

### Effects of Combination of Cereals and Legumes (Millet, Sorghum and Soybean) on the Functional, Fungal, Yeast and Mould Properties of *Ndaleyi* Products: A Fermented Nigerian Food in Borno State

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Abstract: The purpose of this study is to determine the effects of dehulling and fermentation of millet, sorghum and defatted soybean flour as methods of processing of Ndaleyi a traditional Nigerian fermented food and to evaluate minerals, vitamins and copper composition of the raw materials and the finish products. The raw materials (millet, sorghum and soybeans) were purchased from Maiduguri Monday Market on 18<sup>th</sup> October, 2023 and transported to the Department of Food Science and technology, Ramat Polytechnic Maiduguri, Borno State, Nigeria for production and subsequent analysis. All the raw materials were purchased and stored in 25kg polythene bags at room temperature. 2kg of millet, sorghum and soybean were soaked for one week except soybean for three days, continuous addition of fresh water and removal of the older water was constantly maintained and allowed for fermentation. After fermentation and dehulling of millet, sorghum and soybean are allowed to dry and mixed sorghum, millet and soybean in ratio of dehulled and undehulled with defatted soybean at 75:25, 90:10 for both millet and sorghum with soybean respectively. The application of AOAC methods were adopted for the analysis. The functional properties of dehulled and fermented samples of millet, sorghum and soybean decreased for oil absorption capacity 84.71% to 83.60%, bulk density 0.656 to 0.613g%, water absorption capacity 85.725 to 84.59%, swelling capacity 18.763 to 17.13%, gelatinization capacity 19.131% to 14.29%, foaming capacity 18.688 to 18.220% and viscosity (Cp) 492.43 to 488.74% respectively. The functional properties of Ndaleyi, Chir and Bran shows an increase in all the parameters for millet, sorghum and soybean. Fungi, Yeast and mould counts in cfu/q was also found in raw material and the fortified samples ranged from  $1 \times 10^{1}$  to  $7.94 \times 10^{2}$  cfu/g found in all samples.

Keywords: Effects, Combination, Cereals and Legumes, Millet, Sorghum and Soybean,

#### Introduction

*Ndaleyi* is basically a fermented sun-dried agglomerated powder made from millet or sorghum. It is a major traditional food of the Kanuri people of the Kanem Bornu Empire in Nigeria. The Kanuri people have an estimated population of over five million (2006 Nigerian Population Census). Until recently, *Ndaleyi* was a food for members of the royal family and the affluent in society. It is consumed during Sallah, naming and marriage ceremonies. It is

marketed in Saudi Arabia in packaged form most especially during the Hajj (Nkama, et al. 1994).

Pearl millet (*pennisefum americanum leeke*) is one of the two most popular staple cereals (the other being sorghum) grown in Northern Nigeria which is situated in the arid and semi-arid regions (SAR) of the tropics, Nkama *et al.* 1994. This is because the millet plant can withstand drought and acidity. Over three million metric tonnes of the grain were produced in Nigeria in 1984 which accounted for about 10% of the total world production.

In Nigeria, millet is consumed mainly as human food, in many forms, one of which is *Ndaleyi* is basically a fermented, sun-dried, agglomerated powder made from millet or sorghum. Production of millet in Nigeria was estimated to be two million metric tonnes. Between 2005 and 2020 the millet crop in the country decreased due to adverse effects. The largest drop in millet production was registered in 2011 when the crop volume experienced a decrease by 75% compare to 2010 going from 5.2 million metric tonnes to 1.3 million metric tonnes due to adverse weather conditions Varrella, *et al.* (2020).

Soybeans have been one of the five main plant foods of China along with rice; soybeans, wheat, barley and millet. Soybeans production was localized in China until after the Chinese-Japanese war of 1894-95 when Japanese began to import soya bean oil cake for use as fertilizer, shipments of soy beans were made to Europe about 1908 and the soybean attracted world attention. Europeans had been aware of soya beans as early as 1712 through the writing of a German botanist. The cereals and legumes are rich in carbohydrates and proteins and many other nutrients beneficial for health. These are known as poor man's meat and good sources of complex dietary carbohydrates (starch and dietary fiber), protein, minerals and B vitamin Sangeetha, etal., (2017). The climate change and excessive use of chemical pesticides/fertilizers lead to concern over quality food production, Verma *et al.*(2016).

Nutrition plays a vital role in prevention of diseases. The richest source of proteins and nutrients are those that can be derived from cereal grains presented abundant in developing countries (Verma *et al.* 2016). Some soybean seed may have been sent from China by missionaries as early as 1740 and planted in France. Soybean in U.S. literature was in 1804, however, it is through that soybean was first introduced to Nigeria in 1908 and was cultivated as an export crop in a small area in Benue State where the introduced variety 'malayan' was adopted. The crop is usually grown in small holdings in mixed cropping with sorghum or maize or as an intercrop in citrus orchards.

Mutual supplementation of cereals with legume/oil seed has been advocated by several workers because of amino acid complementation, hence further improvement of nutritive value of the products can be done by the addition of defatted soy flour. Defatted soyflour is rich in high quality protein and an excellent source of iron, calcium and B vitamins.

Soy foods are also rich in nonnutrient functional component such as flavons. Soybean provides both soluble and insoluble fibre, which may help lower serum cholesterol level, control blood sugar, increase stool bulk, prevent colon cancer and relief symptoms associated with some digestive disorders. Other health benefits of soybeans in diet include cancer prevention, cholesterol lowering, combating osteoporosis and menopause regulation (Carroll, 1991).

Pearl millet is an important source of energy in the form of starch, but it can also contribute significant amount of fiber, minerals and other nutrients to diet. The nutritional composition of the grain shows that it is equal to wheat in its protein and superior to wheat in fats and minerals particularly iron and calcium (Asha, 1999). But information regarding the use of pearl millet in product development is scanty. The major constraints for wide spread utilization of

pearl millet are its coarse fibrous seed coat, coloured pigment, characteristics astringent flavor and poor keeping quality of the processed products (Archana *et al.*, 2001). Some of the constraints associated with pearl millet use for product development may be overcome by malting the grain. Malting has been reported to be beneficial in improving nutritive value and sensory quality of grains (Asha, 1999).

#### **Statement of the Problem**

Protein energy malnutrition (PEM) has been identified as one of the most endemic notational problems in sub-saharan Africa including Nigeria. Attempts have been made to device certain strategies for combating this menace. Highly nutritious food rich in protein and high calorie value promoted this research. Cereal grain and legume complementation has been suggested. In Africa, traditional food with adequate nutritional value like *Ndaleyi* is recommended. Nutritional deficiency disease like kwashiorkor and marasmus can be equally controlled.

