IJABR Vol. 11(1): 90 - 100 (2020)

Original article

MODIFICATION OF CEREAL BIOPOLYMER BY FERMENTATION AND PREGELATINIZATION AND ITS IMPACT ON COOLING, HARDENING PATTERN AND ACCEPTABILITY OF MAIZE *TUWO*

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Submitted: May, 2020; Accepted: May, 2020; Published: June, 2020

ABSTRACT

Tuwo is a stiff porridge produced from cereal grains and consumed in different forms across Africa and Asia ranging from products with neutral, acidic or alkaline pH. In all these, the keeping quality is of major importance as *tuwo* may be stored overnight, reheated and eaten as *dumame* as referred to by the Hausas of West Africa. Pregelatinization and fermentation of cereal biopolymer result in the modification of the crystalline structure of the biopolymer leading to other changes in the properties of the final product. In his study, modified maize flour was produced and used to prepare *tuwo* and its hardening pattern evaluated. The cooling time of *tuwo* and index of hardness were positively correlated ($r < 0.9$) and fit well into a linear polynomial curve. The hardness of *tuwo* at any temperature can be estimated from the linear equation with good accuracy. Also, the overall acceptability of the *tuwo* was not significantly ($p > 0.05$) affected either pregelatinization or fermentation indicating that modified cereal flours can be well accepted to the consumer.

Keywords: ***Tuwo*, Hardness, Starch Modification, Fermentation, Pregelatinization**

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INTRODUCTION

Tuwo is a stiff porridge produced from cereal grains mainly from sorghum, millet, maize, wheat and rice and also from some other small grains like Fonio (Acha). Different forms of *tuwo* are consumed in different parts of Africa and Asia ranging from products with neutral, acidic or alkaline pH and given different local and regional names like *sangati* (in India), *ugali* (in Kenya, Uganda and Tanzania), *umqo* (in south Africa), *boule* (Mauritania), *tô* (in Burkina Faso and Mali), and *Tuwo* (in Nigeria, Niger, Cameroon, Chad, and Ghana) [1, 2]. The most important qualities of *tuwo* desired by consumers are its thickness and firm texture, non-stickiness and keeping quality. The pH of the medium used in the preparation affects *tuwo* quality [3]. *Tuwo* made in acidic medium is generally firmer in texture and lighter in colour than that made in alkaline medium [4]. Laboratory evaluation of a range of *tuwo* samples produced using a similar set of grain indicated that grains with a highly corneous endosperm and good dehulling qualities are suitable for preparation of thick porridges [5]. Normally grains with floury or soft endosperm texture produce *tuwo* with sticky and mushy texture. The keeping quality is of major importance, because *tuwo* may be stored overnight, reheated and eaten as *dumame*. The actual taste of *tuwo* is usually masked by the soup with which it is eaten and thus might be of little quality importance. The quality and functionality of the *Tuwo* vary from one community to another, and even within a given community, quality and functionality may vary from one household to another [6]. There is thus a need for quality specification from the raw materials, process conditions and the final product characteristics.

In starchy foods, fermentation is reported to modify crystalline structure of the amylose leading to reduced gelling and other functional properties of the starch [7] resulting in final product of thin consistency. *Tuwo* produced from such flours exhibits modified physical properties including variations in the cohesiveness and softness indexes of maize *tuwo* due to fermentation [8]. The study of the effects of grain fermentation on the cooling and hardening pattern of *tuwo* and its acceptability thus forms a vital part of the overall efforts towards raw material specification and product quality standardization. Modification of cereal starches is done to alter the properties of the native starches in order to make them more suitable ingredient for use in different food products. Several methods have been developed to produce modified starches with a variety of characteristics and applications including fermentation [9], thermal modification [10] and chemical methods [11]. All of these techniques alter the starch polymer, making it highly flexible and changing its physicochemical properties and structural attributes to increase its value for food and non-food industries [12]. Modifications of starch can be achieved by physical, chemical and enzymatic methods [13]. Physical methods involve the use of heat and moisture, and chemical modifications include etherification, esterification, crosslinking, hydrolysis and oxidation [14]. Commonly used modified starches in food applications include oxidized starches, cross-linked starches, starch phosphates, starch acetates and pregelatinized starch (PGS). Pregelatinized starches are native starches that are subjected to precooking, followed by drying, resulting in complete granular fragmentation and the loss of birefringence. Properties of PGS important in food processing include increase in swelling capacity, solubility and cold water dispersion. In addition, a greater weight of PGS is required to produce a given viscosity than the weight native starch needed to get the same viscosity. One of the largest uses of PGS is in instant pudding packaged powders that offers uniform powder consistency and reduced pudding preparation time. These properties of PGS, when applied in traditional foods preparation, can impart beneficial changes in the physical and sensory attributes of the final product; especially that the preparation of most traditional cereal-based foods involve heating of cereal biopolymer in the presence of water and then cooling to variable degrees before consumption. The unique character of many of these foods results from the changes that starch undergoes during heating and subsequently cooling processes especially stiff porridges like *tuwo*. The cooling process is important as it determines the final eating texture of the product. Upon cooling the gelatinized starch undergo retrogradation and losses moisture which reduce the stickiness of the product and increase the *tuwo* hardness. The desired final *tuwo* hardness determines the water: flour ratio to be mixed during production, taking into consideration the moisture loss during cooling and the subsequent texture modification. However, research on the cooling behavior of *tuwo* was not given enough attention probably due to fact that *tuwo* preparation is still artisanal and yet to be industrialized. The aim of this work is therefore to assess the impact of fermentation and pregelatinization on the cooling and hardening pattern of maize *tuwo* and to evaluate its consumer rating.

