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Original Research Article

Rheological and Microbiological Assessment of Complementary meal produced from Sprouted and Fermented Sorghum (*S. bicolor*) blended with Cowpea (*Vignia unguiculata*) and Groundnut (*Arachis hypogea)*

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Abstract

A variety of cereals (sorghum) was used singly and in combination with legumes to produce a number of fermented and sprouted complementary foods. The study investigated the effect of sprouting and fermentation singly and in combination on some functional properties and microbiological quality of the food formulations produced from sorghum (*S. bicolor*), cowpea (*Vigna unguiculata*) and groundnut (*Arachis hypogea*) in a ratio of (70:20:10) respectively. Viscosity, functional properties and microbiological quality of the food formulations were evaluated using standard laboratory methods. Sprouting, fermentation singly and in combination significantly (P<0.05) decreased gruel viscosities ranging from 1978±25.1cps – 225.0±19.4cps for red sorghum and 19520±22.9cps – 2300±18.6cps for white sorghum variety respectively. The results of the bulk density ranged from 0.70±0.01g/ml – 0.60±0.02g/ml for red sorghum and 0.71±0.02g/ml – 0.60±0.02g/m) for white sorghum, while an increase in water absorption capacity in sprouted and fermented sorghum samples for (FCR 6.47±0.00g/ml, FCW 5.43±0.01) and SCR 6.03±0.00g/ml, SCW 4.41±0.02g/ml) compared to raw and the composite blends. Total bacterial counts reduced significantly with sprouting and fermentation. The dominant micro organisms isolated in this study were all fermenters non pathogenic micro-organisms which are safe for consumption staphylococcus species dominant only in the non-fermented products.

Keywords: Blend, Cowpea, Fermentation, Groundnut, Sprouting

INTRODUCTION

In most cultures habits are based in the available agricultural raw materials. Traditionally cereals and grain legumes play an important role in achieving the dietary pattern/habit of many people in Africa and Asia, thus they form the major sources of proteins, carbohydrates, vitamins and mineral (FAO, 2012).In developing countries, both commercial and traditional, weaning foods are sourced and prepared from cereals (rice, maize and sorghum e.t.c) and legumes (soya-beans and cowpea) (Modu *et al*., 2005) in the northern and southern parts of Nigeria, flour from various cereals forms the main raw materials used in production of popular food products like; ogi, fura, ndaleyi e.t.c) with high acceptability, good storage characteristics and affordable cost (Nkama *et al*., 1989). One such food product is "ogi", a fermented product cereal porridges made from sorghum, millet and maize product using simple processing methods.

A variety of cereals (sorghum) are used either single

or combined to produce a number of fermented beverages and foods many brands of low cost proprietary weaning foods have been developed from locally available high caloric cereals and legumes in tropical Africa (Living stone *et al*., 1993, Sanni *et al*., 2001). This was proposed by the integrated child development scheme (ICDS) and FAO to combat malnutrition among mothers and children of low socio-economic group.

The major constrain in the development of sorghum based foods is the levels of tannins that made them to be inferior, in terms of digestibility and bioavailability of mineral elements, hence the need to process these cultivars of cereals to enhance their nutritional value and subsequently introduce them as weaning meals singly or when supplemented with legumes. Some of the processing methods are sprouting, fermentation, dehulling and soaking e.t.c since food constituents are enriched nutritionally upon processing other have their nutritional content depleted or completely removed upon processing. This conforms the use of sprouting and fermentation methods singly or in combination in order to achieve the set objectives. Earlier studies have documented that combined sprouting and fermentation significantly reduce the antinutritional contents of cereals (sorghum) when compared to using either sprouting, fermentation, dehulling and soaking alone (Modu *et al*., 2010). However, information on the combined processing techniques such as sprouting/fermentation and supplementation of grain legumes such as cowpea and groundnut food formulations are in adequate in this part of the country, Nigeria. Thus, this research is an attempt to formulate a cereal-based weaning food for infants.

