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Effect of Production Method on the Dietary Fibre Composition of *Akamu*- A Nigerian Fermented Maize Product

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Abstract This study utilized the Integrated total dietary fibre assay in evaluating the total dietary fibre (TDF) components: insoluble dietary fibre (IDF), soluble dietary fibre precipitates (SDFP) and soluble dietary fibre soluble (SDFS), of porridges from ground maize slurries fermented by *Lactobacillus plantarum* strain and porridges from *akamu* sample. The traditional process was modified by skipping the soaking, wet milling and wet sieving stages to fermenting ground whole maize slurries with pure culture of *L. plantarum* strain. The aim was to investigate the effect of the production method on the dietary fibre composition of the new in comparison to *akamu* sample. The samples had similar physical and microbiological characteristics: pH of 3.43 – 3.95, titratable acidity of 1.05 – 1.59% and lactic acid bacteria count of 8.56 CFU mL^{-1}. The most available sugar from the SDFS was glucose (43 - 54 mg mL^{-1} for 24 h *L. plantarum* fermentation and the *akamu* sample, respectively). The *akamu* sample had the lowest IDF and the highest SDF (50.80 mg g^{-1}) with particular reference to its SDFS (35.40 mg g^{-1}) content. This study has brought to knowledge the dietary fibre component of a Nigerian fermented maize food.

Keywords: *Lactobacillus plantarum, Maize porridge, fermentation, akamu, insoluble dietary fibre, soluble dietary fibre precipitates, soluble dietary fibre soluble*

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1. Introduction

Dietary fibre (DF) comprises of chemically heterogeneous edible plant components that are resistant to digestion and absorption in the human small intestine but undergo complete or partial fermentation in the large intestine [1,2]. Although fibres of animal sources and modified or synthetic non-digestible carbohydrates are now been considered as dietary fibres [3], majority of the dietary fibre intakes worldwide are obtained from cereals and cereal-based foods.

In cereal grains dietary fibre are concentrated in the bran, representing 18.1 - 86.7% of the grain weight for oat and maize respectively with only about 13.1 - 19.6% in whole maize grain [4]. The two basic categories of dietary fibre depending on solubility in water are insoluble and soluble dietary fibres [5]. The water-insoluble dietary fibre (IDF) consists mainly of cellulose, hemicellilulose and lignin. Although the solubility of components of water-soluble dietary fibre (SDF) is demonstrated under conditions which may not exist in the human gastrointestinal tract (GIT), the soluble dietary fibre comprises of dietary fibre that are precipitated in a solution of one part water and four part ethanol (soluble dietary fibre precipitates) and those that remain soluble (soluble dietary fibre soluble) in the aqueous ethanol [6].

Some health enhancing benefits such as regulation of body weight through the inhibition of food intake by increasing satiety [7,8], lowering plasma lipid concentrations, improvement of glycemic control and decreased hyperinsulinemia [9,10], promotion of intestinal health and prevention of infective diarrhoea [11], has been attributed to dietary fibre. In addition to the physiological benefits, dietary fibre can improve the functional, textural, shelf-life and sensory characteristics of food products [12]. Before consumption, cereals grains are usually processed in one form or another and the processing technique may lower the fibre composition of the final product. While cereal such as barley, millet, rye, sorghum, wheat, rice and oat and their products have received wider attention [1], [5,7,13,14,15,16,17], there are limited studies on the dietary fibre composition of whole grain maize and its products. Although, as reviewed by Achi [18] the traditional fermentation of maize foods have been upgraded to high technology production systems that undoubtedly has improved the general well-being of the people as well as the economy, the dietary fibre composition of *akamu* have not been reported.

Akamu is a Nigerian traditional lactic acid fermented cereal-based meal, made basically from maize (*Zea maize*), and other cereals; sorghum or millet [19,20]. The

traditional process of *akamu* production involves steeping of the grain in excess water for 2 - 3 days, washing, wet milling and wet sieving. The extracted solids are allowed to sediment overnight, during which fermentation by various microorganisms associated with the raw material and utensils take place. The resultant product (*akamu*) varies in colour from white to yellow or dark brown depending on the variety of the cereal used. Addition of an equal part of boiling water to the fermented slurry with vigorous stirring yields a nearly gelatinized lump-less porridge. Its' porridges, which has a smooth texture from the sieving process and sour flavour from the lactic fermentation, are consumed by all ages as complementary food for infants and children, adult main meals, food for the convalescents and when cooked to a stiff gel is a convenient food for travellers [19,21,22].