MATERIALS AND METHODS

Cereal Grains: Maize sample (34) was collected from the Institute of Agricultural Research (IAR), Zaria.

Sample Formulation: Three flour samples were produced from the maize grains with native (unmodified) flour sample as the control against which pregelatinized and fermented maize flours were compared. The notation of the three samples is such that the first letter represents the Grain (M = Maize), the second letter represents the biopolymer modification method (C = Control, P = Pregelatinization, F = Fermentation), while the third letter N stands for normal *tuwo* preparation procedure. Thus sample MFN, for example means the sample is of fermented maize grain in normal *tuwo* preparation, MPN stands for sample of pregelatinized maize in normal *tuwo* preparation, while MCN is the control maize sample.

Sample Preparation

Raw Flour: Sample grain was first tempered with water using a quantity of 3 - 4% (v/w) followed by decortication of the grains in commercial dehulling machine (previously cleaned), where the germs and hulls of the grains were removed. The decorticated grains were aspirated manually to remove adhering hulls and then ground into flour using a Laboratory Hammer mill. The sample flour thus obtained was sieved using a standard sieve with 300μm aperture and then kept in airtight polythene bags until needed.

Fermentation-Modified Flours: Natural fermentation was carried out at the prevailing ambient temperature (34 ± 1^0c) for 48 hours. Fermentation water was being discarded after every 12 hours to avoid undesirable microbial succession and the development of putrid odor. The fermented grains were decanted and then spread on a canvass material for drying. The dried grains were then ground into flour using a Laboratory Hammer mill. The fermented flour thus obtained was sieved through a standard sieve with 300μm aperture and then kept in airtight polythene bags until needed.

Pregelatinized Flours: Three kilograms of decorticated and aspirated maize grains were introduced into a basin containing two litres of boiling water. The grains were then gently stirred to achieve even distribution of the grains in the water. The water was first brought to boil before adding the grains to maintain constant temperature for uniform thermal treatment and was allowed to stay in the boiling water for 10 minutes for complete gelatinization of the starch granules. The grains were then decanted and dried in a solar dryer. The dried grains were then milled in a laboratory hammer mill and sieved through a standard sieve with 300µm aperture and then kept in airtight polythene bags until needed.

***Tuwo* Preparation:** *Tuwo* was prepared by traditional method as described in literature [15]. The overall ratio of flour to water used was 1:3.5 (w/v). Cold slurry of the flour was first prepared by mixing 20% of the desired quantity of flour (1 kg) with 25% of the desired quantity of water (3.5 l). This was followed by bringing 60% of the water into boiling and the cold slurry initially prepared was added to this boiling water coupled with vigorous stirring, using a wooden stirrer to form a consistent gruel. The remaining quantity of the flour (80% of the desired total) was then added incrementally to the boiling gruel with continuous stirring so as to avoid lumps formation and to ensure a homogenous gel is obtained. The remaining quantity of water (15% of the desired total) was finally added to the formed gel, covered properly without stirring, and allowed to cook for about 5 - 7 min, after which it was stirred vigorously to ensure smoothness of the gel. The final product so obtained is the desired *tuwo*.

