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Moisture Transfer Characteristics and Microbiological Stability of Fermented Pre-gelatinized Plantain and Soybean Based Complementary Food Flours

Agbor Evelyn Agbor [a,b*], Charles Chukwuma Ariahu [a,c], Peter A. Adie [a,b] and E.C. Ariahu [d]

[a] Centre for Food Technology and Research (CEFTER), Benue State University, Makurdi, Nigeria.
[b] Department of Chemistry, Benue State University, Makurdi, Nigeria.
[c] College of Food Technology & Human Ecology, Joseph Sarwuan Tarka University- Makurdi, Benue State, Nigeria.
[d] Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Bingham University, Nasarawa, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author AEA carried out the practical work and did the write-up, Author CCA designed and supervised the work together with authors PAA, and ECA organized the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The effect of gelatinization and fermentation on the moisture sorption characteristics and microbial stability of complementary foods from unripe plantain fruits and soybean seeds was evaluated. The plantain was heated to obtain 10%, 18%, 23%, 33%, 74% and 100% starch gelatinization. The samples were fermented, dried and ground to flour. Plantain and soybean flour were mixed by material balance to produce 16% protein complementary foods. Moisture sorption isotherms

**Corresponding author: Email: eyungong81@gmail.com;*

wereinvestigated at 10, 20, 30, and 40 ° C using the gravimetric method. The BET equation was fitted to the experimental sorption data, and the Clausius-Clapeyron equation was used to determine the isosteric heat of sorption. The microbial stability of the gruels was evaluated using the microbial challenge test with *Staphylococcus aureus.* With better results from fully gelatinized samples, gelatinization and fermentation reduced the hygroscopic properties of the dehydrated products, with the gelatinized fermented samples having the least equilibrium moisture contents, monolayer moisture values, surface area of sorbent, and net isosteric heats of sorption, an indication that these samples could be stored at temperatures as high as 40 ° C with extended shelf life. The microbiological stability of foods was enhanced by fermentation, since microbial growth was suppressed in fermented samples, offering perspectives for microbial stability of the products.

Keywords: Gelatinization; fermentation; moisture sorption; microbiology.

1. INTRODUCTION

Food processors face the problem of reducing food spoilage. The water in food is responsible for the spoilage that appears in most foods. Reducing the moisture content of food has been associated with reducing microbial activity, reducing enzyme action, and extending the shelf life of the food. Starch gelatinization has been reported to increase the drying rate of food, leading to a decrease in the moisture content of the food [1].Oluwalana [1] and Olatunde et al. [2] also stated that pre-gelatinization reduced the moisture content of plantain flour. The joint effect of fermentation and extrusion was reported to decrease the moisture content of plantain-cowpea blends.

“Complementary foods introduced to infants in addition to breast milk after the 6th month of age usually consist of foods that are rich in protein, energy and micronutrients (especially iron, zinc, calcium, vitamin A, vitamin C and folates) and free from contaminants (pathogens, toxins and harmful chemicals)” [3]. According to the Standard Organization of Nigeria (SON), a maximum moisture content of 10 % is recommended for safe keeping quality of flours.

Much work has been done on production of complementary food from plantain and soybean. Gelatinization and fermentation have individually been used to increase the quality of complementary foods produced from these raw materials. However, information on the effect of degree of gelatinization and the optimization effect of gelatinization and fermentation on the quality of these foods is rare. This work was thus aimed at evaluating the effect of different degrees of starch gelatinization and fermentation on the moisture sorption characteristics and microbiological properties of complementary foods from plantain and soybean.

2. MATERIALS AND METHODS

2.1 Sourcing of Raw Materials and Preliminary Handling

Five big bunches (approximately 10 kg) of unripe matured plantain fruits, cultivar *Big Ebanga*(False ‘Horn’), and 4 kg of dry soybean seeds were purchased from the Wurukum market in Makurdi, Benue State- Nigeria. The raw materials were sorted and cleaned prior to use for experiments.