A healthy diet incorporates all nutrients in moderation. Low protein intake has several health consequences and a severe lack of protein in the diet causes death (UNICEF, 2008).

The common legumes such as soybean, mung bean, black bean, lentils and chick pea increase the protein content and improving protein quality of cereal-based complementary foods. Both legumes and cereals however are rich in phytic acid which is a potent inhibitor of mineral and trace element absorption. However, a combination of cereals, millet and oil seeds provides most of the amino acids which complement each other to provide better quality proteins.

Aim and Objective

The aim is to produce *Ndaleyi* from millet, sorghum and soybean while the objective is to

- i. produce *Ndaleyi* from cereals and soybean.
- ii. determine the functional properties of the raw material and the finished products.
- iii. determine the fungal, yeasts and mould counts of the raw materials and finish products.

#### LITERATURE REVIEW

#### Cereal and Grain Legumes

The major cereals cultivated throughout the world are wheat, rice, barley, maize, rye, sorghum, oat and millet. The total cereal production in the world in 1991 was about 1.883,888,000 metric tonnes. Africa accounted for about 5.2% (99,397,000 metric tonnes) of the total cereal production in Africa during the same period (FAO, 1991).

Some of the important cereals grown in Nigeria are sorghum, millet, rice and maize. The country was the largest producer of sorghum and millet in Africa in 1991 and it produced about 14% and 8% of the world total production of millet and sorghum, respectively. (FAO 1991) Cereals are food from the cultivated grasses, member of the monocotyledonous family Gramineae. Cereals have been an important crop for thousands of years. The successful production, storage and use of cereals have contributed in no small measure to the development of modern cultivation (Kent, 1994).

A cereal is any of the edible components of the grain. Botanically, a type of fruit called caryopsis of cultivated grass, composed of endosperm, germ and bran. Cereal grains are grown in greater quantities and provide more food energy worldwide than any other type of crop (IDRC, 2016) and therefore, are staple crops for significant number of people. In their natural, unprocessed, whole grain form, cereals are rich source of vitamins, minerals, carbohydrates, fats, oils and proteins. When processed by the removal of the bran and germ, the remaining endosperm is mostly carbohydrate. In developing countries, production and consumption of grains such as rice, wheat, millet, sorghum or maize constitute majority of

daily sustenance. In developed countries, cereal consumption is moderated and varied but still substantial (IDRC, 2016).

#### **Production of Cereal Grains**

Cereal remains the most important single food in many rural areas of Asia and African providing more than 70% of the common diets (Stanley, et al. 1975). Cereal grains are relatively low in proteins and lack some of the essential amino acids. Considerable quantities of cereal grains such as maize, sorghum, rice among others is produced in various part of the world (Kent, 1983). Global production statistics (FAOSTAT, 2018) indicated that China is the highest producer of cereals in the world. As of 2016, cereal production in China was 580 million metric tons which accounts for 20.40% of the world's cereal production, followed by the United States of America, India, Russian Federation and Indonesia collectively accounting for 55.04% of the world's total cereal production which was estimated at 2,846 million metric tonnes .(FAOSTAT,2015)

Close examination of the production figures between 1961 and 2013 (Fig. 2.1) indicated that significant annual increase is recorded in developing countries than developed countries indicating huge dependence of developing countries on cereal grains for sustenance. The Food and Agricultural Organisation (FAO) recent projected global cereal production in 2018 to be 2.591 million tons which is 2.4% less than the 2017 production figure. Corn is currently the leading cereal in terms of quantity produced followed by wheat and rice (Fig. 2.1).

World wheat production in 2018 is forecast at 722.4 million tons representing the lowest level since 2013. The current reduction may mostly be as a result of lower yields because of reduced rainfall and flood in many parts of the world. Global rice production in 2018 therefore is anticipated to amount to 513 million metric tonnes up 1.3 percent from 2018 all-time high. These increased production in 2018 follows evidence of greater Asian land area planted with rice, particularly in India even though short water availabilities for irrigation might have dampened the expected harvest in Mali, Pakistan and the Philippines.

World production of coarse grain (cereal grains other than wheat and rice) in 2018 is forecast at 1,356 million tons which is 35.6 million tons (2.6 percent) below the 2017 recorded production.

#### Millet

Millet is an important food grain and major source of calorie proteins and vitamins for a large segment of population in the semi and tropical (SAT) regions of Africa and India. More than 400 million people rely on millet for their sustenance. The important classes of millet grown in Africa are *pennisetum americanum* pearl millet (Africa millet, bulrush cattaril, candle, bajra or cumbru in India; eleusine corcana finger millet (ragi birds foot, millet, marica and Teff which is confined to the highlands of Ethiopia.

Cereals are the most important staple foods for mankind worldwide and represent the main constituent of animal feed. Most recently, cereals have been additionally used for energy production for example, by fermentation, yielding biogas or bioethanol. The major cereals are wheat, corn, rice, barley, sorghum, millet, oats and rye. They are grown on nearly 60% of the cultivated land in the world. Wheat, corn and rice take up the greatest part of the land cultivated by cereals and produce the largest quantities of cereal grains (FAOSTAT, 2012). Botanically, cereals are grasses and belong to the monocot family poaceae. Wheat, rye and barley are closely related as members of the sub-family poideae and the tribus trticeae, oats are a distance relative of the triticeae within the subfamily poideae, whereas rice, corn, sorghum and millet show separate evolutionary lines. The family of all cereals is, in principle, similar. They are annual plants and consequently, one planting yields one harvest. The demands on climate, however, are different. Warm-season cereals (corn, rice, sorghum, millet) are grown in tropical low-lands throughout the year and in temperate climates during the frost-free season. Rice is mainly grown in flooded fields and sorghum and millet are adapted to arid conditions. "Cool" season cereals (wheat, rye, barley and oats) grow best in a moderate climate; wheat, rye and barley can be differentiated into winter or spring varieties. The winter type requires vernalization by low temperatures; it is sown in autumn and matures in early summer. Spring cereals are sensitive to frost temperatures and are sown in springtime and mature in mid-summer, they require more irrigation and give lower yields than winter cereals.

#### Physical, Chemical and Nutritive Characteristics of Millet

Millet is the smallest of the cultivated cereal, in size, 2mm length by 1-2.5m in width with average grain weight of 7g per width, with average grain weight per 1000 grains. The grain comprised of the seed coat, germ and endosperm. Each main part of the grain is further divided in various layers, tissues or regions. The pericarp and test are hard and indigestive. The aleurone layer contains a high proportion of protein than the flavor. The germ and scutelum are rich in protein and fat. The endosperm comprising about 70-80% of the whole grain consist of mainly starch and protein (Kent, 1994).