Sprouting and fermentation are among the simple and easily adaptable technologies for reduction of bulk densities (high viscosity) and increasing shelf life of cereals and legume based food formulation (Gema *et al*., 2011, Oluwamukomi *et al*., 2003). Since the traditionally processed foods from cereal/legume blends have short storage stability and spoilage organisms (e.g. Bacillus cersus) were found in stored non fermented traditional complementary foods (Gernah *et al*., 2012); while severe contamination of Kenyan children's food with enterobacteriaceae and staphylococcus aureus was reported by van steenbergen *et al*., (1983). Challenge tests using food formulations from Africa bread fruit (Treculia Africa) and soyabean (Glycine max) gave growth of enterobacteriaceac and staphylococcus in non fermented products. Therefore, there is the need to assess the traditionally prepared weaning foods to ascertain their microbiological quality. Even though many work has been conducted on the effect of malting and fermentation of the physicochemical and microbiological quality of food formulations from other cereals/legumes (Gernal *et al*., 2012; Sefa-Dedeh *et al*., 2001; Obasi *et al*., 2009). Information on the combined sprouting and fermentation of sorghum cowpea and groundnut food formulations is very scanty.

MATERIALS AND METHODS

Materials

Source of Materials and Preliminary Treatments

Sorghum (*S. bicolor*), cowpea (*Vigna unguiculata*) and groundnut (*Arachid hypogea*) were used for this study, they were obtained from seed store at Lake Chad Research Institute Maiduguri Borno State and were identified by a seed breeder Mr. Angrawa in same Institute. (Nutrend' (a maize-soybean based instant food made Nestle Food, Nigeria Plc, Lagos) was purchased from a local supermarket in Maiduguri. Wister albino rats of weaning age were obtained from the department of Biochemistry University of Maiduguri Animal breeding unit. Most chemicals used for this analysis were purchased from local stores in Nigeria and were of Analar grade (British Drug House chemicals, poole England).

Pre – Treatment of Samples

All the grains and legumes samples were manually cleaned by removing the ones that were mouldy or broken. The grains were sprouted as described by Kulkurni *et al*., (1991). Five hundred grams each of the sorghum varieties (mere and chakalari white) were soaked in plastic bucket containing 300 ml of distilled water and were steeped in water for one hour at room temperature ($28^oC \pm 2^oC$). The steep water was discarded by decantation and the steeped grains were germinated for seventy-two hours by spreading on a clean grease free tray pan and thereafter, it was sundried for two - three days by putting it in a sterilized tray pan. The sorghum grains was then milled using a disc attrition mill (Hunt No. 2A Premier Mill Hunt and Co, UK) to an average particle size of less than 0.3mm. The milled grains were then sieved through a fine mesh (0.5 µm) to obtain the sorghum flour.

Fermentation of the Sorghum Samples

The two sorghum grains samples (mere and chakalari white) were washed and soaked in water, about three times its weight by volume for seventy-two hours i.e approximately three days. The sorghum grains were covered and kept for seventy-two hours. The fermented samples were washed thoroughly in water and sundried for three days. The dried sorghum grains were milled and sieved through a 0.5µm mesh screen to obtain the flour.

This was done according to the method of kulkarni *et al*., (1991).

Preparation of the Cowpea Flour

Cowpea Flour

Cowpea seeds, about (two hundred grams) of the seeds were cleaned, washed and then soaked in ordinary water for twenty minutes. The seeds were dehulled, washed to remove the husk, after which it was dried to a constant weight. The cowpea seeds were roasted and then milled into fine powdered flour. (.Kulkarni *et al*., 1991).

Preparation of Groundnut Sample

Two hundred grams (200g) of groundnut was roasted to golden brown colour at 30 for 30 minutes on cooling; the skin was removed by rubbing between palms. Formulation of Sprouted and Fermented Sorghum flour with Cowpea and Groundnut: groundnut, cowpea and sorghum flours were mixed together in a ratio of 70:20:10 (W/W). Flow chart for production of traditional weaning foods from cereal/legume grains described by kulkarni *et al*.,(1991) was adopted with slight modifications.