2.2Preparation of Complementary Foods

The unripe plantain was processed into flour by a modification of the method described by Folorunso & Ayodele, [4](Fig.1). Following this method, mature unripe plantain fruits were washed, peeled with a knife, and the pulp cut into small round slices of about 2mm thick using a portable plantain slicer. A portion of the plantain was heated to obtain 10%, 18%, 23%, 33%, 74% and 100% starch gelatinization at 80 ° C for 45 seconds, 20, 40, 60, 120 and 150 minutes respectively. The non-gelatinized-nonfermented and non-gelatinized fermented samples were used as control. The flour obtained was subjected to accelerated natural fermentation by back slopping as described by Kim et al. [5] through four cycles to a final pH of 4.5 and a total titratable acidity (TTA) of 0.05 % when the products remained fairly stable with no changes in pH. Dry soybean seeds were processed into soybean flour by a modification of the methods used by Kohli et al. [6] and Aduke, [7]. Following this method, the soybean seeds were sorted and soaked in water containing 1.25% baking soda for 12 hours (1 kg soybean per 2 liters of water). The seeds were then boiled for 30 minutes to remove antinutrients, oligosaccharides, and beany flavor, and to reduce the viscosity of the resulting porridge [8]. The seeds were then

dehulled (to further reduce the antinutrient content) by rubbing them between the palms and dried in a hot air fan driven oven at 50 ° C for 48 hours. When dry, the seeds were milled and sieved using a 100-um particle size sieve (Fig. 2). Following material balance calculations, the proportions of plantain flour were mixed with soyabean flour to obtain a 16 g/100 g protein food, as recommended by the Protein Advisory Group (PAG). The fourteen (14) complementary foods produced were preserved in zip-lock cellophane bags prior to analysis.

2.3 Moisture sorption studies

The water sorption isotherms were determined gravimetrically using sulfuric acid solutions of 10 %, 20 %, 30 %, 40 %, 50 % and 60 % to provide water activities ranging from 0.15 to 0.95. A thermostatically controlled incubator and 500 ml containers were used for temperature and humidity controls, respectively. Acidic solutions (100 ml each) were carefully introduced into plastic containers. A wire gauze screen was forced into the plastic containers to form a support for the samples.

According to the method used by Sengev et al., [9] 0.25% sodium azide powder was mixed with samples to prevent mold growth. Triplicate samples (0.5 g each) were weighed in crown corks and placed on the wire gauze above the salt solution. The containers were tightly covered and placed in the incubator at selected temperatures of 10 $^{\circ}$C, 20 $^{\circ}$C, 30 $^{\circ}$C and 40 $^{\circ}$C. The samples were removed and weighed at 2-day intervals until the differences between consecutive readings were <0.5% of the sample weight (equilibrium). The total time forremoval, weighing, and return of samples into air-tight containers was about 2-5minutes.

For desorption studies, weighed samples were wetted by sprinkling with distilled water followed by mixing with a spatula. The moistened samples were allowed to equilibrate in a refrigerator before being placed in the incubator and monitored as for adsorption. Sorption isotherms were obtained by a graph of the equilibrium moisture content (EMC) against water activity [9].