#### **Chemical Composition of Millet**

The mature millet grain consists of carbohydrates, proteins, lipids, minerals matter and water in addition to small amounts of vitamin and enzymes (Table 2). The carbohydrates are the most important constituent forming about 77-87% of the total dry matter, carbohydrates present in millet are starch, cellulose, hemicellulose, pentosans, dextrins and sugars. The protein content of pearl millet is from 8.6 to 17.4% depending in part on variety and class but more largely on environmental factors during growth. The removal of some of the outer layers of millet grain by dehulling lowers protein content but increases the digestibility of the dehulled product (Nkama and Ikwelle, 1997).

The protein content of pearl millet and foxtail millet is comparable with other cereal. Fearly, millet has higher protein content than sorghum or maize. Finger millet appears to be adequate in all the essential amino acids compared to sorghum, which is deficient in lysine, tryptophan and sulfur containing amino acids.

Millet is rich in the B-complex vitamins with levels of thiamine, niacin, pantothenic acid and pyridoxine familiar to that of wheat. Finger millet is high in calcium (344mg/100g fresh basis). Millet has been shown to possess anti-pellagra properties. It has been observed that incidence of pellagra was lower in areas where millet are consumed than were maize is the sole cereal food (Kent, 1983, Nkama, 1994, Ahmed and Dominguez, *et al.* 1993).

#### **Anti-nutritional Factors of Millet**

Millet contains significant amounts of polyphenols (tanning and phytic acid). The occurrence, chemical nature and mechanism of anti-nutritional or toxic effect of such components are well documented. Phytate bind Cu, Zn, N, Co, Mn and Ca make them unavoidable. Therefore, their reduction or elimination becomes very important in millet-based foods through suitable processing methods like fermentation, germination and dehulling which have been found to reduce the levels of phytic acid and tannins in food (Khetar, Paul and Chauhan, 1989; 1991 (a) and 1991 (b)).

#### **Millet Legume Combination**

The nutritional complementarity of cereals and legumes proteins seems well known in Africa. Millet or sorghum with cowpea or groundnut is used in the Sahelian countries. Chemical analysis has shown that amino acids deficient in the millet are generally adequately compensated by the proteins of legumes and vice versa. The mutual compensation is closer to deal when the ratio by mass of cereal to legume is about 70:30 in which proportion each provides about equal part by mass of protein (Nkama, 1993; Amleida Dominguez, *et al*, 1993; Merero *et al*. 1988).

#### MATERIALS AND METHODS

#### Production of Flours

The cereal grains (millet) flours were processed according to the method demonstrated by Ihekoronye and (Ngoddy 1985). Essentially, the grains were clean to remove extraneous matters such as stones, chaffs, sands and broken grains conditioned to moisture content of 14% and mill with a hammer mill (Meadows Model 35). The flour was sieved using sieves of 315 microns to separate the bran from the endosperm to produce fine flour ready for use in composite blending and the soybean flour was steeped in water for some time.

The flours produce millet, sorghum and soybeans are package and stored at room temperature for both processing and laboratory analysis in the demonstration kitchen of the Department of Hospitality Management and Department of Food Science and technology, Ramat Polytechnic Maiduguri respectively.

#### **Determination of Physical and Functional Properties of Millet**

A portion of the millet sample (2kg) was placed in an earthen ware pot and about 2.5 litres of step water (fresh borehole water or kadal) added the sample was steeped for 6 days at room temperature ( $30\pm2^{\circ}C$ ), then removed, washed with fresh water and briefly sun-dried for 1 hour to remove surface moisture. Clean soybean grains were placed in an earthen ware pot measuring 500g of step water (fresh borehole water) was fermented for one week by steeping at room temperature then removed, washed with fresh water and briefly dried for 2 hours to remove surface moisture. The steeped millet and steep soybean were mixed in ratio of 4:1 and millet homogenous using a No.2A premier grinding mill (Christy Hunt Engineering Ltd, Atlas Wors Earls Colne, U.K.). A very thin slurry was prepared from the mixed millet and soybean by adding excess water (1:10w/v). This was then sieved using a very fine cheese cloth. The bran fraction (over tail or biria) was removed, sun-dried and reserved for analysis. The filtrate was transferred into a larger earthen ware pot. Vigorously stirred by hand and allowed to settle for two or three days. Excess water was removed and a portion of it reserved for analysis.

The top-lighter layer chir was carefully removed and placed on a mater in thin sheets and the bottom denser layer *Ndaleyi* was then recovered and placed on another mat. Both samples (*Ndaleyi* and chir) were sun-dried for 6 to 8 hours.

Determination of Ndaleyi and by-product (chir and bran). Each of these was expressed as a percentage of dry matter content of cleaned dried mixed of millet and soybean grain (Nkama *et al.* 1994).

#### Physical Analysis of Millet, sorghum and Soybeans Flours

The moisture content, crude protein (m x 6.25), crude fat and ash content of millet and soybean grain, *Ndaleyi*, chir, millet and soybean mixed ogi and bran was determined using relevant AOAC methods. Titratable acidity (TA) and pH of millet grain and soybean grain during steeping, the steep liquor, *Ndaleyi*, chir and millet and soybean ogi mix was determined according to AOAC methods. Reducing sugars in raw millet grain, soybean *Ndaleyi*, chir and millet ogi were determined according to Lane and Erynon method as described by (Pearson and Nkama, 1994).

# Functional Properties of the Flour Blends and the *Ndaleyi* Determination of Bulk Density

The method described by (Onwuka 2005) is adopted. Ten (10ml) capacity graduated measuring cylinder will be pre-weighted. The cylinder was filled gently with the sample. The cylinder was tapped gently several times on the laboratory beach until no further reduction of the sample level after filling to the 10ml mark, it was weighted and calculated as follows:

Bulk density (g/ml) =  $\frac{weight of sample (g)}{volume of samples}$ 

The bulk density (g/cm3) will be determined as weight of the sample (g) divided by the sample volume (cm<sup>3</sup>) as reported by (Adejuyitan et al. 2009). Fifty-gram flour of the complementary foods will be placed into a 100ml measuring cylinder and tapped to a constant volume. The bulk density (g/cm3) was determined as weight of developed complementary food (g) divided by developed complementary food (g) divided by developed complementary food volume (cm3) as reported by (Adejuyitan *et al.*, 2009).

