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*Full Length Research Paper*

# **African yam beans (*Sphenostylis stenocarpa*): A review of a novel tropical food plant for human nutrition, health and food security**

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**This review investigated African yam beans (AYB) as a novel tropical food plant for human nutrition. AYB plants food potentials, its nutritional and amino acid compositions were also examined. In the same vein, the health benefits, utilization of AYB in starch and composite flour technology as well as its toxicological analysis were explored. Also, strategies to minimize processing difficulties and antinutritional factors were highlighted. It was revealed that AYB plant products are very rich in protein, minerals and antioxidants with low glycemic index (GI). Furthermore, viable processing techniques like fermentation, boiling and roasting can reduce AYB antinutritional factors and its toxicological effects. The result suggests that AYB plant products can meet dietary needs of people suffering from malnutrition, kwashiorkor, diabetes and other cardiovascular diseases, thus improving food security. Full utilization of AYB plants in food, drugs and health industries should be explored by carrying out further studies on ethno-pharmacological claims of AYB leaves and tubers. The findings revealed that AYB plant products hold great potential in improving food security globally.**

**Key words:** African yam beans, food security, nutritional factors, health benefits, limiting factors.

## **INTRODUCTION**

Plant foods still maintain the greatest possible fountainhead of basic nutrients for major population globally. They are major sources of food for mankind that cannot be replaced. Plant foods are more advantageous over other sources of food due to the fact that they are readily available, cheap, affordable and generally acceptable. In Africa, especially in Nigeria, most of these plant food sources have gone to extinction, ignored and

underutilized (Abdulkareem et al., 2015; Barrett, 2010). African yam beans (AYB) is one of such neglected and underutilized foods. AYB is a tropical leguminous plant cultivated mainly in West African countries (Frank et al., 2016). Above the ground, AYB produces a good yield of edible seeds. It could be found in forests, open wooded grasslands, rocky fields, and marshy grounds as weed and cultivated crops. It grows on wide range of soils

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including acid and highly leached sandy soils at altitudes from sea level to 1950 m (Frank et al., 2016). AYB is cultivated mainly for home consumption and only about 30% of the dry grain produced is sold (Osuagwu and Nwofia, 2014). The noticeable attracting flowers are mauvish-pink, purple or greenish-white in colour, which are about 2.5 cm in length and borne on stout auxiliary peduncles. The smooth, hairless seed pods are linear, flat, with both margins raised, 25 to 30 cm long and 1.0 to 1.5 cm broad, containing 20 to 30 seeds which may be ellipsoid, rounded or truncated, and show considerable distinct in size and colour; the largest are usually about 1.0 cm long and 0.7 cm wide (Adewale et al., 2010). AYB tubers are small and spindle-shaped usually about 5.0 to 7.5 cm in length and weighing on average 50 to 150 g (Adewale et al., 2010). Also, it can weigh up to 0.3 kg under favorable conditions. The tubers are rich in protein, and thus, regarded as an important source of amino acids. AYB dry matter is about 35% in which 14% are protein while 80% are starch (Frank et al., 2016; Ojueoderie and Balogun, 2019). The FAO/WHO (1991) have also reported the AYB amino acid profile to be higher than those in other legumes including soybean, and affirmed that this same amino acid profile compares favorably with whole hen's egg and met the organizations daily requirement of food. It was reported that the tubers can be cooked and eaten in the same manner with other tubers like yams and potatoes. The flavor of the tuber is similar to that of potatoes (Olisa et al., 2010; Okoye et al., 2019). The colour of the seed may differ from creamy-white or brownish-yellow to dark brown, occasionally with black steaky appearance. Previous work done on the AYB seeds showed that the dry matter is approximately (90.50%), which comprise protein (24-28%), fat (1.5-2.0%), total carbohydrate (74.10%), fiber (5.2-5.7%) and ash (2.80-3.20) (Frank et al., 2016; Nwokeke et al., 2013; Osuagwu and Nwofia, 2014; Olisa et al., 2010). The amino acid content of the protein depicted similar value to that of soya bean but richer and higher in histidine and iso-leucine (Frank et al., 2016; Nwokeke et al., 2013; Osuagwu and Nwofia, 2014; Olisa et al., 2010). The energy content of the seeds per 100 g dry matter was reported to be 1.640 kJ. It was reported that the seeds can be eaten without harm, non-toxic to humans, suitable for consumption but must be soaked in water for about 12 h before being cooked or processed (Olasoji et al., 2011; Ajayi and Akande, 2010). The plant has beautiful flowers that are cultivated as ornament in many countries like Europe. There are different varieties of AYB with different sizes, seed coat colors and shades; they include grey, white, black, brown and stripped brown AYB (Gbenga-Fabusiwa, 2019). Nigerian synonyms of AYB are: *Hausa* (Girigiri), *Yoruba* (Sese), *Igbo* (Okpodudu), and *Ibibio* (Nsama) while the International synonyms of AYBs are: *China* (Fan-ko), *India* (Sankalu), *German* (Knollige Bohne), *Malawi* (Nkhoma), and *Ghana* (Kulege). There

are two edible products of AYB; they include its tuber that grows as the root and the seeds that develop in the pods (Olasoji et al., 2011). Some underutilized legumes similar to AYB including pigeon pea and baobab have been reported to be useful in the dietary management of cardiovascular diseases due to its high protein and fiber contents (Gbenga-Fabusiwa et al., 2019). However, AYB is deficient in sulphur containing amino acids, methionine and cysteine but very rich in lysine and thus could be utilized as a complementary diet (Abioye et al., 2015). The crop is highly underexploited due to the fact that little is known about it. Another reason for its underutilization is due to its relatively low farm yield and long cooking time which is about 140 min (Frank et al., 2016). It is one of the ideal sources of protein supplementation of starchy foods. This will improve the use of this lesser known and underutilized legume in a number of food preparations especially in the developing countries. Supplementation equally has resulted in products with high nutritional values. In line with the aforementioned, the effect of incorporation of AYB on the pasting, proximate and sensory attributes of cassava was studied by Nwokeke et al. (2013). Frank et al. (2015) studied the effect of AYB on serum calcium, inorganic phosphate, alkaline phosphatase and uric acid concentration on male albino rats. It was observed that there were significant reductions of the serum concentration of calcium and uric acid of the test group compared to those of the Baseline and Control groups. The reduction in uric acid level is an indication that AYB consumption could be a good remedy for gout and potential in mopping up urate crystals. Also, some gouty conditions usually led to arthritis. This also means that the AYB can be used as a local analgesic under this condition.

The reduction in serum uric acid concentration of AYB compares well with that of soybean (Frank et al., 2016). Sam (2019) stated that the seeds of AYB contribute to nutrient intake by the consuming populations in Nigeria and contain some pharmacological evidence for the treatment of stomach aches and acute drunkenness which can serve as an antimalaria, antidiabetic, fertility agent, anti-ulcer and cardioprotective agent. In Togo, Ghana and Nigeria, the lectin content of AYB seed is used as insecticide. The pastes made from the seeds are used as a cure for stomach ache/antacid. Also, when water is added to this paste, it becomes an anti-alcohol abuse (antabuse) which is natural unlike the drug disulfiram with its adverse drug reaction antecedents (Micheal et al., 2018). An increase in dietary protein has a negative effect on bone calcium level. The proposed mechanism is that increased protein intake (especially proteins with sulfur containing amino acids) leads to an increased glomerular filtration rate, reduced renal reabsorption of calcium, hypercalciuria and thus leaching of calcium out of the bone (Kumar et al., 2013). Food chemists are greatly concerned about oxalate due to its

adverse effect on the availability of minerals. Food having high level of oxalate can result in kidney dysfunction as its high levels tantamount to increased absorption of calcium in the kidneys (Michae, 2018; Okonkwo et al., 2013). Oxalate builds complex if taken by animals and also great amount of oxalate diets can increase the risk of absorbing renal calcium (Sam, 2019). Also, dietary oxalate can form complex with calcium, magnesium and iron resulting in insoluble oxalate salts and oxalate stone. However, polyphenols such as tannins have anticancer properties, so foods such as green tea that contain large amounts of these compounds might be good for the health of some people despite their antinutrient properties. The present unpredictability in the global food supply, the increased demand in searching for other food sources with general accessibility and willingness of weary consumers to consume functional food that will add values to their health prompted this study. A lot of researchers like Adegunwa et al. (2012), Adefegha and Oboh (2013), and Abioye et al. (2015) have investigated the nutritional potentials of plant seeds less known as other food sources. Developing nations' desire to advance in eradicating severe destitution and famine specifically necessitates doing great study in certain under-utilized native crops and tree plants. Due to the transformations all over Africa, wild plants are at risk of going into extinction and might affect the nutrition of the local people. The blood glucose responses of carbohydrate foods can be classified by the glycemic index (GI) and glycemic load (GL), which are considered to be valid indices of the biological value of dietary carbohydrates, and the measure of the potential of carbohydrate to raise blood glucose concentration after a meal (Abioye et al., 2015; Gbenga-Fabusiwa et al., 2019; Okonkwo et al., 2013). In short, carbohydrates that decompose rapidly during digestion have a high glycemic index due to the fact that blood glucose response is fast and high, while those that break down slowly have a low glycemic index (Okonkwo et al., 2013). Many starchy staple foods produced traditionally possess low glycemic indices and they include cracked wheat or barley, parboiled rice, dried peas, beans, pasta and lentils (Oboh et al., 2010). Michael et al. (2018) investigated the antidiabetic activity of seed milk extract in alloxan-induced diabetic rats. The oral glucose tolerance test was also carried out using animal experimental method. The phytochemical analysis of the milk extract revealed the presence of flavonoids, isoflavones, saponin, tannin, phytosterol, lignin and anthocyanidine at moderate concentrations. It was equally reported that the seed milk extract of AYB possessed anti-diabetic activity like the reference drug glibenclamide, and have blood glucose-lowering effect in a time and concentration-dependent manner. AYB is being increasingly consumed by diabetics, hypertensive and cardiovascular patients in some Nigerian communities. However, the processes

involved in making it ready to eat are cumbersome and time consuming, thereby denying vulnerable groups instant consumption access. Therefore, it is of great industrial and commercial importance to develop the technology for its processing into ready-to-eat breakfast cereals, in combination with other local food materials, which will eliminate the associated drudgery and add value to the products. Efforts are being made to promote the use of composite flour in which flour from locally grown crops and high protein seeds replace a portion of wheat flour for use in baking industries, thereby decreasing the demand for imported wheat and producing nutrients enriched flour baked products (Gbenga-Fabusiwa et al., 2018a; Adefegha and Oboh, 2013). Global nutrition survey revealed that the most dietary deficiencies are of protein and of high biological value (Chikwendu et al., 2014; Ajayi, 2011; Christian, 2010). Therefore, the introduction of affordable and readily available plant-derived alternatives to wheat protein could be one of the dietary interventions required to close a nagging nutritional gap in meal planning for diabetic clients. AYB including pigeon pea and baobab have been reported to be of great importance in the management of cardiovascular diseases due to its high protein and fiber contents (Gbenga-Fabusiwa, 2019). It possesses high metabolizable energy, low true protein digestibility and high mineral contents (Abioye et al., 2015). AYB is one of such legumes with a protein, fat and carbohydrate content of 22, 2.5 and 62.5%, respectively (Ikemefuna and Julian, 2010; Okonkwo et al., 2013). Despite its nutritive value and increased production in Akoko area and other South Eastern states of Nigeria, consumption has not increased as with cereal products. Food insecurity is a major challenge to be tackled globally as one billion people are suffering from starvation and malnutrition (Padulosi et al., 2013; Lama, 2017). The trends include the continuing addition of 70 million people per year to the earth's population and the desire of some 4 billion people to move up the food chain and consume livestock products. In China, for instance, annual *per capita* consumption of meat has risen from 20 to 50 kg in less than 30 years (Sasson, 2012, Chikwendu et al., 2014; Frank et al., 2016; Morales, 2011). About half of the grains produced in the world are used to feed the livestock. That is why the increase in cereal and fodder prices has a strong impact on livestock products. For example, milk rose from 80 to 200%, while poultry rose by 10%. Food self-sufficiency is the first challenge developing countries need to tackle and resolve (Sasson, 2012). Africa, where people under 15 years old represent some 45% of the whole population, will have to feed a population that is expected to increase from 832 million in 2002 to more than 1.8 billion by 2050. The agricultural sector, which employs about 60% of the whole population, represents some 20% of the gross domestic product (GDP) and provides more than 10% of the export



**Plate 1.** Varieties of African yam beans seeds.

revenues. It should become the driving force of economic and social development (Sasson, 2012; Abdulkareem et al., 2015; Ojuederie and Balogun, 2019; Oshomoh and ilodigwe, 2018). Food insecurity and malnutrition is a serious problem in Nigeria, despite all the efforts to combat them. This is because animal food which are rich sources of protein are very expensive and beyond the rich of majority of the population (Morales, 2011; Godfray et al., 2010). There is relatively little information about clinical trials of AYB plant products on kwashiorkor and diabetic clients. Therefore, this study intends to highlight the nutritive composition of AYB, its antioxidants potentials, glycemic response, other health benefits, antinutritional limitation factors, toxicological analysis and suggests viable ways to maximize its utilization in both food and pharmaceutical industries. This could be a veritable way of establishing and maximizing AYB health benefits and its utilization as well as improving food security globally.

## MATERIALS AND METHODS

AYB samples were procured from interior farm of Oka-Akoko, Ondo-State, Nigeria. The beans were sundried and the picture taken as shown in Plate 1. The tubers of AYB were extracted from the international grain legumes information center (Adebowale IITA, 2010) while the leaves and the flowers pictures were sourced from Wikipedia, the free encyclopedia. These were done for a period of four (4) months. Effect of processing on nutritional composition of AYB was sourced from the reports of Abioye et al. (2015), Adewale et al. (2013), and Ngwu et al. (2014). This spanned for a period of one (1) month. Mineral composition of raw and processed AYB were extracted from the report of Uche et al. (2014) and Adegunwa et al. (2012) for one (1) month. Antinutrients of unprocessed and processed (boiled and roasted) AYBs with their respective permissible limits were reported according to Sam (2019), Chai and Liebman (2004), Uche et al. (2014), and Adegunwa et al. (2012) for

a period of two (2) months. It is worthy of note that amino acids compositions of raw and processed AYBs were extracted from the reports of Esan and Fasasi (2013) for one (1) month. Also, physical and chemical properties of AYB seed milk were obtained from the reports of Michael et al. (2018) and this took place for one (1) month. The gathering of other valuable information and resources took place for a period of four (4) months while the write up of the paper took four (4) months. It took total period of 18 months to complete the study. 78 papers (2010-2020) were reviewed, 10% of which are on AYB plants, 30% on nutritive composition of AYB, 20% on food security, 10% on health benefits, 20% on composite flour technology, 5% on toxicological analysis of AYB 3% on conclusion, 1% on recommendation and 1% on limitation of the study.

