Rheological Properties and Sugar Profile of a Maize-Based Complementary Food for Ugandan Children 12 to 23 Months of Age

Joel Tumwebaze¹, Peter Gichuhi¹, Vijay Rangari², Alfred Tcherbi-Narieh²,
Adelia Bovell-Benjamin^{1,*}

¹Department of Food and Nutritional Sciences, Tuskegee University, Tuskegee AL, USA

²Department of Materials Science and Engineering, Tuskegee University, Tuskegee AL, USA

Email address:

JTeomwebaze0414@mytu.tuskegee.edu (J. Tumwebaze), pgichuhi@mytu.tuskegee.edu (P. Gichuhi),
rangariv@mytu.tuskegee.edu (V. Rangari), atcherbi-narteh@mytu.tuskegee.edu (A. Tcherbi-Narieh),
acbenjamin@mytu.tuskegee.edu (A. Bovell-Benjamin)

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Abstract: In this study, the rheological properties and sugar profiles of a maize-based (maize flour, sweet potato leaf and root flour) complementary food for 12 to 23 month-old Ugandan children were evaluated. Two foods were formulated. Treatment 1 (T1) was made of 70% maize meal, 15% sweet potato root flour and 15% sweet potato leaf flour, while Treatment 2 (T2) consisted of 70% maize meal, 20% sweetpotato root flour and 10% sweetpotato leaf flour. Both formulations had equal amounts of sugar, oil and added water. The viscosity of the complementary foods was determined at 45°C by two different methods (rheometer and line spread test). The viscosity decreased with increasing temperature for all the samples. The viscosity at 45°C for the Control, T1 and T2 were 1.35, 1.96 and 0.86 Pa, respectively. There were significantly ($P<0.05$) different line spread measurements among the samples; however, the control sample flowed the greatest distance. The highest level of sugars was found in T2 (5.1 ± 2.7 g/100g) versus the control, which had the least amount of total sugar (2.9 ± 1.6 g/100g). Supplementation of a maize-based complementary food with sweetpotato root and sweet potato leaf flours improves the viscosity and sugar profile.

Keywords: Viscosity, Sugar Profile, Total Sugar, Complementary Food

1. Introduction

Complementary foods should be added to the diets of children when breast milk is inadequate to meet their nutritional needs. The changeover from exclusive breastfeeding to family foods is referred to as complementary feeding. Complementary feeding, which usually covers the period from 6 to 24 months of age is a very vulnerable period. It is the time when malnutrition begins in many infants, contributing significantly to the high prevalence of malnutrition in children less than five years of age globally. Globally, 161 million under-five year olds were estimated to be chronically malnourished or stunted in 2013 [1]. Roughly 33% of these children reside in Africa [1].

The World Health Organization (WHO) recommends the use of local staples for complementary feeding, as these are

most likely to be available, easy to prepare from family foods, and more affordable [2]. However, most staple-based complementary foods in developing countries are starchy [3-4]. Starchy foods form a highly viscous porridge on cooking and this necessitates dilution with large volumes of water for effective infant feeding [5-6]. The over-dilution, leads to a watery, reduced energy and nutrient food, which is generally referred to as nutrient thinning [5, 7]. Nutrient thinning is one of the major causes of poor growth during the weaning period [8]. Apart from nutrient thinning, the sugar content of complementary foods also affects its rheological properties such as viscosity.

A number of studies have focused on the increase in energy density and nutritional value of complementary foods in

developing countries [9-12]. Some studies suggest that energy value can be improved by increasing the amount of flour but, generally, an increase in flour concentration leads to production of very thick complementary foods on cooking [13-16]. Highly viscous foods are not usually acceptable to children [17]. It follows therefore that a child has to consume a lot of watery food to meet the daily nutrient requirement. However, infants' stomachs are too small (30 to 40 mL per kg) to accommodate large volumes of food [18-19].