#### Foaming Capacity and Stability of Millet, Sorghum and Soybeans Flours

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The procedure of (Lawhom *et al.* 1971) in determining forming capacity and stability of millet, sorghum and soybean flour was adopted. Two grams of flour blends sample and 50ml distilled water were mixed in a Braun blender at room temperature. The suspension will be mix and shake for 5 minutes at 1600rpm. The content along with the foam was poured into a 100ml graduated measuring cylinder. The total volume was recorded after 30 seconds. Then the content was allowed to stand at room temperature for 30 minutes and the volume of foam only was recorded.

Foaming capacity (Fc) (%)

Where: AW = After whipping

BW = Before whipping

P<sub>5</sub> = The volume of foam only (total volume – liquid volume) after the 30 min standing is taken as foam stability

#### Oil Absorption Capacity of Millet, Sorghum and Soybeans Flours

Oil absorption capacities of the sample of millet sorghum and soybean were determined by the centrifugal method elicited by (Beuchat 1977) in the slight modifications. One gram of sample were mixed with 10ml of pure canola oil for 60 sec, the mixture then was allowed to stand for 10 min at room temperature, centrifugal at 400g for 30 min and the oil that separated was carefully decanted and the tubes will be allowed to drain at a 45° angle for 10 min and then weighed. Oil absorption is expressed as percentage increase of the sample weight.

#### Water Absorption Capacity (WAC) Millet, Sorghum and Soybeans Flours

Water absorption capacity of millet, sorghum and soybean flour was determined using (AOAC 1990) official methods of analysis. About 2g each of the flour blends of ingredients and soybean and millet blends was weighed into a centrifuge tube. Five milliliters of water were added and mixed well. The mixture was allowed to stand for 30 minutes and centrifugal at 600rpm for 15 minutes. The supernatant was decanted, and the new weight of the sample was taken as water absorbed and the result is expressed on weight (g) of water per 100g dry samples. The experiment was repeated, and triplicate determination was made for each *Ndalayi* blend.

% WAC = weight of sample after centrifuge wei of the original samples

**Swelling Power and Solubility Index Determination of Millet, Sorghum and Soybean Flours** The swelling power and solubility index method as described by (Hirsch and Kokini 2002). One gram of the flours of ingredients of soybean and millet blends was poured into pre-weighed graduated centrifuge tube appropriately labelled. Then 10ml of distilled water was added to the weighed sample. In the centrifuge tube and the solution was stirred and placed in a water bath heated at different temperature of 85oC for one hour while shaking the sample gently to ensure that the starch granules remained in suspension until gelatinization occurred. The samples were cooled to room temperature under running water and centrifugal for 15 min at 3000rpm. After centrifuging, the supernatant was decanted from the sediment into a preweighed petri-dish; the supernatant in the petri-dish was weighed and dried at 105°C for 1h. The sediment in the tube was weighed and the reading recorded. The starch swelling power and solubility were determined according to the equations below:

Swelling power	_	Weigh of swollen sediment		
Swening power		Weight of sta		
Solubility =	Weigh	of dry supernatar at of starch sample	$\frac{1t}{100}$ (%)	
Solubility -	- Weigh	t of starch sample	-X 100 (78)	

#### Determination of viscosity in Millet, Sorghum and Soybean Flours

The hot paste viscosity of the samples was determined with Brookfield Viscometer RV Model (Brookfield Engineering Laboratories Stoughtonn, U.S.A) as described by (Badau *et al.* 2006). The viscosity of the developed complementary foods was determined by first preparing the gruel. The slurry of the complementary *Ndaleyi* was prepared by dissolving 40g in 200ml and 20g in 200ml of distilled water to give 20% and 10% w/v) concentration respectively. The slurry was heated in water bath timed to reach a cooking temperature of 95% for 7 minutes. The viscosity of the flours and complementary *Ndaleyi* foods measured appropriate ambient temperature of 32.1%, revolution per minutes (RPM) of 2.5 for 10% w/v concentration using spindle "L" and 28.9°C RPM of 6 for 20% w/v concentration using spindle (Mosha and Svanberg, 1983; Badau, *et al.* 2006).

#### **Microbial Analysis**

The pour plate technique (Hanigan and McCane, 1976) was used for the inoculation. Ten grams (10g) of the samples of dehulled, undehulled fermented and fermented millet, sorghum, soybean and its foritificants were weighed and dissolved in 10ml of sterile peptone water. It was allowed to stand for 10-15 minutes after shaking vigorously. Media such as Eosine methylene blue agar, monnisol salt agar, McConkey Agar, Deoxychlolate citrate Agar, salmonella, Shigella Agar and Selenite "E" were all prepared and used. The suspension wase poured to each of these media and allowed to cover it completely for 10-15 minutes, the excess water discarded. The plates were incubated at 37°C aerobically and anaerobically for 48 hours. Observations were made for the growth of colonies appearing on the plates with digital colony counts. The mean values of the triplicate platescounts, were expressed as colony forming units per grams (cfu/g). Microorganism associated with the complementary food formulations were determined by Harrigan and McCancer (1976) and AOAC (2000).

#### Preparation of the Nutrient Agar

Nutrient agar should be prepared and kept at pH 5.8. The colony forming unit (cfu/g) of the millet samples were determined using plate count agar (PCA) or nutrient and potato dextrose

agar (PDA) for bacteria and fungi respectively via pour plate method after serial dilution  $(10^{-1} - 10^{-5})$ . 100µl of serially diluted *Ndeleye* sample was dispensed into petri-dishes with plate count agar (PCA) or nutrient agar and potato dextrose agar (PDA) and incubated at ambient temperature (20-29°C) for 24-36h and 48-72h for bacteria and fungi respectively (Eruteva and Odunfa, 2017). After incubation, the colonies were enumerated, and distinct colonies will be selected and sub-cultured successively to obtain pure cultures.

#### Identification of Bacterial Isolates

Pure cultures of bacteria isolated was characterized and identified on the basis of their cultural (size, pigment, opacity, edge, elevation, shape, consistency) morphological (gram staining and spores staining) and bio-chemical properties (catalase, citrate, urease, coagulase, gelatinase, starch, hydrolysis, sugar fermentation oxidate test). Bergey's manual of Determinative Bacteriology (Holt *et al.* 1994). Pure cultures were preserved in slant and also stored in 20% glycerol broth at 70°C for further analysis.

#### **Preparation of Samples for Microbial Analysis**

Samples of the complementary food formulations was sealed in plastic bags and kept in dry plastic containers for analysis.