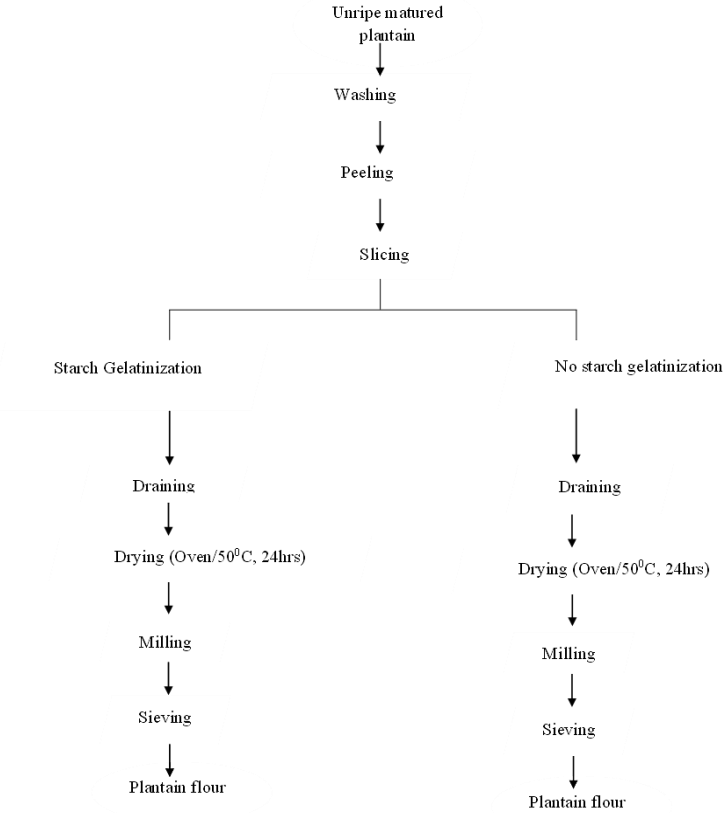

Fig. 1. Flow chart for plantain flour production
Source: [4]

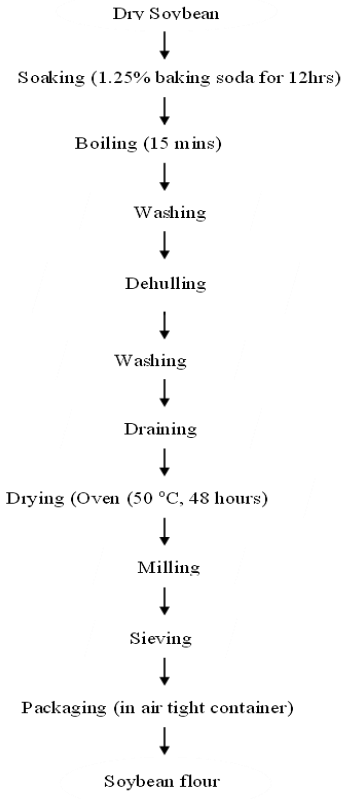

Fig. 2. Flow chart for soybean production
Source: [6] and [7] with modifications

2.3.1 Moisture sorption models

The equilibrium moisture data were fitted using the Brunauer–Emmett–Teller (BET) model**.** The BET isotherm equation [10] was chosen for its reported fit for starchy foods. It is the most widely used model that provides a good fit to data for a variety of foods in the a_w region of 0.05 to 0.45 [11]. The model is

$$\frac{a_w}{(1-a_w)m} = \frac{1}{M_0 C} + \frac{(C-1)a_w}{M_0 C} \quad (1)$$

Where;

M = equilibrium moisture content, M_0 = monolayer moisture content and C = a constant related to heat of sorption, a_w = water activity

2.3.2Sorption data analysis

From the data generated, equilibrium moisture versus water activities for each temperature were plotted. The acquired sorption data were analyzed using the BET model (Equation 1). The parameters of the BET model were calculated by linear regression analysis.

The BET monolayer values [10] were calculated from the regression equation of the BET plot using moisture sorption data up to 0.46aw, since the BET equation holds well below 0.5a_w[12].

2.3.3 Isosteric heat of sorption

Net isosteric heat of sorption provides a measure of the water-solid binding strength. The determination of the sorption of the differential molar quantities derived from the temperature dependence of the sorption isotherm was calculated by applying Clausius-Clapeyron equation:

$$\ln a_w = C_{st} - \frac{\Delta H_{st}}{R}\frac{1}{T} \quad (2)$$

Where Hst = net isosteric heat, Cst = constant related to entropy of sorption, T = temperature under absolute conditions (° C) and R = constant molar gas (0.008314 kJ/mol °C) and a_w= water activity.

