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Effect of Co-fermentation Duration onthe Nutritional Composition and Anti-nutritional Contents of Sorghum-Cowpea Flours and Sensory Properties ofTheir Gruels

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Abstract:*The present study was aimed at assessing the effect of co-fermentation time on the nutrients and anti-nutrient contents in sorghum-cowpea flours and sensory attributes of gruels prepared from the flours. Five different portions of sorghum-cowpea mixture (70:30) were prepared and used for the experiment. Portion I was processed into flour without fermentation and served as control sample. Portions 2, 3, 4 and 5 were separately co-fermented for 24, 48, 72 and 96h respectively before processing into flours. The flours were analyzed for proximate composition, amino acid profile, mineral and anti-nutrient contents. Gruels were prepared from the different flours and used for sensory evaluation. The results showed that crude protein, fat, total amino acids and total essential amino acids progressively increased with co-fermentation time upto 72h of fermentation and then declined while the carbohydrate content decreased with co-fermentation time up to 72h and then increased slightly in the sample that was co-fermented for 96h. The ash, crude fibre, minerals (K, Ca, Mg and Zn) and anti-nutrients (phytate, tannin and trypsin inhibitor) progressively decreased with co-fermentation time till the end of fermentation period (96h). Gruels prepared from 48h and 72h co-fermented samples were the most preferred by the panelists in terms of colour, flavour, texture and overall acceptability*.

Keywords:*Sorghum, cowpea, co-fermentation time, nutrients, anti-nutrients, sensoryevaluation.*

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I. Introduction

In developing countries where there is limited access to animal based foods, childhood malnutrition is highly related to poor nutritional quality diet [1]. For rural dwellers and low to middle income earners in Nigeria, the usual first complementary food (ogi) is made by fermentation of maize (Zea mays), millet (*Pennisettumamericanum*) or guinea corn (*Sorghum spp*) [2]. The homemade complementary foods based on cereals alone are high in carbohydrate but low in protein quantity and quality. They therefore lack adequate nutrients required for proper growth and development. Where such complementary foods form themain source of nutrients to infants and children, they may lead to protein – energy malnutrition [3]. Malnutrition in childhood could lead to higher risk of diabetes, hypertension and dyslipidaemia among other impairments later in life [4, 5].

Sorghum (*Sorghum bicolor* L. *Moench*) is one of themost important food constituents of semi – arid parts of Africa and Asia [6]. Sorghum contains 9.28% protein, 2.27% fat, 1.27% ash, 2.01% crude fibre and 85.20% carbohydrate [7]. It is a rich source of B-complex vitamins [8]. Like other cereal grains, sorghum is low in protein quality as a result of being limited in some essential amino acids, particularly lysine and tryptophan which are necessary for the growth and development of infants and young children [2, 9].

A number of strategies have been developed to improve the nutritional value of cereal based complementary foods in low and the middle income countries. One of the strategies is the supplementation of cereals with legume products in such a way that will maximize the efficiency of the protein for weaning children [10]. One of such legume is cowpea (*Vignaunguiculata*) with 20 – 25% protein content [11]. Like other legume crops, cowpea is a good source of lysine and tryptophan but is poor in sulphur containing amino acids (methionine and cystine) [12]. According to Kumar and Sangeetha[13], the overall protein quality, nutritional value and health promotion are enhanced when cereals and legumes are combined together as composite mix, supplementary food mix and other ready-to-cook flour mix. Both cereals and legumes contain anti-nutritional factors such as phytate, tannin, hemagglutinins, saponin, and trypsin inhibitors that affect the digestion,

absorptionand bioavailability of some essential nutrients in foods [10, 11]. Soaking, boiling, sprouting, and fermentation are among the common traditional processing methods used to reduce or eliminate these anti-nutrients in foods and improve the nutritive value of the final product [11].

When cereals and legumes are used in complementary food preparation, the common practice is to ferment them separately before blending to have a composite mix. This is labour intensive, time consuming, require more energy and may lead to loss of more nutrients. Considering the fact that the low socio-economic status mothers who prepare the homemade complementary foods usually carry over-load of work daily, coupled with the financial constraint in obtaining commercial complementary foods, co-fermentation (cereals and legumes jointly fermented) is highly desirable. According to Omenna*et al.*[14], supplementation prior to co-fermentation led to improvement in protein, fat, ash and lower crude fibre content. The duration of co-fermentation usually varied from one processor to another and may range from 24 – 96h. However, there is paucity of information on the effect of co-fermentation time on some quality attributes of the final product. The present study was aimed at assessing the effect of co-fermenting sorghum and cowpea for 0-96h on the proximate composition, amino acids profile, mineral and anti-nutritional contents of the flours and sensory characteristics of gruels prepared from the flours.