Measurement of Cooling Time: 10 equal quantities (30g) of each *tuwo* sample were taken immediately after preparation with the aid of a stainless-steel die to obtain uniform sample size and surface area. The die was lined with vegetable oil to avoid sticking of the *tuwo* sample on the inner surface of the die. The samples were kept in covered sample holders at room temperature (34±1^{o}C). Successive temperatures of the *tuwo* samples were recorded with a thermometer at intervals of 10 minutes for the first six readings and then twenty minutes subsequently until constant temperature is attained.

Measurement of Index of Hardness: Index of hardness was estimated from the depth of penetration data (recorded as mm of penetration) obtained by Hand Penetrometer while maintaining constant force of penetration. Ten equal quantities of each *tuwo* sample were taken immediately after preparation with the aid of a stainless steel die to obtain uniform sample size and surface area. The die was lined with vegetable oil to avoid sticking of the *tuwo* sample on the inner surface of the die. The samples were kept in covered sample holders at room temperature (34±1^{o}C). Successive index of hardness were measured using a Hand Penetrometer respectively at 10 minutes intervals for the first one hour and 20 minutes intervals subsequently until constant values were recorded.

Determination of Cooling and Hardening Pattern: The measured temperature and index of hardness data were correlated and fitted into a linear polynomial curve to enable prediction of the hardening pattern of *tuwo.*

Sensory Evaluation: A group of twelve panelists generally familiar with *tuwo* were trained for the sensory evaluation as described by [16]. A seven point hedonic scale was used to rank the degree of like and dislike of the tuwo samples by the twelve semi-trained panelists. Point seven represented like extremely and point one represented dislike extremely. Samples were coded with random numbers and presented to the assessors.

Data Analyses

The temperature and index of hardness data of the *tuwo* samples were correlated by multiple regression analysis, curve fitted by linear polynomial curve and the constants evaluated from the curves were used to predict the index of hardness between 30 to 80°C. The predicted values were used to plot the regression lines to show the impact of the modification on the cooling behavior of the samples. Sensory evaluation results were analyzed by 3-way analysis of Variance (ANOVA) and mean separation carried out by the Tukey-Kramer honestly significant difference (hsd) at $P < 0.05$. Both were done with MATLAB statistical software [MATLAB 7.12.0 (R2011a)]

RESULTS

Cooling Behavior: Tables 1 and 2 show the temperature fall recorded during the cooling process of the tuwo samples and the penetrometer readings taken during the same process respectively under the prevailing ambient conditions of 34±1°C. The two sets of data were correlated to determine the relationship between the two sets of data as the cooling behavior of the samples. Product-Moment Correlation Coefficients (r) of temperature and index of hardness data were shown in table 3 where positive correlation was observed with coefficient values > 0.9. It was observed that both the temperature and index of hardness of the samples decreases with increasing cooling time (Table 1).

Table 1: Temperature Readings (°C) with Time

Flour	Cooling Time (min)									
	0	10	20	30	40	50	70	90	110	130
Native	81.5	73.5	67	62.5	57.5	50.5	44.5	36.5	34	33.5
Fermented	79.5	73	67	61.5	56	51	45	37.5	34.5	33.5
Pregelatinized	81.5	73	66	60	55	49.5	45.5	37	34.5	34.5

Values are means of two readings

Table 2: Penetrometer Readings in mm as Index of Hardness

FLOUR	Cooling Time (min)									
	0	10	20	30	40	50	70	90	110	130
Native	15.5	14.2	11.7	9.8	9.2	7.7	6.7	5.1	4.6	5.1
Fermented	14.4	13.4	11.9	10.2	9.6	8.5	6.2	5.4	5.1	5.1
Pregelatinized	15.6	14.4	12.9	11.4	10.2	9.4	7.4	5.4	5.5	5.3

Values are means of two readings

Table 3: Product-Moment Correlation Coefficients (r) of Temperature & Hardness Index

Sample	1	2	3	4	5	6
MCN(P)	1					
MCN(T)	0.98670	1				
MPN(P)	0.99449	0.99305	1			
MPN(T)	0.98650	0.98995	0.98933	1		
MFN(P)	0.98957	0.98944	0.99820	0.98961	1	
MFN(T)	0.99061	0.99912	0.99625	0.99397	0.99362	1

(P) = Penetrometer readings, (T) Temperature readings

Curve Fitting [Index of Hardness (mm) Against Temperature (°C)]

Table 4 shows the constants for the linear regression curves fitted from the temperature and index of hardness data.

A linear polynomial curve was fitted to enable the prediction of hardness at any given temperature. The slope of the curve β and the intercept ε were evaluated, while r-square indicated the goodness of fit of the data to the curve. The values of r-square for all the samples approached 1 indicating that curves were all well fitted to the data.

