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Chemical Composition of an East African Traditional Beverage, Togwa

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Togwa is a starch-saccharified nonalcoholic traditional beverage consumed in sub-Saharan Africa. In the southern part of Tanzania, togwa is usually made from maize flour and finger millet malt. In this region, togwa is consumed by working people and also used as a refreshment as well as a weaning food. Togwa was prepared in the laboratory using a specific reactor and analyzed for its general chemical composition in both laboratory and field forms. From the analysis of the trace elements and amino acid contents in togwa and its materials, it has been clarified that togwa contains many kinds and a high quantity of minerals derived from finger millet malt. It was concluded that togwa should be nourishment and a major source of minerals as nutrients for the inhabitants of this region.

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

INTRODUCTION

In sub-Saharan Africa, the traditional alcoholic and nonalcoholic beverages prepared using cereal malt flour are widely consumed (1-3). Togwa is one such traditional nonalcoholic beverage consumed in East Africa. In the southern part of Tanzania, togwa is made from maize flour and flour from finger millet malt (finger millet sprout flour) (4). The former is a starch source, and the latter is an amylase one. For the preparation of togwa, finger millet sprout flour was added to maize porridge and the mixture was then left at room temperature overnight. Togwa is opaque and brownish in color due to the solid particles suspended in the solution. The taste of togwa is sweet, occasionally sour, and it usually spoils within 2-3 days at room temperature (4).

In the previous study based on fieldwork (4), which was done in a rural village in the southern part of Tanzania, it has been shown that togwa is, in particular, consumed as a popular energy source during hard work in the fields on the slope of an escarpment as well as in daily life. Furthermore, adequate sweetness and sourness of togwa would be favorable to people in this region. It is probable that this technique of preparing togwa using malt amylase could be considered to be one of the oldest methods of food processing. Farmworkers as well as sick people or pregnant women also consume togwa in their daily life (4-9). As it is difficult to analyze togwa on the spot, we prepared reproducible togwa in our laboratory to study its nutritional value and analyzed the general components, trace elements, and amino acids in order to demonstrate the role of this traditional beverage.

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 from 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. Field togwa was collected at a rural village in the southern part of Tanzania. Togwa was collected in plastic bottles and immediately heated in boiling water for 5 min to inactivate enzymes at the spot of the field. Sodium azide (0.1% w/v) was added to prevent microbial growth. The togwa was then lyophilized and powdered in our laboratory in Japan before long-term storage.

Reagents. General bacteria count paper was purchased from Sibata Science Technology, Ltd. (Tokyo, Japan). Benzyl penicillin potassium (crystalline penicillin G potassium Meiji) and streptomycin sulfate (streptomycin sulfate Meiji) were purchased from Meiji Seika Kaisha, Ltd. (Tokyo, Japan). Other chemicals were purchased from Wako Pure 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 conventional method learned from the fieldwork at a rural village in southern Tanzania as shown in the previous paper (4). To monitor the temperature and the consistency of the sample during cooking, we used a specific reactor (TMV-1000, Taiatsu Scientific Glass Co., Tokyo, Japan) equipped with a stirrer (DC-2RT, Tokyo Rikakikai Co., Ltd., Tokyo, Japan), a thermal monitor, and a torque meter (Figure 1). Three hundred milliliters of water was added to 53 g of maize flour in a glass vessel reactor. The slurry was then continuously stirred at 400 rpm with two propellers, which were a turbine and an anchor type, followed by heating of up to 90 °C. The torque value was regarded as the slurry consistency and was recorded by the pen recorder connected with the torque meter. A thermal sensor was attached to the reactor, and the temperature of the slurry was monitored. When the temperature reached 90 °C, heating was stopped and the slurry was cooled to 50 °C with continuous stirring. Then,

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Figure 1. Equipment for preparing togwa. A reacting glass vessel was put into the main body of the equipment, and a mixing shaft with propellers was set. The rate of the rotation shaft was controlled, and the torque on the shaft and temperature in the vessel were monitored and recorded.

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 left at room temperature for 9 h in the reactor and lyophilized. Subsequently, togwa flour was stored in a deaerated polyethylene bag at 4 °C until analysis. Some togwa samples were incubated for 36 h, and during incubation the changes in glucose concentration, lactic acid concentration, and pH value were measured periodically.

Measurement of Glucose, Lactic Acid, and pH. The concentrations of glucose and lactic acid in togwa were measured using small portable devices, Glutest Ace (Arkray, Inc., Kyoto, Japan) and Lactate Pro (Arkray, Inc.) as described elsewhere (4). The pH values were measured by a pH meter (B-212, Horiba Ltd., Kyoto, Japan).