To the isosteres obtained at constant moisture contents up to 30 g of H2O / 100 g of solids following the procedure reported by Ariahu et al. [12]. “Re-plotting the experimental sorption isotherm in the form In(aw) versus I/T, for a specific moisture content, Hst was determined from the slope (-Hst / R). This procedure is based on the assumption that Hst is invariant with temperature and requires measurement of the sorption isotherms at more than two temperatures” [9]. “Predictive models for the relationship between moisture content and net isosteric heats were proposed using an empirical equation to describe the relationship between Hst and equilibrium moisture content: Hst = Ho exp. (M/Mc) where M is the equilibrium moisture content, Ho is the isosteric heat of sorption of the first molecule of water; Mc is a characteristic moisture content of the food material” [10].

2.4 Assessing the Potential of Microbial Growth in Gruels from Formulations Through a Microbiological Challenge Test

The microbiological stability of cooked gruels was investigated by challenge tests as described by Serraino et al., [13]. Approximately 10 g of each duplicate sample from each product was mixed with 100 ml of sterile distilled water into smooth slurry. Slurries were cooked for 10 min at 90 C into smooth pastes in 250 ml glass beakers by boiling and continuously stirring with glass rods. Immediately after cooking, the beakers were covered with aluminium foil and allowed to cool prior to incubation.

Duplicate 25 ml gruel samples from each product were inoculated with a culture of *Staphylococcus aureus positive for coagulase* at an infestation level of 10^2 CFU / ml followed by incubation at 37±2 °C for 24 hours. Staphylococcus aureus was enumerated using MSA. Within a three-day monitoring period, plate observations with resulting colonies on cultures counted were appropriately used for calculation of respective microbial loads as:

$$Microbial\ load = \frac{Total\ number\ of\ colonies}{Inoculum\ size \times Dilution\ factor} \quad (3)$$

3. RESULTS AND DISCUSSION

From analyses, results of six samples; 2 non gelatinized, 2 pregelatinized, and 2 fully gelatinized, were reported.

3.1 Moisture Sorption Characteristics of Plantain and Soybean Complementary Foods Influenced by Gelatinization and Fermentation

3.1.1Adsorption and desorption isotherms

The adsorption and desorption isotherms of the unripe plantain and soybean formulations at 10, 20, 30 and 40 ° C are shown in Fig. 3. These temperatures were selected as possible storage conditions. The J-shaped shapes shown by the isotherms show an increase in the equilibrium moisture content with an increase in the water activity at all temperatures. For all samples, the values for EMC were always higher for desorption than for adsorption.

Moisture content of foods influences their texture, storage stability and susceptibility to microbial spoilage, since most microorganisms have specific water activities below which they cannot grow.

The type III (J-shaped) moisture isotherm shown by the formulations is relatively rare. As stated by Blahovec and Yanniotis in Ocheme et al. [11], this type of isotherm is common with solids such as sugar that are soluble in water. The J shape could be due to the presence of glucose sugar broken down from starch during fermentation. The increase in equilibrium moisture content with increased water activity is consistent with findings reported by other researchers such as [10,14] and [9]. For both adsorption and desorption, although the equilibrium moisture content increased with increasing water activity, the increase was more visible at water activities above 0.88 This implies that at a water activity >0.88, microbial growth, enzymatic reactions, and lipid oxidation will occur much faster leading to rapid spoilage of formulations.

Igbabul et al. [10] reported that for most dry foods, an increase in water activity of 0.1 can decrease the shelf life by a factor of two to three. The highest equilibrium moisture content values recorded by the NGNF control sample could therefore be an indication that pretreatments such as gelatinization and fermentation would enhance storage stability of the products by reducing equilibrium moisture content of products. Similar findings of higher moisture content in non-blanched non-fermented arrowroot lily tubers than in blanched and fermented samples were made by [10]. They suggested that heat may have damaged some

active binding sites, while fermentation may have modified tissue structures, thus reducing the number of active binding sites available for water molecules. The higher sorptive capacity of the NGNFPS control sample could be due to the availability of relatively more undamaged and unoccupied binding sites.