II. Materials and Methods

2.1 Material Procurement: Cowpea and yellow variety of sorghum used for the study were purchased from Itam market in Uyo metropolis, AkwaIbom State, Nigeria.

2.2 *Preparation of Sorghum and Cowpea Co-fermented Flour*: The method described by Offiah*et al.*[15] was followed in the preparation of flour from sorghum and cowpea seeds that were co-fermented with slight modification. The two grains were separately cleaned to remove infected seeds and other unwanted materials. The sorghum (3.5kg) and cowpea (1.5kg) were thoroughly mixed together to obtain a blending ratio of 70:30 (sorghum: cowpea). The mixed grains (5kg) were divided into five equal portions of 1kg each. Portion 1 was processed into flour without fermentation and served as the control sample. Portions 2, 3, 4 and 5 were separately steeped in distilled water at the ratio of 1:4 (w/v) and allowed to co-ferment by endogenous microflora at ambient temperature (27 ± 2^{o}C) for 24, 48, 72 and 96h respectively. At the end of fermentation periods, the water was decanted and the seeds were thoroughly washed in potable water. While washing, they were decorticated. The washed seeds were dried in a conventional air oven (model pp. 22 US, Genlab, England) at 60^{o}C for 36h. The dried seeds were milled using hammer mill and sieved through a mesh of 425 micrometer pore size sieve to obtain the flour. Each of the flours was separately packaged in air tight plastic container, labeled and stored at 4^{o}C for various determinations.

2.3 *Methods of Analysis*:

2.3.1 *Proximate Composition Determination*:The moisture,crude protein, fat, ash, and crude fibre were determined following the methods described in AOAC [16]. Carbohydrate was calculated by difference [17]. Energy value was calculated using Atwater's factor formula [18].

2.3.2 *Amino Acids Determination*: Amino acid profile of the flours was determined using the method described by Benitez [19]. The samples for amino acid determination were dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator (Labatorums Technic AG, Model CH – 9230) and loaded into the applied biosystem PTH amino acid analyzer (Model 120A PTH, serial No. 704520, USA). Essential amino acid scores were computed using the FAO/WHO/UNU reference amino acid pattern [20].

2.3.3 *Mineral Determination*: The minerals (Ca, K, Mg and Zn) were determined using atomic absorption spectrophotometer (UNICAM, Model 939, UK) as described in AOAC [16].

2.3.4 *Determination of Anti-nutrients*:Phytate was determined following the method described by Oberleas[21]. Tannin was determined following the method described in AOAC [16]. The spectrophotometric method described by Arntfield*et al.*[22] was followed in the determination of trypsin inhibitor.

2.3.5 *Sensory Evaluation:* Gruels which were neither too thin nor too thick were prepared by mixing 100g of each flour sample with 300ml of water followed by gentle heating with constant stirring for about 15 min. One teaspoon of granulated sugar was added to each sample. The prepared gruel was cooled to about 45^{o}C and served in identical coded containers to 20 semi-trained panelists drawn from the Department of Food Science and Technology, University of Uyo, Nigeria for sensory evaluation using a 9-point hedonic scale ranging from 1 (disliked extremely) to 9 (liked extremely)[23]. The panelists were provided with questionnairefor entering

scores and potable water to rinse their mouths in between tasting of the gruels to avoid carry over effect. Each panelist evaluated the gruels for colour, flavor, texture and overall acceptability.

2.3.6 ***Statistical Analysis***

Data obtained were subjected to a one-way Analysis of Variance (ANOVA) using the software statistical package for social sciences (SPSS) version 18 (SPSS, Inc., Chicago, USA) to determine significant difference at $P<0.05$, Means were separated using Duncan's Multiple Range Test (DMRT).

