluble fraction of togwa was <4. The amount of glucose in the field togwa was larger than that in the laboratory togwa. This could be due to the difference of the incubation time after the preparation of togwa (**Figure 3**). In the case of the field togwa, the sample kept for 30 h was collected and heated, and sodium azide was added to cease bacterial activity, followed by lyophilization. On the other hand, the laboratory togwa was lyophilized after 9 h of standing. Maltose is a dominant saccharide in both the field and laboratory togwas. According to the measurement of the peak area of the chromatogram of laboratory togwa, maltose accounted for 20% of total saccharide in togwa.

Change of Carbohydrate Composition during Incubation of Togwa. The togwa samples periodically collected during incubation were lyophilized, and the powder obtained was dissolved in DMSO/DMF. They were then analyzed using a GPC-MALLS system (**Figure 4**). In each panel, the observed molecular weight of the eluted fraction was plotted against the eluted volume (extent of dots). RI values of the eluted samples are also shown in the same figure (solid line). The calculated molecular weight was distributed from 10^5 to 10^7, indicating that there were many different varieties of the carbohydrates in togwa. The RI values of the elution volume from 5.5 to 7.0 mL were not changed throughout the incubation. This implies that the carbohydrate in the fraction within 5.5−7.0 mL was resistant to α-amylase hydrolysis derived from the finger millet malt.

According to the result of GPC-MALLS, the fraction from 5.5 to 7.5 mL of 9 h togwa contains high molecular weight ($>1 \times 10^7$) molecules (**Figure 4D**). The peak of the RI pattern has shifted to the low molecular side and a large peak appeared at 9.5 mL (**Figure 4A**−**D**). This demonstrates that enzymes including α-amylase in the finger millet malt should hydrolyze starch throughout the incubation until 9 h and degraded saccharides were formed. The peak of the position of ∼9.5 mL

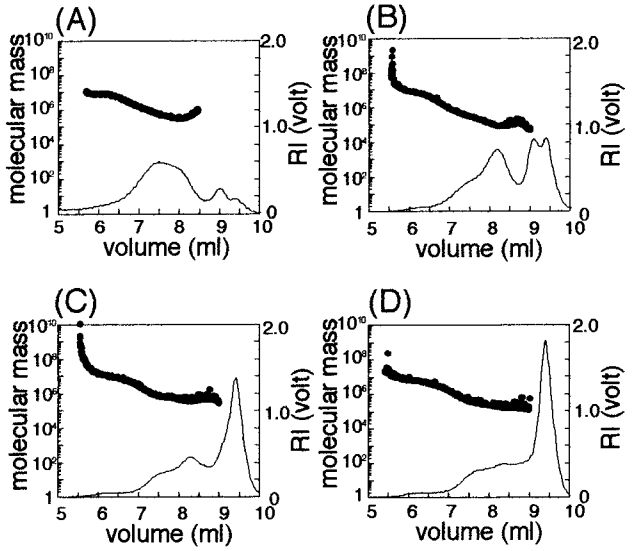

Figure 4. Chromatograms of reflective index (solid line) and the calculated molecular mass for each eluate (0.4 mL) (dots) for togwa samples obtained 10 min (A), 1 h (B), 3 h (C), and 9 h (D) after the addition of finger millet malt flour. Lyophilized and powdered togwa samples (20 mg/mL) were dissolved in DMSO/DMF eluent. The molecular masses of polymer at each eluted volume were calculated on the basis of the light scattering intensities and value of the refractive index value using the software ASTRA.