## RESULTS AND DISCUSSION

### African yam beans plants

AYB (*Sphenostylis stenocarpa*) is a perennial climbing bush, 1-3 m high, that is cultivated annually. Its leaves comprise three leaves with oval leaflets (2.7 to 13 cm long and 0.2 to 5.5 cm broad) (Abuldukareem et al., 2011; Chikwendu et al., 2014; Chinedu and Nwonyi, 2012). AYB is cultivated for its edible tubers, which resemble elongated sweet potatoes, and for its seeds, which are contained in hard and tough pods of about 20-30 cm long. It is exclusively utilized as food for feeding livestock (Ajayi et al., 2010; Heuze, 2016; Ikemefuna and Julian, 2010). It grows on deep, loose sandy and loamy soils with good organic content and good drainage. It flourishes better in regions where annual rainfall lies between 800 and 1400 mm, and where temperatures ranged from 19 to 27°C. The plant springs up flowers after 90 days and the pods become mature in 140 to 210 days. The tubers are ready to harvest as from 150 to 240 days after planting (Ohanmu et al., 2018; Heuze, 2016;



**Plate 2.** African yam beans tubers. Source: Adebowale (2010).

Ikemefuna and Julian, 2010). AYB plant yields small spindle-shaped tubers that are about 5.0 to 7.5 cm tall (Olisa et al., 2010; Okoye et al., 2019; Aremu and Ibirinde, 2012). The seeds are usually ground and substituted to other flour to produce composite flour so as to improve its functional potentials. Seeds can also be used in the preparation of soups, sauces and starch (Olasoji et al., 2011; Ajayi and Akande, 2010, Ajayi et al., 2010). They are important source of calcium and amino acids (Frank et al., 2016; Nwokeke et al., 2013; Osuagwu and Nwofia, 2014; Olisa et al., 2010). Greater attention is being paid by the researchers on the exploitation of starch from under-utilized legumes seeds like pigeon pea and AYB so as to alleviate protein-energy deficiency in food and to improve products diversification of legumes. The picture of two varieties of African yam beans is as shown in Plate 1. They can cause giddiness if consumed in excess; however, it was believed that they can cure drunkenness when mixed with water (Sam, 2019; Yusuf et al., 2013). The high protein content of AYB makes it an important source of protein in the human diets of many tropical countries (Elsie and David, 2016). Furthermore, the high protein bean flour fractions could be substituted for wheat flour to produce good quality biscuits and bread in food industries (Gbenga-Fabusiwa et al., 2018b; Yusuf et al., 2016; Nneoma et al., 2012; Amakoromo et al., 2012). It could also be used in preparing porridge, moimoi and beans cake. The flesh is white and watery, the tubers can be eaten fresh and thus saving the cost of fuel and fire woods (Heuze, 2016; Ojueoderie and Balogun, 2019; Popoola et al., 2011). The researchers that worked on AYB food processing reported that soaking overnight is a vital key to minimize cooking period (Heuze, 2016; Ojueoderie and Balogun, 2019; Popoola et al., 2011). The tubers could be eaten raw or processed, however, the details are still not well

understood, therefore, more work is expected to be carried out on this. Some countries like Mexico process the raw AYB in the following ways: raw tubers are sliced into sticks, sprinkled with lime juice and chill; the sliced tuber could be added to salads for dinner; cooked tuber could be used with or without vegetables for soup preparation; they can also be grated and boiled in milk to create a tasty drink; the tuber could be sliced, diced and pickled with onion and chill to form a popular snack food (Heuze, 2016; Ojueoderie and Balogun, 2019; Okoye et al., 2019).

Furthermore, it was reported that AYB are preserved in vinegar and used as a variety of three-bean salad. The picture of AYB is as shown in Plate 2. AYB should be cultivated industrially to meet the need of people in dearth need of food especially during this corona virus compulsory vacation. AYB leaf and flower are shown in Plates 3 and 4, respectively. The plant is adaptable and can be cultivated anywhere. The plant climbs, twines to heights higher than 3 m, thrives in a region where the rainfall is extremely high and tolerates and grows even in acidic, leached and infertile soil (Heuze, 2016; Osuagwu and Nwofia, 2014). However, there is dire need of information on the health benefits of the AYB leaf and flower. AYB plant has mutual symbiosis with bacteria that fix nitrogen from the atmospheric air and thus adding nutrient such as nitrogenous fertilizer to the soil as well as saving the cost of procuring fertilizers (Osuagwu and Nwofia, 2014; Uwaegbute et al., 2012). In Nigeria, the plant is cultivated from the tropical forests to at least the savanna by the primitive farmers. The plant has great potential feed for livestock and green manure for rejuvenating the soil fertility. It is believed that the leaf is edible but the mode of processing it and the safety of eating it has not been established experimentally. The protein-rich leafy vegetables left-over after harvesting





**Plate 3.** African yam beans leaf. Source: Wikipedia.



**Plate 4.** African yam beans flowers. Source: Wikipedia.

serve as useful fodder and are nutritionally beneficial to livestock. However, the utilization of AYB protein-rich-leaf has not been investigated and could be useful in the formulation of dietary supplement.

#### **African yam beans as a solution for the food security problem**

Food security occurs when mankind gain access physically, socially and economically to adequate, safe

and nutritious meal to meet their dietary demands and functional food preferences for healthy and active living (Sasson, 2012; Adewale and Dumet, 2010). Sasson (2012), Petrikora et al. (2017) and Adewale and Dumet (2010) identified food availability, access to food, its utilization and stability as the core four pillars of food security. Food insecurity is a major challenge to be tackled globally as one billion people are suffering from starvation and malnutrition (Padulosi et al., 2013; Kandala et al., 2011; Godfray et al., 2010; Lama, 2017). Previous work done revealed that 11.11% people are malnourished, more than 70% of these human beings reside in developing countries. Severe wasting of 52 million children occurs globally due to undernutrition (Hickel, 2019). This results to 45% of deaths in children under five that is 3.1 children die in a year due to malnutrition (Kinyoki et al., 2020). Food and Agricultural Organization of the United Nation (FAO) is expertizing action on attaining sustainable development goals (SDGs) to terminate hunger, accomplish food security and ameliorate sustainable agriculture. Globally, an estimated 155 million children are influenced negatively by acute malnutrition. It also hinders the normal development of children's brain and promotes the risk of death (Fan and Polman, 2014). The International Food Policy Research (2013) reported that emphasis of the SDGs should not be to terminate poverty by 2030, but to abolish hunger and malnutrition by 2025 (Fan and Polman, 2014). Agriculture, social protection and dietary intervention or a combination of any two methods stated are major tracks to eliminate hunger and malnutrition (Fan and Polman, 2014). The number of persons experiencing pains from hunger is estimated at 239 million in sub-Saharan Africa, and this number could shoot up rapidly in the nearest future. The major identified source of food insecurity is insufficient food production. There has been great increase in awareness to cultivate more and better functional foods like AYB to enhance food security since the global food insecurity of 2007 to 2008 (Padulosi et al., 2013). The food insecurity the world is experiencing is motivated by the accumulation of successive confirmed phenomena that influence demand and supply globally (Sasson, 2012; Chikwendu et al., 2014; Frank et al., 2016; Morales, 2011). The phenomena comprise continuing inclusion of 70 million people annually to the world population and the demand for 4 billion populaces to go up the food chain and consume animal products. For example, meat consumption per capital per year in China rose from 20 to 50 kg in less than 30 years (Sasson, 2012; Chikwendu et al., 2014; Frank et al., 2016; Morales, 2011). Livestock are fed with about 50% of the grains produced globally, as a result of this higher prices of cereal and fodder have a strong competitive impact on livestock products. Consequently, the prices of milk rose from 80 to 200%, while poultry rose by 10%. Food self-sufficiency is the

most eminent difficulty task that the developing countries need to tackle and resolve (Sasson, 2012). In Africa, people with less than 15 years old typify 45% of the total population; this is anticipated to rise from 832 million in 2002 to above 1.8 billion by 2050 which have to be fed. The agricultural industry hires about 60% of the total population which exhibits 20% of the gross domestic product (GDP) and furnish with greater than 10% of the export turnovers. This should emerge the driving force for economic and social growth (Sasson, 2012; Abdulkareem et al., 2015; Ojuederie and Balogun, 2019; Oshomoh and Ilogigwe, 2018). Food insecurity and malnutrition is a serious problem in Nigeria, despite all the efforts to combat them. Global nutrition survey displayed that the most dietary inadequacies are protein of high biological value (Chikwendu et al., 2014; Petrikova and Hudson 2017). This is because animal food which are rich sources of protein are very expensive and beyond the reach of majority of the population (Morales, 2011; Godfray et al., 2010). However, vegetable proteins complement each other, for example, a combination of legume and cereal protein will possess a nutritive value as good as animal protein. Chikwendu et al. (2014) and Frank (2015) reported that protein inadequacy cannot be surmounted by using animal products alone. However, it can be improved in combination with vegetable protein consumption, which makes up to 70% of world's protein production. Masika (2014) reported that food insecurity is associated with increased risk of non-adherence to antiretroviral therapy among HIV-infected clients. Mankind are endowed with plant resources for meat before their arrival on earth. Availability of functional plants is not a challenge in developing countries like Nigeria, which is having rich agroclimatic, cultural and ethnic biodiversity. However, full utilization of these functional plants in food, pharmaceutical and health industries is a big challenge. AYB has been reported to have the potential for supplementing the protein requirement of many families throughout the year (Ikemefuna and Julian, 2010; Okonkwo et al., 2013). Several processing methods have been used to enhance its acceptability and nutritional values (Uwaegbute et al., 2012; Abioye et al., 2015). The blood glucose responses of carbohydrate foods can be classified by the glycemic index (GI) and glycemic load (GL), which are considered to be valid indices of the biological value of dietary carbohydrates, and the measure of the potential of carbohydrate to raise blood glucose concentration after a meal (Abioye et al., 2015; Gbenga-Fabusiwa et al., 2019; Okonkwo et al., 2013). AYB potential to scavenge free radicals and to act as a chelating agent of metal ions, or act as hydrogen donor enable it to neutralize the effect of free radical in the body. AYB is usually cooked and eaten either alone or with yam, maize and rice. It can be used to replace cowpea in food preparation especially during the lean period when food is scarce among rural farmers

(Frank et al., 2016; Nwokeke et al., 2013; Osuagwu and Nwofia, 2014; Olisa et al., 2010; Kandala et al., 2011). It is one of the ideal sources of protein supplementation of starchy foods. This will improve the use of this lesser known and underutilized legume in a number of food preparations especially in the developing countries. Supplementation equally has resulted in products with high nutritional values. In line with the aforementioned, the effect of incorporation of AYB on the pasting, proximate and sensory attributes of cassava was studied by Nwokeke et al. (2013). It was reported that incorporation of AYB into cassava gives the product referred to as AYB fufu flour. The feasibility of using the legume (AYB) and guinea corn to produce a weaning food formulation has also been investigated (Chikwendu et al., 2016). Michael et al. (2018) and Yusufu et al. (2016) produced cookies from maize, AYB and plantain composite flour and reported that cookies had higher levels of proteins, fat, moisture, beta-carotene (provitamin A), vitamin C, and iron but reduced carbohydrate when compared with the control (100% Wheat). Cookies produced from 70% maize, 20% AYB and 10% plantain composite flour had the highest score for general acceptability and compared favorably with the control cookies for almost all sensory attributes. Microbiological analysis revealed that all the cookies produced were free of microorganisms for up to two months of storage under ambient conditions. Uche et al. (2014) studied the level of anti-nutrients composition of raw and processed AYB. It was observed that the processed AYB registered significant reduction in levels of hydrogen cyanide, trypsin inhibitor, phytate, oxalate, and tannins compared to the unprocessed. AYB is being increasingly consumed by diabetics, hypertensive and cardiovascular patients in some Nigerian communities. However, the processes involved in making it ready to eat are cumbersome and time consuming, thereby denying vulnerable groups instant consumption access. Therefore, it is of great industrial and commercial importance to develop the technology for its processing into ready-to-eat breakfast cereals, in combination with other local food materials, which will eliminate the associated drudgery and add value to the products. Researchers who did a nutritional evaluation of 44 genotypes of AYB reported that the crop is well balanced in essential amino acids and has higher amino acid content than pigeon pea, cowpea, and bambara groundnut (Chikwendu et al., 2014; Ajayi, 2011; Magdi, 2010). Apart from the use of soybean as an alternative to animal protein, protein from other plant sources is not often exploited. Thus, AYB incorporation in formulating new food products may suggest a viable way of food security. Another researcher reported on positive contribution of AYB to food security due to presence of lectin identified in the seeds, which could be a potent biological control for most leguminous pests (Chikwendu et al., 2014; Ajayi, 2011; Magdi, 2010). AYB farming is



**Table 1.** Effect of processing on nutritional composition of AYB (g/100 g).

Sample	Moisture	Ash	C. Fiber	Fat	C. Protein	Carb
UAYB	10.58±2.01 <sup>ab</sup>	2.79±0.30 <sup>a</sup>	9.40±0.24 <sup>a</sup>	2.84±0.05 <sup>a</sup>	21.54±1.51 <sup>a</sup>	62.92±1.05 <sup>d</sup>
AYBB	12.61±0.01 <sup>ab</sup>	2.64±0.04 <sup>a</sup>	7.98±0.00 <sup>a</sup>	1.88±0.00 <sup>a</sup>	18.95±1.06 <sup>a</sup>	64.90±1.06 <sup>c</sup>
AYBR	9.83±2.62 <sup>ab</sup>	2.64±0.01 <sup>a</sup>	8.84±0.50 <sup>ab</sup>	2.41±0.03 <sup>a</sup>	20.47±0.00 <sup>b</sup>	68.62±1.52 <sup>bc</sup>

Source: Abioye et al. (2015), Adewale et al. (2013), and Ngwu et al. (2014).

**Table 2.** Mineral composition of raw and processed AYB (mg/100 g).

Sample	UAYB	AYBB	AYBR
Ca	245.00±5.0 <sup>b</sup>	220.95±0.30 <sup>a</sup>	249.40±6.24 <sup>c</sup>
P	252.61±0.0 <sup>b</sup>	226.40±0.04 <sup>a</sup>	257.98±0.00 <sup>c</sup>
Mg	89.83±2.62 <sup>a</sup>	91.84±0.50 <sup>b</sup>	90.41±0.03 <sup>b</sup>
K	478.47±0.00 <sup>b</sup>	452.62±1.52 <sup>a</sup>	580.62±1.52 <sup>c</sup>
Na	352.47±0.00 <sup>a</sup>	392.62±1.52 <sup>c</sup>	389.62±1.52 <sup>b</sup>
Mn	3.57±0.00 <sup>b</sup>	3.26±1.52 <sup>a</sup>	6.0062±1.52 <sup>c</sup>
Fe	19.47±0.00 <sup>a</sup>	19.22±1.52 <sup>a</sup>	19.62±1.52 <sup>a</sup>
Cu	2.28±0.00 <sup>a</sup>	2.42±1.52 <sup>a</sup>	2.49±1.52 <sup>a</sup>
Zn	6.05±0.00 <sup>b</sup>	3.75±1.52 <sup>a</sup>	3.79±1.52 <sup>a</sup>

Source: Uche et al. (2014) and Adegunwa et al. (2012).

not capital intensive; hence, it could provide new vista of opportunities for farmers. It is a remarkable wholesome quality crop, since is mostly cultivated by primitive farmers which constitute about 70% of African population. It provides a functional way to economically empower the poor farmers, thus improve the standard of living of mankind. The seeds are drought resistant and perfect for intercropping and crop rotation; they do not require huge land space but do enhance nitrogen fixation and consequently soil fertility. Food insecurity and malnutrition is a serious problem in Nigeria and the globe at large, despite all the effort to combat them. The food industries should subscribe to the production, processing and utilization of this under exploited AYB leguminous plant as they could be valuable in the persistent fight against hunger, malnutrition, diabetes and food insecurity.