Food Products Formulation

Ten different food formulations from two varieties of sorghum i.e. red and white sorghum were made by blending the different sorghum flours using laboratory method shown below. Formulate composite (1) consisted of sorghum flour (70 %), cowpea (20 %), Groundnuts (10%), Formulate composite (2) was made up of sorghum flour (70 %), cowpea (20 %), groundnut (10 %).All the products were milled and using a 0.5mm me

METHODS

Determination of Functional Properties

Water Absorption Capacity

Water absorption capacity was determined by the method of (Bhattacharya *et al*., 1986). Eighty (80ml) of tap water at 28^0C and 50^0C were added to Ten grams (10g) of the sample. Pulverized extrudates allowed standing for one hour in a One Hundred and Fifty (150ml) beaker. The hydrated extrudates was collected by inverting the beaker over a 20 mesh screen (Bs) for 60sec. The percentage hydration was defined as

$$\% \text{ hydration} = \frac{\text{wet sample} - \text{intial weight}}{\text{intial weight}} x\ 100$$

Determination of Bulk Density

The bulk density was determined using the method of Okezie and Bello (1988). Ten grams of the sample material were placed in a Twenty Five Mills (25ml) graduated cylinder and packed by gentle tapping of the cylinder on a bench top ten times from a height of 5 – 8cm. The final volume of the test material was recorded and expressed as g/ml.

Viscosity

Viscosity (AV) The apparent viscosity of slurries were determined by the methods of Beuchat, (1977) and was determined by placing Twenty grams of the sample in measuring cylinder of 100ml of water in a boiling water bath of 75 – 80^0C. The slurry was constantly stirred and until boiling which was continued for five minutes. The slurry was cooled to room temperature 23 – 25^0C and their viscosity were measured with a cannon viscometer.

Microbiological Analysis (Harrigan and Mc Caine, 1976)

Appropriate dilutions of final dried samples were enumerated for counts of bacteria and yeast using nutrient agar, MacConkey agar, sabouraud dextrose agar and blood agar base. Inoculated plates were incubated at appropriate time and temperature combinations. Colonies of respective microbial types appearing in inoculated plates was counted and expressed as colony forming units per gram (Cfu/g). Colonies of bacteria and yeasts was isolated and subculture to obtain pure cultures.

Determination of Total Viable Count

After inoculation the plates were incubated at 37□ for twenty-four hours. The colonies were obtained and counted with an electric colony counter (Gallen kemp Colony counter).

Isolation and Identification

One gram of the sample was smeared over one corner of the solidified medium which was sufficiently dried. A ninchrome wire loop was sterilized over a spirit lamp allowed to cooled and was made to parallel streaks from the main inoculums. The plates were incubated at 37°C for twenty-four hours.

The colonies were separated from one another based on the difference of colony monopoly. One of the separated colonies was taken using a sterilized wire loop and inoculated in another media then was incubated for

Table 1. Ingredients for Weaning Food Designed Formulations (%)

	RCR	SCR	FCR	SFCR	SFCRF1	RCW	SCW	FCW	SFCW	SFCWF2
Sorghum,	100	100	100	100	70	100	100	100	100	70
Cerelac	100	100	100	100	100	100	100	100	100	100
Cowpea	–	–	–	–	20	–	–	–	–	20
Groundnut	–	–	–	–	10	–	–	–	–	10

Source: (Kulkarni *et al*, (1991)
Keys: RCR: Red Chakalari red, SCR: Sprouted chakalari red, FCR: Fermented chakalari red SFCR: Sprouted/fermented chakalari red and SFCF: Sprouted fermented fortified; RCW: Raw chakalari white, SCW: Sprouted chakalari, FCW: fermented chakalari white SFCW: Sprouted / fermented chakalari white and SFCWF: Sprouted fortified.

Table 2. Some Functional Properties of Sprouted, Fermented, Combined Sprouted/Fermented Unfortified and Fortified Sorghum Composite Blends