3.1.2 Effect of temperature on the moisture characteristics of the formulations

The equilibrium moisture content of the food formulations decreased with increasing temperature. From the EMC versus a_w curves (Fig. 3), the increase in temperature leads to decreased water activity at a constant equilibrium moisture content.

Decrease in equilibrium moisture content with increasing temperature implies that increasing temperatures will cause the product to be less hygroscopic [11]. Yan et al. in Ocheme et al. [11], suggested that "the decrease in equilibrium moisture content observed with increasing temperature at constant relative humidity may be due to higher active state of water molecules at higher temperatures at which the attractive forces between them decrease". "Elevated isotherm curves realized at high temperatures and constant equilibrium moisture content imply that at high storage temperatures with equilibrium moisture content remaining constant, water activity values remain within the critical level for storage of the product. Consequently, at constant equilibrium moisture content, the product remains safe even at high temperatures, as it remains less susceptible to microbial change" [11].

3.1.3 Surface area of sorption

The surface area of sorbent (S_o) followed the same trend as that of the monolayer moisture content, decreasing with an increase in temperature. The non-gelatinized non-fermented control sample recorded the highest surface area of sorption (S_o) (Table 1).

The decrease in surface area of sorbent with increase in temperature implies that at low temperatures, the sorbent molecules are exposed to more areas for sorption. The surface area of sorption (S_o) of the non-gelatinized non fermented control sample was higher than the values of 100 – 250m^2/g solid exhibited by most foods as reported by Igbabul et al., [10]. The higher values obtained by the NGNFPS control sample could be due to the fact that during gelatinization, starch molecules absorb water, swell and rupture. When these molecules rupture, there is loss of water leading to shrinkage. Hence, there is a lesser surface area than in the nongelatinized sample. Also, water molecules can plasticize the various long-chain polymers that make up the structural matrix, thus exposing more sites for sorption.

3.1.4 Monolayer moisture content

The monolayer moisture content calculated using the BET model decreased with an increase in temperature at constant water activity, with the non-gelatinized nonfermented control sample recording the highest monolayer values. Therefore, the amount of water adsorbed by the formulations decreased with an increase in temperature at constant relative humidity (Table 1).

"The monolayer moisture content (M_0), represents the moisture content of the material when the entire surface is covered with a unimolecular moisture layer. It is a measure of the moisture content for maximum stability of a food material" [10]. "With the exception of the oxidation of unsaturated fats, the rate of biochemical reactions is minimal below the monolayer moisture content. Consequently, the safest water activity is that corresponding to the monolayer moisture content at a given storage temperature" [11]. "A decrease in monolayer moisture content with an increase in temperature was also reported" [14]. "This decrease in monolayer moisture content was attributed to an increase in the energy level of water molecules activated by high temperatures, which causes the water molecules to become less stable and to break away from the water binding sites of the food material, thus decreasing the monolayer moisture content" [11]. The decrease in monolayer values in the gelatinized fermented samples could be an indication that pretreatments (gelatinization and fermentation) might have destroyed some active sites and/or affected the tissues, leading to a decrease in its sorptive ability and subsequently the monolayer moisture content.

3.1.5 Isosteric heat of sorption

For both adsorption and desorption, the isosteric heat of sorption decreased with increasing EMC. These heat values were highest for the non-gelatinized nonfermented control sample. Gelatinization and fermentation led to a decrease in isosteric heat of sorption (Fig. 4).