III. Results and Discussion

3.1 *Effect of co-fermentation time on proximate composition of the flours*

The effect of varying the co-fermentation periods on the proximate composition of sorghum-cowpea flours is shown in Table 1. The results showed that the crude protein, fat, ash, crude fibre, carbohydrate and caloric value varied with the co-fermentation period. The crude protein contents of all the flours from co-fermented samples were significantly ($P<0.05$) higher than that of unfermented sample. According to Cui *et al.*[24], microorganisms usually utilize carbohydrates as an energy source and produce carbon dioxide as a by-product. This causes the nitrogen in the fermenting sample to be concentrated, and thus the proportion of protein in the total mass increases. In the present study, the crude protein increased from 13.09% for the flour from unfermented sample (control) to a maximum level of 15.98% for the flour from 72h co-fermented sample. The highest crude protein level for 72h co-fermented flour suggests that 72h co-fermentation is the optimum time for sorghum-cowpea blend. Chavan*et al.*[25] similarly reported that the crude protein of sorghum and green gram increased from 14.9% on day 0 to 17.6% on day 3 and then declined to 16.8% on days 4 and 5 of fermentation.Onweluzo and Nwabugwu[26] also made similar report for fermented millet and pigeon pea flour. The observed decline in the crude protein content after 72h of co-fermentation to 15.40% for 96h fermented sample could be attributed to possible increase in the number of microorganisms that use protein for metabolism [26]. An adequate supply of protein in the daily diet is essential for normal growth and development and for maintenance of health. Proteins build and repair body tissue, play major role in regulating various body functions, and provide energy if there is insufficient carbohydrate and fat in the diet [23]. The improvement in protein content of the flour as a result of co-fermentation of sorghum – cowpea is of nutritional significant in a country like Nigeria where many people especially children, can rarely take adequate foods with high protein content because of cost [27].

Table 1: Effect of co-fermentation time on proximate composition of sorghum – cowpea flours (dry matter basis)

	Co-fermentation Time (h)				
Parameters	0	24	48	72	96
Crude protein (%)	$13.09^{b}\pm0.11$	$15.13^{a}\pm0.08$	$15.75^{a}\pm0.03$	$15.98^{a}\pm0.05$	$15.40^{a}\pm0.02$
Fat (%)	$2.65^{b}\pm0.06$	$2.90^{b}\pm0.04$	$3.46^{a}\pm0.05$	$3.96^{a}\pm0.02$	$3.89^{a}\pm0.06$
Ash (%)	$2.17^{a}\pm0.03$	$1.84^{a}\pm0.00$	$1.40^{a}\pm0.09$	$1.15^{b}\pm0.01$	$1.08^{b}\pm0.04$
Crude fibre (%)	$4.06^{a}\pm0.05$	$3.93^{a}\pm0.13$	$3.72^{a}\pm0.08$	$3.40^{a}\pm0.10$	$3.11^{b}\pm0.06$
Carbohydrate (%)	$78.03^{a}\pm0.10$	$76.20^{b}\pm0.06$	$75.67^{b}\pm0.04$	$75.51^{b}\pm0.08$	$76.52^{b}\pm0.05$
Caloric value (Kcal/100g)	$388.31^{b}\pm0.09$	$391.42^{a}\pm0.10$	$396.82^{a}\pm0.06$	$401.60^{a}\pm0.04$	$402.69^{a}\pm0.03$

Values are means ± SD (standard deviation) of triplicate determinations. Means on the same row with different superscripts are significantly different at $P<0.05$.

The trend recorded for fat content in the present study was similar to that of crude protein. The fat content increased from 2.65% in the flour from unfermented sample (control) to 3.96% in the flour from the sample that was co-fermented for 72h and then declined slightly to 3.89% in the flour from the sample that was co-fermented for 96h. This finding is in agreement with the report by Onweluzo and Nwabugwu[26] for millet and pigeon fermented flour. Fat is insoluble in water. Hence, the increase in fat content with co-fermentation time upto 72h could be due to concentration effect caused by the removal of soluble carbohydrates and other water soluble materials during fermentation [28]. Also, it is possible that within the first 72h of fermentation, the fermenting organisms did not utilize the fat in the samples as energy source. Igbabul*et al.*[29] had earlier reported on the increase in fat content of cocoyam flour from 1.83% at 0h to 2.61% at 72h fermentation. Chavan and Kadam[30] also recorded a slight increase in the fat content of sorghum and sorghum plus green gram blend during natural fermentation. In the present study, it is possible that the fermenting organisms started to use part of the fat for energy production after 72h of co-fermentation thereby leading to its decline. Besides acting as carriers of fat soluble vitamins, fat in foods acts as favour retainer, increases the mouth feel of foods and contributes to improved palatability of food [31].