### Nutritional composition of AYB

Animal food which are rich sources of protein are very expensive and beyond the rich of majority of the population (Chikwendu et al., 2014; Ajayi, 2011). However, vegetable proteins complement each other, a combination of legume and cereal protein will have a nutritive value as good as animal protein. According to Ajayi (2011) protein deficiency cannot be overcome by using animal products alone. He maintained that the only

alternative is to push up the vegetable protein intake, which already makes up 70% of world's protein production. This study revealed that AYB have many nutritional benefits which could improve the level of malnutrition, boost food security and serve as a good functional food in formulating composite flour that possess some health benefits. Tables 1, 2, and 3 depicted the proximate, mineral and antinutrient compositions of processed and unprocessed AYB. It was revealed that the nutritional composition of AYB consists of carbohydrate (63.39), ash (3.41), fat (0.54), protein (20.37) and crude fiber (1.54). The average mineral composition comprised Na (496), K (620), Ca (92), Mg (103), P (251), Zn (6), Fe (53), Mn (12), Cu (1.3), Cd (2.1) but Hg, As, Ni were found below detection limit (Abioye et al., 2015; Adewale et al., 2013; Ajayi et al., 2010; Ngwu et al., 2014). Yusufu et al. (2013) evaluated complementary food produced from sorghum, AYB and mango mesocarp flour blends and reported an increase in the protein and fat level of the formulated complementary food from 8.90 to 17.40 and 1.50 to 3.50, respectively. However, a significant decrease in the carbohydrate level (78.00 to 67.59%) was observed. The sensory evaluation revealed that the formulated complementary food had good overall acceptability. In addition to this, it is revealed that the food produced is a protein-rich product with good functional potentials. Yusufu et al. (2016) produced cookies from maize, AYB

**Table 3.** Antinutrients of unprocessed and processed AYB.

Antinutrient	UAYB	AYBB	AYBR	PL
Hydro cyanide (mg/kg)	18.58±2.01 <sup>a</sup>	44.29±1.30 <sup>b</sup>	49.40±1.24 <sup>c</sup>	50
Tannin (mg/g)	16.40±0.01 <sup>b</sup>	3.64±0.04 <sup>a</sup>	3.45±0.00 <sup>a</sup>	20
Phytate (mg/100 g)	429.30±2.62 <sup>b</sup>	125.34±0.01 <sup>a</sup>	122.84±1.50 <sup>a</sup>	50
Oxalate (mg/kg)	8.41±0.03 <sup>b</sup>	2.22±0.00 <sup>a</sup>	3.17±1.52 <sup>a</sup>	3-5
Trypsin Inhibitor (mg/100 g)	6.67±0.03 <sup>b</sup>	1.10±0.00 <sup>a</sup>	1.64±0.00 <sup>a</sup>	0.7-3

Unprocessed African yam beans (UAYB), African yam beans boiled and African yam beans roasted ( $P < 0.05$ ). PL-Permissible limits. Source: Sam (2019), Chai and Liebman (2004), Uche et al. (2014), Adegunwa et al. (2012), and Hession et al. (2009).

and plantain composite flour and reported that cookies had higher levels of proteins, fat, moisture, beta-carotene (pro-vitamin A), vitamin C, and iron but reduced carbohydrate when compared to the control (100% Wheat). Cookies produced from 70% maize, 20% AYB and 10% plantain composite flour had the highest score for general acceptability and compared favorably with the control cookies for almost all sensory attributes. Microbiological analysis revealed that all the cookies produced were free of microorganisms for up to two months of storage under ambient conditions. The use of plant protein sources in local food formulations could be vital in upgrading their nutritional, functional and sensory properties. Previous work done on the effect of processing on the nutritional levels of AYB depicted that protein content, crude fiber and lipid are significantly higher in raw, unprocessed than the processed AYB (Uche et al., 2014; Adegunwa et al., 2012; Sam, 2019). The level of crude protein and crude fiber for the processed AYB were lower than that of unprocessed AYB (Uche et al., 2014; Adegunwa et al., 2012). The loss in protein content could be due to denaturation that took place during roasting process. Contrary to this, Chikwendu et al. (2014) reported that fermentation process improved protein (24.70-28.70) and ash levels of AYB flour. However, carbohydrate content of the flour sample was decreased by fermentation process. Roasting increased the % levels of Ca, K, Cu, Fe, Mn, Mg, P but Zn level was reduced (Uche et al., 2014; Adegunwa et al., 2012). Boiling processing resulted in reduction in Ca, K, Fe, Mn and Zn, however there was increased in moisture and carbohydrate levels of AYB (Uche et al., 2014). This assertion could be attributed to the dehydration that took place during roasting process, hence, improve the concentration of the mineral content in the raw or unprocessed AYB. The presence of water during boiling will definitely increase moisture content of AYB, enhance hydration, hydrolysis, thus the conversion of some mineral to other compounds which may likely result to the reduction in mineral levels of processed AYB when compared to raw AYB. Antinutrient compounds are toxic, harmful and may impair digestibility of protein and some

vital minerals into the body system. However, the good news is that they are labile and thus may be reduced or inactivated by processing methods such as boiling, roasting and frying (Nwosu, 2010). The antinutrient contents of AYB were significantly reduced by processing methods such as roasting, baking and boiling (Uche et al., 2014; Adegunwa et al., 2012). It was revealed that roasting process is more effective and efficient in the reduction of phytate levels in AYB than boiling process. However, boiling process reduced oxalate better than roasting (Uche et al., 2014). Table 4 reveals that roasting and boiling process increase the hydro cyanide content of AYB. However, the increment is still below the permissible limit recommended by FAO/WHO. Conversely, the processes lower the tannin, phytate, oxalate and trypsin inhibitor. This is in agreement with the report of Chikwendu et al. (2014) where fermentation reduced the tannin levels in both flours as against the control from 0.51 to 0.31 and 0.16 to 0.02 mg, respectively. The phytate content was also reduced from 0.49 to 0.25 and 0.26 to 0.06 mg. It has been reported that AYB processing has both positive and negative correlation with its nutritional potentials. For example, it was reported that roasting increased the % of mineral levels of AYB and decreased its Zn content. Boiling had negative correlation with AYB mineral levels but resulted to positive correlation with the levels of Cu, Mg, P and Na (Uche et al., 2014). The levels of oxalate in unprocessed AYB (16.40) were higher than the boiled AYB (3.64) and roasted AYB (3.45) (Sam, 2019). Ikemefuna and Julian (2010) reported that AYB has high quality protein and minerals which could be much improved by adequate preparation and supplementation for incorporation into various traditional dishes for both children and adults. All the articles reviewed pointed to the fact that, adequate processing like soaking, parboiling, boiling, cooking and roasting is required to improve antinutrient content of AYB seeds. Table 4 depicted the basic amino acids present in unprocessed AYB and FAO/WHO suggested amino acid contents for children and adults. It was also revealed that processing like boiling, cooking and roasting increase the Alanine, Glycine and Valine

**Table 4.** Amino acids composition of raw and processed AYB (mg/100 g).

Amino acid	UAYB	AYBB	AYBR	FAO/WHO
Morlencine	4.00	6.86	6.63	-
Glutamic acid	12.10	10.44	10.64	-
Aspartic acid	10.00	9.89	7.87	-
Serine	5.04	4.48	3.52	0.5
Threonine	4.58	5.00	4.27	-
Alanine	3.85	5.35	5.53	-
Glycine	4.11	6.65	6.31	-
Valine	-	4.92	4.04	-
Cystine	5.44	1.79	1.69	-
Methionine	5.44	0.97	1.12	1.90
Isoleucine	4.89	2.81	2.28	13.00
Leucine	7.90	5.07	4.34	19.00
Tyrosine	3.80	2.19	2.88	-
Phenylalanine	6.13	1.78	2.72	-
Histidine	5.51	3.41	3.58	1.60
Lysine	8.80	6.95	7.09	1.60
Arginine	6.39	3.41	3.49	-

Unprocessed African yam beans (UAYB), African yam beans boiled and African yam beans roasted (P<0.05). Source: Esan and Fasasi (2013).

contents of AYB. Esan and Fasasi (2013) reported that AYB raw flour (unprocessed) had threonine (4.58 g/100 g) which was far above (0.9 g/100 g) required by FAO/WHO for adult. Amino acids are essential components for wound healing and protein production. Lack or deficiency in these vital essential amino acid components will inhibit the healing and recovery processes (Michael et al., 2018; Okonkwo et al., 2013). Polypeptide that enhance growth and wound healing are produced from glycine and other essential amino acids such as alanine, proline, arginine, serine, isoleucine and phenylalanine (Esan and Fasasi, 2013; Sarwar et al. 2010). AYB contains all the essential amino acids in higher proportion compared with FAO requirement for adults (Rajapakse et al., 2005), and contains sulphur amino acid found to be deficient in other legumes (Ikemefua et al., 2010). AYB may contribute adequate proportions of total essential amino acid since the value obtained is similar to the recommended (39%) considered to be adequate for ideal protein food for infants above that recommended (26%), and considered to be adequate for ideal protein for children and (11%) for adults (FAO/WHO/UNU, 1985). Amino acid composition of AYB suggests that it has adequate protein content and are excellent for mankind consumption. However, there is dire need of information on how to improve the hygroscopic nature of AYB protein concentrate and its production as a dietary supplement or tablet. Improvement of the palatability of protein hydrolysates,

while maintaining nutritional value and safety is required.

### Health benefits of African yam beans

Consumption of legume seeds such as AYB, pigeon pea and soya beans have many health benefits in that they are rich in food components such as vitamins, flavonoids, total polyphenols, fibers and lipids (Esan and Fasasi, 2013; Chikwendu et al., 2014; Ajayi, 2011; Magdi, 2010; Antangwo et al., 2013; Amakoromo et al., 2012). Legumes ability to prevent disease is based on its antioxidant, anti-inflammatory, anti-ageing and detoxification potentials (Esan and Fasasi, 2013; Atangwho et al., 2013). Natural antioxidants are more desirable because they do not have harmful side effect associated with the use of synthetic equivalent even at higher concentration (Soetan et al., 2018). The following phytochemicals are reported: total phenolic (0.925 mgGAE/ml); DPPH scavenging ability of AYB (69.16-87.83), total antioxidant capacity (3.9795 mgAsAE/ml); reducing capacity (1.4985) (Soetan et al., 2018). The antioxidant activities observed in aqueous extract are generally low (Soetan et al., 2018; Enujiugha et al., 2012). Phenolic compounds play vital roles in human pathogenic infections (Doughari, 2012). Nwankwo and Ekeanyanwu (2011) reported that the phytochemicals present in AYB such as flavonoids, isoflavones, anthocyanides, saponins, phytosterols, and lignin have

the potential health benefits functioning as anti-cancer, and heart disease, lower blood cholesterol, reduce risk of heart disease, anti-hypertensive, anti-diabetic, anti-osteoporosis as well as anti-inflammatory agent. In 2016, an estimated 1.6 million deaths were directly caused by diabetes. Another 2.2 million deaths were attributable to high blood glucose levels in 2012 (Sarwar et al., 2010). The dietary glycemic index (GI) and glycemic load (GL) concepts indicate an expected behavior for the rate of carbohydrate assimilation in the prevention, management and treatment of chronic disease such as diabetes and high blood pressure. Diabetes mellitus (DM) is a complex metabolic disorder affecting 171 million people worldwide, and this number is projected to reach 366 million by 2030. About 50 to 70% diabetic wound resulted to limb amputation globally. WHO report that diabetes will be 7th foremost reason for death in 2030. In 2014, 9% adults had diabetes and resulted to 1.5 million death in 2012. The number of people with diabetes has increased from 108 million in 1980 to 422 million in 2014. The global prevalence of diabetes among adults over 18 years of age has increased from 4.7% in 1980 to 8.5% in 2014 (Basith et al., 2020). Diabetes prevalence has been rising more rapidly in middle-and low-income countries. Diabetes is a major cause of blindness, kidney failure, heart attacks, and stroke. The good news is that diabetes can be treated and its consequences avoided or delayed with diet, physical exercise, medication, regular screening and treatment for complication (Sarwar et al., 2010). Oboh et al. (2010) worked on the glycemic response of some boiled legumes commonly eaten in Nigeria and reported that AYB had the lowest GI (17). The GI of AYB is very low when compared with cow pea (41), pigeon pea (24), and groundnut nut (24) (Oboh et al., 2010). Legumes produce relatively low glycemic responses in both healthy individuals and diabetic clients (Gbenga-Fabusiwa et al., 2018a, 2019). The components present in legumes, particularly the dietary fiber, the protein contents and the type of starch can influence the rate by which glucose is released from starch and rate at which it is absorbed from the small intestine. This makes legumes especially the one with low GI like AYB suitable as functional meal in regulating postprandial rise in blood glucose levels. Many researchers have reported epidemiological importance of AYB in the management and prevention of several diseases. The medicinal properties of plants secondary metabolites are many and well documented (Frank et al., 2016; Michael et al., 2018; Okonkwo et al., 2013; Sarwar et al., 2010; Akpan et al., 2012). AYB are very nutritious in that they contain vital nutrients and if well utilized can reduce the number of people suffering from diabetes due to its low GI. AYBs are very high in antioxidants such as vitamin C at 44% of the RDI (Soetan et al., 2018). Tables 5 and 6 reveal the physical and chemical properties of AYB milk.

Antioxidants fight against free radicals and the release

of oxidative stress which is responsible for ill-health like cancer and cardiovascular diseases. They therefore, help protecting cell damage. AYBs are very rich in dietary fiber, potassium, iron and nitrate which exchanges LDL (bad cholesterol) to HDL cholesterol (good cholesterol) improves heart health, blood pressure and red blood cells. The presence of antioxidant and dietary fiber in AYB helps prevent colon cancer (Michael et al., 2018). AYB are very rich in dietary fiber which helps to improve bowel movements by 31% while the moisture content of the beans ease constipation thus, aids and boost digestion (Michael et al., 2018). They contain inulin (a prebiotic fiber) which may not be absorbed by the digestive system but can be fermented into good bacteria for the gut. The presence of many good bacteria in the gut could help reduce the risk of cardiovascular diseases. AYB contains low carbohydrate contents, low calories, high fiber content and water content. Previous work done revealed that regular consumption of legumes leads to decrease blood sugar levels (Gbenga-Fabusiwa et al., 2019), improve insulin sensitivity and it makes stomach feel full longer (Michael et al., 2018). AYB is an important source of protein rich diet which can help to fight ill-health resulted from malnutrition such as kwashiorkor.

### **African yam beans, starch and composite flour technology**

Composite flour can be defined as a mixture of two or more flours obtained from roots, tubers, cereals and legumes with or without the addition of wheat flour with the sole aim of improving its nutritional values. In the tropic sub-Saharan region where protein inadequate and deficiency in diet is common leading to endemic protein malnutrition with its attendant health challenges in mankind especially in children. Therefore, production of complementary food from protein-rich legumes like AYB is inevitable. Complementary food are foods consumed other than breast milk introduced to an infant or ill-health clients in order to meet or supply basic nutrients needed in the body. In developing countries like Nigeria, Algeria, Ghana, and Cameroun, complementary foods are majorly based on carbohydrate-rich tubers and grains like yam, potatoes, maize, millet and sorghum. Children and ill-health clients are normally given this staple food in form of pap by mixing the gruels in boiled water or boiled with water (Igyor et al., 2011). Gbenga-Fabusiwa (2019) investigated starch extracted from pigeon pea and African yam beans. It was reported that the flour was rich in minerals, but no fiber was detected in the starch samples. Iwe et al. (2016) investigated composite flour produced from FARO 44 rice, AYB and brown cowpea seeds and reported that incorporation of AYB increases the protein, fiber and ash contents of the composite flour. The composite flours were used in the production of

**Table 5.** Physical properties of African Yam Bean (AYB) seed milk.

Parameter	Results
Color	Milk color
Aroma	5.8
Taste	6.4
Mouth feel	6.2
Overall acceptability	6.8

Source: Michael et al. (2018).

**Table 6.** Chemical properties of African Yam Bean (AYB) seed milk.

Parameter	Results
Titration acidity	25.24
pH	4.76
Ca	0.12605 ppm
Fe	0.05605 ppm
Cu	0.0009 ppm
Pb & Cd	Not detected

Source: Michael et al. (2018).

functional bakery food products like bread, biscuits and enriched pap (Atinuke, 2015). Obasi et al. (2012) evaluated biscuits produced from AYBs and wheat composite flour and stated that the biscuit had 0.88-2.28% crude fiber, 12.75-22.00% protein, and 63.30-73.01% carbohydrate. Sensory evaluation showed that all the biscuits had high rating for general acceptability. AYB proportions at 75:25% and 50:50 compared favorably with 100% wheat flour (control). Anoshirike et al. (2018) made biscuits from wheat, AYB and cocoyam composite flours and reported that the biscuit produced had higher energy, protein and fiber contents than the control (100% wheat flour). The biscuits were higher in calcium (0.19%), magnesium (0.22%), zinc (1.91 mg) and iron (5.98 mg), richer in nutrients and had high general acceptability. Ogbonnaya et al. (2018) and Aniedu and Aniedu (2014) examined the blends of cassava fufu, cooking banana, and AYB, with the sensory. It was reported that overall protein content and fat content of the blended flour samples increased with increase in the AYB component of the blended samples. Also, the fibre contents of blended samples significantly increased with increase in both cooking banana and AYB. The sensory evaluation revealed that sample that contains 33.3% CF, 33.3% CB, and 33.4% AYB scored the highest in texture (7.08) and aroma (7.00) but did not carry out the glycemic index and texture profile analysis of the work. Ukegbu et al. (2015) reported nutritional potential of AYB flour enriched

complementary foods. It was revealed that enrichment of complementary foods with AYB had good acceptable organoleptic attributes when incorporated in infant formulations (Okafor and Usman, 2010; Nwosu, 2010). The results in this study suggest that utilization of AYB in starch and composite flour formulation technology could be a viable way of overcoming the hard to cook utilization limiting factor.