Table 4: Constants for the Linear Regression Curves

FLOUR	β	E	r^2	Adjusted r^2
Native	0.2682	-6.1930	0.9736	0.9670
Fermented	0.3107	-6.5720	0.9873	0.9841
Pregelatinized	0.2360	-2.2360	0.9788	0.9735

Linear Regression Lines for the *Tuwo* Samples:

Fig. 1 show the regression lines of index of hardness against temperature for the control, pregelatinized and fermented of *tuwo* samples.

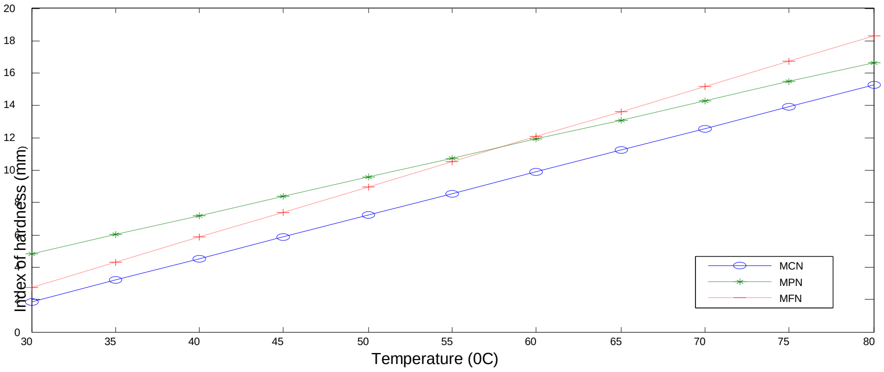

Figure 1: Regression Lines Showing the Effects of Processing Methods on the Hardening pattern of Maize *Tuwo*

Both the *tuwo* samples from the fermented and pregelatinized flours exhibited higher index of hardness than the tuwo from native flour at all temperatures. However, at low temperatures pregelatinized flour *tuwo* showed higher index of hardness than fermented sample. But curiously, at higher temperatures the reverse is the case where pregelatinized flour *tuwo* showed lower hardness index the fermented sample, while at temperatures between 55 to 60°C the two *tuwo* samples have the same hardness index.

Sensory Properties of *tuwo:* The results of the sensory evaluation of the samples are as presented in Table 5. Compared to control samples, both modification methods reduced the consumer ratings of the *tuwo*.

Table 5: Sensory Scores of Native, Fermented and Pregelatinized Maize *tuwo*

Sensory Attribute	Native Grain	Fermented Grain	Pregelatinized Grain
Colour	5.75 ± 1.13^{dg}	4.75 ± 0.97^{dg}	4.83 ± 0.72^{dg}
Aroma	5.41 ± 0.79^{dg}	5.08 ± 1.08^{dg}	4.67 ± 0.98^{dg}
Taste	5.67 ± 1.15^{dg}	4.58 ± 1.24^{fg}	4.36 ± 0.81^{eg}
Texture	6.08 ± 0.9^{dg}	5.25 ± 1.36^{fg}	4.92 ± 1.38^{eg}
Overall Acceptability	6.25 ± 0.75^{dg}	5.25 ± 1.14^{dg}	5.67 ± 1.23^{dg}

Mean values in the same row with different superscript differ significantly ($p < 0.05$)

DISCUSSION

Cooling Behavior of *Tuwo:* The index of hardness is the extent of the penetration of the penetrometer spindle into the sample it means that the harder the sample the less the penetration. Therefore the positive correlation observed between decreasing temperature and decreasing index of hardness means a negative correlation between the temperature drop and actual hardness of the sample, i.e. as the temperature of the sample decreases, its actual hardness increases. One of the most important changes that take place after *tuwo* is prepared is the hardening of the texture as the temperature drops, and a left-over *tuwo* is normally considered to be of reduced quality because of its increased hardness. Table 1 shows the temperature drop of the food samples recorded at regular time intervals. *Tuwo* from the fermented pregelatinized grain samples tend to lose temperature faster than the control sample as can be observed from table 1: after the first 30 minutes, the temperature of the modified flour tuwo samples are lower than the control. This may be due to the starch modification during fermentation and pregelatinization which reduces the flour's bulk density and water absorption capacity. This may mean that the *tuwo* from such flour exhibits less dense cellular structure leading to higher surface area for energy and mass transfer [12].