The ash and crude fibre contents in the flours decreased with increase in co-fermentation time ranging from 2.17% and 4.06% in the flour from unfermented sample to 1.08% and 3.11% in the flour from sample that was co-fermented for 96h (Table 1). Ash content of a food is an index of the mineral element in such food. The

observed decreased in ash content with co-fermentation time could be attributed to leaching of some of the inorganic matter into the aqueous medium used for fermentation which was discarded at the end of the fermentation period [32]. The decrease in ash content with co-fermentation time could also be due to metabolic activities of microorganisms in the fermenting medium [29]. This finding is in consonance with the reports by other researchers[26, 29, 33]. The decrease in crude fibre content with co-fermentation time might be due to partial degradation of cellulose and hemicellulosic type of materials by microbial enzymes [28, 34].

The carbohydrate content in the samples decreased from 78.03% in the flour from unfermented sample to 75.51% in the flour from 72h co-fermented sample and then increased to 76.05% in the flour from sample that was co-fermented for 96h. Carbohydrate, particularly starch and soluble sugars are principal substrates for fermenting microflora[35]. The decrease in carbohydrate with co-fermentation up to 72h period may therefore be due to the use of carbohydrates as energy source by fermenting organisms [36, 37]. The observed increase in carbohydrate content in the flour from sample that was fermented for 96h could be attributed to reduction of other constituents since the percentage carbohydrate was estimated by subtracting other constituents (protein, fat, ash and crude fibre) from 100%. Similar reductions in carbohydrate content cause by fermentation process have been reported by other researchers[28, 34, 36, 37].

The caloric value of the flours increased with co-fermentation time ranging from 388.31kcal/100g for the flour from unfermented sample to 402.69kcal/100g for the flour from sample that was co-fermented for 96h. The protein, fat and carbohydrate constituents contributed to the calculated caloric value of the samples with fat as the major contributor (9kcal/g) while protein and carbohydrate have about 4kcal/g. This explains why the co-fermented samples had higher values than the unfermented sample.

3.2 *Effect of Co-fermentation Time on Amino Acid Profile of the Flours*

Amino acids are component subunits of protein. Essentially, the quality of protein food is nutritionally judged by its protein content and the number and amounts of essential amino acids it contains. The effect of co-fermentation time on the amino acid profile of sorghum-cowpea flours is presented in Table 2. The result showed that the amino acids yield followed a trend similar to that of crude protein (Table 1). Glutamic acid was the highest contributor to the total amino acids (TAA) and was followed by aspartic acid while leucine was the highest contributor to the total essential amino acids (TEAA). The TAA and TEAA increased with co-fermentation time upto 72h fermentation and then declined in the sample that was co-fermented for 96h. The TAA and TEAA progressively increased from 81.08g/100g protein and 33.05g/100g protein for the flour from unfermented sample to the maximum levels of 86.52g/100g protein and 35.75g/100g protein respectively for the flour from the sample that was co-fermentation for 72.

Table 2: Effect of co-fermentation time on the amino acid profile of sorghum – cowpea flours (g/100g protein)