### Toxicological analysis of AYB

Toxicity is referred to the effect on a whole organism (humans, animal, bacterium, or plant), as well as the effect on a substructure of the organism, such as a cell or an organ like liver and kidney. It is the degree to which a chemical substance (toxin, poison) or a particular mixture of substances can damage an organism. The distribution of heavy metal in soils and sediments indicates the potential harm to the environment through their chemical associations since a buildup of heavy metals at high concentration can cause serious risk to human health when food plants are consumed. Cadmium (Cd) is one of the most toxic pollutants found in the air, water, and soil, and is nonessential for plants (Gallego et al., 2012). Cd has a negative influence on photosynthetic, respiratory, and nitrogen (N) metabolism in plants, resulting in a poor growth and low biomass (Gallego et al., 2012). AYB have provided a good source of anti-infective agents and phyto-medicines that have shown great promise in the treatment and management of numerous kinds of infections and diseases. Most people in rural areas utilize AYB to bring about cure and relief to disease conditions with little or no knowledge about the safety and toxicity of AYB. Okonkwo et al. (2013) investigated acute toxicity (LD50) test and hematological parameters of anaemic rats treated with the methanol extract of the plant seeds and stated that the extract was not toxic up to 5000 mg/kg, indicating the safety of the plant for both human and animal consumption. Oshomoh and Ildigwe (2018) studied ameliorative potential of biocharcoal on sodium azide toxicity in AYB. It was reported that the growth of AYB was significantly improved by the application of the biocharcoal as higher plant height, number of leaves, percentage seedling emergence and number of branches were observed when compared with control. Biocharcoal proved to have ameliorative potential on AYB, however, more work is needed to understand the mechanism by which it operates. Ohanmu et al. (2018) examined the nitrogen assimilation and distribution pattern of AYB exposed to cadmium stress by partitioning the plant accessions into root and leaf, and N-nitrate and N-ammonia analysis was carried out during the seedling and flowering stage. It was reported that Cd toxicity reduced the total nitrogen assimilation of AYB accessions. Okoye et al. (2019) reported the growth responses of

AYB (Hochst. ex A. Rich.) harms to cadmium pollution. It was observed that cadmium pollution significantly delayed seedling emergence in all tested AYB accessions by at least one day. Overall, decrease in total chlorophyll content seems to be the major reason of injury in Cd-exposed plants. Michael et al. (2018) reported the acute toxicity test showed there was no lethality in AYB seed milk up to a concentration of 5000 kg/body weight. The reviewed studies revealed that raw AYB possess some levels of hydrogen cyanide, trypsin inhibitor and oxalate which can be reduced below detection limit when it undergoes certain process techniques such as parboiling, cooking, fermentation, boiling and roasting.

### Strategies to eliminate or minimize the processing difficulties of AYB

The major challenge in the utilization of AYB is the fact that it is very hard to cook. Therefore, different dehulling methods are required to remove the hulls. Locally, the dehulling process is done by soaking the beans and dehulling manually. This process is time consuming, labor-intensive and thus inhibits the effective utilization of AYB. Under cooked AYB seeds may cause diarrhea and when it is over-done results to constipation (Oyedele et al., 2019). Steeping may improve the dehulling process of AYB. In addition to this, processing of AYB into flour and composite flour will be a viable way of improving AYB utilization. Another limitation to the utilization of AYB is poor storage capacity as result of adverse effect of seed-borne fungi. Oyedele et al. (2019) reported that the Jute bag is the most effective method of preserving AYB seeds. Anti-nutritional factors like flavonoids and phytate, contributed greatly to underutilization of AYB in that they reduce beans' digestibility, swelling bellies and causing flatulence when they are not properly processed. However, studies revealed that roasting, cooking, formulation of flour, composite flour, pre-cooking treatments and fermentation could reduce the processing difficulties of AYB (Olisa et al., 2010; Chikwendu et al., 2014; Ajayi, 2011; Magdi, 2010). Uche et al. (2014) studied the level of anti-nutrients composition of raw and processed AYB. It was observed that the processed AYB registered significant reduction in levels of hydrogen cyanide, trypsin inhibitor, phytate, oxalate, and tannins compared to the unprocessed.

### CONCLUSION

African yam bean is going to extinction and reported as one of the underutilized legumes in Africa. However, AYB holds great potentials in improving food security globally. The results obtained in this study indicate that AYB is rich in protein, minerals and antioxidants with low GI and therefore, can serve as nutraceutical. It is of great

importance to explore its starch, flour and composite flour in producing functional food such as AYB paste called "Amala", bread, biscuit as dietary intervention for prevention and management of diet-related diseases like diabetes, obesity and kwashiorkor. Feeding infants with the blend flour of AYB will contribute to preventing infant malnutrition problems in developing countries. The reviewed papers revealed that pretreatment of AYB by soaking, parboiling before other processing like cooking and roasting has had great improvement on its hard-to-cook and antinutritional factors.

### RECOMMENDATION

The government should make a policy that will support the cultivation and more researches on AYB so as to achieve SDG goal 2. The media house and health institutions should stress more on health benefits of AYB to mankind. Further research should be carried out on ethno-pharmacological claims of AYB tubers, leaves, flowers and clinical trials of AYB composite flour blends formulated on humans. The mode of processing the leaf of AYB and the safety of eating it should be explored experimentally. Toxicological analysis of the plant should also be established.

### LIMITATIONS OF THE STUDY

AYB is cultivated majorly by the primitive farmers in small scale. This limits its industrial utilization and the ethno-pharmacological studies on AYB seeds, leaves and tubers. Governments as well as Food and Agriculture Organization should encourage and promote the industrial cultivation of the novel grain called AYB. This will enhance its maximum utilization to meet dietary needs of mankind and improve food security as well as health status of the people. In addition, there are limitations on the clinical trials of AYB products on kwashiorkor and people suffering from diabetes.

### CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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*Review*

# Nutritional, health benefits and usage of chia seeds (*Salvia hispanica*): A review

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As we enter the 3rd millennium, with improved life expectancy, and high media coverage of health care issues, people are becoming more aware of and interested in the potential benefits of nutritional support for disease control or prevention. The review aimed to gather published information regarding nutrition and health benefits associated with the use of chia seeds for human health. Researchers have reported chia seeds to have high nutritional content in the form of protein (15-25%), dietary fibre (18-35%), fat (15-35%) and ash content (4-6%). Apart from their rich nutritional content, chia seeds can improve the nutritional content of various food products when they are blended or mixed. This article reviews the nutritional content, bioactive compounds, and nutraceutical functionality of chia seeds and their use in the development of functional foods. Also highlights functional properties of chia seeds, usage in the food industry and fortification of food with chia seeds. The results showed the potential use of chia seeds with blending with other food to produce more nutrient dense food products that can be regarded as a functional food.

**Key words:** Chia seeds, functional foods, bioactive compounds, nutraceuticals, fortification.

## INTRODUCTION

Suitable nutrition is a critical element in the prevention of numerous diseases related to civilization condition. The leading killer diseases due to unhealthy food today are cancer, depression, diabetes, and coronary heart disease (CHD) (Wang et al., 2016). However, the main challenge today, and even in the future, will be providing people with enough, safe, and healthy food. Foods from animals and fish, which contain essential ingredients such as

protein, unsaturated fat, and minerals, will be incredibly scarce (Kaale and Eikevik, 2014). With increased health awareness globally as a result of an increase in the number of non-communicable diseases, the demand for a healthier lifestyle through the consumption of foods that prevent and control these non-communicable diseases has increased (Berner et al., 2014; Chadare et al., 2019). The chia seed is an oilseed that is native to Central and

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South America (Nieman et al., 2009). It is considered as a pseudo-cereal, which is cultivated for different usage and commonly grown in several countries. The main chia producing countries are Bolivia, Ecuador, Guatemala, Mexico, and Peru (Ixtaina et al., 2008). It has been a significant food crop in the Aztec, Mayan, and Incan civilizations of the past. It was used as food and medicine, as well as in making paint. In recent years, chia seeds have attracted much interest in the following countries; Mexico, Argentina, Chile, Japan, the USA, Canada, Europe and Australia. In these countries, it is part of different food products (Porrás-Loaiza et al., 2014). Chia seeds were introduced into East Africa recently, and it is cultivated in Kenya, Rwanda, Uganda, and Tanzania. In Tanzania, chia seeds were firstly introduced in Kagera Region on the western shores of Lake Victoria in 2013 to improve rural farmers' income. Chia seeds in Tanzania used in raw form and added to drinking water, fruit juices, blending with other cereals for the formulation of composite flour and occasionally taken as nutritional supplements. The crop is increasingly earning popularity in East Africa because of its economic and human-health benefits (Kibui et al., 2018). It is referred to as "the seed of the 21st century", "new gold, super nutrient and superfood" (Dinçoğlu and Yeşildemir, 2019; Suri et al., 2016). The shape of the chia seed is flat and oval. It is 2.0-2.5 mm long, 1 to 2 mm wide, and 0.8-1.0 mm thick (Ixtaina et al., 2008; Suri et al., 2016). The colour of the coat of the seed varies from grey, black- and white-spotted, which are slightly different from each other. However, black seeds are more common, and white seeds are slightly larger than black seeds (Ixtaina et al., 2008; Segura-Campos et al., 2014; Ayerza, 2013). Increased consumption of chia seeds and a greater variety of plant foods provides most of the lost minerals and vitamins, in addition to phytochemicals.

The capacity and value of these safe, healthy, and high-quality foods, as well as other alternative sources of rich foods such as plants, are required. Various research studies have been conducted to identify inexpensive and sustainable solutions for supplementing the needed nutrients from different plant sources such as soybeans (SB), *Moringa oleifera* (Rweyemamu et al., 2015), and chia seeds (Otondi et al., 2020). Such sources of nutrients have shown positive results, especially in fighting micronutrient deficiency in developing countries (Rweyemamu et al., 2015). Chia seeds have great nutritional potential because of their composition. Their composition depends on genetic factors and on the effect of the ecosystems where the plants are grown (Marcinek and Krejpcio, 2017).

Current trends in healthier nutrient-dense foods show chia seeds are becoming more popular to researchers because of their nutritional composition, and benefits to the human-health. Chia seeds reported to contain protein (15-25%), fat (15-35%), ash (4-6%) dietary fibre (18-35%)

and carbohydrates (18-31%) (Coelho and Salas-Mellado, 2014; Ixtaina et al., 2008; Muñoz-González et al., 2019; Nieman et al., 2009; Porrás-Loaiza et al., 2014). Furthermore, in several developing countries, both micronutrient malnutrition (MNM) and protein-energy malnutrition (PEM) have increased, particularly among children. These are related to food insecurity and hence non-communicable diseases. Chia seeds might be used to combat these pandemics in many societies. Sub-Saharan African countries including Tanzania, child malnutrition and availability of healthy food is still a common problem in rural areas, and urban regions as the high number of population are of earners that have low-income. This review aimed to gather information about chia seeds nutritional benefits and potential usage with other food products to enhance nutritional composition.

## THE CHEMICAL COMPOSITION OF CHIA SEEDS

Various studies have shown that chia seeds consist of vital macronutrient and micronutrient components (Coelho and Salas-Mellado, 2014; Ixtaina et al., 2008; Muñoz-González et al., 2019; Nieman et al., 2009; Porrás-Loaiza et al., 2014; Segura-Campos et al., 2014). The most common macronutrients are proteins, fats with a good quality profile of fatty acids, ash, carbohydrates, and dietary fibre (Table 1).

Suri et al. (2016) reported that different ecosystems influence the nutritional composition of protein, fatty acids, and fibre in chia seeds. Ixtaina et al. (2008), mentioned chia seeds comprise 90 to 93% of dry-matter. Coelho and Salas-Mellado (2014) stated that the dietary-fibre content was higher than 30% of the total weight of the seed, and approximately 19% of the seed contains proteins of high biological-value. Ullah et al. (2016) reported the chia seeds are good sources of vitamins, antioxidants and other important minerals. Suri et al. (2016) reported chia seeds contained protein content (15 to 23%), total dietary fibre (36 to 40%) and lipids average of 30.74%. The human health benefits of chia seeds have drawn much attention from researchers and consumers because of the essential fatty acids and a high level of proteins and other components like phytochemicals such as phenolic compounds, flavonoids, tocopherols, steroids, and carotenoids (Valdivia-López and Tecante, 2015; Prakash and Sharma, 2014). These include increased blood levels, improved blood sugar, lower blood pressure, and improvement of the health of guts (Marcinek and Krejpcio, 2017; Suri et al., 2016).

### Fat composition of chia seeds

There are different classifications of fats and oils, depending on the structure of the seeds. Chia seeds oil

**Table 1.** Proximate composition of chia seeds.

Country of origin	Protein	Fat	Ash	Carbohydrate	Dietary fibre	Source
Salta, Argentina	15 - 25	30 - 33	4 - 5	26 - 41	18 – 30	Ixtaina et al. (2008)
Ecuador, Brazil	20 - 24	16 - 33	3.5 - 4.6	-	27 - 36	Carrillo et al. (2018); Coelho and Salas-Mellado (2014)
India	15 - 23	25 - 34	-	37 - 45	23 - 35	Many and Sarasvathi (2016); Suri et al. (2016)

**Table 2.** Composition of omega 3 and omega 6 for different types of vegetable oil.

Type of oil	Country of origin	Omega 3	Omega 6	Source
Chia	Mexico, Ecuador, Brazil and Argentina	54 - 67	17 -20	Álvarez-Chávez et al. (2008), Carrillo et al. (2018), Coelho and Salas-Mellado (2014), Ixtaina et al. (2011).
Sunflower	Czech Republic and Poland	0.16 – 0.50	55 - 65	Orsavova et al. (2015); Marcinek and Krejpcio (2017)
Pumpkin	Cameroon, Czech Republic and Poland	0.12 – 0.50	47 - 69	Fokou et al. (2009); Orsavova et al. (2015); Marcinek and Krejpcio (2017)
Wheat germ	Czech Republic and Poland	1.2 – 2.90	45 - 65	Orsavova et al. (2015); Marcinek and Krejpcio (2017)
Rapeseed	Czech Republic and Poland	1.2 – 9.80	18 - 20	Orsavova et al. (2015); Marcinek and Krejpcio (2017)

has been reported to contain an excessive amount of fatty acids, especially polyunsaturated fatty acids (PUFA), which consist of more than 60%  $\alpha$ -linoleic acid and more than 20% linoleic acid (Di Marco et al., 2020; Segura-Campos et al., 2014).

Increasing environmental concerns on water sources in terms of pollutants, overfishing, and the use of un-approved fishing gear have prompted the use of plant origin sources, which give polyunsaturated-fatty-acids (PUFAs). Among the common PUFAs of great interest to researchers are omega -3 fatty acids, which have certain nutritional benefits. Rajaram (2014) reported that  $\alpha$ -linolenic acid, which is derived from plant origin, has the potential to reduce cardiovascular disease, fracture, and Type 2 diabetes. Table 2 shows the composition of omega 3 and omega 6 fatty acids from different seeds, including chia seeds.

Chia seeds have a substantial lipids amount

that is around 40% of the entire weight of chia seeds (Table 2). Carrillo et al. (2018) used a soxhlet method to extract oil from chia seeds and used a gas chromatography-mass selective detector (GC-MSD) to profile fatty acid. Carrillo et al. (2018) reported the composition of omega 3 as 54.08%, the content of omega 6 as 18.69%, and omega 9 as 10.24%. All are from the total fats that are present in chia seeds. Coelho and Salas-Mellado (2014) used acid hydrolysis to extract oil from chia seeds and used gas chromatography (GC) to profile fatty acids.

The high content of PUFAs, primarily linolenic acid and linoleic acid, in chia seeds, has attracted the attention of many researchers. Chia seeds contain a prodigious amount of PUFAs, primarily linolenic acid and linoleic acid (Enes et al., 2020a; Ghafoor et al., 2020) which are beneficial to the human-health. The fatty acids found in chia seeds also contain several phenolic compounds,

antioxidants that play a significant role in human health (Oliveira-Alves et al., 2017). In recent healthy diet trends, the consumption of unsaturated oils has increased because of an increase in the number of non-communicable diseases like cardiovascular diseases and hypertension, and because of people's consciousness about health. Subsequently, physicochemical characteristics and the nutritional composition of chia seeds influenced the use of the seeds to enhance human health (Coates 2011; Ixtaina et al., 2011).