In this study, the traditional method of production of the fermented maize was modified by skipping the soaking, wet milling and wet sieving stages to fermenting irradiated ground whole maize grain slurry with selected starter culture of *Lactobacillus plantarum* strain. The aim was to investigate the effect of the production method on the dietary fibre composition: insoluble dietary fibre (IDF), soluble dietary fibre precipitates (SDFP) and soluble dietary fibre soluble (SDFS) of the new product in comparison to *akamu* sample.

2. Materials and Method

2.1. Materials

2.1.1. Traditionally Fermented Maize Sample

The traditionally fermented maize sample otherwise called '*akamu*' was purchased from Port Harcourt in Rivers State, Nigeria, West Africa. The freshly prepared *akamu* (24 h) at the point of sale in the market was sealed in well labelled polyethylene bag and transferred into a plastic container that was packaged in a cardboard box and delivered the next day to the Food and Nutrition unit of Plymouth University, UK. On the receipt of the sample it as refrigerated at 4°C overnight. Thereafter, the number of samples required for physicochemical and microbiological analysis were taken and the rest of the samples were stored frozen at -80°C until required again for analysis. The *akamu* sample as ascertained from the producer was prepared according to the traditional method described by Obinna-Echem *et al*. [23]. Briefly, the key steps involved, cleaning and sorting, steeping, wet milling, wet sieving and fermentation of the resultant slurry overnight at 28 – 30°C.

2.1.2. Maize Flour and Fermentation Slurry

Organic maize flour was obtained from Health Food Shop, Rickard lanes, Plymouth City Centre in UK. About 50±0.01 g flour was weighed into cellophane bags, sealed and irradiated with ^{60}Co at 25.88±0.79 kGy (Becton and Dickinson, Plymouth, UK). The sterility of the irradiated sample was confirmed with no microbial growth observed when 10 g of the irradiated sample was homogenised in 90 mL of Phosphate buffered saline (PBS) and streak plated on Nutrient agar plates with incubated at 30 and 37°C for 3 - 7 days. Although maize grains for *akamu* production would not ordinarily be irradiated, the essence of irradiation in this study was to ensure that the fermentation was that of the inoculated starter culture. The slurry for fermentation was thereafter prepared by adding 100 mL of sterile distilled water containing 1 mL of the microbial inocula.

2.1.3. Inoculum Preparation

The *L. plantarum* strain was previously identified from the traditionally fermented *akamu* sample using both traditional microbiological and molecular methods. The lactic acid bacteria was conventionally isolated from the sample and its identity was confirmed using polymerase chain reaction (PCR) and sequencing analysis in a previous study by Obinna-Echem *et al*.[24].

The identified *L. plantarum* strain was cultivated by streaking on de Man, Rogosa and Sharpe (MRS) agar incubated at 37°C for 24 h. Thereafter, a distinct colony was grown in MRS broth at the same growth condition. Cells were harvested by centrifugation (Hettich Zentrifugen Rotina 46 S, Tuttlingen, Germany) at 4000 *xg* for 10 min and washed twice in phosphate buffered saline (PBS) and re-suspended in the diluent such that 1 mL of inoculum produced 10^9 CFU mL^{-1}.

2.1.4. Chemicals and Reagents

The sodium hydroxide and the phenolphthalein indicator used for determination of samples titratable acidity were obtained from Oxoid Limited (Basingstoke, England). The chemical reagents used for the total dietary fibre analysis were supplied in the Megazyme integrated total dietary fibre assay kit K-INTDF 06/12 [25]. Ethanol and the HPLC analytical grade sugars: glucose, maltose, arabinose, xylose were obtained from Fisher Scientific (Loughborough, UK).

2.2. Methods

2.2.1. Fermentation of the Ground Whole Maize Slurries

The fermentation slurry was prepared by inoculating 1 mL of the microbial inoculum into 100 mL of sterile distilled water that was thoroughly mixed with 50 ± 0.01 g irradiated ground maize. The inoculated slurries were distributed in 30 mL quantity into sterile transparent 50 mL plastic pots (Fisher Scientific, Loughborough, UK) with lids and incubated at 30°C. After 0, 24, and 72 h, samples were aseptically withdrawn for the determination of pH, titratable acidity and Lactobacillus count while the rest of the samples for dietary fibre analysis were preserved at -80°C until needed. Un-inoculated ground maize (GM) was also analysed as control.