	Co-fermentation Time (h)				
Amino Acids	0	24	48	72	96
Leucine	6.60^{a} ±0.04	6.71^{a} ±0.10	6.83^{a} ±0.02	6.95^{a} ±0.05	6.80^{a} ±0.03
Lysine	3.42^{a} ±0.01	3.69^{a} ±0.05	3.74^{a} ±0.08	3.80^{a} ±0.02	3.76^{a} ±0.06
Isoleucine	3.57^{a} ±0.06	3.60^{a} ±0.03	3.67^{a} ±0.02	3.72^{a} ±0.10	3.68^{a} ±0.02
Phenylalanine	4.37^{b} ±0.03	4.61^{a} ±0.02	4.70^{a} ±0.05	4.79^{a} ±0.04	4.21^{b} ±0.01
Tryptophan	1.08^{b} ±0.02	1.13^{a} ±0.03	1.16^{a} ±0.10	1.18^{a} ±0.00	1.10^{a} ±0.05
Valine	3.89^{b} ±0.04	4.01^{a} ±0.04	4.09^{a} ±0.02	4.16^{a} ±0.04	4.12^{a} ±0.02
Methionine	1.65^{b} ±0.02	1.82^{a} ±0.06	1.93^{a} ±0.03	2.01^{a} ±0.06	1.85^{a} ±0.04
Proline	3.78^{a} ±0.05	3.96^{a} ±0.02	3.96^{a} ±0.04	4.00^{a} ±0.02	3.55^{b} ±0.10
Arginie	5.63^{a} ±0.03	5.85^{a} ±0.02	6.02^{a} ±0.03	6.17^{a} ±0.05	5.99^{a} ±0.02
Tyrosine	3.61^{a} ±0.02	3.61^{a} ±0.10	3.75^{a} ±0.05	3.78^{a} ±0.01	3.27^{b} ±0.03
Histidine	2.31^{b} ±0.05	2.56^{a} ±.00	2.54^{a} ±0.02	2.62^{a} ±0.06	2.32^{b} ±0.04
Cystine	1.45^{a} ±0.02	1.49^{a} ±0.04	1.57^{a} ±0.06	1.63^{a} ±0.02	1.39^{b} ±0.05
Alanine	5.01^{b} ±0.01	5.35^{a} ±0.06	5.50^{a} ±0.01	5.61^{a} ±0.04	5.08^{b} ±0.02
Glutamic Acid	16.32^{a} ±.0.03	16.59^{a} ±0.03	16.73^{a} ±0.04	16.79^{a} ±0.02	16.62^{a} ±0.06
Glycine	3.40^{b} ±0.04	3.54^{a} ±0.02	3.52^{a} ±0.03	3.64^{a} ±0.05	3.45^{a} ±0.10
Threonine	3.41^{b} ±0.06	3.56^{a} ±0.05	3.61^{a} ±0.02	3.73^{a} ±0.03	3.52^{a} ±0.04
Serine	4.26^{a} ±0.02	$4..32^{a}$ ±0.10	4.40^{a} ±0.04	4.45^{a} ±0.02	4.30^{a} ±0.10
Aspartic Acid	7.32^{a} ±0.05	7.34^{a} ±0.06	7.41^{a} ±0.02	7.49^{a} ±0.05	7.35^{a} ±0.03
TAA	81.08^{b} ±0.03	83.77^{a} ±0.04	85.13^{a} ±0.06	86.52^{a} ±0.02	82.36^{b} ±0.05
TEAA	33.05^{b} ±0.04	34.23^{a} ±0.02	35.05^{a} ±0.05	35.75^{a} ±0.04	33.70^{a} ±0.02
TEAA/TAA (%)	40.76	40.86	41.17	41.32	40.92

Values are means ± SD (standard deviation) of duplicate determinations. Means on the same row with different superscripts are significantly different at $P<0.05$. TTA = total amino acids; TEAA = total essential amino acids

Extension of co-fermentation time from 72 – 96h resulted in reduction of both the TAA and TEAA to 82.36g/100g protein and 33.70g/100g protein respectively in the flour made from 96h co-fermented sample. Apena*et al* [32] had earlier reported that fermentation of millet and sorghum for 72h resulted in increased

concentration of some selected amino acids (tryptophan, alanine, arginine, cystine, histidine, isoleucine, lysine and phenylalanine). Okafor*et al.*[38] similarly reported of increases in essential amino acids in maize – pigeon pea that was co-fermented for 48h.

All amino acids in food have different role to play that help the body to grow and function normally. However, essential amino acids are of main concern as they are not synthesized in the body and must be supplied in adequate amount through diets. From the result of the present study, it is evident that in order to derive maximum amount of essential amino acids, sorghum-cowpea blend (70:30) should be co-fermented for 72h. The percentage ratios of TEAA to TAA increased with co-fermentation time up to 72h fermentation ranging from 40.76% for unfermented sample to 41.32% for the 72h co-fermented sample and then declined to 40.92% for the sample that was co-fermented for 96h.The percentage ratios of TEAA to TAA for all the samples were above 39% considered to be adequate for ideal protein for infants, 26% for children and 11% for adults [20].

Comparison between the essential amino acids composition of the samples and the reference values [20] showed that most of the essential amino acids would meet the recommended range of amino acids required for children aged 2-5 years and adults. The essential amino acid scores as presented in Table 3 revealed that co-fermented samples had higher scores than the unfermented sample. The scores for all the essential amino acids progressively increased with co-fermentation time up to 72h of fermentation and then declined for the sample that was co-fermented for 96h. Except for lysine that the scores ranged from 63.62% for 24h co-fermented sample to 65.52% for 96h co-fermented sample, the scores for the rest of essential amino acids for the co-fermented samples were above 100% with 72h co-fermented sample recording the highest scores for all the essential amino acids.