Ullah et al. (2016) reported a high intake of  $\alpha$ -linolenic acid by humans reduces the risk of heart failure. This may be due to the anti-inflammatory, anti-thrombotic and anti-arrhythmic role that chia seeds, as a natural source of omega 3 and 6 fatty acids, play (Oliveira-Alves et al., 2017; Arredondo-Mendoza et al., 2020). A study conducted by Koh et al. (2015) showed that increased intake of

**Table 3.** Essential amino acid composition of chia seeds (g per 100 g).

Country of origin	Amino acid	Quantity	Health/nutritional benefit	Source
Poland and Slovakia	Leucine	1.37- 1.42	Regulates blood sugar, promotes growth, and repairs muscles and bones. It is also responsible for wound healing.	Nitrayová et al. (2014); Kulczyński et al. (2019)
Poland and Slovakia	Phenylalanine	1.02– 1.6	It plays a vital role in specific brain functions and the structure and function of other proteins and enzymes.	Nitrayová et al. (2014); Kulczyński et al. (2019)
Poland and Slovakia	Lysine	0.93- 0.97	Immunity functions of the body, energy production, collagen, and elastin, which support body tissues.	Nitrayová et al. (2014); Kulczyński et al. (2019)
Poland and Slovakia	Valine	0.79- 0.95	Responsible for muscle growth, regeneration, and energy productions	Nitrayová et al. (2014); Kulczyński et al. (2019)
Poland and Slovakia	Isoleucine	0.74- 0.80	Production of haemoglobin and energy regulations	Nitrayová et al. (2014); Kulczyński et al. (2019)
Poland and Slovakia	Threonine	0.54- 0.71	Responsible for healthy skin and teeth, fat metabolism and immune functions	Nitrayová et al. (2014); Kulczyński et al. (2019)
Poland and Slovakia	Methionine	0.59- 0.67	Health flexibility of skin and hair. It is responsible for tissue growth and facilitates the absorption of vital minerals.	Nitrayová et al. (2014); Kulczyński et al. (2019)
Poland and Slovakia	Histidine	0.53- 0.61	Acts as a protective barrier which surrounds nerve cells and facilitates growths	Nitrayová et al. (2014); Kulczyński et al. (2019)

omega 3 fatty acids from marine or plant sources by humans reduces the risk of cardiovascular diseases, which are among the most chronic non-communicable diseases in the world is facing at present. There are other sources of PUFA like flaxseed and marine products. These other sources have disadvantages or shortcomings which are associated with their usage like digestive problems and a fishy flavour (Tur et al., 2012).

### Proteins composition of chia seeds

Knowledge of protein in any food compound is essential as proteins contribute to the physical and functional properties, which can also improve the sensory properties of food and, therefore, consumer acceptability (Otondi et al., 2020). Most food products, like cereals and grains, are

consumed after being subjected to any form of heat treatment. During this process, protein denaturation is likely to occur and contributes to the functional properties of a particular food item (Boye et al., 2017). Being the main building block in the human body for blood, bones, cartilage, muscles, and skin, protein must be sufficient in the human body for all these processes to take place. With a 20% protein content, chia seeds have the potential to correct and prevent protein-energy malnutrition. Ullah et al. (2016) noted that chia seeds do not have gluten in which gluten intolerance people can utilize the seeds so that they do not suffer from coeliac diseases. Chia seeds contain 18 out of 22 amino acids, of which 9 are essential which contribute significantly to human health (Ullah et al., 2016). Table 3 shows that chia seeds contain the following essential amino acids: phenylalanine, valine, threonine, tryptophan, methionine, leucine, isoleucine, lysine,

and histidine.

The consumption of foods that are rich in protein contributes to weight loss because of the reduction of fat in the body (Ullah et al., 2016). The protein content of chia seeds was reported to be in the range of 15-26% (Table 1), oats (15.3%), wheat (14%), corn (14%), barley (9.2%) and rice (8.5%) (Coates, 2011). These variations are justified by different seed sources due to geographical, agronomic, and environmental conditions. However, the level of protein in chia seeds is still significantly higher than that of other kinds of grain (Ullah et al., 2016).

### Carbohydrates and dietary fibre

Among the nutritional contents of chia seeds are carbohydrates and fibre. The nutritional benefits of dietary fibre include the regulation of bowel health.

**Table 4.** The micronutrient composition of chia seeds (g per 100 g).

Country of origin	Micronutrient	Quantity	Nutritional benefit	Sources
Poland and the USA	Niacin – B <sub>3</sub> (mg)	8.83	Healthy skin, blood cells, brain and nervous system	Jin et al. (2012); Kulczyński et al. (2019)
Poland and the USA	Ascorbic acid – C (mg)	1.6	Improve immune system, building connective tissues, antioxidant	Jin et al. (2012); Kulczyński et al. (2019)
Poland and the USA	Thiamine - B <sub>1</sub> (mg)	0.62	Healthy hair, brain and skin as well as important for nerve function.	Jin et al. (2012); Kulczyński et al. (2019)
Poland and the USA	Phosphorus (mg)	860 - 919	Protect bones and teeth. It helps shuttle nutrients into and out of cells	Jin et al. (2012); Kulczyński et al. (2019)
Poland and the USA	Calcium (mg)	456 - 631	Builds and protects bones and teeth. Assist nerve impulse transmission, muscle relaxation and contraction, as well as blood clotting. Enzyme activation and hormone secretion. Helps maintain healthy blood pressure	Jin et al. (2012); Kulczyński et al. (2019)
Poland and the USA	Potassium (mg)	407 - 726	Balances blood fluids	Jin et al. (2012); Kulczyński et al. (2019)
Poland and the USA	Magnesium (mg)	335 - 449	Chemical reactions in the bodywork with calcium.	Jin et al. (2012); Kulczyński et al. (2019)
Poland and the USA	Iron (mg)	7.72 – 9.18	Blood production and the making of amino acids, collagen, neurotransmitters, and hormones	Jin et al. (2012); Kulczyński et al. (2019)
Poland and the USA	Zinc (mg)	4.58 – 6.47	Enhance the immune system, sense of taste and smell, and wound healing	Jin et al. (2012); Kulczyński et al. (2019)

There are two types of carbohydrates in chia seeds, namely dietary fibre and starch. Dietary fibre (DF) can be classified into soluble DF (such as, gums, pectins, mucilages, and algal polysaccharides), insoluble DF (such as, cellulose and hemi-celluloses),/mixed (such as bran), fermentable and non-fermentable DF (Coelho and Salas-Mellado 2014; Fernandes and de las Mercedes Salas-Mellado 2017; Suri et al., 2016). Soluble dietary fibre absorbs water, turning it into gel-like, highly hydrated masses that are almost entirely fermented in the colon by microorganisms. The main purpose of the dietary-fibre in the human body is to retain healthy digestive system in the human body (Capitani et al., 2012).

It has been reported that the high content of gum and mucilage in chia seeds is a potential means of application in the food industry (Fernandes and de las Mercedes Salas-Mellado

2017; Suri et al., 2016; Coelho and Salas-Mellado 2014) reported that chia seeds contain 5-6% mucilage which can be used as dietary fibre. Muñoz et al. (2012) noted that chia seeds can form a gelatinous mass when soaked in water. This is due to the occurrence of a large amount of mucilages and gums, which make the seeds strongly hydrophilic and thus capable of absorbing several times their weight in liquids such as water that can absorb up to 12 times its weight (Attalla and El-Hussieny, 2017). The mucilage can be extracted from chia seeds and hydrated to archive water retention of 27 times its weight in water. This property makes chia seeds a functional ingredient that can be used in the food industry as, for example, a thickener and stabilizer. Because of this property, chia also improves the function of the digestive system and helps to improve the health of the guts through a

prolonged time of gastro-intestinal (Anderson et al., 2009; Suri et al., 2016).

#### MICRONUTRIENT COMPOSITION OF CHIA SEEDS

There are different vitamins needed by the human body in different quantities for various metabolic purposes. The human body as vitamin D, can synthesize other vitamins obtained from an additional food source like vitamin C. Researchers have shown that chia seeds consist of some vitamins and minerals (Suri et al., 2016; Ullah et al., 2016). The minerals found in chia seeds are calcium, phosphorus, potassium, iron, zinc, and magnesium. The vitamins found in chia seeds are thiamine, riboflavin, niacin, folic acid, ascorbic acid, and vitamin A (Suri et al., 2016). Table 4

shows the micronutrient composition of chia seeds, as well as their quantities and nutritional benefits.

Muñoz et al. (2012) noted that chia seeds are richer in niacin than in other cereals like corn, soybean, and rice. Vitamin B1 and vitamin B3 are similar to corn and rice, but chia seeds have six times more calcium, eleven times more phosphorus, and four times more potassium than milk, which is only 100 g (Muñoz et al., 2012; Suri et al., 2016).

## CHIA SEEDS AS A NATURAL FUNCTIONAL FOOD

In its unchanged or changed form, chia seed is a functional food (Marcinek and Krejpcio, 2017). An unchanged natural food is a food that has not undergone human interference to improve its nutritional/health benefits. Functional foods are foods that offer additional nutrition and health benefits and can perform three functions. Their primary function is a particular food's nutritional value. Furthermore, their secondary functions are the food's sensory appeal, sensory satisfaction, or organoleptic properties. The tertiary function refers to the food's physiological aspects, such as neutralizing harmful substrates, regulation of body functions and physical conditions, prevention of diseases related to nutrition, and elevation of the mental and physical health of people (Yao et al., 2012).

There is no specific meaning of the functional food concept, which has been proposed. However, the /Functional Food Institute in Dallas or/ Functional Food Centre describes functional foods as "*processed or natural foods that comprise of unknown or known compounds which are biologically-active; which, is defined in non-toxic and effective amounts, that can deliver a documented and clinically proven health-benefit for the treatment, management and prevention of diseases that are chronic*" (Martirosyan and Singh, 2015). The Institute of Food Technology (IFT) considers the term "functional food" as evolving, and it uses it to refer to foods and food components that provide health benefits besides basic nutrition. Functional foods offer important nutrients, usually beyond quantities needed for usual maintenance, development and growth of other biologically active components and/or the target population desirable to provide required physiological effects or health-benefits (Rajaram, 2014). Functional foods can also be beneficial in increasing the number of nutrients by providing particular dietary food components that intensify their palatability and availability (Ullah et al., 2016).

More research should be directed on the provision of functional foods through either enriched or fortified food with the use of natural sources like chia seeds so as to come up with a new formulation of known concentration and reported therapeutic benefits expected. The expected processing conditions and the possible chemical and

biological changes during food preparation, as well as food bioavailability and its bio-accessibility after its consumption was established.

## Phytochemicals in chia seeds

The phytochemical compounds that have been detected in chia seeds include carotenoids, sterols tocopherols, and phenolic compounds, including quercetin, myricetin, caffeic acid kaempferol and chlorogenic (Capitani et al., 2012; Oliveira-Alves et al., 2017). The phytochemicals, either alone or in combination with others, have the therapeutic potential to cure various ailments (Marcinek and Krejpcio, 2017). Epidemiological and animal studies suggest that dietary intake of phytochemicals may have certain health benefits and protect people against human chronic degenerative disorders such as neuro-degenerative diseases, cancer, cardiovascular diseases, kidney diseases, and as well as diabetes (Tokuşoğlu and Hall III, 2011). Majority of foods, such as whole grains, legumes, fruits, vegetables, and herbs, contain phytochemicals (Tokuşoğlu and Hall III, 2011). Tables 5 and 6 shows nutritional bioactive compounds and antioxidant compounds in chia seeds, as reported by various researchers.

## Functional properties of chia seeds

Besides considering the nutritional quality of new food products, consumers have expressed a need to have more knowledge of the functional properties and nutritional quality of the food-products (Limsangouan et al., 2010). The functional properties of any food item shows how their starch, protein, and lipids in it behave during the processing of that particular food item and their effects on the final food product in relation to properties of an organoleptic (smell, appearance, texture and taste). The behaviour of these nutrients is essential in relation to technological and dietary formulation in developing novel functional foods (Brennan et al., 2016).

Food items in flour form, common functional properties that describe how ingredients behave during preparation and cooking are emulsion stability, bulk density and least-gelation concentration, swelling capacity, foam stability, gelatinization temperature, oil absorption capacity, foam capacity, emulsion activity and water absorption capacity. Olivos-Lugo et al. (2010) reported the protein functional properties such as foaming and gelling, oil holding capacity and water holding capacity, are from chia seeds. It was observed that the protein from chia seeds has good water holding capacity and excellent oil retention capacity, which makes chia a useful ingredient in products from the bakery.

Segura-Campos et al. (2014) reported a study

**Table 5.** Nutritional bioactive compounds in chia seeds.

<b>Phytochemical</b>	<b>Nutraceutical value (Health benefit)</b>	<b>Source</b>
Linolenic acid	Cancer preventive, reduce the risk of coronary heart Disease, Coronary Heart Disease, Bone Health, Immune Response Disorders, Weight Gain, Stroke Prevention, Mental Health, Cancer and Visual Acuity	Koh et al. (2015) McClements et al. (2009)
Linoleic acid	It helps the body to build muscle rather than store fat and has anti-inflammatory properties. It also prohibits cancer.	Martínez-Cruz and Paredes-López, (2014)
Tocopherols	Contributes to the antioxidant's composition of chia seeds, which helps to reduce inflammation, promoting anti-cancer cells, anti-ageing, and other antioxidant activities in the stabilization of cell membranes.	Marcinek and Krejpcio, (2017)
Sterols	Plant sterols are effective in inhibiting cholesterol incorporation into mixed micelles, which is an essential step at the intestinal-epithelium for the absorption of cholesterol.	Tokuşoğlu and Hall III, (2011)
Carotenoids	Cancer, Coronary Heart Disease, Macular Degeneration, and Cataracts	Maruyama et al. (2014)
Glutamic acid	Proper brain functioning	Olivos-Lugo et al. (2010)
Dietary-fibre	Treating and preventing diseases of the circulatory and digestive systems, kidney stones, diabetes, haemorrhoids colorectal cancer and disorder in the metabolic system	Valdivia-López and Tecante (2015)
Phenolic compounds	Protective role against cardiovascular illness, cancer, and diabetes; Antioxidant protectants for human beings and play a beneficial role in reducing the risk of hypertension, diabetes, coronary heart disease, and some types of cancer.	Oliveira-Alves et al. (2017) Prakash and Sharma (2014)
Caffeic acid	Hypoglycemic activity, memory protective effect Anti-carcinogenic, antihypertensive, neuron protective effects and antioxidant activity	Oliveira-Alves et al. (2017) Enes et al. (2020b)
Rosmarinic Quercetin	The immuno-regulatory function that includes antimicrobial, antioxidant and anti-inflammatory activities and antidiabetic effect. A potent antioxidant, anti-inflammatory, antibacterial, antiviral, anti-hepatotoxic, reduces LDL oxidation, vasodilator and blood thinner	Oliveira-Alves et al. (2017) Prakash and Sharma (2014)
Myricetin	Exhibits antibacterial activity and has anti-gonadotropic activity	Prakash and Sharma (2014)
Kaempferol	Antimicrobial Antioxidant, anti-cancer, cardioprotective, anti-inflammatory, antidiabetic, anti-osteoporotic, anxiolytic, neuroprotective, anti-estrogenic/estrogenic, analgesic, and anti-allergic activities Reduction in hot flushes and menopausal symptoms	Prakash and Sharma (2014)
Protocatechuic acid	Antioxidant properties, anti-inflammatory, anti-hyperglycaemic, and antimicrobial effects against gram-positive and harmful bacteria and fungi.	Semaming et al. (2015)
Gallic acid	Cytotoxic and anti-oxidative activities, antileukemic, antioxidant, anti-cancer, antineoplastic, anti-inflammatory, antidiabetic	Prakash and Sharma (2014)
Chlorogenic acid	Anti-carcinogenic, antihypertensive, neuron protective effects; Antioxidant activity that can reduce the production of free radicals in the body, inhibit peroxidation of fats.	Oliveira-Alves et al. (2017) Alagawany et al. (2020)

**Table 6.** Composition of antioxidant compounds in chia seeds (mg/g).

Country of origin	Polyphenol	Chlorogenic acid	Caffeic acid	Quercetin	Kaempferol	Source
Mexico	0.51-0.88	0.046-0.102	0.003-0.007	0.15-0.27	0.36-0.51	Reyes-Caudillo et al. (2008)
Bolivia, Ecuador and Paraguay	0.91-0.98	0.214-0.235	0.141-0.156	0.006	0.024-0.025	Ayerza (2019)
Brazil, Mexico	0.64	0.005	0.02 - 0.03	0.1- 0.2	0.0002	Coelho and Salas-Mellado (2014); Martínez-Cruz and Paredes-López (2014)

concerning the functional properties such as water absorption, oil holding capacity and water holding capacity of the chia-seed gum, which they confirmed that good functional and physicochemical properties of chia-seed gum could be useful in food industries. Most of the studies on the functional properties of chia seeds have been conducted on specific items like the amount of protein in the seeds, the gum and mucilage (Iglesias-Puig and Haros 2013; Olivos-Lugo et al., 2010; Pizarro et al., 2013; Segura-Campos et al., 2014; Zettel et al., 2015).