General Bacteria in the Material. To evaluate the extent of the bacterial contamination, we measured the number of general bacteria in finger millet malt flour samples. To measure the number of general bacteria, we used the test paper for general bacteria (Sibata Science Technology Ltd., Tokyo, Japan). The finger millet malt flour sample (5 g) was suspended in sterilized water (100 mL) and mixed thoroughly. After 20 min, the supernatant was collected and serially diluted. Then, each supernatant (1 mL) was applied to the test paper in a plastic bag and incubated for 24 h at 37 °C, and the number of red colonies was counted. The number of contaminated bacteria was expressed as colony forming units (cfu) per gram. Bacterial contamination in maize flour was evaluated using a method of a standard plate count (spc) by the supplier (Sunny Maize Inc.), and the number of bacteria was expressed as spc.

Analysis of Chemical Composition of Togwa. The protein, fat, ash, and dietary fiber contents in the lyophilized powder were measured according to the Kjeldhal method (11), the acid hydrolysis method (11), the direct incineration method (11), and the enzyme-weight method (11), respectively. The amount of carbohydrates was calculated by deducting the amounts of protein, lipid, dietary fiber, and ash from the total weight. Energy was calculated using Atwater's energy conversion factor. Also, moisture content was measured before lyophilization according to the drying method (11) to determine the moisture in the flour with minor modifications. The togwa sample (~1 g) was dried at 105 °C until it reached a constant weight.

Analysis of Trace Elements. To analyze inorganic trace elements, first, the samples were wet-incinerated (*12*), described as follows: lyophilized samples (\sim 2 g each) were weighed and heated with nitric acid (10 mL). When the gas formation ceased, the samples were cooled to room temperature. Then, perchloric acid (2 mL) was added, and the samples were heated at 150 °C. The samples were dehydrated almost completely by evaporation. Finally, the samples were dissolved in 3% HCl with warming and adjusted to 100 mL with 3% HCl.

We measured the content of the trace elements using an inductively coupled plasma (ICP) emission spectrometry analyzer, IRIS Advantage



Figure 2. Changes in temperature and torque profile while cooking togwa (upper). An arrowhead indicates the time when finger millet malt flour was added to maize porridge. The lower chart shows the torque profile when autoclaved finger millet malt flour was added to maize porridge.

ICAP (Nippon Jarrell Ash Co., Ltd., Uji, Japan). The qualitative analysis was preliminary performed to determine the interference effect between the coexistence substances. On the basis of the qualitative analysis to discern the approximate composition of inorganic elements in the sample, we selected the following nine emission wavelengths of the elements to analyze in detail: Ca (393.366 nm), Fe (259.940 nm), K (766.490 nm), Mg (279.553 nm), Mn (257.610 nm), Na (589.592 nm), P (213.618 nm), Si (251.612 nm), and Zn (206.200 nm). These selected elements were recognized as necessary nutrients for health and were not interfered with by other elements' emission in the analysis. Echelle diffraction grating, which was applied in the spectrometry system, develops a diffraction spectrum in two dimensions. The data obtained were analyzed using ThermoSPEC/CID for IRIS Spectrograph software (Nippon Jarrell Ash Co., Ltd.).

Amino Acid Analysis. Lyophilized samples were hydrolyzed in the gas phase with 6 N HCl containing 1% (v/v) phenol for 22 h at 110 °C under a vacuum. After hydrolysis, HCl was evaporated and the residue was dissolved in the sodium citrate buffer, pH 2.2. The sample was then analyzed on an L-8500A high-speed amino acid analyzer (Hitachi, Tokyo, Japan). The amino acid was detected with postcolumn derivatization with ninhydrin, and it was determined using an amino acid standard solution supplemented with *S*-(carboxymethyl)cysteine as a standard.

RESULTS AND DISCUSSION

Changes in Temperature and Viscosity during the Preparation of Togwa. Togwa was prepared using a specific reactor shown in Figure 1. Figure 2 shows the changes in temperature of the togwa inside the reacting glass vessel and the torque value of the shaft during preparation of togwa. The torque value reflects the consistency of the slurry. During heating, the temperature of the slurry reached 90 °C within 10 min. When the temperature of the slurry reached 70 °C, the torque of the shaft drastically increased, which was confirmed by the observation of the change in the slurry in the vessel to a sticky paste, a thick porridge, during the rising of the torque. This was due to the gelatinization of the starch. Although heating was stopped at 90 °C, the temperature of the thick porridge reached ~ 100 °C because of the remaining heat. The torque rose with an increase in temperature until ~90 °C, and then the torque value suddenly dropped. This drop of torque value was due to a breakdown of the starch granule that was soaked in water, gelatinized, and swelled. During cooling, at \sim 30 min after heating, the viscosity rose again (Figure 2). This increase would be attributed to the gelation and/or hardening of the gel structure of the paste during cooling. A change in torque shown here seemed to be a typical one conventionally observed on the amylogram of maize flour (13).