Formulations	pH	Water Absorption Capacity (g/ml)	Bulkdensity (g/ml)	Viscosity (cps) (25% Conc.)
RCR	7.56±0.06^{a}	1.37±0.03^{a}	0.70±0.01^{a}	1936.6±2.91^{a}
RCW	7.55±0.05^{a}	1.21±0.04^{a}	071±0.02^{a}	1955.6±3.01^{a}
FCR	7.37±0.08^{a}	6.47±0.00^{b}	0.53±0.03^{b}	614.7±5.32^{b}
FCW	7.27.±0.05^{a}	5.43±0.01^{b}	0.50±0.02^{b}	625.3±6.20^{ca}
SCR	7.22±0.07^{ba}	6.03±0.00^{c}	0.45±0.06^{c}	375.6±3.81^{d}
SCW	7.16±0.05^{ca}	4.41±0.02^{c}	0.52±0.04^{db}	415.7±3.91^{e}
SFCR	5.51±0.09^{d}	1.79±0.06^{d}	0.47±0.00^{ec}	458.4±3.82^{f}
SFCW	5.53±0.06^{ed}	1.38±0.04^{d}	0.59±0.07^{f}	253.5±2.50^{g}
SFCRF	6.53±0.05^{f}	1.38±0.06^{a}	0.63±0.00^{g}	235.4±3.02^{hg}
SFCWF	6.74±0.03^{gf}	1.60±0.00^{e}	0.60±0.02^{hg}	242.6±2.21^{ig}

Value recorded as mean ± SEM of three determinations, value in the same row with different superscript are significantly different (P<0.05)

Keys: RCR = Raw Chakalari Red FCR = Fermented Chakalari SCR = Sprouted Chakalari Red SFRC = Sprouted / Fermented Chakalari Red SFCRF = Sprouted / Fermented Fortified Chakalari Red RCW = Raw Chakalari White FCW = Fermented Chakalari White SCW – Sprouted Chakalari White SFCW = Sprouted / Fermented Chakalari White SFCWF – Sprouted / Fermented Fortified Chakalari White

twenty-four hours at 37 ^{o}C. Colonies was obtained on the medium after twenty-four hours

Statistical analysis

All determinations were carried out in triplicates. The results obtained were analysed using one way analysis of variance (ANOVA). Using a pre-packaged computer software (MINITAB 15).

Duncan multiple-range test were used to compare the difference between the means.

RESULTS

Table 2 shows the results of Some Functional Properties of Sprouted, Fermented, Combined Sprouted/Fermented Unfortified and Fortified with Cowpea and Groundnut Flour.

The functional properties such as (bulk density, water absorption capacity and viscosity of the formulated foods are presented in table 2. The sprouted and fermented sorghum flour from the two sorghum varieties had lower bulk density, when compared with the raw unprocessed sorghum flour which were significantly higher than that of sprouted, (SCR which was 0.45g/ml and SCW was 0.52g/ml), Fermented (FCR was 0.53 g/ml and FCW was 0.50g/ml), Combined Sprouted/Fermented (SFCR was 0.47 g/ml and SFCW was 0.59g/ml) and Combined Sprouted/Fermented Fortified with Cowpea and Groundnut (SFCRF was 0.63g/ml and SFCWF was 0.60g/ml).

There was an increase in water absorption capacity with (6.03g/ml) sprouted flours, (SCR6.03g/ml, SCW4.41g/ml), Fermented, (FCR6.47g/ml), (FCW 5.43g/ml) combined sprouted/fermented flour (SFCR1.79g/ml), (SFCW1.60g/m). Sprouted/fermented flour has more water absorbing capacity than the raw unprocessed red and white sorghum flour (RCR 1.37g/ml and RCW 1.21g/ml) which had lower water absorption capacity. The results of the viscosity values at 25% concentration due to sprouting and fermentation also recorded a significant (P<0.05) reduction in viscosity for raw unprocessed flour (RCR was 19780cps and RCW was 19520cps), to sprouted (SCR was 3721cps and

Table 3. Micro-Organisms Isolated and Identified in Raw (Unprocessed) and Processed Formulated Sorghum Flour From two Sorghum Varieties