### Uses of chia seeds in the food industry

Chia seeds because of the various nutritional properties, multiple studies have been conducted for different purposes, including their composition, characterization of the compounds, application in the food industry as well as usage in cosmetic industries (Iglesias-Puig and Haros 2013; Many and Sarasvathi 2016; Olivos-Lugo et al., 2010; Pizarro et al., 2013; Segura-Campos et al., 2014; Zettel et al., 2015).

The high content of gum and mucilage in chia seeds makes the seeds potentially useful in the food industry (Fernandes and de las Mercedes Salas-Mellado 2017; Suri et al., 2016). The seeds are mostly used in the baking industry. This is because of the higher amount of carbohydrates in

the baked products compared to other nutrients which are essential (Romankiewicz et al., 2017). Among the products in which chia seeds have been included are bread, pasta, biscuits, and cakes. Chia seeds can also be used in beverages, snacks, and other products (Coelho and Salas-Mellado, 2014; Steffolani et al., 2014).

Also, among the products which have been fortified with chia seeds is wheat bread (Romankiewicz et al., 2017). The presence of bioactive compounds in chia seeds has contributed significantly to the formulation of various functional foods (Bresson et al., 2009). However, the addition of chia seeds to original products made the products have characteristics different from their original characteristics. (Zettel et al., 2015) analyzed the effects of chia on the production of wheat bread and noted a reduction in bread firmness. Coelho and Salas-Mellado (2014) noted that breadcrumbs incorporated with whole chia seeds were softer than chia flour. Romankiewicz et al. (2017) added 4 to 6% of chia seeds to wheat flour during the baking of bread and noted a reduction in the amount of the bread obtained. In addition, 6 to 8% chia seeds to wheat flour reduced baking loss because the high content of dietary fibre in chia combines with water and prevents evaporation during bread baking. This indicates that when chia seeds are incorporated into flour products, they might influence the swelling capacity and water-holding

capacity of the products. Iglesias-Puig and Haros (2013) noted dark colour in the bread baked into which chia seeds had been incorporated because of phenolic compounds.

### Fortification of foods with chia seeds

Subsequently chia seeds are rich of omega-3 fatty acids, protein, fibre, vitamins, minerals, and phytochemicals, they can be used as nutritional additives in food, a nutritional supplement, and a base for beverages. According to Dary and Hurrell (2006), food fortification is the addition of one or more essential nutrients to food so as to reduce or prevent the deficiency of nutrient(s) in a given population (Dary and Hurrell, 2006). Food fortification is precisely stated as the addition of one/more components of another food item that is not naturally occurred to increase its nutritional/health benefits of the newly designed/formulated food product (Świeca et al., 2014). Food fortification is among the techniques commonly used in the development or formulation of functional foods.

There are two principal reasons for fortifying foodstuffs. The first reason is when legislation demands it through public health policy. Examples of this include fortification of baby foods, weight reduction products, and even national programs requiring the staple foods fortification for example



wheat flour, maize flour, and cooking oil, which is common in developing countries (Dary and Hurrell, 2006). The second reason for fortifying foods is market differentiation. The non-compulsory fortification of dairy products, breakfast cereals, and drinks has been effectively used to boost sales through enhanced nutritional claims (Dary and Hurrell, 2006). Certainly, fortified beverages and food products can decrease dietary deficiencies of which is resulting from a progressively reliance on processed food products and busy lifestyle that, without fortification, may be viewed as “empty calorie” products (Dary and Hurrell, 2006; Iwatani and Yamamoto, 2019; McClements et al., 2009; Tokuşoğlu and Hall III, 2011).

### **Boosting the bioavailability of chia seed phytochemicals using a food matrix design**

Contrary to conventional practices, the composition and structure of foods are typically optimized to improve their quality characteristics such as mouth-feel, appearance, taste and texture. Foods have of late been intended to improve their nutritional profiles by plummeting the levels of the macronutrients. These are believed to have adverse health effects. Such effects are saturated fats, digestible carbohydrates, and salt. On the other way, such foods can be improved with other food components that are supposed to have health effects which are beneficial, for instance minerals, nutraceuticals vitamins or dietary fibre (McClements et al., 2009). Nevertheless, the potential advantages of a significant number of these nutraceuticals are not ideally comprehended, as a result of their comparatively low as well as adjustable oral bioavailability (McClements et al., 2015). Both the composition and structure of a food matrix can influence the bioavailability of co-ingested nutraceuticals (McClements et al., 2009; McClements et al., 2015).

Chia seeds have nutritional and nutraceutical benefits. They are a good source of material for the fortification of consumed cereals, roots, and tubers. Foods fortified with chia seeds improve people’s health, especially pregnant women, and children. Some of these deficiencies may result in irreversible effects like stunting for children, and congenital disabilities and death (Coelho and Salas-Mellado, 2014). However, more studies have to be conducted on different food products that are developed to produce functional foods and to confirm its quality characteristics of fortified foods (McClements et al., 2015; Yao et al., 2012).

### **Chia seeds as a vehicle for bioactive components in food products**

Chia is a crucial ingredient for the development of

functional food products (Dinçoğlu and Yeşildemir, 2019). The bioactive components found in chia seeds may be used to fortify food, besides being used as a vehicle for increasing the number or amount of vital nutrient intake (Coelho and Salas-Mellado, 2014). The formulation of new functional foods with the incorporation of chia seeds is a promising and innovative way of protecting and delivering omega-3 fatty acids into a range of food products (Garg et al., 2006; Geelen et al., 2004; Koh et al., 2015).

### **Considerations to ensure the number of bioactive compounds present in commercial food products are satisfactory**

Ideally, it is desirable to design chia-based functional food products that are stable in many different applications. Food ingredient manufacturers usually have functional food formulations designed for different food categories. Examples of common available functional foods already in the market include infant formula, bakery products, dairy, and liquid beverage, of which all have different requirements. There are various challenges relating to the final food formulation with regard to food standard regulations, shelf-life, and quantity of bioactive components present (McClements et al., 2015; Ottaway, 2008). Some of the critical issues that must be considered so that there are enough chia bioactive components in commercial food products include the following:

(i) *Compliance with regulatory standards* – availability of product standards from regulatory bodies to ensure that the ingredients chosen as nutraceutical-rich (health-promoting) foods are approved for usage in each country where a given product will be marketed or sold. This step will minimize the time for seeking regulatory approval when the final product is ready to go into the market (Dary and Hurrell, 2006; Ottaway, 2008).

(ii) *Definition of a final product application to ensure a uniform product – dry blending with other food powders* – it is necessary for the nutraceutical-rich (health-promoting) food ingredient like powder before usage in combination with other food items, to be matched in terms of physical and functional properties like water activity, bulk density, and particle size to achieve the homogeneous end product. Also, to avoid possible physical separation during the transportation, storage, or use of a newly formulated food product. For liquid products, the rehydration and re-dispersion behaviour of the powder is vital if a powder format is chosen (Dary and Hurrell, 2006; Ottaway, 2008).

(iii) *Understanding of the protection and release*

*requirements during processing* – Knowledge and understanding of the processing steps and the unit operations involved in manufacturing the final food product is important to be able to design functional food properties in such a way that the core is protected during the process and released at the desired time (Dary and Hurrell, 2006; Ottaway, 2008).

(iv) *Understanding how and where to add chia* – familiarity with the setup and layout of a food manufacturing plant is also required in order to identify the stage at which it is appropriate to add chia seeds to a food product (such as how and where chia seeds should be added), without any other process modification (McClements et al., 2015).

(v) *Understanding possible interactions with other ingredients* – when the new nutraceutical-rich food ingredient is added to a food product, the final food product properties, including sensory and physical properties, as well as the shelf life of the food may be affected by possible ingredient interactions. Sometimes these interactions may be minimized by making minor modifications to the process or formulation. However, sometimes even small changes cannot be made because of some limitations in the plant's layout/setup or further modifications could add significant capital investment (Majeed et al., 2013; McClements et al., 2015).

(vi) *“Working in partnership”* – all stakeholders or corresponding partners in the value chain should be aware of what is going on and, therefore, work together to ensure that the final product is of good or high quality (Dary and Hurrell 2006; Ottaway, 2008).

## CONCLUSION

Chia seeds richness is due to its high composition of protein (15-25%), fats (15-35%), fat (15-35%), ash (4-6%) and presence of minerals, vitamins, phytochemicals and antioxidants. The nutritional composition of chia seeds varies because of their geographical origins and climatic and environmental conditions. Characterization of chia seeds grown in different locations is crucial before the development of any new food product, and for food fortification or enrichment purposes using other local food products and chia seeds. This review recommends usage of chia seeds on its natural form or in combination with other less dense food products. During the development of new functional foods with the incorporation of chia seeds, consideration of the physical and functional properties of the new food product to be developed is essential. Emphasis on product's attributes like shelf life, usability and consumer acceptability not affected as compared to those of the original product (unfortified product). A systematic approach is important during

product formulation for different food products so that the nutrients needed (recommended dietary intake – RDI) do not negatively affect the acceptability of products. The functional foods to be developed should have supported scientific evidence for the availability of nutritional and health benefits to ensure that they are not lost in the food chain before the food is consumed.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# **Successful value-chain approach for oat industry; development and quality evaluation of oat (*Avena sativa*) incorporated yogurt**

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**Oat (*Avena sativa*) is a cereal rich in fiber and nutrients necessary for good health. This study was conducted to develop oat flakes incorporated yogurt. Oat flake concentrations were used at 2.5, 3.75, and 5 g of oats per 100 ml of milk. The selected oat concentration from a sensory evaluation was compared with a commercial yogurt for consumer preference and proximate analysis. The shelf life of the selected yogurt sample was determined by measuring total soluble solids, pH, titratable acidity, and the rate of syneresis. The microbiological assessment was carried out by the total plate count method and yeast and mold count for 21 days. Statistical analysis of data was done using SSPS and SAS at  $p < 0.05$  significance level. Most consumer preferable oat concentration was 3.75 g oats per 100 ml of milk, and its sensory parameters were similar to the commercial yogurt. The selected oat concentration showed significantly higher values for fat, protein, solids non-fat, and fiber contents than the commercial yogurt. The shelf life of oat yogurt was 14 days from the production according to the yeast and mould count. In summary, oat flakes incorporated set yogurt can be developed with a reasonable shelf life, better sensory attributes, and higher nutrient content than the commercial yogurt.**

**Key words:** Oat, physico-chemical properties, proximate analysis, yogurt, shelf life.

## **INTRODUCTION**

Chronic diseases like cancers, diabetes, and heart-related diseases are widely spreading throughout the world causing a significant number of deaths (Wang et al., 2016). Unfortunately, these diseases sometimes appear in the early stages of life. This prevalence of non-

communicable diseases has become a global crisis due to unhealthy diet plans among humans (Varma et al., 2016). As an attempt to overcome this issue, people around the world give considerable attention to healthy foods and beverages. Healthy foods command increasing

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demand worldwide. Interestingly, cereals are incorporated in developing functional foods as a new trend in food technology to increase the nutritional profile of foods and beverages (Gasmalla et al., 2017).

Oat (*Avena sativa*), a cereal considered as a functional food, belongs to the family *Poacea*. Oat is important in promoting good health among people in numerous ways (Sterna et al., 2016). It is rich in antioxidants like avenanthamides and water-soluble fiber  $\beta$  – glucan (Henrion et al., 2019; Varma et al., 2016).  $\beta$  – glucan shows hypoglycemic properties that stimulate insulin secretion to control the blood sugar level. This soluble fiber, also, contributes to preventing cardiovascular diseases and other obesity-related diseases (Wu et al., 2019). Oat incorporated foods have shown a positive impact on human health such as lowering blood cholesterol, reducing blood sugar level, reducing hypertension or high blood pressure, improving bowel function, and controlling obesity (Tiwari et al., 2017). Yogurt, as a food frequently consumed by people of all age groups, is undoubtedly a promising food item for the incorporation of oats in the formulation of functional food.

According to the Food and Drug Administration (FDA, 2019), yogurt is a fermented dairy product which is produced by culturing one or more basic ingredients (cream, milk, partially skimmed milk, skimmed milk, or other reconstituted versions of these ingredients may be used alone or in combination) and any of the optional dairy ingredient with a characterizing bacteria culture which contains *Streptococcus salvarius* sub sp *thermophilus* and *Lactobacillus delbrueckii bulgaricus*. Yogurt is one of the most easily digestible dairy products (Tamime and Robinson, 2007). Yogurt has a high nutritional profile with protein, milk carbohydrates, vitamins, and minerals. It is an important food as a probiotic carrier to humans to improve human gut health (Pei et al., 2017). Although yogurt itself shows a high nutritional profile and health effects, value addition for yogurt has been researched to further improve its nutrition value. A study has proved that yogurt enriched with  $\beta$ - glucan can be used as an effective dietary therapy for hypercholesterolemia (Ahmed et al., 2017). Incorporation of fibers in yogurt has become a novel technique to enhance the fiber consumption among people (Dabija et al., 2018). However, reports on the incorporation of oat flakes that most probably may give a high nutritional, functional, and sensorial effect to yogurt are lacking in the literature. This study was conducted to develop an oat incorporated yogurt with favorable nutritional and sensory properties with a reasonable shelf life as a functional food.

## MATERIALS AND METHODS

Oat flakes (Stassen group, Colombo, Sri Lanka), full cream fresh milk with acceptable organoleptic and microbial quality, sucrose, non-fat milk powder (Fonterra Brands, Sri Lanka), and potassium sorbate (INS No. 202) were purchased from a local retail shop. The

starter culture with *Streptococcus thermophilus* and *Lactobacillus bulgaricus* was purchased from the Veterinary Research Institute, Gannoruwa, Sri Lanka.

### Preparation of oat incorporated yogurt

Preparation of oat yogurt was done according to the following procedure (Figure 1). Gelatin (7.5 g) was dissolved in slightly warm water (20 ml) at 60- 65°C before mixing up with the mixture. Three different oat concentrations were used to prepare oat yogurts according to Table 1.

### Sensory evaluation

A non-trained panel of 50 members was selected from the university premises for the sensory evaluation. The panel was with 25 men and 25 women. A five-point hedonic scale (1 – Not acceptable, 2 – Poor, 3 – Fair, 4 – Good, 5 - Excellent) was used to evaluate color, appearance, odor, sweetness, sourness, taste, overall quality, and purchasing intension as sensory parameters. The selected oat concentration (T2) from the first sensory evaluation was then evaluated against a commercial yogurt sample using a non-trained panel of 30 members. The panel was with 15 men and 15 women. Age of the both panel members were varied from 24 to 40.

### Proximate analysis of oat yogurt and commercial yogurt

Moisture content (MC %), ash content, and total solid content (TS %) were determined using the methods recommended by the Association of Official Analytical Chemists (2005). Crude protein content was determined using the Kjeldahl distillation method and crude fat content was determined using the Soxhlet extraction method (AOAC, 2005). Solid non-fat content (SNF %) was determined using Sri Lanka standard 824 (1989) and crude fiber content was determined using crude fiber analyzer. A calculation method (Energy (kcal) = weight of carbohydrate  $\times$  4 kcal/g + weight of protein  $\times$  4 kcal/g + weight of fat  $\times$  9 kcal/g) was used to determine the energy content (James, 1995).