Viscosity dropped with the addition of finger millet malt flour. At that time, we observed the thick porridge changed swiftly



Figure 3. Changes in glucose concentration (\bullet), lactic acid concentration ($\mathbf{\vee}$), and pH (+) of togwa during incubation: (A) finger millet malt flour (18.6 g) was added to maize porridge (353 g; water/flour = 300:53); (B) togwa was prepared in the presence of the antibiotics or sodium azide to examine the effect of microorganisms in the starting materials on the properties of togwa [100 units/mL penicillin + 100 mg/mL streptomycin (\Box) or 20 mM sodium azide was added to maize flour slurry (Δ), respectively]. Lactic acid was not detected throughout the incubation when both antibiotics and sodium azide were added to the sample slurry.

to a liquefied slurry. When the malt flour treated at 121 °C for 20 min in advance was added, the liquefaction process was not observed (**Figure 2**), which would be due to the inactivation of α -amylase by heating at 121 °C (*14*). This indicates that the liquefaction ability is due to the hydrolysis of the maize starch by the α -amylase contained in untreated finger millet malt flour. The liquefaction of finger millet α -amylase was so powerful that maize porridge containing 15% solid became liquid with the addition of 5% finger millet malt to the total amount. The activity of saccharifying is higher than that of malted sorghum, pearl millet, or maize (*15*). The liquefied slurry contains a considerable amount of small particles, which would be derived from the hull of seeds and the starch granules escaped from amylolysis.

Changes in pH and Concentrations of Glucose and Lactic Acid during Preparation of Togwa. Changes in pH, glucose concentration, and lactic acid concentration during the preparation of laboratory togwa in a specific reactor are shown in **Figure 3**. After the addition of malt flour, glucose concentration was notably raised. The concentration of glucose reached 50– 100 mM after 9 h of incubation. Some differences of glucose concentration between the preparations of the samples should be attributed to the reaction temperature.

The pH value was ~ 6 at the beginning of the reaction. However, after 9 h of incubation of maize porridge with finger millet malt flour, the pH value of the sample decreased from 6 to 4 and lactic acid was produced. From the result of the sensory test, 9 h togwa, which was prepared in the laboratory by a 9 h incubation, elicited sweetness and little or slight sour tastes. In the field, togwa is usually served for 2 days after the addition of finger millet malt, so we measured the profile of togwa incubated for 36 h in the glass vessel reactor. Glucose concentration in 36 h togwa reached 100-130 mM, pH reached 3.9-4.2, and lactic acid concentration was 21-36 mM. The production of lactic acid continued even after 36 h of incubation with finger millet malt flour. In the laboratory condition, we observed faster saccharification and lactic acid bacteria fermentation than in the field. This would be mainly due to the difference of temperature.

To verify the effect of bacteria or yeast contained in the materials such as maize and finger millet malt flour on the

Table 1. Comparison of Compositions between the Field and Laboratory Togwas a

	field togwa		laboratory togwa	
	powder ^b	liquid ^c	powder ^b	liquid ^c
water (g)	5.6	85	4.0	83
protein (g)	6.8	1.1	6.8	1.2
lipid (q)	1.4	0.2	1.9	0.3
ash (q)	1.4	0.2	1.1	0.2
carbohydrate (g)	78.4	12.5	80.5	14.3
dietary fiber (g)	6.4	1.0	5.7	1.0
energy (kcal)	353	56.1	366	64.8

^{*a*} The amounts are expressed as grams per 100 g of lyophilized togwa powder. ^{*b*} The amount of each component in powder samples was estimated as described in the text. ^{*c*} The contents in liquid togwa were calculated amounts using measured water contents (85 and 83% in the field and laboratory togwas, respectively) and the amounts of each component in lyophilized togwa powder. The amounts are expressed as grams per 100 g of togwa.

changes in glucose concentration and lactic acid concentration, we added antibiotics, penicillin and streptomycin, or sodium azide to the materials to cease the activity of the bacteria and yeast here (**Figure 3B**). When togwa was made in the presence of antibiotics, the concentration of lactic acid was below the detection limit (0.8 mM) of the measuring device. After 20 h of incubation with finger millet malt, the glucose concentration had dropped and a yeast fermentation characteristic odor could be smelled from the slurry. We used 9 h togwa as the laboratory togwa for the following experiments. This togwa was producing lactic acid at an early stage, and it has a sweetness similar to that of field togwa. The glucose concentration of the laboratory togwa measured using the portable device is also similar to that of the field togwa (4).