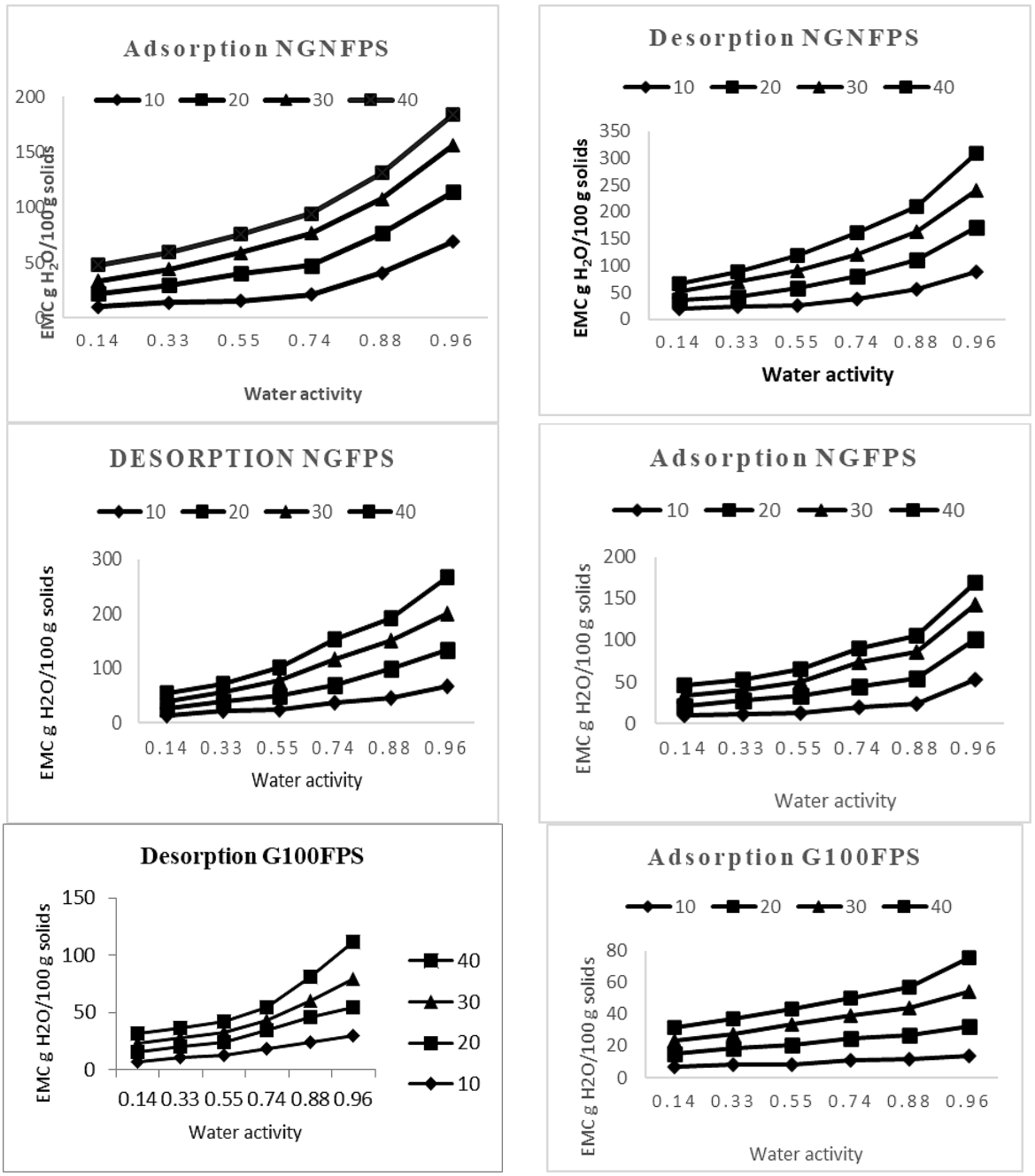

Fig. 3. Experimental adsorption and desorption isotherms of samples NGNFPS, NGFPS, and G100FPS at 10 ˚C, 20 ˚C, 30 ˚C, and 40 ˚C