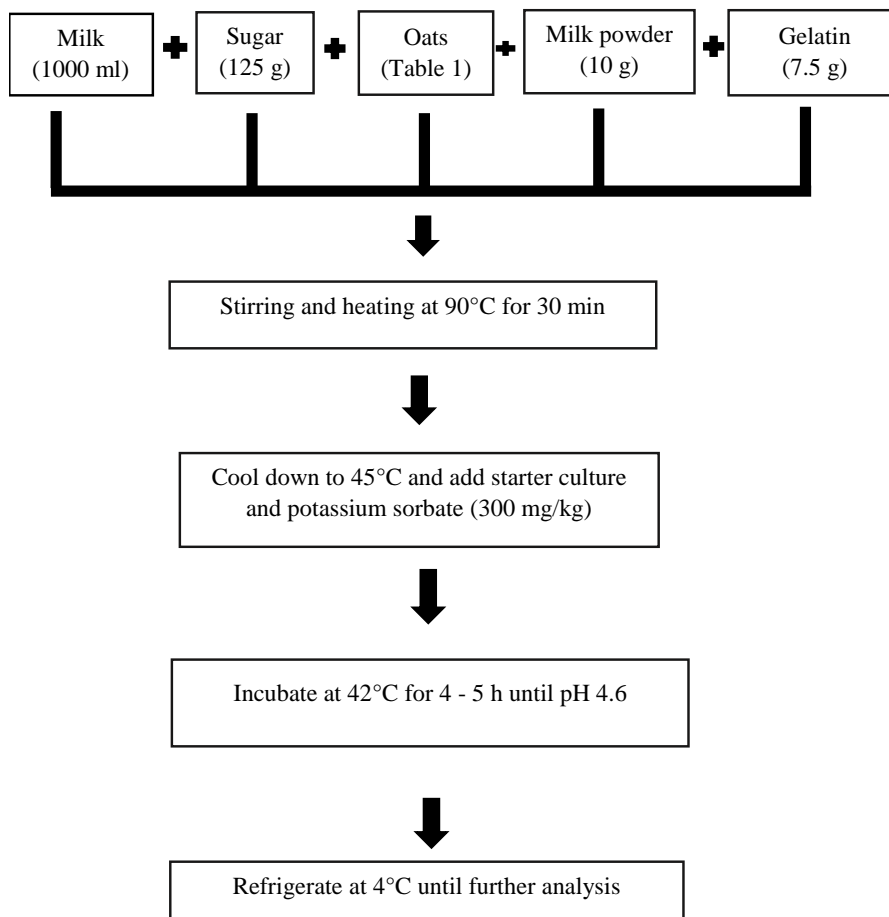
### Shelf-life evaluation of oat yogurt

#### Physicochemical parameters of oat yogurt

Refrigerated (4°C) yogurt samples were used. A digital pH meter (Model: pp – 206, EZODO) was used to measure the pH of the yogurt samples. Total soluble solids (TSS) content was determined by a handheld refractometer (Model: ATAGO N – 46, Japan) as a percent value in Brix%. Titratable acidity (TA %) was determined by titration of yogurt samples against 0.1 N NaOH using phenolphthalein as the indicator according to the AOAC 947.05 method (AOAC, 1990). The previously mentioned tests were conducted at 3-day intervals for 21 days. The rate of syneresis was determined for 21 days with 7-day intervals. Yogurt sample was well stirred and 10 g was weighed. The weighed sample was placed on a whatman filter paper (110 mm) and allowed to drain off for 10 min. Weight loss was measured (Wijesinghe, 2015).

#### Microbiological assessment

The microbiological assessment was carried out for 21 days with 7-day intervals. Yogurts were stored at 4°C and 1 g of a yogurt sample was mixed with 9 ml of 0.1% peptone. A dilution series was



**Figure 1.** Flow diagram for preparation of oat yogurt.

**Table 1.** Three different oat concentrations were used to prepare yogurts.

Concentration	Oat concentration (g per 100 ml of milk)
T <sub>1</sub>	2.5
T <sub>2</sub>	3.75
T <sub>3</sub>	5

T<sub>1</sub> – Oat concentration 1, T<sub>2</sub> – Oat concentration 2, T<sub>3</sub> – Oat concentration 3.

prepared up to  $10^{-5}$ . The dilution  $10^{-5}$  was selected for the bacterial colony count while the dilution  $10^{-1}$  was selected for the yeast and mould count. Nutrient agar was used for the bacterial colony count while potato dextrose agar was used for yeast and mould count as growth media, and 0.1 ml of the dilute was spread on the agar which was then incubated at 37°C (SLS 824, 1989).

#### Statistical analysis

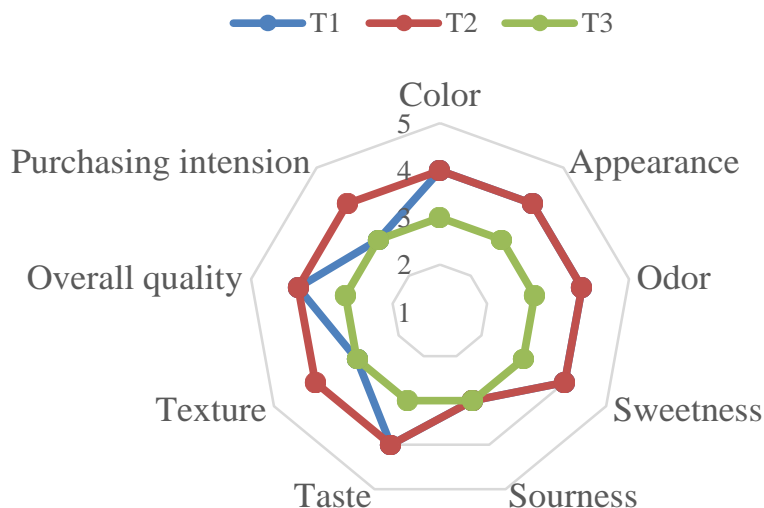
Sensory evaluation data were analyzed by the non-parametric analysis method (Kruskal Wallis test) by SSPS statistical software and parametric data analysis was conducted by Analysis of Variance (ANOVA) using SAS statistical software at  $p < 0.05$  significance level.

## RESULTS AND DISCUSSION

### Sensory evaluation

There was a significant difference ( $p < 0.05$ ) in color, appearance, odor, sweetness, taste, texture, overall quality, and purchasing intension except sourness among the three different oat concentrations (Figure 2). T<sub>3</sub> which contained the highest amount of oats showed the lowest mean ranks for all sensory attributes. There was no significant difference in color, appearance, odor, sweetness, sourness, and overall quality except in texture and purchasing intension between T<sub>1</sub> (2.5 g of





**Figure 2.** Median ranks obtained for sensory attributes of oat incorporated yogurt (T1 – Oat 2.5 g per 100 ml of milk, T2 - Oat 3.75 g per 100 ml of milk, and T3- Oat 5 g per 100 ml of milk).



**Figure 3.** Median ranks obtained for sensory attributes of oat yogurt T<sub>2</sub> (selected through T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>) and commercial yogurt.

oats per 100 ml of milk) and T<sub>2</sub> (3.75 g of oats per 100 ml of milk). Finally, T<sub>2</sub> was selected as the best oat concentration as the intended objective of this study was in line with incorporating the highest quantity of oats possible in yogurt.

The second sensory evaluation was conducted to compare the selected oat concentration from the first sensory evaluation and commercial yogurt. There was no significant difference in color, appearance, taste, texture, overall quality, purchasing intension, odor, sweetness, and sourness between oat yogurt and commercial yogurt (Figure 3).

### Proximate analysis of oat yogurt

Results of proximate analysis of oat yogurt and commercial yogurt are given in Table 2. There was a significant difference ( $p < 0.05$ ) in moisture content between the two yogurt samples. Commercial yogurt had a higher moisture content (70.23%) than oat yogurt (65.77%). This decrease in moisture content was due to the incorporation of oat flakes into the yogurt. Also, the total solid contents of the two yogurt samples were significantly different. Oat yogurt had a higher mean value for total solid (TS %) content ( $36.38 \pm 0.3\%$ ) than



**Table 2.** Variation of proximate parameters between oat yogurt and commercial yogurt.

Proximate component	Commercial yogurt	Oat yogurt
MC%	70.23 ± 1.1 <sup>a</sup>	65.79 ± 1.0 <sup>b</sup>
TS%	30.23 ± 0.7 <sup>b</sup>	36.38 ± 0.3 <sup>a</sup>
Ash%	0.28 ± 0.2 <sup>a</sup>	0.37 ± 0.3 <sup>a</sup>
Protein%	3.00 ± 0.05 <sup>b</sup>	6.20 ± 0.05 <sup>a</sup>
Fat%	3.00 ± 0.0 <sup>b</sup>	6.10 ± 0.8 <sup>a</sup>
SNF%	32.50 ± 0.3 <sup>b</sup>	41.76 ± 0.6 <sup>a</sup>
Fiber%	0.00 ± 0.0 <sup>b</sup>	0.36 ± 0.5 <sup>a</sup>
Carbohydrate%	23.49 <sup>a</sup>	21.18 <sup>b</sup>
Energy(kcal/100 g)	140.33 <sup>a</sup>	164.56 <sup>b</sup>

The same letter in each row represents values not significantly different from each other ( $p < 0.05$ )  
MC – Moisture content, TS – Total solid content, SNF – Solid Non-Fat content

commercial yogurt which had a TS% of 30.23±0.7%. Increment of TS% in oat yogurt is due to the addition of oat flakes that increase the solid content per a given mass. There was no significant difference ( $p > 0.05$ ) in ash content between the two yogurt samples.

The protein content of commercial yogurt showed a mean value of 3.0±0.0% while the protein content of oat yogurt was 6.2±0.0%. Yogurt should contain a minimum of 2.7% of protein content according to the CODEX standard (Codex Stan 243, 2003) and thus oat yogurt has acceptable protein content. Interestingly, oat yogurt had a considerably higher percentage of protein content due to the incorporation of oats as oat is a protein-rich cereal (Varma et al., 2016). The fat contents of the two yogurt samples exhibited a significant difference ( $p < 0.05$ ). Commercial yogurt had a mean value of 3.0±0.0% while oat yogurt had a fat content of 6.1±0.8%. The high-fat content of the oat yogurt was due to the high-fat content of oat grains (Varma et al., 2016). According to CODEX, the maximum fat content of yogurt should be 15%. According to the SLS standard, the minimum fat content of yogurt should be 3%. Oat yogurt is in an acceptable stage concerning fat content and the high-fat content may have contributed to the consistency of the oat yogurt.

The solid-non-fat (SNF) content of the two yogurt samples showed a significant difference. Commercial yogurt had an SNF of 32.5±0.3% while oat yogurt had a SNF of 41.76±0.6%. These results indicate that the SNF content was higher due to the incorporation of oats. SNF mainly contains lactose, protein, and mineral matter (Tamime and Robinson, 2007). Yogurt should contain a minimum of 8.0% of SNF content (SLS standard., 1989). Therefore, oat yogurt consists of an acceptable level of SNF. The high protein content of oats can directly affect the SNF value of the treated yogurt. The fiber content of the two yogurt samples was, also, significantly different ( $p < 0.05$ ) due to the absence of fiber in commercial yogurt. Oat is a fiber-rich cereal (Varma et al., 2016). The fiber content of oat yogurt was 0.36±0.1%. This is the most beneficial point of this product because fiber is one of the

most essential dietary components for human health. The carbohydrate content of oat yogurt was 21.18% while that of commercial yogurt was 23.49%. The 2% decrease in the carbohydrate content of the oat yogurt may be due to the higher quantities of proteins and lipids of this product. Lactose is the major sugar in yogurt (Tamime and Robinson, 2007) contributing much to the carbohydrate content of yogurts. Nevertheless, all carbohydrates, fat, and protein act as the main sources of energy. The energy value of commercial yogurt was 140.33 kcal/100 while that of oat yogurt was 164.56 kcal/100 g. The higher energy value of oat yogurt is due to higher protein and fat content. This proves that consumption of oat yogurt gives a higher energy value. Therefore, this is more suitable as a perfect breakfast meal for people of all age groups (Varma et al., 2016).

### Shelf life evaluation of oat yogurt

#### Physicochemical parameters

The variation of physicochemical parameters with time is given in Table 3. There is a significant difference in variation of pH, TA, and TSS with time. The reduction of pH value and increment of TA is due to the increment of lactic acid production by lactic acid bacteria (Ibrahim et al., 2019). Titratable acidity (TA) and pH values are in the acceptable range for 21 days (SLS 824, 1989). TSS of oat yogurt, expressed in Brix% values, increased with time. This variation may be due to the removal of water during the fermentation process by lactic acid bacteria.

Variation of syneresis with time indicates the variation of whey separation with time. Syneresis has a significant increment with storage time for this treated yogurt (1.96-3.93) (Table 4).

#### Microbiological assessment for oat yogurt

Bacterial colony count and yeast and mold count showed

**Table 3.** Variation of physicochemical parameters of oat yogurt stored under refrigerated temperature (4°C).

Days	pH	TSS (%)	TA (%)
1	4.62 ± 0.0 <sup>a</sup>	31.50 ± 0.5 <sup>f</sup>	0.72 ± 0.0 <sup>d</sup>
3	4.55 ± 0.0 <sup>b</sup>	33.83 ± 0.2 <sup>e</sup>	0.78 ± 0.0 <sup>cd</sup>
6	4.50 ± 0.0 <sup>c</sup>	34.40 ± 0.1 <sup>de</sup>	0.85 ± 0.0 <sup>bc</sup>
9	4.45 ± 0.0 <sup>d</sup>	34.80 ± 0.2 <sup>d</sup>	0.90 ± 0.0 <sup>b</sup>
12	4.41 ± 0.0 <sup>e</sup>	34.83 ± 0.2 <sup>d</sup>	1.04 ± 0.0 <sup>a</sup>
15	4.38 ± 0.1 <sup>f</sup>	36.50 ± 0.5 <sup>c</sup>	1.05 ± 0.0 <sup>a</sup>
18	4.29 ± 0.1 <sup>g</sup>	38.16 ± 0.7 <sup>b</sup>	1.08 ± 0.0 <sup>a</sup>
21	4.18 ± 0.0 <sup>h</sup>	39.16 ± 0.7 <sup>a</sup>	1.08 ± 0.0 <sup>a</sup>

The same letter in each column represents values not significantly different from each other ( $p < 0.05$ ).

**Table 4.** Variation of syneresis with time.

Days	Syneresis (%)
1	1.96 ± 0.01 <sup>d</sup>
7	2.22 ± 0.37 <sup>c</sup>
14	3.23 ± 0.05 <sup>b</sup>
21	3.93 ± 0.02 <sup>a</sup>

The same letter in each column represents values not significantly different from each other ( $p < 0.05$ ).

**Table 5.** Variation of bacterial colony count and yeast and mould count with time.

Days	Bacterial count(log cfu/g)	Yeast and Mould count(log cfu/g)
1	7.91 <sup>d</sup>	—
7	8.55 <sup>c</sup>	2.16 <sup>c</sup>
14	8.85 <sup>b</sup>	2.95 <sup>b</sup>
21	9.26 <sup>a</sup>	3.44 <sup>a</sup>

The same letter in each column represents values not significantly different from each other ( $p < 0.05$ ).

a significantly different increment with time (Table 5). According to SLS 824 (1989), bacterial colony count was at an acceptable level up to 21 days. Yeast count has exceeded the maximum colony count in the third week as per the SLS 824(1989). This indicates that oat yogurt is more suitable to consume until 14 days from the day of production at this preservative level (300 ppm). However, it can be increased by adding potassium sorbate up to 1000 ppm according to Laws and Regulations (Country report) applicable to food and food additives in Sri Lanka.

## Conclusion

A consumer preferable oat incorporated yogurt production is possible with oat flakes up to 3.75 g per 100 ml of milk. This product is similar to the commercial yogurt according to the sensory attributes considered in this study. Strikingly, this product has a considerably high nutrient

profile with high protein, fat, and energy values. The pH, TSS, and TA variation of the developed oat yogurt was in an acceptable range during 21 days. This product stored at 4°C is microbial safe to consume for up to 14 days. The findings can be used to conduct further developments of oat yogurt and to introduce this product to the Sri Lankan dairy industry to fulfill the daily nutrient requirements of people of all age and to use as an effective functional food.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Estimation of nitrite level and effect of processing on residual nitrite level in sausages marketed in Dharan, Nepal

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The study was conducted to examine nitrite levels in processed meat products (sausage, salami, bacon, and ham) marketed in Dharan City and to determine the effect of common methods of processing on residual nitrite levels in sausages. Preliminary survey was conducted to know the popular processed meat products. Total of 44 samples were analyzed for nitrite levels and 10 sausage samples were subjected to two different heat processing methods: Frying and boiling. The nitrite contents in all samples were found to fall within the mandatory limits by the Nepal government with a wide range of variation (3.18-101.26 ppm). Both frying and boiling reduced the residual nitrite level in sausages with greater reduction by boiling at 100°C for 15 min. As long as the measured nitrite level in the sausages produced continues, the level of nitrite is not considered to pose a major risk to human health or safety.