Penicillin (3 \times 10⁴ units) and streptomycin (30 mg) added to the sample slurry repressed the growth of procaryotic bacteria including lactic acid bacteria. When 6 mmol of sodium azide was added to water (300 mL) in the glass vessel reactor before the addition of the maize flour to repress the growth of both procaryote and eucaryote including yeast, no reduction of glucose concentration was observed for 2 days (Figure 3B). In the presence of sodium azide, the concentration of lactic acid was reduced as well when antibiotics were added, and the particular odor from yeast fermentation was not perceived. In this system the growths of both lactic acid bacteria and yeast were inhibited; therefore, an increase in lactic acid concentration and a decrease in glucose concentration were suppressed. These results show that yeast as well as bacteria was contained in the sample. Furthermore, these microbacteria should adhere to maize and/or finger millet seeds.

The number of bacteria in the finger millet malt was counted. When the slurry was diluted 10^4 times with water, the number of colonies of bacteria on the test paper for general bacteria was 34 ± 8.1 /paper. As 1 mL of first slurry contains 0.05 g of finger millet malt flour, the number of bacteria was calculated as $(6.8 \pm 1.6) \times 10^7$ cfu/g of finger millet malt. Values are presented as mean \pm SD. Although we did not identify the species of bacteria in this study, lactic acid bacteria should most likely grow and dominate the growing bacteria, because the pH of the togwa decreases with maturation. That is, lactic acid fermentation lowers the pH of togwa and reduces the contamination with pathogenic bacteria (9). The number of bacteria in the refined maize flour obtained in Japan was 1.9×10^3 spc, which is remarkably smaller than that of the finger millet malt.

Chemical Composition of Togwa. Table 1 shows the composition of lyophilized togwa powder and liquid togwa. In

 Table 2. Trace Elements in Laboratory Togwa, Field Togwa, Maize Flour, and Finger Millet Malt^a

	lab togwa ^b	field togwa	maize flour	finger millet malt
Са	72.2	71.9	3.1	282.7
Fe	8.5	5.7	3.8	33.8
Κ	122.3	96.5	41.6	214.2
Mg	67.3	59.6	11.3	124.0
Mn	10.1	10.4	0.2	33.0
Na	0.2	19.7 ^c	0.1	0.3
Р	152.9	126.7	42.6	264.9
Si	0.4	1.0	0.1	1.0
Zn	1.2	0.9	0.4	2.0

^{*a*} All values are means of duplicate analysis and expressed in milligrams per 100 g of sample. Samples were treated with wet incineration and analyzed using the ICP emission photometer analyzer (IRIS Advantage ICAP). ^{*b*} Lyophilized and powdered samples were used. ^{*c*} Sodium from sodium azide added to the sample for preservation at the field is included.

field togwa (made by three women), the water contents were 79-85%. Difference in the water contents would be due to the difference of the preparation conditions among individuals. The women at the rural villedge in southern Tanzania prepared togwa without weighing the materials on scales. We believe their quantitative and reproducible procedure to prepare togwa was sufficiently accurate.

Carbohydrate contents in the field togwa and the laboratory togwa were 12.5 and 14.3%, respectively. The energy value of the field and laboratory togwas, which was calculated by using Atwater's constant, were 56.1 and 64.8 kcal/100 g, respectively. From these results, we considered that there is no difference between the field and laboratory togwas.

Trace Elements in Togwas, Maize Flour, and Finger Millet Malt Flour. We selected nine elements to quantify from the results of qualitative analysis as mentioned under Materials and Methods. Table 2 shows the results of the trace element analysis of togwa and starting materials, that is, maize flour and finger millet malt. The finger millet malt we used was domestically sprouted and floured at the village in southern Tanzania. Finger millet malt flour contained a much larger amount of minerals than maize flour and was especially rich in calcium, potassium, and phosphorus.