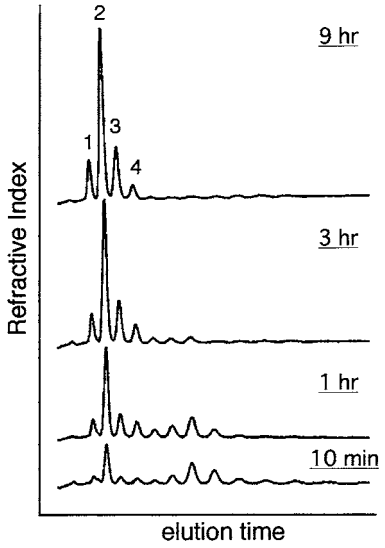

Figure 5. Changes in the normal phase column chromatogram of the laboratory togwa samples detected by the refractive index (RI). Togwa samples were obtained from the inlet hole of the reactor at 10 min and 3, 6, and 9 h after finger millet malt flour had been added. Togwa samples were lyophilized and stored until analysis. Each sample was prepared as described in the text. Peaks: 1, glucose; 2, maltose; 3, maltotriose; 4, maltotetraose.

appeared in time, which should be correspondent with the low molecular weight saccharides, mostly maltose (**Figure 4C**,**D**). Most of the starch in togwa was degraded to low molecular weight oligosaccharides, whereas the large size of dextrin still considerably remained in the position between 7.5 and 9.0 mL (**Figures 4D** and **6**).

A water soluble fraction of togwa samples was analyzed by the NPC, using an Amide-80 column (**Figure 5**). Samples at 10 min and 1, 3, and 9 h incubation times after the addition of finger millet malt flour were lyophilized and powdered. Glucose,

Table 1. Distribution of Carbohydrate in Each Fraction of Togwa Shown in **Figure 1**

	residue[a]
ppt 1	41.3 ± 1.3
ppt 2	16.6 ± 0.5
ppt 3	7.3 ± 0.7

	carbohydrates	
	as glucose equivalent[b]	as polysaccharide[c]
sup 1	59.1 ± 1.3	53.2 ± 1.2
sup 2	26.7 ± 1.6	24.0 ± 1.4
sup 3	3.2 ± 0.5	2.9 ± 0.5
sum	89.0	80.1
total	91.5 ± 2.5	82.4 ± 2.3

[a] Values for precipitates were directly weighed. [b] The amount of carbohydrates in each supernatant fraction was determined by glucose oxidase assay as described in the text and expressed as a glucose equivalent. [c] Conversion factor (0.9) was multiplied to the value of monosaccharide (*13*). Values were examined in triplicate, and the amounts are expressed mean ± SD.

maltose, and oligosaccharides (DP < 10) were clearly separated by the Amide-80 column. As mentioned above, the amount of glucose measured by the Glutest Ace increased at all times during and after the 9 h incubation. The glucose contents obtained from chromatogram data are consistent with those of the Glutest Ace. Furthermore, the major saccharide in togwa was maltose. The amount of maltose in 9 h togwa calculated from the peak area was 35% of water soluble carbohydrate (20% of total carbohydrate).

Distribution of the Carbohydrate in Togwa. The distribution of carbohydrate in the laboratory togwa by solubility in water, organic solvent, and alkaline solvent fractions is shown in **Table 1**. The water insoluble fraction (ppt 1), which may contain relatively large molecular saccharides that have DP > 10, was 41.3 ± 1.3 mg, and the residue was dissolved into DMSO/DMF. The amount of ppt 2 was 16.6 ± 0.5 mg, and the residue remaining after alkaline treatment (ppt 3) was 7.3 ± 0.7 mg. It is supposed that all starch and degraded products were fractioned to the supernatant by extraction with water, DMSO, and alkaline solvent. Finally, ppt 3 may not contain any carbohydrates.