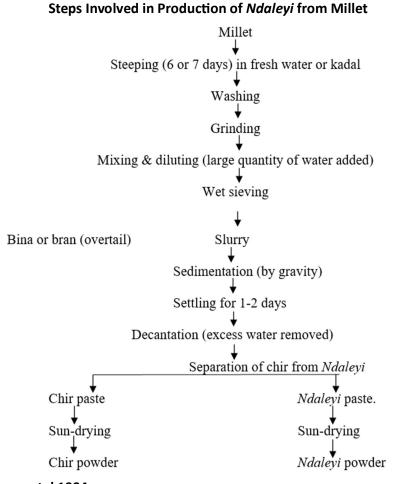
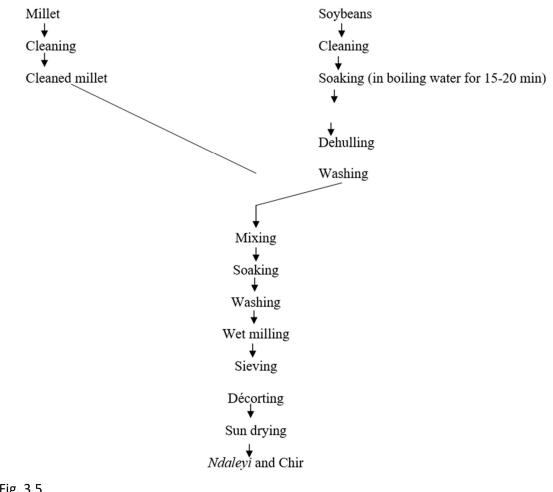


Fig. 3.4 Nkama etal 1994



#### **Ndaleyi** Production Flow Chart



#### **RESULTS AND DISCUSSION**

#### Effects of Dehulling, on the Functional Properties of Millet, Sorghum and Soybean Flour.

The oil and water absorption capacities of the samples increased from dehulled to undehulled millet, sorghum and soybean flours from (80.63% to 82.97%) and (84.26% to 87.63%) for water absorption capacity and (83.37 to 86.30%) (0.16% to 83.63%) respectively. The samples exhibit high degree of viscosity and low bulk density as shown on Table 4.3. The swelling capacity (%) of the sample increases for dehulled for millet, sorghum and soybean flours respectively. Gelatinization capacity of samples of millet, sorghum and soybean increasing from dehulled to undehulled flour, foaming and emulsification capacities also reduced as the samples are dehulled, likewise increases for the undehulled samples.

The results also revealed the decrease in water absorption capacity of the millet and sorghum from 86.2% to 83.40% and 84.36 to 82.58% after dehulling respectively. Th oil absorption capacity also decreased in millet and sorghum from 80.64%, to 76.93% and 87.25% to 84.23% after dehulling respectively. Oil absorption capacity and foaming capacity also decrease for soybean flour from 80.80% to 78.1% and 17.33% to 16.27% respectively. Gelatinization capacity and viscosity also decrease upon dehulling of millet and sorghum from 18.53% to

14.34% and 387.33 to 375.30 respectively. The functional characteristics as shown on the results table 4.3, has evaluated the possibility of the compaction and mixing behavior of various nutrients example, proteins, fats, vitamins, fibre, minerals and carbohydrates in both dehulled and undehulled flour mixed for both cereals and legumes.(Singh 2006, Siddiq et al., 2009). Functional properties are the fundamental physico- chemical properties that reflects the complex intersection between the composition, structure, molecular conformation and the component of food within the environment in which are in associations (Kinsella 1976).

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		Parameters <sup>(1)</sup>				
Samples (CODE) (2)	OAC (%)	BD (g.m3)	WAC (%)	SC (g)	GC (%)	Viscosity (CP)
DMF	80.65 <sup>bc</sup> ±1.0E.02	0.57 <sup>c</sup> ±0.0	83.40 <sup>c</sup> ±0.02	16.57 <sup>d</sup> ±0.03	14.35 <sup>d</sup> ±0.03	375.30 <sup>d</sup> ±0.01
UMF	76.96 <sup>c</sup> ±5.28	0.64 <sup>ab</sup> ±0.02	86.29 <sup>a</sup> ±0.03	17.73 <sup>c</sup> ±1.0E.02	18.54 <sup>a</sup> ±1.0E.02	387.33 <sup>c</sup> ±0.01
DSF	84.25 <sup>ab</sup> ±0.01	0.61 <sup>d</sup> ±0.02	82.58 <sup>d</sup> ±0.02	18.93 <sup>b</sup> ±0.02	17.13 <sup>c</sup> ±0.01	331.36 <sup>b</sup> ±0.01
USF	87.62 <sup>ª</sup> ±5.4E.03	0.64 <sup>b</sup> ±5.4E.03	84.36 <sup>b</sup> ±0.01	20.49ª±0.02 <b>FC (%)</b>	18.34 <sup>b</sup> ±0.02 EC (%)	342.61ª±0.02
DSOF	78.14 <sup>b</sup> ±0.02	0.81 <sup>b</sup> ±0.0252	80.18 <sup>b</sup> ±1.0E.02	6.28 <sup>b</sup> ±0.02	8.35ª±0.03	462.73 <sup>b</sup> ±1.0E.02
USOF	80.81 <sup>a</sup> ±0.02	$0.85^{a}\pm0.01$	83.60 <sup>a</sup> ±1.0e.02	17.33 <sup>ª</sup> ±1.0e.02	6.56 <sup>b</sup> ±0.01	481.34 <sup>a</sup> ±0.01

Table 1: Effects of Dehulling, on the Functional Properties of the Millet, Sorghum and
Soybean flour

 Values are mean ± standard deviation of triplicate determinations. In any column, means bearing similar superscripts are not significantly different (P≥0.05).

2. OAC = 0i1 Absorption Capacity; BD = Bulk Density; WAC = Water Absorption, SC = Swelling Capacity; GC = gelatinization Capacity (CP); Viscocity, FC = Foaming Capacity, EC = Emulsification Capacity

 DMF = Dehulled Millet Flour; UMF = Undehulled Millet Flour; DSF = Dehulled Sorghum Flour; USF = Undehulled Sorghum Flour

DSOF = Dehulled Soybean Flour; USOF = Undehulled Soybean Flour

## Effects of Fermentation on the functional properties of Millet, Sorghum and Soybean Flours

The importance of starch in foods depends on its technological properties wherein, it confers several attractive textured qualities to recipe formulation and food product development. Starches are generally regarded as the most important constituents of cereals in terms of pasting behavior, gelatinization retrogradation and other functionality attributes which influence the product quality (Hagenimana and Ding, 2005).