The calcium content in finger millet is much higher than in many other cereal grains (16). Our result of calcium content in finger millet malt is lower than that reported by Barbeau and Hilu (16). Lyophilized togwa powder contains 72 mg of Ca/ 100 g of togwa powder (**Table 2**), and liquid togwa had ~ 10 mg of Ca/100 g of liquid togwa. The iron content of finger millet is less than that of other cereals (16, 17). However, the iron content of finger millet malt flour was higher (34 mg of Fe/100 g) than that of finger millet grains. The iron content of liquid togwa was calculated at \sim 5 mg/100 g of liquid togwa (Table 2). As we collected the finger millet sample malted in the field of southern Tanzania by their own people's procedure in a rural village (4), some kinds of minerals in the sample including iron were contaminated from the soil. Therefore, the iron content of liquid togwa would be higher than expected from finger millets. The inhabitants of this village seem to take in iron from the soil through togwa. Sodium was detected only in the field togwa sample; this was due to the sodium azide added to the togwa sample collected in the field to prevent the bacteria growth during delivery to the laboratory (Table 2).

Amino Acid Composition of the Proteins in Togwa, Maize Flour, Finger Millet Malt Flour, and Finger Millet Flour. The amino acid contents in togwa and the starting materials

 Table 3. Amino Acid Contents in Laboratory Togwa, Maize Flour,

 Finger Millet Malt, and Finger Millet Grain^a

	lab togwa ^b	maize flour	finger millet malt	finger millet grain
Asp	362 ± 34	330 ± 43	648 ± 250	431 ± 157
Thr	207 ± 21	182 ± 26	396 ± 105	227 ± 80
Ser	278 ± 33	250 ± 41	501 ± 119	269 ± 80
Glu	1144 ± 103	994 ± 207	2037 ± 430	1013 ± 249
Gly	211 ± 25	192 ± 30	407 ± 223	290 ± 110
Ala	449 ± 46	400 ± 78	621 ± 174	354 ± 113
Val	268 ± 19	236 ± 29	548 ± 109	281 ± 99
Met	85 ± 11	66 ± 15	179 ± 24	88 ± 41
lle	186 ± 15	166 ± 17	365 ± 58	184 ± 72
Leu	648 ± 64	603 ± 86	833 ± 116	397 ± 151
Tyr	35 ± 16	42 ± 19	55 ± 16	31 ± 26
Phe	214 ± 25	198 ± 27	375 ± 23	180 ± 91
Lys	147 ± 13	141 ± 23	308 ± 206	251 ± 134
His	182 ± 12	174 ± 35	275 ± 110	161 ± 58
Arg	176 ± 34	161 ± 8	256 ± 50	227 ± 146
Pro	464 ± 68	481 ± 78	438 ± 9	257 ^c

^{*a*} Amino acid analysis was performed by an amino acid analyzer. Values are means \pm SD of triplicate analyses except for Pro in finger millet grain. ^{*b*} Lyophilized togwa was used for amino acid analyses. ^{*c*} Results of single analysis.

are shown in **Table 3**. The values for Trp and Cys are not shown in **Table 3** as they were degraded during hydrolysis. The content of amino acid in finger millet malt is greater than that in finger millet grain (**Table 3**). Finger millet was rich in Leu and Glu, whereas Tyr content was very low in all samples. These results are not in accordance with the reported results (16, 17). Barbeau and Hilu (16) reported that the protein content varies between 7.5 and 11.7% among the cultivars they estimated. The finger millet malt was purchased from the market in Mbinga, Ruvuma, Tanzania, which would be a mixture of different varieties with the composition of the grain possibly varying depending on the seasons.

Togwa is a liquid containing many small particles and is used as a beverage without filtration. As we noted, the main component of togwa is carbohydrate, and it has high energy density. Togwa is easier to consume than a thick porridge, and the hydrolyzed and liquefied starch in togwa is digestible. Nevertheless, togwa seems to contain a certain amount of starch and dextrin. Furthermore, finger millet malt enriches the mineral and amino acid contents in togwa. Togwa is undoubtedly an important source of energy and nutrients for the people in this area. Moreover, from a food safety point of view, lactic acid produced during fermentation lowers the pH of togwa and suppresses the growth of pathogenic bacteria (8).

Recently, the benefits of togwa were reevaluated in Tanzania. The Ministry of Food and Health of Tanzania published leaflets promoting the usage of togwa as a weaning food, showing the recommendation to take one cup of togwa for each child at a meal (*Matumizi ya togwa katika kulikiza*). Nowadays, many kinds of so-called health drinks are commercially available, particularly in the developed countries. Those drinks are produced from a variety of ingredients to accomplish a nutritional purpose. Togwa is not a fabricated drink, but it appears to be a very indigenous and traditionally successful healthy one.

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