In developing complementary foods, strategies to improve the consistency and energy density include; i) modification of the dry matter composition by malting and fermentation [13]; ii) addition of sugar and oil [20]; iii) chemical and enzymatic starch modification; and iv) extrusion cooking [21]. To improve the energy density of complementary foods, studies that relate energy density with consistency have to be done [22]. The viscosity of complementary foods depends on factors such as, complementary food temperature, shear time, shear rate, dry matter concentration, pH and ionic strength, and the viscosity measurement systems [23-25].

The rheological properties of complementary foods maybe assessed using either instrumental or sensory methods. Instrumental methods involve a number of parameters whereas sensory parameters are based on visual and tactile perception prior or during consumption [25]. A line spread test that measures the distance a food/ beverage flows across a flat surface can also be used to determine the thickness of a food or beverage [26]. The line spread test is a simple test, which could be used for training caregivers to ensure that infants receive foods of the right consistency. Line spread measures are advantageous over rheometers and viscometers in that they are inexpensive and more practical, therefore they can be used at the household level [27-28].

Besides viscosity, the level of sweetness (sugar content) of complementary foods determines their acceptability and quantity consumed [29-30]. Sugars are mostly used in pastries, juices and drinks where they impart different attributes including, color (Maillard reaction), flavor, mouthfeel, leavening capacity, sweetness, stability and moisture retention [31-32]. In reference to complementary feeding, children are born with an innate preference for sweet tastes [33]. The fetus responds to sweet tastes from the amniotic fluid as early as 6 weeks and it has been shown that injection of sweet fluids increase fetal swallowing while sour fluids decrease swallowing [34-39]. At birth, most infants are fed breast milk that contains the milk sugar lactose [40-41]. The possibility exists that sweeter complementary foods will be more acceptable to children (sweeter does not always mean too much sugar that would possibly pose a health risk). Other than breast milk, studies reported that infants consumed more cereal-based porridge when sweetened [42]. Additionally, the sugar content of complementary foods has an effect on their viscosity as reported by [43]. The objective of this study was to evaluate the rheological properties and sugar profile of a maize-based (maize flour, sweet potato leaf and root flour) complementary food for 12 to 23 month-old Ugandan children.

2. Materials and Methods

2.1. Flour Formulations

Fresh Whatley/Loretan sweet potato [*Ipomoea batatas* (L.) Lam] roots from the George Washington Carver Agricultural Experiment Station, Tuskegee University were processed into flour. The roots were trimmed, weighed, hand washed and drained on a rack in open air for 30 minutes, as proposed by [44]. The first four leaves on a vine of Whatley/Loretan cultivar sweet potato were picked, sorted, weighed, washed and blanched. This was followed by draining, immersion in an ice bath for 10 minutes, drained again, and then dehydrated in a mechanical convection incubator (Thermo Fisher Scientific, Waltham, MA) at 65°C until brittle. The dried leaves were cooled, milled, weighed and stored at -14°C until use.

Four flour formulations were prepared from the processed sweet potato root and leaf flours, organic white maize (*Zea mays*) meal, oil and sugar. The maize meal was sourced from Hodgson Mill Inc. (Effingham, IL) whereas maize oil and sugar were purchased from a local grocery store. Several combinations represented in Table 1 were developed using Microsoft Excel 2011 predictive statistics with the intent of selecting those that provided as close to the recommended dietary allowance of protein, energy and β -carotene for children 12 to 23 months-old [45].

Table 1. Various Prototype Combinations of the Complementary Food.

Energy and Nutrients	Control*	Treatment 1 (T1)	Treatment 2 (T2)
Energy	463.8 kcal	503.0 kcal	502.8 kcal
Protein	18.3 g	21.3 g	20.2 g
Fat	4.5 g	9.9 g	9.7 g
Carbohydrate	257.9	249.7	251.1
Dietary fiber	10.0 g	21.4 g	19.8 g
PUFA	0.0 g	3.1 g	3.1 g
Vitamin A	5.0 μ g	3.8 μ g	3.8 μ g
Carotene	0.0 mg	1.6 mg	1.8 mg
Sodium	0.0 mg	1.2 mg	1.0 mg
Potassium	718.7 mg	505.0 mg	504.8 mg
Phosphorus	525.0 mg	422.8 mg	425.8 mg
Iron	3.7 mg	8.9 mg	8.2 mg
Zinc	5.5 mg	4.1 mg	4.1 mg
Total Vitamin A	5.0 μ gRAE*	137.1 μ gRAE	153.8 μ gRAE