2.2.2. pH and Titratable Acidity

The pH of both the traditional *akamu* obtained from Nigeria and the starter culture fermented ground maize slurries were determined with a pH meter (Accumet^R AB10, Fisher Scientific, Loughborough, UK) The pH was calibrated against standard buffer solutions (Fisher Scientific, Loughborough, UK) according to the method of AOAC, [26].

The amount of lactic acid as total titratable acidity (TTA) produced in the fermentation was determined

according to the method Annan *et al.* [27]. Samples from pH analysis were titrated against 0.1 mol L^{-1} NaOH with phenolphthalein as indicator. Results were expressed as percentage lactic acid using the formula: TTA (%) = (Titre × Normality of acid × Equivalent weight of acid × 100)/(Volume of sample × 1000); where equivalent weight of lactic acid is 90.08 g.

2.2.3. LAB Enumeration

Ten-fold dilutions of 10 g of the traditional sample in 90 mL of PBS (pH 7.3±0.2) and 1 mL of the *L. plantarum* fermented slurries in 9 mL PBS were prepared and plated out using the drop method by Miles and Misra [28] on de Man, Rogosa and Sharpe (MRS) agar with incubation at 37°C for 24 - 48 h. The number of colony forming unit per millilitre was computed as: CFU mL^{-1} = *(Average number of colonies for a dilution × Dilution factor)/Volume of sample plated*.

2.2.4. Dietary Fibre Analysis

The insoluble dietary fibre (IDF), soluble dietary fibre precipitates (SDFP) and soluble dietary fibre soluble (SDFS) of the samples were determined using an integrated total dietary fibre assay kit (K-INTDF 06/12 Megazyme International, Co. Wicklow, Bray, Ireland) according to the integrated enzymatic-gravimetric and chromatographic procedure of AOAC Method 2011.25 [25]. Blanks without any sample were analysed simultaneously with the samples and served as controls. Briefly, the samples were digested according to the Megazyme procedure. After the vacuum filtration of the digest the protein content of the dried residue was determined by Kjeldahl method and ash was determined gravimetrically following the AOAC [29]. Values for the protein and ash determinations were used in correcting the insoluble dietary fibre content.

Deionization was performed using 20 mL syringes loaded with thoroughly mixed 4 g each of freshly prepared Amberlite FPA 53 (OH-) and Ambersep 200 (H+) (Megazyme International, Co. Wicklow, Bray, Ireland) that were mounted on the column of vacuubrand vacuum pump (GMBH+Co, Wertheim, Germany) and eluted at the rate of 1.0 mL min^{-1} into 50 mL Duran bottle. This stage is necessary for obtaining a quality chromatographic data for soluble dietary fibre soluble.

Chromatographic determination of the soluble dietary fibre soluble was performed using Ultra-high performance liquid chromatography (UHPLC), Dionex-Ultimate 3000 UHPLC+Focused (Dionex Softron GmbH, Germering, Germany). The UHPLC was equipped with WPS-3000 autosampler, LPG-3400SD pump, and TCC-3000 column compartment, RI-101 refractive index detector and MWD-3000 Ultra violet detector. The chromatographic separation of 50 µL injected sample volume was achieved with an elution phase of Milli-Q water containing Na_2Ca-EDTA (50 mg L^{-1}) in a PL Hi-Plex H Guard column 50x7.7 mm (Agilent Technologies, Waghaeusel-Wiesental, Germany). The eluent was pumped at a flow rate of 0.5 µL min^{-1} within a pressure limit of 5 - 100 bars at a column oven temperature of 75°C. The sugars were detected using the RI.The retention times of the standards were noted and used in the identification of the sample peaks. The sugars were from the solubilisation and hydrolysis of non-resistant starch to D-glucose, maltose and maltodextrin by the two enzymes: pancreatic α-amylase (E-PANAA) and Amyloglucosidase (E-AMGDF) [25]. All data obtained were processed using Chromeleon® 7.1 Chromatography Data System Software (Dionex Softron GmbH, Germering, Germany). Calculations were made using Microsoft Excel 2010 package following the formulas provided in the Megazyme instructional manual.