Oil absorption capacity, water absorption capacity and viscosity of the fermented samples of cereals and legume based on this research are all high, especially the undehulled millet, sorghum and soybean increased after fermentation. The Bulk density, swelling capacity, gelatinization and emulsion capacity for soybean are all low. The functional properties of millet as shown on the table 4.6 indicate an increase in oil absorption capacity, bulk density, gelatinization capacity and also viscosity, on fermentation and dehulling from 84.71% to 83.60%, 0.656% to 0.613%, 19.13% to 4.24% and 398.74% to 3.96% respectively. The fermented sorghum flour shows little changes in the oil absorption capacity from the control sample. Swelling capacity and water absorption capacity shows decrease on dehulling and fermentation because of their high moisture content. (Kinsella 1976). Increase in oil

absorption capacity makes the flours suitable in facilitating enhancement in mixing and mouth feel when used in food preparations (Jones *et al* 2000).

The results also showed decrease in oil absorption capacity, bulk density, water absorption capacity, emulsion capacity, foaming capacity and viscosity of the fermented and dehulled samples of soybean flour from 82.64% to 80.43%, 0.94% to 0.92%, 84.57% to 83.76%, 9.51 to 8.71%, 18.68% to 18.26% and 497.43% to 488.74% respectively. Results obtained, is in conformity with the available literatures.

Table 2: Effects of Fermentation on the Functional Properties of the Millet, Sorghum and
Soybean Flours

			Parameters (1)			
Sample	OAC)	BD	WAC	SC	GC	СР
(Code)(2)						
FDMF	83.60 <sup>d</sup> 0,03	0.61 <sup>d</sup> ±0.02	84.59d±1.E-02	17.11 <sup>d</sup> ±0.02	14.24 <sup>d</sup> ±0.01	395.96 <sup>b</sup> ±0.01
FUMF	84.71 <sup>c</sup> ±1.0E-02	0.66 <sup>c</sup> ±0.01	85.78c±1.0E-02	18.76 <sup>c</sup> ±0.02	19.13 <sup>b</sup> ±0.02	398.74 <sup>ª</sup> ±0.02
FDSF	87.55ª±0.02	0.75 <sup>b</sup> ±0.01	87.57±0.02	18.92 <sup>b</sup> ±0.02	18.92 <sup>b</sup> ±0.02	367.97 <sup>d</sup> ±0.01
FUSF	87.21 <sup>b</sup> ±1.0E-02	88.24 <sup>a</sup> ±0.02	88.24a±0.02	21.14 <sup>a</sup> ±5.E	21.61 <sup>a</sup> ±0.02	386.65 <sup>c</sup> ±5.4E-03
				EC (%)	FC (%)	
FDSOF	80.44 <sup>a</sup> ±54E-03	0.92a±0.01	83.77 <sup>b</sup> ±0.02	8.71 <sup>b</sup> ±0.01	18.26 <sup>b</sup> ±1.0E-	488.74 <sup>b</sup> ±0.02
					02	
FUSOF	82.65 <sup>b</sup> ±5.74E-03	0.94a±10E-	84.57 <sup>ª</sup> ±1.0E-02	9.51ª±0.01	18.69ª±0.01	497.43 <sup>ª</sup> ±0.02
		02				

1. Values are means ± standard deviatyion of triplicates determination in any column, means bearing similar superscripts are not significantly different (P≥0.05)

2. OAC = Oil Absorption Capacity, BD = Bulk Density, WAC = Water Absorption Capacity, SC = Swelling Capacity, GC = Gelatinization Capacity, CP = Viscosity, EC = Emulsion Capacity, FC = Foaming Capacity

 FDMF = Fermented Dehulled Millet Flour; FUMF = Fermented Undehulled Millet Flour FDSF = Fermented Dehulled Sorghum Flour; FDSOF = Fermented Dehulled Soybean Flour FUSOF = Fermented Undehulled Soybean Flour

## Functional Properties of Fortified *Ndaleyi* and Defatted Soybean from Dehulled and Undehulled Millet and Sorghum Flour

The functional properties of undehulled millet *Ndaleyi* and soybean at (75:25) fortification ratios with oil absorption capacity at 82.93%, bulk density at 0.63%, water absorption at 85.82%, swelling capacity at 16.20%, gelatinization capacity at 34.59% and viscosity at 421.75% are all higher when compared to the dehulled millet *Ndaleyi* and soybean fortification at (90:10) ratios, for oil absorption capacity at 80.95%, bulk density at 0.58%, water absorption capacity at 82.48%, swelling capacity at 14.53%, gelatinization capacity at 30.66% and 410.91 viscosity which are all low, when compared to (75:25) sorghum *Ndaleyi* flour fortified with defatted soybean flour. As shown on table 4.15

Functional properties of the fortified samples of cereals and legumes are all affected by the processing. The functional properties of the defatted soybean with the dehulled and undehulled millet *Ndaleyi* flour at (75:25) and (90:10) remains high than the dehulled and undehulled sorghum *Ndaleyi* flour at (75:25) and (90:10) levels of fortification samples as shown for oil absorption capacity, Bulk density, water absorption capacity, and viscosity at 84.18%, 0.63%, 84.33% and 421.75 respectively. High in water absorption capacity and bulk density of the fortified *Ndaleyi* from ratios (75:25) for dehulled and (90:10) for undehulled millet and sorghum flours are suitable for products that are mixed to produced paste and

harden products like *Ndaleyi* (Singh 2006, Siddiq et al 2009)). Functional properties of flours help to developed new products by understanding the molecular conformation and physicochemical properties that reflects the complex intersection between composition and structure (Kinsella 1976). Oil absorption capacity at 84.18% for sorghum *Ndaleyi* at (90:10) fortification ratio, makes the flour suitable in facilitating enhancement in flour and mouth feel when used in food preparations (Jones et al 2000).