The amounts of carbohydrate in the supernatant fraction and the total carbohydrate amount were each measured by the glucose oxidase assay. In **Table 1**, we show the distribution of carbohydrate in each fraction shown in **Figure 1**. In the case of supernatant, the carbohydrate amount was measured by two methods: glucose equivalent by the glucose oxidase assay and polysaccharide equivalent by the concentration factor method. The amounts of the monosaccharide measured by the glucose oxidase assay were converted to that of the polysaccharide using a conversion factor (0.9) (*13*). In the water soluble fraction (sup 1) containing the saccharides with low molecular weight, 59.1 ± 1.3 mg of carbohydrate derived from 100.0 mg of lyophilized togwa powder, which represents a glucose equivalent, corresponds to a low molecular mass carbohydrate including glucose and maltose. In the DMSO/DMF soluble fraction (sup 2), 26.7 ± 1.6 mg of carbohydrate exists as a glucose equivarent, and 3.2 ± 0.5 mg of carbohydrate was dissolved in 2 N KOH (sup 3) as polysaccharide. Therefore, the sum of carbohydrate in each fraction, that is, the total carbohydrate amount in 100.0 mg of lyophilized togwa, was 80.1 mg as polysaccharide. The total carbohydrate amount in 100.0 mg of lyophilized togwa was 91.5 ± 2.5 mg as a glucose equivalent, that is, 82.4 ± 2.3 mg as polysaccharide. These results of enzymatic analysis are consistent with our previous results obtained by the analysis of the chemical composition of laboratory togwa (*2*).

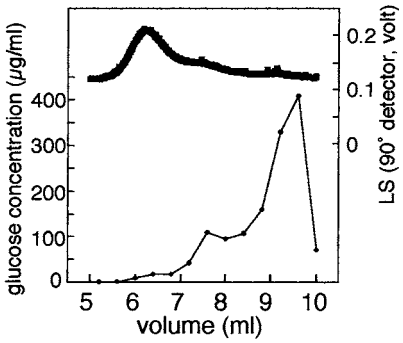

Figure 6. Amount of carbohydrate in eluate (each 0.4 mL) from GPC-MALLS and the elution pattern of the togwa sample dissolved in DMSO/DMF. A lyophilized and powdered togwa sample (20 mg/mL) was used. The elution pattern was detected by a laser light scattering detector at an angle of 90°. The sequential line shows the amount of total carbohydrate in each fraction determined by using the phenol–sulfuric acid method.

Figure 6 shows the GPC-MALLS elution patterns of carbohydrate of the laboratory togwa detected by both the light scattering (LS) method and the phenol−sulfuric acid method. The pattern detected by the LS method showed a peak at the position of 6.0 mL. About 6% of the total amount of carbohydrate was observed in the fraction between 5.5 and 7.0 mL by the phenol−sulfuric acid method. The carbohydrate in the fraction from 5.5 to 7.0 mL that contains a considerably large molecular mass would be resistant against amylase activity in the finger millet malt of togwa.

Dietary carbohydrate content has been usually calculated by subtracting the contents of other constituents from the total content of the sample. We directly measured carbohydrate content using HPLC and the method by fractionation and enzymatic analysis. The HPLC method makes it easy to understand the change of carbohydrate components in the sample over the course of time, whereas the enzymatic analysis reveals the distribution of the starch in the sample from the view of solubility. Another advantage of enzymatic analysis is specificity, that is, this analysis is able to exclude nonstarch carbohydrate components.

Togwa is a liquid containing many small particles and is used without filtration in the field. Carbohydrates, the main component of togwa, consist of various kinds of starches and starch-degraded products from monosaccharide to high molecular weight ($>1 \times 10^7$) molecules. Hydrolyzed starch would be easily taken, digested, and absorbed in the small intestine, whereas macromolecules such as starch may be slowly digested and absorbed. As togwa contains these different types of carbohydrates, it should be supposed that both fast and slow supplies of energy to the body are fulfilled by taking togwa, which might sustain the hard work of the farmers at the hillside field. Because cereal-based togwa contains minerals, vitamins, and proteins as well as carbohydrates, we believe that togwa is a kind of nutritional and functional food which is able to provide sustainable energy to the workers on the farm and takes a key role in their food habits.

Prolonged fermentation of togwa produces lactic acid by lactic acid bacteria derived from the contaminant of botanical materials. This lactic acid also contributes to the sour taste of togwa and a lower pH. Moreover, lactic acid bacteria inhibit other pathogenic bacteria (*7*, *14*). Some other researchers have

revealed the advantageous effect of togwa on the prevention of diarrhea and enteropathogenic bacteria (*6*−*8*).

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