Table 3:Effect of co-fermentation time on essential amino acids scores of sorghum – cowpea flours

	Co-fermentation Time (h)					FAO/WHO (1985)
Parameters	0	24	48	72	96	Reference Value (g/100g protein)
Leucine	99.85	101.51	103.33	105.14	102.87	6.61
Lysine	58.97	63.62	64.48	65.52	64.83	5.80
Isoleucine	127.50	128.57	131.07	132.86	131.43	2.80
Phen. + Tyro	126.67	130.48	134.13	136.03	118.73	6.30
Tryptophan	98.18	102.73	105.46	107.27	100.00	1.10
Valine	111.14	114.57	116.86	118.86	117.71	3.50
Met. + Cyst.	124.00	132.40	140.00	145.60	129.60	2.50
Threonine	100.29	104.71	106.18	109.71	103.53	3.40

* Reference amino acid pattern of pre-school children (2-5 years) (FAO/WHO/UNU, 1985). Phen = phenylalanine; Tyro = tyrosine; Met = methionine; Cyst = cystine.

3.3 *Effect of Co-fermentation Time on Mineral Content and Anti-nutritional Factors in Sorghum – Cowpea Flours*

The effect of varying the co-fermentation time on the mineral content in sorghum – cowpea flours is presented in Table 4. Minerals are essential nutrients that are needed to facilitate proper functioning of certain organs in the body. The result showed that K, Ca, Mg and Zn contents in the flour from unfermented sample were 144.85mg/100g 54.27mg/100g, 118.73mg/100g and 4.09mg/100g respectively. It was observed that the concentrations of these mineral elements in the flours from co-fermented samples were significantly ($P<0.05$) lower than the values recorded for unfermented flour. This could presumably be attributed to leaching of soluble inorganic mineral elements into the aqueous medium which was discarded at the end of fermentation period. This result agrees with the report by Oyarekua[6] that co-fermented sorghum/cowpea and millet/cowpea exhibited lower K, Ca, Mg and Zn contents than its unfermented counterparts. Omenna*et al.*[14] similarly reported on significant reduction of K, Ca, Mg and Zn in millet/soybean and maize/soybean blends as a result of co-fermentation process. Other researchers [32, 39] also reported that fermentation caused a general reduction of all the minerals tested.

There were progressive reductions in all the mineral elements with increased in co-fermentation time. The percentage reductions in K, Ca, Mg and Zn ranged from 9.08%, 7.57%, 12.04% and 8.31% for the flour from the sample that was co-fermented for 24h to 36.71%, 28.93%, 41.20% and 33.25% respectively for flour from sample that was co-fermented for 96h. Considering the fact that mineral elements are vital for maintenance of healthy body, it is evident from the result that co-fermentation period should not be unnecessarily prolonged in order to reduce mineral losses. The intake of potassium is required in relatively large amount in the body because it functions as an important electrolyte in the nerve system and has been shown to have a powerful, dose-dependent inhibitory effect on sodium sensitivity [40]. Calcium is important mineral for bone and teeth formation, blood clotting, muscle contraction and in certain enzymes in metabolic processes [41].Magnesium is important for bone health; is needed as a co-factor for numerous reactions in the body and is also essential for

nerve and muscle conductivity [41]. High amount of potassium, calcium and magnesium have been reported to reduce blood pressure in humans [42]. Zinc is involved in cellular growth and differentiation. It is a limiting factor in the growth of severely malnourished infants in developing countries because the diets are low in animal products and high in phytate[6, 14].