 Table 3: Functional Properties of Fortified Ndaleyi Samples and Defatted soybean from

 dehulled and undehulled millet and sorghum flour

			(Parameters) (1)			
Sample Code (2)	OAC	BD (%)	WAC	SC (%)	GC (%)	СР
MNSO Dehulled (90:10)	80.95 <sup>d</sup> ±0.04	0.58ª±0.19	82.48 <sup>d</sup> ±0.28	14.53 <sup>d</sup> ±0.05	30.66°±0.05	410.91°±0.11
MNSO (undehulled (75:25)	82.92 <sup>b</sup> ±0.05	0.63ª±0.02	85.82ª±0.17	16.2 <sup>c</sup> ±0.05	34.59 <sup>b</sup> ±0.52	421.75°±0.06
SNSO Dehulled (90:10)	84.18ª±0.05	0.61ª±0.05	84.33 <sup>b</sup> ±0.05	16.43 <sup>b</sup> ±0.11	36.53ª±0.32	393.65 <sup>d</sup> ±0.11
SNSO (undehulled) (75:25)	82.28 <sup>c</sup> ±0.11	0.69ª±0.56	83.59°±1.0E-02	14.54 <sup>d</sup> ±0.05	36.50°±0.02	417.02 <sup>b</sup> ±0.06
DSOF	74.11 <sup>e</sup> ±0.01	0.60ª±0.02	81.19 <sup>e</sup> ±0.03	19.42ª±0.0153	22.15 <sup>d</sup> ±0.02	348.37 <sup>e</sup> ±0.03

 Values are means ± standard deviation of triplicates determination in any column, means bearing similar superscripts are not significantly different (P≥0.05)

- OAC = Oil Absorption Capacity; BD = Bulk Density; WAC = Water Absorption Capacity; SC = Swelling Capacity; GC = Gelatinization Capacity; CP = Viscosity
- MNSO = Millet Ndaleyi and Soybean (90:10) dehulled MNSO = Millet Ndaleyi and Soybean (75:25) undehulled SNSO = Sorghum Ndaleyi and Soybean (90:10) dehulled SNSO = Sorghum Ndaleyi and soybean (75:25) undehulled DSOF = Defatted Soybean Flour

## Table 4.22: Fungal, Yeasts and Mould Counts of Sample of Raw Materials (Millet, Sorghum and Soybean in (cfu/g)

The results of fungal, yeasts and mould count on samples of raw materials millet, sorghum and soybean flour Shows that, the total fungal count, on the sample of millet, sorghum and soybean was detected on all the sample of the raw materials under investigation on table 4.22. The total fungal count on the soybean flour is about  $4.54 \times 10^3$  cfu/g which is higher than both millet and the sorghum flour at  $3.44 \times 10^2$  cfu/g and  $4.42 \times 10^2$ cfu/g respectively. Among the dangerous fungi found in cereal and legumes are *Aspergillus, Fusarium* and *Penicillium*. The acceptable level of fungi contamination in cereals and legumes are in maximum of Log 5cfu/g (world food programme 2020).

Yeast count on the millet, sorghum and soybean flours ranges from  $6.34 \times 10^2$  cfu/g,  $6.24 \times 10^2$  cfu/g and  $4.83 \times 10^2$  cfu/g respectively. The highest yeasts count found in soybean flour followed by sorghum flour and lastly millet flour, the acceptable limit of contamination of cereals and legumes in molds and yeast is <1000cfu/g. Mould count on the raw material as indicated on the table, revealed that soybean flour is high in mould counts at  $4.43 \times 10^2$  cfu/g while  $2.62 \times 10^2$  cfu/g was found in millet flour which is lower. High amount of mould count was detected in sorghum flour at  $4.33 \times 10^2$  cfu/g. The level of contamination by yeasts and moulds on millet, sorghum and soybean grain flours are above the threshold level.

F	Parameters (cfu/g)	
Total Fungal Count TFC	Yeast Count	Mould Count
3.44x10 <sup>3c</sup> ±0.01	4.83x10 <sup>2a</sup> ±0.01	2.62 <sup>b</sup> x10 <sup>2</sup> ±1.000E-04
4.42x10 <sup>2b</sup> ±1.0E-02	$6.24 x 10^{2a} \pm 0.01$	4.33x10 <sup>2a</sup> ±1.0E-04
4.54x10 <sup>3a</sup> ±1.000E-02	6.34x10 <sup>2a</sup> ±0.01	4.43x10 <sup>2c</sup> ±0.01
	Total Fungal Count TFC 3.44x10 <sup>3c</sup> ±0.01 4.42x10 <sup>2b</sup> ±1.0E-02	$\begin{array}{ccc} 3.44 \times 10^{3c} \pm 0.01 & 4.83 \times 10^{2a} \pm 0.01 \\ 4.42 \times 10^{2b} \pm 1.0 \text{E-}02 & 6.24 \times 10^{2a} \pm 0.01 \end{array}$

### Table 4: Fungal, Yeasts and Mould Counts of Samples of Raw Materials (Millet, Sorghum and Soybean (cfu/g)

 Values are means ± standard deviations of triplicate determination in any column, means bearing similar superscripts are not significantly different (P≥0.05)

2. MF = millet flour; SF = sorghum flour; SOF = soybean flour

#### Fungal, Yeast and Mould Counts of Ndaleyi, Chir and Bran from Millet and Sorghum Flour

The results of total fungal counts on the millet *Ndaleyi* flour is less than the millet chir flour at  $5.66 \times 10^2$  cfu/g and  $5.87 \times 10^3$  cfu/g respectively, these may be due to the fact that the chir is Porteous part of the *Ndaleyi* by-product and hence is susceptible to fungal attack. Millet bran flour is also high in fungal contamination at  $6.22 \times 10^3$  cfu/g than both the millet and chir *Ndaleyi*.

Sorghum bran flour is also rich in fungal colony counts, then both the *Ndaleyi* and chir from sorghum fermented flour at 7.56x103 cfu/g. This is due to the concentration of fungal organisms found in the sorghum bran flour. Yeast counts of the millet *Ndaleyi* flour is less than both its chir and bran at  $6.74x10^2$  cfu/g, while the sorghum bran flour is high in yeast counts at  $6.35x10^2$  cfu/g than the *Ndaleyi* and its chir extracts. Mould counts of the millet bran flour is also higher than the *Ndaleyi* and its chir extracts at 5.85x102 cfu/g while millet and chir are at  $4.64x10^2$  cfu/g and  $5.72x10^2$  cfu/g respectively. Also the sorghum *Ndaleyi* flour is high in mould count at  $4.23x10^3$  cfu/g than chir flour at  $4.34x10^2$  cfu/g and the bran at  $4.45x10^2$  cfu/g respectively. The level of contamination by yeasts and moulds on the sorghum *Ndaleyi* and Bran are above the required acceptable level of <1000cfu/g (World Food Programme 2020).