2.2.5. Statistical Analysis

Data obtained were statistically analysed using Minitab (Release 16.0) Statistical Software English (Minitab Ltd. Coventry, UK). Statistical differences and relationship among variables were evaluated by analysis of variance (ANOVA) under general linear model and Tukey pairwise comparisons at 95% confidence level.

3. Results and Discussion

3.1. pH, Titratable Acidity and Microbial Analysis

The pH, titratable acidity and the LAB count *akamu* sample and the *L. plantarum* strain in the modified samples are shown in Table 1. Although the *L. plantarum* fermented sample had significantly ($p \leq 0.05$) lower pH, the titratable acidity of the traditional sample was significantly higher. This could be attributed to the activities of other fermenting microorganisms present in the spontaneously fermented sample as reported by Obinna-Echem *et al.* [24].

Table 1. pH, Titratable acidity (%Lactic acid) and LAB count (Log_{10} CFU mL^{-1}) of *akamu* sample and ground maize slurries fermented by *Lactobacillus plantarum* strains (NGL5)

Samples	Time (h)	pH	TTA (%Lactic acid)	Viable LAB count (Log_{10} CFU mL^{-1})
AL5	0	5.52 ± 0.04^{a}	0.12 ± 0.05^{d}	7.23 ± 0.08^{b}
	24	3.51 ± 0.03^{c}	0.69 ± 0.05^{c}	9.09 ± 0.06^{a}
	72	3.43 ± 0.01^{d}	1.05 ± 0.05^{b}	8.56 ± 0.10^{a}
Akamu		3.95 ± 0.01^{b}	1.59 ± 0.05^{a}	7.32 ± 0.41^{b}

Values with the same superscript in the same column do not differ significantly ($p \leq 0.05$), n=3±SD
AL5 - Sample fermented by the *L. plantarum* strain (NGL5)
Akamu- Traditionally fermented samples.

The number of the *L. plantarum* increased with progression in fermentation time. No significant difference was observed in the viable count of the *L. plantarum* fermented sample and the traditional sample. The acidity and microbial number of both samples indicated that the samples further analyzed were of similar physical and microbiological characteristics. Decrease in pH is been established as an important parameter for assessing how fast a process will reach conditions (pH<4.5) which can inhibit the growth of pathogenic

organisms [30]. The pH (3.95) of the *akamu* sample obtained from Nigeria was such that would not permit the survival of most unwanted microorganisms. AL5 sample had pH ≥5.31 after 24 h, a condition that would not favour the inhibition of pathogenic growth. Similar results for *L. plantarum* were reported in the studies by Teniola and Odunfa [31] and Mugula *et al.* [32].

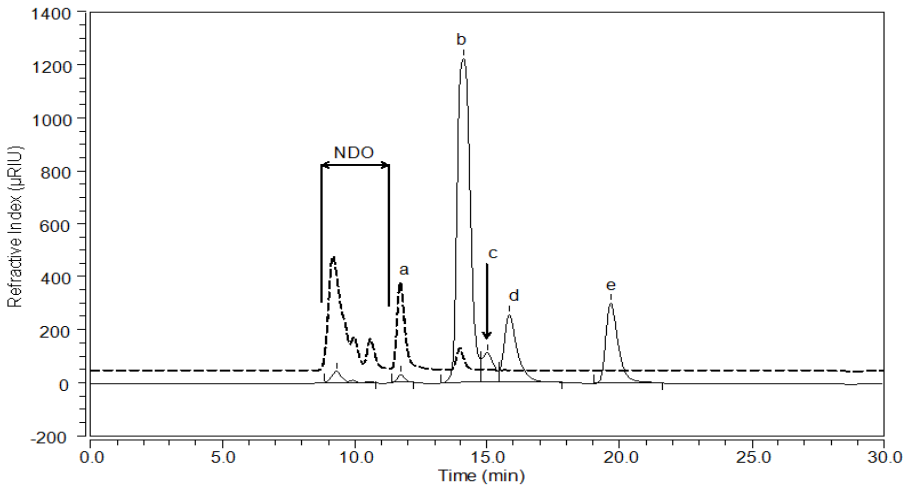

Figure 1. Chromatograph of the internal standard and the Soluble Dietary Fibre Soluble (SDFS) of the Samples

Solid line - Chromatography of 72 h *Lactobacillus plantarum* fermentation sample extract overlaid with the broken line - Chromatograph of the internal standard retention time (LC RT)
a = Maltose; b = Glucose; c = Xylose; d = Sorbitol (Internal Standard) and e = Glycerol
NDO - Non-digestible oligosaccharide.