Table 4: Effect of co-fermentation time on mineral content and anti-nutritional factors in sorghum-cowpea flours

	Co-fermentation Time (h)				
Parameters	0	24	48	72	96
K (mg/100g)	146.85^{a} ±0.06	133.52^{b} ±0.11 (9.08)	122.36^{c} ±0.04 (16.68)	109.24^{d} ±0.13 (25.61)	92.94^{e} ±0.05 (36.71)
Ca (mg/100g)	54.27^{a} ±0.11	50.16^{b} ±0.05 (7.57)	47.33^{c} ±0.08 (12.79)	43.84^{d} ±0.04 (19.22)	38.57^{d} ±0.20 (28.93)
Mg (mg/100g)	118.73^{a} ±0.08	104.44^{b} ±0.08 (12.04)	93.61^{c} ±0.10 (21.16)	82.83^{d} ±0.08 (30.24)	69.81^{e} ±0.08 (41.20)
Zn (mg/100g)	4.09^{a} ±0.05	3.75^{a} ±0.10 (8.31)	3.30^{c} ±0.03 (19.32)	2.98^{b} ±0.10 (27.14)	2.73^{b} ±0.03 (33.25)
Phytate (mg/g)	2.81^{a} ±0.13	2.22^{b} ±0.06 (21.00)	1.74^{c} ±0.04 (38.08)	1.25^{d} ±0.11 (55.52)	0.84^{e} ±0.06 (70.12)
Tannin (mg/g)	0.68^{a} ±0.09	0.54^{b} ±0.21 (20.59)	0.43^{c} ±0.10 (36.77)	0.36^{d} ±0.06 (47.06)	0.24^{e} ±0.04 (64.71)
Trypsin inhibitor (TUI/mg)	1.16^{a} ±0.04	0.99^{b} ±0.08 (14.66)	0.83^{c} ±0.05 (28.45)	0.60^{d} ±0.14 (48.28)	0.49^{e} ±0.06 (57.76)

Values are means ± SD (standard deviation) of triplicate determinations. Means on the same row with different superscripts are significantly different at $P<0.05$. Values in parenthesis indicate percentage losses.

The nutritional importance of a diet depends not only on the nutrient composition of such diet, but also on the presence of anti-nutritional factors. The presence of anti-nutritional factors in diets decreases the digestion, absorption and utilization of some essential nutrients in the diets and may have adverse effects on human nutrition [43]. Hence, inactivation or elimination of anti-nutrients in foods is absolutely necessary to improve the nutritional quality of such foods and ensure effective utilization of their nutrients. Phytate, tannin and trypsin inhibitors are among the anti-nutritional factors of prime concern for human nutrition and health management.

Fermentation is one of the important processes that decrease the level of anti-nutrients in food grains and increase mineral extractability, in vitro protein digestibility and nutritive value of grains [44]. The effect of co-fermentation duration on the phytate, tannin and trypsin inhibitor concentrations in sorghum – cowpea flour is presented in Table 4. Result showed that flour from unfermented sample had significantly ($P<0.05$) higher pyhtate, tannin and trypsin inhibitor contents than values found in flours from co-fermented samples. It was observed that as co-fermentation progressed, the anti-nutritional factors kept decreasing until the end of fermentation. Progressive reduction in anti-nutrients in the flours with co-fermentation time is advantageous in view of their negative influences on nutrients digestion, absorption and utilization. The reduction in anti-nutritional factors in the samples as a result of co-fermentation process is in agreement with the reports by other researchers [26, 45, 46, 47].

The phytate content in unfermented sample was 2.81mg/g while the values in co-fermented samples ranged from 0.84-2.22mg/g. Percentage reduction of phytate levels ranged from 21.00% for the flour from the sample that was co-fermented for 24h to 70.12% for the flour from sample that was co-fermented for 96h. Ejigui*et al.*[33] similarly reported of 60.50% reduction of phytate level in corn flour after 96h of fermentation. It has been suggested that the loss of phytate during fermentation could be the result of activity of native phytase and fermentative microflora which hydrolyze phytate into inositol and orthophosphate [33, 46, 47]. The decrease in phytate with co-fermentation time is desirable in view of its ability to form complexes with nutritionally important essential divalent cations such as Fe, Ca, Mg and Zn as well as with protein thereby reduce their digestion, absorption and utilization in the body [43, 44].

The tannin content in unfermented sample was 0.68mg/g while the values in co-fermented samples ranged from 0.24 – 0.54mg/g. Percentage reduction of tannin in the flours from co-fermented samples increased with increase in co-fermentation time ranging from 20.59% for the flour from sample that was co-fermented for 24h to 64.71% for the flour from sample that was co-fermented for 96h. Onweluzo and Nwabugwu[26] and Nnam [45] similarly reported of decrease in tannin content in cowpea flour and cereals/cowpea ogi respectively with fermentation time. The observed decrease in tannin due to co-fermentation could be attributed to the hydrolysis of polyphenolic compounds or tannin complexes during fermentation [26]. The decrease in tannin content is desirable in view of its potential to bind and precipitate protein thereby interferes with protein digestion, absorption and utilization [48]. However, recently, there is considerable interest in the antioxidant

activity of tannins and their potential health benefits, especially in the prevention of cancer and cardiovascular disease in adults [49].