	Parameter (cfu/g)				
Sample	Total Fungal Count (TFC)	Yeast Count	Mould Count		
Code					
MNF	5.66x10 <sup>2d</sup> ±0.01	6.74x10 <sup>2b</sup> ±1.0E-04	4.64x10 <sup>2c</sup> ±1.0E-02		
MCF	5.87x10 <sup>3d</sup> ±0.01	7.84x10 <sup>2a</sup> ±1.0E-02	5.72x10 <sup>2a</sup> ±1.0E-02		
MBF	6.22x10 <sup>3c</sup> ±0.01	7.93x10 <sup>2a</sup> ±0.01	$5.85 \times 10^{2a} \pm 0.01$		
SNF	6.35x10 <sup>3c</sup> ±0.01	6.15x10 <sup>2b</sup> ±5.4E-03	4.23x10 <sup>3d</sup> ±0.01		
SCF	7.45x10 <sup>3b</sup> ±5.4E-03	6.23x10 <sup>2b</sup> ±0.01	4.34x10 <sup>2d</sup> ±0.02		
SBF	7.57x10 <sup>3a</sup> ±0.02	6.35x10 <sup>2b</sup> ±0.01	4.45x10 <sup>3d</sup> ±1.0E-02		
1	Values are means + standard deviati	ons of triplicate determination	in any column means hearing similar		

#### Table 5: Fungal, Yeast and Mould Counts of Ndaleyi, Chir and Bran from millet and sorghum flour

 Values are means ± standard deviations of triplicate determination in any column, means bearing similar superscripts are not significantly different (P≥0.05)

2. MNF = millet *Ndaleyi* flour; MCF = millet chir flour; MBF = millet bran flour

SNF = sorghum Ndaleyi flour; SCF = sorghum chir flour; SBF = sorghum bran flour

## Fungal, Yeast and Mould Counts of Fortified *Ndaleyi* with the Soybean in certain Ratios for both Dehulled and Undehulled Millet and Sorghum Flour

The results of the fungal counts for fortified Ndaleyi with undehulled millet and soybean flour indicated an increase in contamination, with levels at 7.83 cfu/g compared to the dehulled millet Ndaleyi, which had a lower fungal count of 7.65 cfu/g. Specifically, the fungal counts for fortified millet at a 90% fortification level were recorded at 7.65 x 10^3 cfu/g, while the undehulled millet at 75% fortification showed higher contamination at 7.83 cfu/g. In contrast, sorghum with a 90% dehulled flour fortification level had a high fungal count of 6.94 x 10^3

cfu/g, whereas the undehulled sorghum at 75% fortification had a lower count of 3.31 x 10^3 cfu/g. All contamination levels remained within the acceptable limit of <1000 cfu/g, as per the World Food Programme standards of 2020.

Yeast counts for fortified millet flour were notably higher in dehulled samples at 7.65 x 10<sup>2</sup> cfu/g compared to the undehulled sample at 90% fortification with 10% soybean flour. In sorghum, the yeast count for dehulled flour at a 90% fortification level was relatively low at 3.62 x 10<sup>2</sup> cfu/g. Mould counts for dehulled fortified millet flour were high at 4.67 x 10<sup>2</sup> cfu/g at a 90% fortification level, while undehulled millet had a lower mould count of 4.32 x 10<sup>2</sup> cfu/g. The mould count for fortified dehulled sorghum flour was also high at 4.47 x 10<sup>2</sup> cfu/g compared to undehulled sorghum flour at 75% fortification, which had a count of 2.52 x 10<sup>2</sup> cfu/g. Despite variations, all fungal, yeast, and mould counts in the fortified Ndaleyi samples of dehulled and undehulled millet and sorghum flours were within the legally acceptable limits.

## Table 6: Fungal, yeast and Mould count of fortified Ndaleyi with the soybean in certain ratios for both dehulled and undehulled millet and sorghum grain flours

otal Fungal Count (TFC)	Yeast Count	
	reast count	Mould Count
.65x10 <sup>3b</sup> ±5.4E-03	6.44x10 <sup>2b</sup> ±0.01	4.67z10 <sup>2a</sup> ±1.0E-02
.8310 <sup>3b</sup> ±1.0E-04	7.65x10 <sup>2a</sup> ±0.01	4.32x10 <sup>2a</sup> ±5.4E-03
.94x10 <sup>3c</sup> ±0.01	7.44x10 <sup>2a</sup> ±0.01	4.47x10 <sup>2a</sup> ±0.01
3.08x10 <sup>3a</sup> ±0.01	$3.62 x 10^{2b} \pm 0.01$	$2.52 x 10^{2b} \pm 0.02$
	8310 <sup>3b</sup> ±1.0E-04 .94x10 <sup>3c</sup> ±0.01 3.08x10 <sup>3a</sup> ±0.01	.8310 <sup>3b</sup> ±1.0E-04         7.65x10 <sup>2a</sup> ±0.01           .94x10 <sup>3c</sup> ±0.01         7.44x10 <sup>2a</sup> ±0.01

 Values are means ± standard deviations of triplicate determination in any column, means bearing similar superscripts are not significantly different (P≥0.05)

2. DMSO = dehulled millet sorghum flour; UMSO = undehulled millet sorghum flour DSSO = dehulled sorghum soybean flour; USSO = undehulled sorghum soybean flour

#### Conclusion

The proximate composition of Ndaleyi derived from both millet and sorghum was characterized by low levels of protein, fats, fibre, ash, and moisture content, as detailed in Table 4.10. However, it was high in carbohydrate content. Compared to Ndaleyi, the chir and the bran or overtail were found to be richer in protein. In terms of functional properties, Ndaleyi exhibited high oil absorption capacity, bulk density, water absorption capacity, swelling capacity, gelatinization, and viscosity, whereas chir had lower values and bran had higher values. Traditional Ndaleyi was low in most B-vitamins, while the chir and bran were comparatively richer. Fortified Ndaleyi also showed high carbohydrate content, with its oil absorption capacity, water absorption capacity, and viscosity exceeding 50%, though its bulk density, swelling capacity, and gelatinization was low.

#### Recommendations

The research work reveals the nutritional significance of *Ndaleyi*, fortified *Ndaleyi* and products:

- 1. Dehulling of cereals and legumes reduces the amount of micronutrients e.g. magnesium, phosphorus, calcium, potassium, sodium, iron and zinc. Dehulling of cereals and legumes should be discouraged in *Ndaleyi* processing.
- 2. Undehulled cereals and legumes contains high amount of protein, fats, crude fibre, ash and carbohydrates than the dehulled samples, it is also recommended that dehulling of cereals and legumes should be discourage in order to safeguard the availability of this essential nutrients in *Ndaleyi* processing.
- 3. *Ndaleyi* food product is highly recommended for a patient of celiac disease (protein intolerance) because of its lower protein content.

4. Fermentation, had been reported to improve sensory and nutritional quality of cereals, because of the lactic acid bacteria that participated in the process which decreases carbohydrate content increase in amino acids and B-group vitamin contents, i.e leusine, arginine, alanine, aspartic acid, glycine and lysine.

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