The heat emitted or absorbed during the adsorption or desorption of water molecules on a solid material is known as the isosteric heat of sorption. It is a thermodynamic parameter that is important to food science and can help to understand how moisture interacts with food to alter attributes, including texture, shelf life, and stability. The heat of sorption is a good indicator of the intermolecular forces of attraction between food and the sorptive sites of water vapor [15]. The maximum net isosteric heat of sorption recorded in this study was higher than the 50 KJ / mol realized by Igbabul et al. [10] in arrowroot lily. Variations in the chemical and structural makeup of food ingredients may be the cause of isosteric heat variations. Igbabul et al. [10] reported that Palou et al. (1997) observed low net isosteric heats of sorption in products that were subjected to heat treatment. In this study, gelatinization, being a heat treatment, may have

damaged some sorptive sites resulting in lower net isosteric heats. This is possibly the reason for the highest net isosteric heats observed for the NGNFPS control sample that did not receive any pretreatment. The decrease in fermented samples implies that fermentation decreases the isosteric heat as well.

The amount of binding energy or availability of polar sites in food to water vapour as sorption progresses can be studied using a heat vs moisture content graph. As the moisture content increased, the heats of sorption dropped. At lower moisture concentrations, the net heat of sorption was significantly higher; As the moisture content increased, it decreased to approach the latent heat of vaporization of pure water (Hst = 0). This suggests that a significant amount of heat was either absorbed or lost during the adsorption or desorption of the initial layers of water molecules, respectively, compared to the heat generated by the condensation of pure water. This was most likely caused by the hydrophilic groups of food and the powerful interactions of water molecules. The lower heat of sorption with increasing moisture content is a good marker of the microbiological, chemical, and physical constancy of food products [15].

3.2 Assessment of the Microbial Growth Potential in the Different Blends

With an initial inoculum level of 10^2 CFU / ml, microbial growth was visible after 48 hours of incubation. This growth was suppressed in fermented samples. In samples where there was microbial growth, the values were within the 1.5 x 10^3 acceptable limit for microbiologically safe foods. These results can be seen in Table 2.

Table 1. Monolayer moisture content and surface area of sorption of plantain and soybean complementary foods as influenced bygelatinization and fermentation

Sorption index	Temperature (ºC)	Product					
		NGNFPS	NGFPS	G18PS	G18FPS	G100PS	G100FPS
M_o(g H_2O/ g solid)	10	6.78	5.56	4.57	4.51	3.30	3.22
	20	11.15	8.96	6.87	6.29	4.81	4.24
	30	7.86	7.20	6.29	6.23	4.66	3.76
	40	6.72	5.82	4.98	4.76	3.66	3.72
S_o(m^2/g)	10	238.33	195.49	160.63	158.6	115.9	112.99
	20	391.64	314.78	241.28	218.74	169.14	148.92
	30	276.18	252.92	221.08	221.08	163.78	131.97
	40	235.93	204.6	175.04	167.37	128.59	130.55

key: M_o = monolayer moisture content, S_o = surface area of sorption
NGNFPS = non-gelatinized, non-fermented plantain-soy blend, NGFPS = non-gelatinized, fermented plantain-soy blend, G18PS=18% gelatinized plantain-soy blend, G18FPS=18% gelatinized and fermented plantain-soy blend, G100PS= 100% gelatinized plantain-soy blend, G100FPS= 100% gelatinized and fermented plantain-soy blend