*Traditional complementary food

*RAE refers to Retinol Activity Equivalent

2.2. Complementary Food Production

Several cooking trials were made using the formulations treatment 1 (T1) and treatment 2 (T2) by varying water, cooking-temperature and time to come up with a complementary food of the right consistency, and flavor. For each trial, T1 and T2 were dissolved in known quantities of cold water to form a paste with no lumps, poured in a known

quantity of boiling water and stirred continuously while cooking. The consistency was determined by cooling the porridge to approximately 45°C on a plate, tilting the plate to one side to observe the ease of flow.

The acceptable consistency was obtained when the food moved down slightly without running down the plate. Another test for consistency was done by pouring a small quantity of the cooled food (approximately 45°C) into water at room temperature (22±2°C), observing the time it took for the lump to dislodge. Once the required consistency was obtained, temperature and time were varied until standard cooking conditions were set.

2.3. Line Spread Test for Viscosity

A line spread test was used to objectively evaluate the viscosity based on the spreadability of the complementary foods. The viscosity of porridges; that is, the control, T1 and T2 was measured using a line spread method modified from [26]. Under this method, concentric circles of radius 2.5 to 7.5 cm and 0.5 cm apart were drawn on a hard clear plastic surface. Lines radiating from the center to intersect the circles were drawn to divide the circles into four equal quadrants at 90° intervals. For each sample, the porridge at 45°C was held in a hollow cylinder of height 3.5 cm and 5 cm diameter positioned at the center of the concentric circles for a setting time of 30 seconds. To ensure that equal volume of porridges were dispensed in the cylinder for each sample and replicate, the cylinders were overfilled and leveled out with a metal spatula. After 30 seconds of sitting, the cylinder was lifted off the plastic to allow the sample spread for 60 seconds. For each sample 10 measurements were done for which the mean measurement in centimeters averaged across bisecting lines at four quadrants represented the degree of thickness of each porridge.

2.4. Instrumental Viscosity Measurement

The viscosity for the three porridge samples was also determined in triplicate using the AR-2000 rheometer (TA Instruments, New Castle, Delaware). The porridge samples were applied to the lower plate and the rheometer calibrated to 1000 µm minimum gap space. Excess porridge sample that protruded from the plates was scrapped off. To determine how viscosity changes with temperature, the rheometer was set to heat the porridge from 25°C to 65°C and values of viscosity were recorded. The change in viscosity with increasing time at a constant temperature was also measured. For this measure, the porridge at 25°C was heated up to 45°C and held for 20 minutes. The change in viscosity with time at constant temperature (45°C) was recorded.

2.5. Total Sugar Analysis

The assay for total sugars was done using a modified Sucrose, D-Fructose and D-Glucose Assay kit with a detection limit of 1.38 mg/L and following the manufacturer's procedure (Megazyme International, Wicklow, Ireland).

2.5.1. Sample Clarification

Carrez I solution was prepared by dissolving 3.6 g potassium hexacyanoferrate (II) trihydrate {K₄ [Fe (CN)₆].3H₂O} supplied by Sigma-Aldrich (Missouri, USA) in 100 mL of distilled water and stored in a closed glass bottle at room temperature (22 ± 3°C). Carrez II solution was made by dissolving 7.2 g Zinc sulfate heptahydrate (ZnSO₄.7H₂O) in 100 mL distilled water and stored in a glass bottle at room temperature (22 ± 3°C). Zinc sulfate heptahydrate was supplied by Sigma-Aldrich (Missouri, USA). Sodium hydroxide (100 mM) was prepared by dissolving 4 g of NaOH in 1L distilled water and stored at room temperature (22 ± 3°C).