Formulations	0hrs	Day 1	Day 2	Day 3
RCR	No growth	*Staphylococcus albus* *Corynebacteria spp*	*Streptococcus lactics* *Lactobacillus*	*Saccharomyces cerevisae* *Lactobacillus*
RCW	No growth	*Bacillus subtilis* *Staphylococcus albus*	*Corynebacteria* *Streptococcus lactic*	*Sacchromyces cerecisae* *Lactonacillus*
FCR	No growth	*Bacillus subtilis* *Corynebacteria spp*	*Corynebacteria spp* *Saccharomyces cerevisae*	*Sacchromyces cerevisae*
FCW	No growth	*Lactobacillus* *Streptococcus lactic*	*Saccharomyces cerevisae* *Lactobacillus*	*Sacchromyces cerevisae* *Lactobacillus*
SCR	No growth	*Lactobacillus* *Streptococcus lactic*	*Saccharomyces cerevisae* *Corynebacteria spp*	*Sacchromyces cerevisae* *Lactobacillus*
SCW	No growth	*Corynebacteria spp* *Bacillus subtilis*	*Saccharomyces cerevisae* *Corynebacteria spp*	*Sacchromyces cerevisae* *Lactobacillus*
SFCR	No growth	*Streptococcus lactics* *Bacillus subtilis*	*Streptococcus lactics* *Lactobacillus*	*Sacchromyces cerevisae*
SFCW	No growth	*Corynebacteria spp* *Lactobacillus*	*Sacchromyces cerevisae*	*Sacchromyces cerevisae*
SFCRF	No growth	*Streptoplastic*	*Sacchromyces Cerevisae*	*Sacchromyces cerevisae*
SFCWF	No growth	*Bacillus subtilis* *Streptococcus lactic*	*Sacchromyces Cerevisae*	*Sacchromyces Cerevisae*

Key: RCR = Raw Chakalari Red FCR = Fermented Chakalari SCR = Sprouted Chakalari Red SFRC = Sprouted / Fermented Chakalari Red SFCRF = Sprouted / Fermented Fortified Chakalari Red RCW = Raw Chakalari White FCW = Fermented Chakalari White SCW – Sprouted Chakalari White SFCW = Sprouted / Fermented Chakalari White SFCWF – Sprouted / Fermented Fortified
Chakalari White

SCW was4216cps), fermented (FCR was 6052cps and FCW was 6217cps), combined sprouted/fermented sorghum flour (SFCR was 4540cps and SFCW 4216cps) and Combined sprouted/fortified (SFCRF was 2250cps and SFCWF was 2300cps).

Table 3 Shows the results of micro-organisms isolated and identified in raw (Unprocessed), processed unfortified and fortified sorghum flour from two sorghum varieties.

The micro-organisms isolated are shown in Table 3. No growth or moulds were observed at zero hour. However in the first day of fermentation, *Staphylloccus aureus* established itself in the raw samples (RCR) and (RCW) and later disappeared after the secondary of fermentation. The micro –organisms isolated in the second and third days of fermentation were all fermenters. *Saccharomyces cerevisae* and *Lactobacillus* appeared in the second day of steeping. Although, *corynebacteria* species disappeared in the third day. These are the dominant micro-organisms in sorghum flour isolated and identified (table 3). When the sorghum grains were subjected to the combined processing techniques i.e sprouted/fermented. After the grains were dried to a constant weight, the number of micro-organisms reduced consecutively

In table 4, Total Bacterial Count in the Unprocessed, Processed and Processed Fortified Sorghum Composite Blends from two sorghum varieties.

The total bacterial count in the first day of fermentation (24hrs) was 20 x 10^3 (cfu/ml) for Raw Chakalari Red (RCR) which dropped to 16 x 10^3 (cfu/ml) by the second day of fermentation (48hrs) and 14 x 10^3 (cfu/ml) in the day 3 (72hrs). The same trend was also observed for Raw Chakalari White where the highest bacterial count recorded at day 1 (24hrs) was 25 x 10^3 (cfu/ml) which dropped to 18 x 10^3 (cfu/ml) by the second day (48hrs) and 11 x 10^3 (cfu/ml) in the third day of fermentation (72hrs). The total bacterial count in the fermented samples dropped from 5.0×10^3 ,$3.0\times10^3$to 1.5×10^3 in first, second and third days of fermentation for FCR and that of FCW also dropped from 2.0×10^3,1.5×10^3 to 1.0×103 respectively. The total bacterial count was found to be higher in the raw samples than in the processed samples. Significant reduction in the total bacterial count, were recorded in the fermented, combined sprouted/fermented and combined sprouted/fermented samples. This indicates that fermentation, sprouting and combined fermentation and

Table 4. Total Bacterial Count in the Raw, Processed and Processed Unfortified and Fortified Chakalari Red and White Sorghum Composite Blends