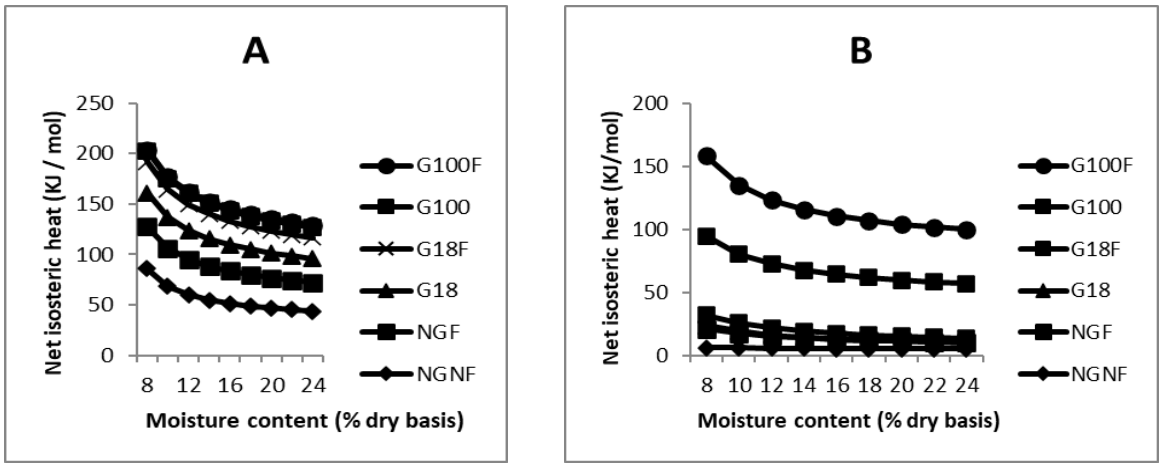

Fig. 4. Isosteric heat of sorption for adsorption (A) and desorption (B) of plantain and soybean complementary foods

Table 2. Microbiological challenge test on gruels of plantain and soybean complementary foods as influenced by gelatinization and fermentation

Parameter	Period (days)	Product NGNFPS	NGFPS	G18PS	G18FPS	G100PS	G100FPS
Staphylococcus aureus(cfu/mL)	0	10^2	10^2	10^2	10^2	10^2	10^2
	1	-	-	-	-	-	-
	2	1.3×10^2	-	1.7×10^2	-	1.5×10^2	-
	3	1.0×10^3	-	1.3×10^3	-	1.2×10^3	-

Key: NGNFPS = non-gelatinized, non-fermented plantain-soy blend, NGFPS = non-gelatinized, fermented plantain-soy blend, G18PS=18% gelatinized plantain-soy blend, G18FPS=18% gelatinized and fermented plantain-soy blend, G100PS= 100% gelatinized plantain-soy blend, G100FPS= 100% gelatinized and fermented plantain-soy blend

A microbial challenge test assesses the ability of a food product to support the growth of microorganisms. It involves artificial inoculation of the food with pertinent pathogenic or spoilage microorganisms in order to determine their behavior in the product. This test also gives an idea of whether organisms present a risk to microbial safety or quality of the product. From the results obtained, all the samples analyzed are considered to pass the challenge test, as in the cases of strive, microbial growth recorded in the samples is below the recommended 1.5 x 10^3cfu/g hence safe for human consumption as stated by Tsehayneh et al., [16]. The reduction in growth levels in fermented samples could be attributed to their low pH (average 4.5), which did not support microbial growth. Lactic acid bacteria (LAB) have been found to inhibit the growth of other organisms during solid state fermentation by three means: 1) by producing lactic acid and other organic acids that lower the pH of food; 2) by producing bacteriocins that inhibit other microorganisms and 3) by producing H_2O_2 which are harmful to many microorganisms [17]. Activities of LAB should be the reason why Staphylococcus could not thrive easily in food samples.

4. CONCLUSION

Gelatinization and fermentation reduced the hygroscopic properties of the dehydrated products, with the gelatinized fermented samples having the least EMC, monolayer moisture values, surface area of sorbent, and net isosteric heats of sorption, an indication that these samples could be stored at high temperatures with their shelf life extended. These qualities led to the microbial stability of the foods.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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