2.5.2. Clarification

For each sample (control, T1 and T2), 1 g was weighed into each of the three 100 mL volumetric flask. To each volumetric flask, 60 mL distilled water was added and incubated at 70°C for 15 minutes in a water bath with occasional shaking after every five minutes. To clarify the samples, 5 mL Carrez I solution, 5 mL of Carrez II and 10 mL NaOH prepared in step 1, were added to each volumetric flask and mixed after addition of solution. The contents of the volumetric flask were cooled to room temperature (22 ± 2°C) after which distilled water was added to the mark. The volumetric flasks were sealed with parafilm to enable thorough mixing. The clarified samples were vacuum filtered using a Buchner funnel and Whatman filter paper no.2. (Sigma Aldrich, Missouri, USA) to obtain a clear filtrate for the assay. All samples including the control, T1 and T2 were prepared in triplicate.

2.5.3. Total Sugar Assay

For each sample (control, T1 and T2) four plastic cuvettes of 1 cm light path were set in order of; blank sucrose sample, Sucrose sample, Blank D-glucose/ D-fructose sample and D-glucose/D-fructose sample. All reagents in the assay were part of the Sucrose, D-Fructose and D-Glucose Assay kit supplied by Megazyme International (Wicklow, Ireland). Absorbance was read at 340 nm using a Shimadzu UV-1201 UV-VIS Spectrophotometer (Shimadzu, Kyoto, Japan).

Step 1: β-fructosidase (0.2 mL) was pipetted into the blank sucrose sample and sucrose sample cuvettes. To the sucrose sample and D-glucose/D-fructose sample cuvettes, 0.1 mL sample solution was pipetted, vortexed for 1 minute and thereafter incubated for 5 minutes. After 5 minutes, 2, 1.9, 2.2 and 2.1 mL distilled water were added to the blank sucrose, D-glucose/ D-fructose, and D-glucose/D-fructose samples, respectively. To each of the four cuvettes 0.1 mL buffer solution and 0.1 mL NADP⁺/ATP were added, vortexed for 30 seconds and after 3 minutes of incubation, the absorbance (A1) was read and recorded.

Step 2: Hexokinase/glucose-6-phosphate dehydrogenase (0.02 mL) was pipetted into each of the four cuvettes, vortexed for 30 seconds and the absorbance (A2) read after 5 minutes of incubation.

Step 3: Phosphoglucose isomerase (0.02 mL) was pipetted into the blank and sample cuvettes D-glucose/ D-fructose and vortexed for 30 seconds. The absorbance (A3) was read after

incubation for 10 minutes. All sample analysis was done in triplicate.

Calculation of total sugars: The concentration of total sugars were calculated from the absorbance differences (A2-A1) and (A3-A1) for both blanks and samples. From the absorbance differences, the changes in absorbance due to D-glucose, D-fructose and Sucrose were obtained and used to calculate the concentration of D-glucose, sucrose and D-fructose.

2.6. Statistical Analysis

Data for rheological measures was analyzed using Analysis of Variance (ANOVA) to compare: i) how viscosity changes with time among the control, T1 and T2; (ii) the viscosity at 45°C for the three formulations; and (iii) how viscosity changes at a 45°C with change in time for the three formulations. Pearson's correlation was used to determine the relationship between the viscosity at 45°C of the line spread test and the rheometer. Fisher's LSD was used to determine where the differences exist using a 95% confidence interval.

3. Results

3.1. Line Spread Measures

Line spread measurements showed that the control food flowed the greatest distance followed by T1 then T2 (Figure 1). Results showed significantly ($P < 0.05$) different line spread measurements among the samples.

Figure 1. Line Spread Test for the Maize-based Complementary Food.

3.2. Viscosity and Temperature

Viscosity measures were determined on heating the porridges from 30°C to 65°C. The viscosity decreased with increasing temperature for all the samples as shown in Figure 2. However at all temperatures, the viscosity of T1 remained higher than the other two samples. The viscosity at 45°C for the Control, T1 and T2 were 1.35, 1.96 and 0.86 pascals per second [Pa.s], respectively.

Figure 2. Flow Curve for the Maize-based Complementary Food.