3.2. Dietary Fibre Composition

3.2.1. The Soluble Dietary Fibre Soluble (SDFS) Chromatogram

Figure 1 shows an example of the chromatogram for the internal standard and the SDFS of the samples. The highest peak was observed for glucose and a much smaller peak for the non-digestible oligosaccharide (NDO) also known as malto-oligosaccharide. The peak for maltose, glucose, xylose, D-sorbitol and glycerol were confirmed with their respective standard solution (0.5, 5 and 10 mg mL^{-1}) peaks and average retention times of 11.72, 14.02, 14.91, 15.89 and 19.67 min, respectively.

Glycerol in the sample may have originated from the digestion reagents: amyloglucosidase (AGM) stock and protease solution that had their basic components in 50% v/v glycerol while sorbitol was from the D-sorbitol internal standard added at the end of sample digestion. The observed high peak area of the glucose residue in comparison to the non-digestible oligosaccharide (NDO) (Figure 1) confirmed the hydrolytic activity of the AMG added during sample digestion as one unit of AMG has the capacity to release 1 micromole of D-glucose from soluble starch [33]. Although it had been reported that for high starch containing food samples the combined pancreatic α-amylase and AMG hydrolysis were unable to convert quantitatively all non-resistant starch and maltodextrin into glucose [33]. This was evidenced by the NDO peak in of the samples in Figure 1. The quantification for the soluble dietary fibre soluble (SDFS) however, involved the Response factor (Rf) for the NDO as well as the SDFS peak area since the NDO also make up the SDFS of the samples.

3.2.2. Sugars, Ash and Protein

Table 2 showed the sugar components: xylose, glucose and maltose of the hydrolysed polysaccharides.

Generally, the sugar levels of the *L. plantarum* fermentation decreased with increase in fermentation time. This may suggest the effect of microbial utilization. The quantified glucose levels that were released ranged between 42.75±0.29 to 54.20±0.16 mg mL^{-1} of sample extract for 24 h *L. plantarum* fermentation and the traditional *akamu* sample respectively.

Table 2. Sugar content (mg mL^{-1}) of the sample extracts

Sample	Time (h)	Xylose	Glucose	Maltose
GM		$4.39±0.09^{a}$	$47.25±0.22^{b}$	$0.93±0.00^{a}$
AL5	0	$4.57±0.04^{a}$	$46.38±0.23^{c}$	$0.89±0.00^{b}$
	24	$3.37±0.02^{b}$	$42.75±0.29^{d}$	$0.42±0.00^{d}$
	72	$2.99±0.03^{b}$	$43.16±0.02^{d}$	$0.51±0.01^{c}$
Akamu		$2.97±0.22^{b}$	$54.20±0.16^{a}$	$0.38±0.00^{e}$

Values with the same superscript in the same column do not differ significantly (p≤0.05), n=3±SD
GM – unfermented ground maize
AL5 - Sample fermented with the *L. plantarum* strain (NGL5)
Akamu - Traditionally fermented sample.

The disaccharide maltose was also produced from the hydrolysis of starch by the α-amylase. This was significantly (p≤0.05) higher in the unfermented samples, indicating the combined effect of microbial hydrolysis and that of the added enzyme in the fermented samples. The available carbohydrate from the integrated dietary fibre analysis was therefore composed of glucose and maltose. Xylose, may have originated from the enzymatic hydrolysis of the cell wall polysaccharide where it constituted the cell wall building blocks [34]. This may explain why the whole unfermented ground maize sample had higher level of xylose.

The ash content was 0.006, 0.010, and 0.039 g for the *L. plantarum* fermented samples at 0, 24 and 72 h respectively. While the *akamu* and the uninoculated sample had ash content of 0.01 and 0.02 g. These values were from the ash determination of the residue of 1 ±0.002g of the initial sample that was digested and vacuum filtered. Hence these ash values were not

comparable with the ash content of *akamu* samples reported by Obinna-Echem *et al*. [24]. The ash values of the dried residue after vacuum filtration was used in the calculation of the insoluble dietary fibre content as required in the dietary fibre procedure for analysis [25]. Protein was not detected in the digested residues. The non-detectable level of protein could be attributed to hydrolytic activity of the protease enzyme.