Index of Hardness: As *tuwo* is subjected to natural cooling process, moisture is being lost to the environment as starch retrogradation also takes place causing increase in the forces of attraction between the molecules leading to increase in hardness with time. Table 2 gives the penetrometer readings of the *tuwo* samples with time. The penetrometer readings recorded was observed to vary in inverse proportion to the cooling time until a constant value was attained. Hardness of cereal flour meals is attributable to the inherent associative forces within the starch molecules such as amylose/amylopectin ratio, and the level of chemical transformation during *tuwo* preparation [17]. It was reported that higher amylose content and longer amylopectin chains could contribute to the hardness of a food gel from maize and that flour preparation methods could affect the inherent associative forces within the starch molecules and by extension that of the food prepared from such flour [18]. This is seen in the differences in penetrometer readings between supplemented and non-supplemented samples.

Relations of Temperature and Hardness of *Tuwo:* The temperature and index of hardness of non-supplemented samples recorded with time all show positive correlation. That means as the temperature of the samples decreases with time so does the index of hardness. But the index of hardness is the extent of the penetration of the spindle into the tuwo sample meaning that the harder the sample the less the penetration. Therefore the positive correlation observed between decreasing temperature and decreasing index of hardness means a negative correlation between the temperature drop and actual hardness of the sample: meaning as temperature of the sample decreases, the actual hardness increases. This is attributable to several factors [18]; at lower temperatures, the forces of attraction between the molecules are higher than at elevated temperatures leading to harder texture. Starch retrogradation as an inherent property of plant starches also lead to harder textures at lower temperatures.

Curve Fitting [Index of Hardness (mm) Against Temperature (°C)]: As strong correlation was established between index of hardness and temperature, a linear polynomial curve was fitted to enable the prediction of hardness at any given temperature. The slope of the curve β and the intercept ε were evaluated, while r-square indicated the goodness of fit of the data to the curve. These constants were presented in Table 3. The values of r-square for all the samples approached zero indicating that curves were all well fitted to the data. This means that the values of hardness predicted from these constants will approximate closely to experimental data. The constants evaluated from the curves were used to predict the index of hardness between 30 to 80°C to observe the effects of grain type, processing methods and cowpea supplementation on the hardening pattern of *tuwo*.

Linear Regression Lines: Both the *tuwo* samples from the fermented and pregelatinized flours exhibited higher index of hardness than the tuwo from native flour at all temperatures (Fig. 1). However, at low temperatures pregelatinized flour *tuwo* showed higher index of hardness than fermented sample. But curiously, at higher temperatures the reverse is the case where pregelatinized flour *tuwo* showed lower hardness index than the fermented sample, while at temperatures between 55 to 60°C the two *tuwo* samples have the same hardness index. This observation may be due to…

Sensory Evaluation: Sensory evaluation data are important in market survey and product formulation [19]. Pregelatinization and fermentation did not significantly affect the color and aroma ratings of the *tuwo* due the fact that tuwo color and aroma do not matter much to consumers since both the color and aroma are associated with the grain used and the *tuwo* is normally eaten with soup. Pregelatinization has significantly reduced the taste rating of the tuwo probably due to the loss of soluble substances without generating any taste inducing substances. Fermentation, on the other hand, has significantly reduced the taste ratings probably due to flavor modification induced by the fermentation process. However, in their work on improved maize varieties, Oladeji and his co-workers [9] reported that fermentation improved all the attributes measured as fermented samples were more preferred compared to unfermented samples. Both pregelatinization and fermentation have significantly reduced the texture ratings of the samples probably due to the starch modification and increased solubility of the amylose content of the biopolymer.

The *overall acceptability* of the *tuwo* was not significantly affected either pregelatinization or fermentation indicating that modified cereal flours can be well accepted to the consumer.

CONCLUSION

The cooling rate of *tuwo* and Index of hardness are positively correlated and fit well into a linear polynomial curve. The hardness of tuwo at any temperature can be estimated from the linear equation with good accuracy. Both the tuwo samples from the fermented and pregelatinized flours exhibited higher index of hardness than the tuwo from native flour at all temperatures. However, at low temperatures pregelatinized flour *tuwo* showed higher index of hardness than fermented sample. But curiously, at higher temperatures the reverse is the case where pregelatinized flour tuwo showed lower hardness index the fermented sample, while at temperatures between 55 to 60°C the two tuwo samples have the same hardness index. Pregelatinization and fermentation did not significantly affect the colour and aroma ratings of the *tuwo* but have significantly reduced the taste and texture ratings of the sample. The overall acceptability of the *tuwo* was not significantly affected either by pregelatinization or fermentation indicating that modified cereal flours can be well accepted to the consumer.

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