3.3. Viscosity, Temperature and Time

Changes in viscosity after holding the porridge samples at 45°C for 20 minutes were determined. This part of the study mimicked the changes in viscosity on storage of the porridge in a thermos for later use. Generally, the viscosity decreased slightly with increased time for all the three porridge samples from 11 to 20 minutes of holding (Figure 3). The point decline in viscosity was highest in T2, followed by T1 and the Control with values of 1.2, 0.85 and 0.4 pascals-second, respectively.

Figure 3. Flow Curve for the Maize-based Complementary Food.

3.4. Line Spread and Rheometer Relationship

The correlation coefficient for viscosity measurements at 45°C between the line spread and rheometer was significantly different ($P \leq 0.05$) for the three samples. Based on the results, there was a strong relationship between the line spread test and the rheometer measurement ($r = 0.8$) for the control sample, meaning both tests yielded similar results. Treatment 1 showed a moderate, positive correlation ($r = 0.4$) between the measurement methods. Treatment 2 displayed a very weak positive correlation ($r = 0.1$) between the line spread and rheometer.

3.5. Sugar Profile

According to the findings, the total sugar content was significantly different ($P \leq 0.05$) for each sample (Table 2). The highest level of sugars (g/100 g sample?) was found in T2 (5.1 ± 2.7) versus the control, which had the least amount of total sugar (2.9 ± 1.6). For all samples, sucrose was the predominant sugar, while fructose was absent in the control (Table 2). According to our results T2 had significantly ($P \leq 0.05$) higher amounts of glucose and fructose than the control and T1. The control and T1 had equal amounts of glucose.

Table 2. *D-Glucose, Sucrose and D-Fructose Contents of Complementary Foods.*

Sample	Sugar Concentration (g/100 g sample)			
	D-Glucose	Sucrose	D-Fructose	Total Sugars
Control	0.1±0.03a	2.8±0.1a	0.0±0.0a	2.9±1.6a
Treatment1	0.1±0.03a	4.2±0.3b	0.1±0.02b	4.4±2.4b
Treatment2	0.2±0.1b	4.8±0.3b	0.2±0.04c	5.1±2.7c

Numbers in each column with the same letters are not significantly different from each other

4. Discussion

4.1. Line Spread Measures

Lower line spread values reflect more viscous products whereas higher mean values reflect less viscous products. Based on the results of the current study, at 45°C, the control and Treatment 1 samples had viscosities within the range recommended for infant and young child feeding (1 to 3 Pascal-Second) [17]. The viscosity at 45°C was of interest because it is the temperature at which viscosity measurements for complementary foods are taken [46].

Studies show that complementary foods prepared from cereals alone always have a high viscosity unless modified by substitution with less starchy foods, extrusion, fermentation or enzymatic action [5, 7, 47]. Orange-fleshed sweet potato flour and maize flour have approximately the same starch content, starch particle size and amylose content [48-50]. However, according to [5, 51], sweet potato-based formulations have lower starch content and higher sugar content, and therefore a lower apparent viscosity at 45°C.

In the current study, Treatments 1 and 2 had sweetpotato root flour and higher sugar concentrations. The presence of more total sugars in Treatments 1 and 2 could have been responsible for the lower viscosities observed. Treatment 2 had more sugar and less starch than any of the other samples, which could be responsible for its very poor viscosity at 45°C. The effect of sugar on thermal and physical properties of starch-based foods was studied by other researchers [43, 52]. Having more water in the control when compared to Treatment 1 may have interfered with the formation of a thick gel when the starchy food was heated, hence a reduced viscosity [16]. Consequently, having less water than the Control, the viscosity of T1 was within the range recommended for complementary feeding (1 to 3 Pascal-Second)[17].

4.2. Viscosity, Time, Shear Rate and Temperature

Changes in viscosity after holding the porridge samples at 45°C for 20 minutes were determined. This part of the study mimics the changes in viscosity on storage of the porridge in a thermos flask for later use. However, data on holding the samples from 0 to 10 minutes was excluded in the analysis because of temperature fluctuations within the system. Generally, for all the samples, viscosity decreased slightly with increase in time (between 11 and 20 minutes of holding). It is speculated that the decline in viscosity with time at

constant temperature and shear rate, that is thixotropy, was due to the progressive breakdown of forces holding suspended particles under a given shear stress, resulting in reduction in particle size of structural particles, which offer lower resistance to flow during shear [53].