Table 3. Dietary Fibre Composition (mg g^{-1} DM) of Ground Maize Slurry Fermented by *Lactobacillus plantarum* Strain and *akamu* Sample

Sample	Time (h)	Insoluble Dietary Fibre (IDF)	Soluble Dietary Fibre Precipitates (SDFP)	Soluble Dietary Fibre Soluble (SDFS)*	Soluble Dietary Fibre (SDF)	Total Dietary Fibre (TDF)
GM		65.75	24.00	13.50 ± 0.00^{e}	37.50	103.25
AL5	0	60.50	10.68	16.28 ± 0.02^{d}	26.97	87.47
	24	62.16	14.57	17.94 ± 0.05^{c}	32.51	94.66
	72	82.05	23.16	21.73 ± 0.10^{b}	44.89	126.94
Akamu		35.10	15.40	35.40 ± 0.04^{a}	50.80	85.90

*The HPLC analysis was performed in duplicate and all the values differed significantly ($p<0.05$). N=2±SD
Values with the same superscript in the same column do not differ significantly ($p\leq0.05$), n=3±SD
GM – unfermented ground maize
AL5 - Sample fermented with the *L. plantarum* strain (NGL5)
Akamu - Traditionally fermented sample.

3.2.3. The Dietary Fibre Fractions: Insoluble Dietary Fibre (IDF) and Soluble Dietary Fibre (SDF)

The range of values for the insoluble dietary fibre (IDF), soluble dietary fibre precipitates (SDFP), soluble dietary fibre soluble (SDFS), soluble dietary fibre (SDF) and total dietary fibre (TDF) content of the samples were shown in Table 3. The *akamu* sample had the lowest IDF and the highest SDF (50.80 mg g^{-1}) with particular reference to its SDFS (35.40 mg g^{-1}) content. The TDF of sample fermented by the *Lb. plantarum* strain after 72 h was the highest.

The insoluble dietary fibre (35.10 - 82.05 mg g^{-1} DM) and soluble dietary fibre (26.97 - 50.80 mg g^{-1} DM) values reported in this study were inconsistent with the IDF and SDF values of 9.42 and 0.18 mg g^{-1} respectively reported for unfermented yellow corn by Guo and Beta [17]. The variation could be a confirmation that fermentation resulted in increased dietary fibre content. The enzymatic-gravimetric method used in this study has been reported to be the most suitable for nutritional labelling and quality control purposes as loss of a considerable portion of dietary fibre occur with the use of non-enzymatic gravimetric methods [35]. The insoluble dietary fibre content in this study was comparable with the report of Huang *et al*. [35]. In cereal products there is high variation in the content of dietary fibre depending on the proportion of the individual kernel parts [36]. The lowest insoluble dietary fibre content of the *akamu* sample may be attributed to the removal of most of the bran fragment during wet sieving process. However, the significantly ($p\leq0.05$) highest content of soluble dietary fibre soluble of the *akamu* sample suggested the influence of any other production process. Although, the process of soaking has been reported to cause increases in resistant starch content [37]. It was observed that there were increases in the dietary fibre fractions of the *L. plantarum* starter culture samples with progression in fermentation.

The soluble dietary fibre constituted the majority of the dietary fibre content of the traditional and the *L. plantarum* fermented sample after 72h. The dietary fibre content of the fermented samples is important due to the physiological benefits attributed to dietary fibre. The insoluble fraction of dietary fibre activates intestinal peristalsis and is capable of binding bile acids and water which is significant in prevention of diet-induced diseases [36], while the soluble fractions have the capacity to increase viscosity, reduce glycemic response and involved in lowered blood cholesterol [12].

4. Conclusion

This study has brought to knowledge the dietary fibre component of a Nigerian fermented maize food. It has also revealed likely influence of sieving and soaking in the traditional production process on the insoluble dietary fibre and soluble dietary fibre soluble (SDFS) content of the traditional sample. A comparative evaluation of the dietary fibre content of the different maize fraction and the effect of variation in processing methods may help in properly attributing the cause of changes in dietary fibre composition of processed maize products.

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