Trypsin inhibitor exhibited similar trend recorded for phytate and tannin. Trypsin inhibitor progressively decreased from 1.16TUI/mg in the unfermented sample to 0.49TUI/mg in the sample that was co-fermented for96h. Percentage reduction of trypsin inhibitor ranged from 14.66% for the sample that was co-fermented for 24h to 57.76% for sample that was co-fermented for 96h. The observed reduction in trypsin inhibitor caused by co-fermentation is desirable in view of the fact that the presence of trypsin inhibitor in the diet leads to the formation of an irreversible trypsin enzyme – trypsin inhibitor complex, causing a trypsin drop in the intestine and a decrease in the diet protein digestibility leading to slower growth [50]. Osman [51] and Mumba*et al.*[52] have earlier reported on reduction of trypsin inhibitors as a result of fermentation process.

3.4 *Effect of Co-fermentation Time on Sensory Attributes of Gruels made from Sorghum-Cowpea Flours*

The effect of co-fermentation time on sensory attributes of gruels made from sorghum-cowpea flours is presented in Table 5.

Table 5: Effect of co-fermentation time on the mean sensory score values of sorghum – cowpea gruels made from the flours

	Co-fermentation Time (h)				
Sensory Attributes	0	24	48	72	96
Colour	6.50^{c}±0.14	7.43^{b}±0.31	8.25^{a}±0.19	8.20^{a}±0.10	7.00^{b}±0.15
Flavour	5.36^{c}±0.20	7.24^{b}±0.15	8.06^{a}±0.25	7.99^{a}±0.19	7.04^{b}±0.31
Texture	5.20^{c}±0.13	6.15^{b}±0.22	7.40^{a}±0.17	7.25^{a}±0.15	7.13^{b}±0.12
Overall Acceptability	6.08^{c}±0.11	7.40^{b}±0.19	8.31^{a}±0.31	8.14^{a}±0.20	7.20^{b}±0.14

Means on the same row with different superscripts are significantly different at $P<0.05$.

The result showed that co-fermentation time had significant ($P<0.05$) effect on the colour, flavour, texture and overall acceptability of gruels prepared from sorghum-cowpea flours. Gruel made from unfermented flour was judged as the least preferredsample in terms of colour, flavour, texture and overall acceptability as their mean score values were the lowest when compared with the rest of the samples. Gruel from sample that was co-fermented for 48h was ranked best in terms of colour, flavour, texture and overall acceptability with the highest mean scores of 8.25 for colour, 8.06 for flavour, 7.40 for texture and 8.31 for overall acceptability. However, the mean score values for 48h co-fermented sample were not significantly ($P>0.05$) different from the mean score values for 72h co-fermented sample in terms of colour, flavour, texture and overall acceptability but were significantly ($P<0.05$) higher than the rest of the samples. The result revealed that co-fermentation enhanced the sensory attributes of the gruels but gruels made from 48h and 72h flours were the most preferred by the panelists.

IV. Conclusion

The study has shown that nutrient composition of co-fermented sorghum-cowpea flours varied with co-fermentation time. Crude protein, fat, total amino acids, and total essential amino progressively increased with co-fermentation time upto 72h and then declined while carbohydrate content progressively reduced with co-fermentation time upto 72h and then increased slightly for 96h co-fermented sample. Crude fibre, ash, mineral elements (K, Ca, Mg and Zn) as well as anti-nutrients (phytate, tannin and trypsin inhibitor) progressively decreased with co-fermentation time till the end of fermentation period (96h). Despite the reduction in mineral content with co-fermentation time, the 72h fermented sample with optimal protein and amino acid contents still contained appreciable quantity of the mineral elements. Reduction of anti-nutrients with co-fermentation time suggests that protein and mineral bioavailability would be improved. Sensory evaluation of gruels made from the flours revealed that gruels made from 48 – 72h co-fermented samples were the most preferred in terms of colour, flavour, texture and overall acceptability.

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Competing Interest

There is no competing interest

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