As the number of disrupted inter-particle bonds increases, the viscosity drops [24]. After some time the number of breakdown of particles is similar to aggregation and a straight curve is obtained [54]. At low concentration of the sample, thixotropy is almost non-existent as in the case of the control, which was more diluted with water compared to T1 and T2. Thixotropic behavior is exhibited more at higher temperatures of holding [55]. Our findings are consistent with those reported by Abu-Jdayil[55] as both studies were held at 45°C.

4.3. Line Spread and Rheometer Measures

The relationship between the line spread and rheometer measure at 45°C was assessed for its significance as well as its strength. The correlation coefficient for viscosity measurements at 45°C was significant ($P \leq 0.05$) for the three samples. This could imply that the line spread test could be used to test the consistency of complementary foods at a similar level of accuracy as one using a rheometer. As a rheometer is more expensive and requires highly technical skills for operation, a line spread test would be a better alternative for training caregivers in developing countries.

4.4. Sugar Content Analysis

During the formulation of the complementary mixes, all samples had 12 g sucrose added therefore the difference in sugar concentration after analysis is attributed to endogenous sugars. Since the concentration of sucrose varied considerably among samples, it was assumed that sucrose contributed largely to the total sugars of the samples. Results from this study are consistent with those reported by other researchers [56]. According to these authors, sucrose was the major sugar in six sweetpotato cultivars. The control had only maize meal whereas Treatments 1 and 2 had sweetpotato leaf and root flour in addition to maize meal. This could imply that maize meal formulation had less endogenous sugars than the other two ingredients.

The low sugar content in the control could also be due to the additional water that was added to bring the viscosity to within the recommended range for complementary feeding. The difference in sugar content between Treatment 1 and 2 could be due to the difference in quantity of sweetpotato root flour. T2 had 25% more sweetpotato root flour than T1. Orange-fleshed sweet potato root flour has been reported to contain endogenous sugars that improved the sweetness of complementary foods [5]. Sweet potato roots were found to have a total sugar content ranging between 2.2 and 2.8 g/100 g [57]. Studies show that foods with more sugar were more accepted for complementary feeding [29, 58]. Children are born with an innate preference for sweet taste; sweetness is an important determinant of food preferences [33]. It is therefore highly likely that children will prefer sweeter complementary

foods. However, complementary foods with higher sugar contents are more likely to be less the viscosity [43]. Although T2 had the highest amount of sugar, its viscosity was unacceptable for complementary feeding [17]. However, it was noted that the level of sweetness after cooking of carbohydrate foods was not determined by the level of sucrose but by the enzymatic conversion of starch to maltose [59-60]. Contradictory to the previous statement, [61] reported that the sugar composition of a cultivar, especially the sucrose values gives a reliable indication of its sweetness. It is therefore recommended that tests of acceptability should be carried out along with total sugar analysis tests for complementary foods supplemented with sweet potato root flour.

5. Conclusion

Using the line spread test, the viscosity at 45°C increased from the control, Treatment 1 to Treatment 2. Rheometric determinations however showed that at 45°C, Treatment 1 had the highest viscosity and the least was recorded in Treatment 2. The viscosity at 45°C also showed that Treatment 1 and the control had viscosities within the range recommended for complementary feeding. Viscosities for all the three formulations decreased when temperatures were increased from 30 to 65°C. Pearson's correlation showed that the rheometric and line spread test viscosity determinations were highly correlated for the control and Treatment 1. The correlation coefficient declined as the viscosity decreased implying that the line spread test may not be a useful tool for highly viscous foods. The line spread test could be a practical, feasible option for training caregivers in developing countries to measure the viscosity of complementary foods, especially because the rheometer is quite expensive and requires highly technical skills for operation.

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