Sample	Time Hours	RCR	FCR	SCR	SFCR	SFCRF
Total Bacterial count (cfu/ml)	24 hrs	20×10^3	2.0×10^3	9.0×10^3	7.0×10^3	3.0×10^3
	48 hrs	16×10^3	1.5×10^3	4.0×10^3	3.0×10^3	2.0×10^3
	72 hrs	14×10^3	1.0×10^3	2.0×10^3	1.6×10^3	1.0×10^3
Total Bacterial count (cfu/ml)		RCW	FCW	SCW	SFCW	SFCWF
	24 hrs	25×10^3	5.0×10^3	9.0×10^3	6.0×10^3	4.0×10^3
	48 hrs	18×10^3	3.0×10^3	7.0×10^3	3.0×10^3	2.5×10^3
	72 hrs	11×10^3	1.5×10^3	4.0×10^3	1.6×10^3	1.2×10^3

Key: RCR = Raw Chakalari Red FCR = Fermented Chakalari SCR = Sprouted Chakalari Red SFRC = Sprouted/Fermented Chakalari Red SFCRF = Sprouted/Fermented Fortified Chakalari Red RCW = Raw Chakalari White FCW = Fermented Chakalari White SCW – Sprouted Chakalari White SFCW = Sprouted/Fermented Chakalari White SFCWF – Sprouted/Fermented Fortified Chakalari White.

sprouting significantly reduced the number of total bacterial count.

DISCUSSIONS

Functional Properties

The significant reduction ($P<0.05$) in bulk density, due to sprouting and fermentation could be as a result of the absorption of water that tends to soften the seeds, this making milling easier with smaller particle sizes than that of unsprouted and unfermented grain, hence the reduction in bulk density. The significance of this is that the less bulky flours will have higher nutrient density, since more flour can be packed in the same given volume. The increased solubility could be a result of the increase in amount of soluble sugars present in the malted and fermented flours. Eneche (2009) also reported water absorption capacity with soaking of maize grains. The significant ($P<0.05$) decrease in viscosity due to sprouting, fermentation could be due to starch degradation caused by the action of hydrolytic enzymes (α and β amalyses) that developed during the sprouting process this hydrolyzing some of the starch into limit dextrin and maltose, which do not swell when cooked. Fermentation on further decreases the total amount of carbohydrates and other nutrients, since microbial activity requires energy and nutrients (Onimawo *et al*., 2001). Flour from sprouted and fermented grains can therefore be used in greater amount to give the same viscosity as flour from unsprouted grains, thereby given higher nutrient and energy density. This is in line with an earlier work reported by Gernah *et al*., (2012

Microbiological Analysis

Data on the microbiological analysis of the unprocessed and processed samples are presented in Table 3 and 4. The microbiological analysis was carried out to ascertain the safety of the products for consumption. The consecutive reduction in the total bacterial count in the steep water and processed flour could be due to acid production, in the fermentation medium due to microbial activity, since not all micro organisms can survive in the severe acidic medium. The result of the bacterial counts in this study is in accordance with the values recorded by (Mensah, *et al*., 1990).

In a related fermentation study for the production of cereal based fermented products. The micro-organisms identified in this study are all similar with earlier bacterial isolated and identified in similar fermentation study for production of other indigenous fermented food products. (Modu, 2003; Modu *et al*., 2012; Laminu *et al*., 2011). The consecutive reduction of micro-organisms isolated in this study could be as a result of the various processing techniques which sorghum were subjected to and could also be possibly due to the acid production since acidity increase as fermentation time progresses. (Mbata *et al*., 2006).

The predominant micro-organisms in this study are *Lactobacillus plantarum, Streptococcus, Coryne-bacterium* and *Saccharomyces cerevisae.* This agreed with the findings of (Modu *et al*., 2012). Most of these isolates are non-pathogenic and may not be of public health concern. These isolates may also have been introduced from environment, processing equipment and water used (Oboh, 2006). However, the high temperature of cooking is expected to reduce the micro-organisms.

CONCLUSION

Sprouting, fermentation and sprouting/fermentation significantly ($p<0.05$) decreased gruel viscosities, leading to improved nutrient density. There was also significant ($p<0.05$) decrease in bulk density while water absorption capacity increased. Natural lactic fermentation significantly ($p<0.05$) affected the microbiological composition and enhanced microbiological safety of the food products by increasing the dominance of lactic acid

bacteria and inhibiting growth of pathogenic micro-organisms.

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