**Key words:** Nitrite level, processed meat, sausages, heat processing, Dharan.

## INTRODUCTION

Nitrites and nitrates are food additives (preservatives) with wide applications in the meat industry. They improve the quality, durability and safety of products, and, above all, inhibit the growth and reproduction of bacteria *Staphylococcus aureus* and *Clostridium botulinum*. On combining with myoglobin nitrites give the stable nitrosomyoglobin, a heat stable bright red color compound, which is most desirable in the meat products (Heinz and Hautzinger, 2007). Nitrates are relatively non-toxic, but nitrites and nitrite metabolites such as nitric oxide and N-nitrosocompounds have potential adverse

health effects. According to the International Agency for Research on Cancer (IARC), nitrates or nitrites consumed are likely to be human carcinogens under conditions favouring endogenous nitrosation. Legal limits for the addition of nitrates and nitrites have been set by several countries (Govari and Pexara, 2018). Nitrite has an acceptable daily intake (ADI) of 0.07 mg kg<sup>-1</sup> body weight and is dependent upon harmful effects in rodents on the lungs and cardiovascular system (Merino et al., 2016). The government of Nepal has prescribed the legal limit of nitrite in food to be 200 ppm (DFTQC, 2017).

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However, Codex Alimentarius has limited the residual nitrite content as 80 mg/kg in processed comminuted meat, poultry and game products (Codex Alimentarius, 2017). Despite such regulations, the chance of a high amount of nitrate and nitrite in cured meat cannot be ruled out. It should be kept at a minimum level as possible to care the health of consumers. There have been numerous attempts to reduce the use of nitrites in the meat industry due to their proven carcinogenic effects of nitrosamines (Lee et al., 2018).

The intake of per capita meat in Dharan was 13 kg while national meat consumption per capita was just 9 kg (Ekantipur, 2014). Dharan is thus one of the largest consumers of meat and meat products in the Nation, yet detailed study in the preferences on processed meat products is not available. As the consumption of meat has been growing rapidly among the urban population, and the country has been importing processed meat products. However, strict regulation and monitoring for processed meat are still lacking. The ignorance along with the haphazard use of additives might cause adverse effects on consumer's health.

Sausages like meat products are generally processed before consumption. The amount of residual nitrite in sausages decreases with storage time and preprocessing before consumption. More than 80% of the nitrite is already degraded within two days (Qadar, 2013). The greater the temperature and the duration of cooking, the more is the reduction (Wang et al., 2012) and leaching of nitrite on boiling water also reduces the nitrite level (Mckenzie, 2017). Research shows that the amount of residual nitrite decreases with several cooking conditions, but there has been less research in Nepal on residual nitrite levels of sausages after treatment. This study is conducted to examine nitrite levels in processed meat products (sausage, salami, bacon and ham) marketed in Dharan City and to determine the effect of the common method of processing on residual nitrite levels.

## MATERIALS AND METHODS

### Study area and time

The study area is a Dharan Sub metropolitan city of Province-1, Nepal. It is situated in the foothills of the Mahabharat range in the north and the Terai region in the south at an altitude of 349 m. It is a commercial center between the hilly region and the Terai plains of the eastern development region. The study was conducted from October 2018 to January 2019.

### Preliminary market survey/observation

A preliminary observation/survey was carried out before sample collection to identify the popular processed meat product, traded places, way of consumption and methods of processing. A total of 110 meat lovers and street vendors were asked for their favorite processed meat products and processing method preferred before consumption.

### Sample collection

A total of 44 processed meat samples (sausages, salami, bacon and ham) were collected from different parts of Dharan city. Samples were sent to the laboratory by maintaining the cold chain and stored in a refrigerator at 4°C. If the analysis could not be completed within two days, the leftover samples were frozen in polyethylene bags at -18°C in unopened condition before the analysis could begin.

### Processing methods applied to the sausages

After raw sample analysis, sausage samples having nitrite levels greater than 10 ppm were randomly selected and coded as A1, A2..., and A10. Then samples were subjected to two different thermal processing methods; frying and boiling. Each treatment was carried out in triplicate manner for all 10 samples. The samples used for the treatments were of dimension 21 to 22 mm diameter and 120 to 150 in length.

#### Frying

The samples were fried at  $149\pm 2^\circ\text{C}$  for 4 min (2 min on each side) on a frying pan.

#### Boiling

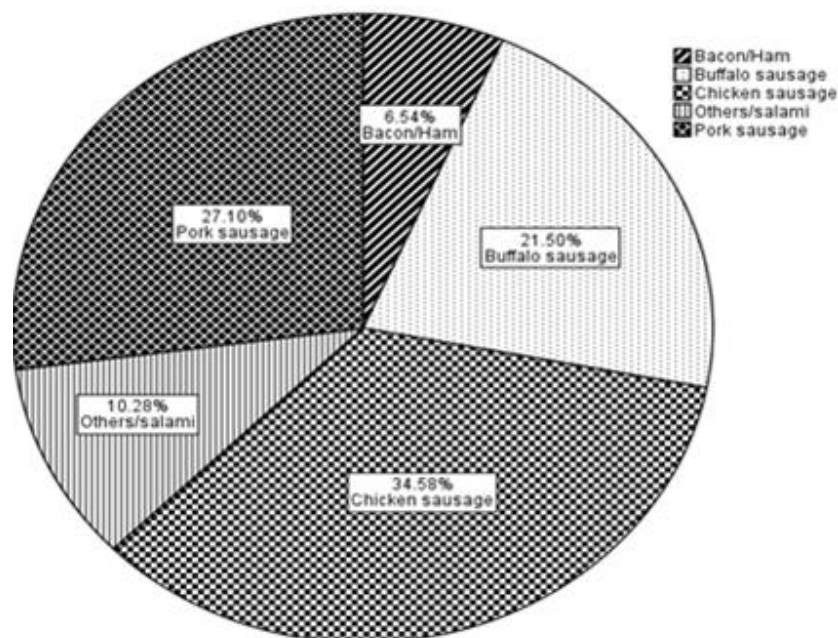
The sausages were boiled with potable water at  $100^\circ\text{C}$  for 15 min in a stainless-steel bowl of capacity 2 L. The ratio between sausages to the amount of water used was 1:10 by weight. After heat processing, samples were cooled to room temperature and again analyzed for the residual nitrite level.

### Determination of nitrite in meat samples

Nitrites contents in the samples were determined by the method described in AOAC (2016), with slight modification. The sample was extracted with hot distilled water and the extract was filtered. Nitrite contained in the filtrate produces on reaction with sulphanilamide and N-1-naphthylethylene diamine dihydrochloride a red color and its absorbance was measured at 540 nm in a UV-vis spectrophotometer. In brief, 5 g of crushed sample was mixed with distilled water in a 100 ml volumetric flask. The extraction was carried out by placing the flask in a shaker water bath at  $80^\circ\text{C}$ . After 2 h of extraction, solution was cooled and filtered. 10 ml of filtrate was then taken for color development in 50 ml volumetric flask followed by recording the absorbance for each sample. The concentration for the corresponding absorbance was found from the standard curve obtained by plotting absorbance vs. different concentrations of nitrite. Each sample was analyzed in triplicate. Results were expressed as  $\text{NaNO}_2$  in ppm. Values are shown in terms of mean, estimated standard error of the estimated mean, minimum and maximum value.

### Data analysis

MS Excel and *GenStat Release 12.1* were used for data analysis. Descriptive analysis and analysis of variance, were performed for statistical analysis. Fischer's Least Significance Difference (LSD) was used further to compare sample means. Statistical analysis was performed at 5% level of significance.



**Figure 1.** Consumer preferences for processed meat products in Dharan (N=107).

**Table 1.** Amount of nitrite in raw sausages samples.

Product type	Sample size (n)	Mean nitrite content (ppm)
Chicken sausage	13	29.08±22.10 (3.18-62.20)
Buffalo sausage	11	49.96±28.14 (15.26-83.12)
Pork sausage	9	64.83± 31.15 (26.01-101.29)
Others/salami	7	49.64±27.40 (17.27-83.12)

Values in the parenthesis are minimum-maximum amount of that product type.

## RESULTS AND DISCUSSION

### Survey results

One hundred and seven respondents have responded positively towards the survey. Their preferences on processed meat products are shown in Figure 1. Sausages, ham, bacon and salami were the popular products available. Sausages are more popular among the consumers while other meat products are still in the preliminary phase of introduction in the Dharan market. These processed meat products were found to be manufactured by 11 different manufacturers. On observation, cured meat products were found to be sold at departmental stores in refrigerated conditions. Besides these, sausages could be found in some street food stalls and major food outlets as well.

While observing the processing methods used by street food stalls, it was found that sausages were generally fried for 4-5 min around 125-150°C and if boiled sausages were asked for, sausages were boiled at 100-

105°C for 12-15 min. The time-temperature profile for processing of sausages in Dharan market showed close resemblance with Hill et al. (1973) and Li et al. (2016) for frying and boiling respectively.

### Nitrite level on raw samples

The amount of nitrite in the sausage samples ranged from 3.18 ppm (chicken sausages) to 101.29 ppm (pork sausage) (Table 1). Similarly amount of nitrite on bacon/ham found in the range of 15.26-37.82 ppm (Table 2). Less than one third (27.27%) of the analyzed samples had nitrite content less than 20 ppm. Nearly half (43.18%) samples had the nitrite content falling between 20-50 ppm and 29.55% had the nitrite contents above 50 ppm. The average nitrite content (64.83) of pork sausages was found slightly higher than other types of products.

However, this variation did not appear to be related to the type of cured meat product and all the samples had nitrite contents within the limits specified by mandatory

**Table 2.** Amount of nitrite in bacon/ham.

Product type	Sample size (n)	Mean nitrite content (ppm)
Bacon/Ham	4	30.52±10.52 (15.26-37.82)

Values in the parenthesis are minimum-maximum amount of that product type.

**Table 3.** Residual nitrite level in sausages after frying and boiling.

Product type	Sample code	Raw (ppm)	Fried (ppm)	Boiled (ppm)	Percentage decrease	
					Frying (%)	Boiling (%)
Sausage	A1	13.2 <sup>a</sup> ±1.72	10.50 <sup>b</sup> ±1.37	6.66 <sup>c</sup> ±0.87	20.45	49.55
	A2	33.07 <sup>a</sup> ±1.06	28.99 <sup>b</sup> ±1.02	23.02 <sup>c</sup> ±0.38	12.34	30.39
	A3	55.15 <sup>a</sup> ±0.50	49.42 <sup>b</sup> ±0.45	41.89 <sup>c</sup> ±0.38	10.39	24.04
	A4	63.20 <sup>a</sup> ±0.99	51.83 <sup>b</sup> ±0.95	15.23 <sup>c</sup> ±0.21	17.99	75.23
	A5	72.9 <sup>a</sup> ±0.50	56.67 <sup>a</sup> ±0.38	38.42 <sup>b</sup> ±0.93	22.28	47.31
	A6	81.69 <sup>a</sup> ±1.24	60.07 <sup>b</sup> ±0.91	60.87 <sup>c</sup> ±0.93	26.47	25.49
	A7	17.27 <sup>a</sup> ±2.01	15.56 <sup>a</sup> ±1.81	7.36 <sup>b</sup> ±0.86	9.90	57.38
	A8	35.61 <sup>a</sup> ±2.21	33.07 <sup>ab</sup> ±2.05	32.82 <sup>b</sup> ±2.04	7.13	7.83
	A9	28.10 <sup>a</sup> ±2.08	25.18 <sup>a</sup> ±2.05	16.84 <sup>b</sup> ±1.25	10.39	40.07
	A10	99.63 <sup>a</sup> ±1.54	57.92 <sup>b</sup> ±0.90	50.97 <sup>b</sup> ±0.79	41.86	48.84

The same letters in the superscript, row-wise, signify no significant difference ( $p > 0.05$ ) between the means of triplicate determination.

standards of Nepal Government (DFTQC, 2017). While about 18% (N=8) of the samples had crossed the codex standard of 80 ppm. From the result, we can conclude that most of the cured meat products had added nitrite/nitrate. However, the level is within that prescribed by government regulation.

The comparatively lower value of nitrite in the samples might be due to the depletion of nitrite during storage of the product from the time of manufacture to the analysis. It is a common practice to use 50-100 mg kg<sup>-1</sup> sodium nitrite in cooked cured type meat products against growth and botulism toxin by *C. botulinum* (Bardhi et al., 2014). Oxidation-reduction reactions converting nitrite to nitrous oxide could be the reason for the observed decrease in residual nitrite level in the products (Hill et al., 1973). The decrease in nitrite level from 116 to 9 ppm residual nitrite after 30 days of storage was observed by Bardhi et al. (2014). The loss of nitrite in a product with time is dependent on several factors including the heat process, pH of the product, storage temperature and addition of ascorbic acid or other reducing agents (Bardhi et al., 2014).

#### Effect of frying and boiling on sausage residual nitrite

All the sausages samples showed reduced nitrite level by both boiling and frying treatments, both the treatments significantly reduced the nitrite level with wide variation in

percentage decrease values (Table 3). There was a maximum of 41.86% reduction on nitrite level was observed by frying with a minimum reduction of 7.13% in sample A8. Similarly, for boiling treatment maximum reduction of up to 75.23% was observed while the lowest reduction observed was 7.83%. The greater range of variability in percentage reduction in sausage nitrite levels upon both types of heat processing is in agreement with the results obtained by Hill et al. (1973). The decrease in nitrite caused by cooking ranged from 3.4 to 41.0% which did not appear to be related to a common parameter such as emulsion type, product size, or quantity of nitrite added initially (Hill et al., 1973).

Regarding frying treatment, Soleimani et al. (2015) observed that initial nitrite content of 33.57 mg kg<sup>-1</sup> in the sausage was decreased to 26.46 mg kg<sup>-1</sup> after frying at 120°C for 5 min. Mean residual nitrite content was significantly different at other temperatures and cooking durations. The mean residual nitrite content reached to 1.42 and nil after frying at 220°C for 5 and 10 min, respectively. With regards to this, the greater the temperature and duration of cooking, the more the reduction in residual nitrite content of the final product. This decreased nitrite upon frying can be attributed to the formation of nitrosamine (Wang et al., 2012).

Regarding boiling treatment, Li et al. (2016) have found boiling as method to remove the residual nitrite in sausage. And suggested that the optimal conditions for processing are 100°C for 15 min, and the solvent/material ratio of 20, which resulted in the 19.47% removal

of nitrite in sausage. This is because of the solubility of nitrite in water. Since sodium nitrite is soluble in water it is easy to extract it from meat with hot water. Boiling and frying of the sausages are two common processing steps before consumption principally for the taste and safety concern. But at the same time the added advantages of such processing can be implicated though reduction in residual nitrite level in the products.

## Conclusions

The level of nitrite in processed meat products marketed in Dharan is within the mandatory limits imposed by the Government of Nepal. As long as this level is maintained in the production of sausages, it will not pose health risks to a human. Furthermore, the processing methods used are also responsible for lowering the nitrite level significantly in the diet. Boiling of sausages before consumption is the best practice to be followed to reduce the nitrite level in the product.

## Practical applications

Amount of nitrite in cured meat products is of great concern because of its potential carcinogenicity. The level of nitrite in commercially available meat products must be monitored regularly to safeguard the consumers' health. Results of the present study can serve as a useful parameter for regulatory agencies and producers. A similar study can be replicated to a different part of the country. The findings can be compared and used for policies formulation and action to be taken.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

## **Content of minerals and antinutritional factors in moin-moin (steamed cowpea food)**

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**Moin-moin (steamed cowpea food; MM) is one of the most popular local dishes in Bahia-Brazil. The present study was carried out to improve the knowledge of type of cowpea used in the preparation of moin-moin and its influence on mineral content and antinutritional factors. MM samples were collected from 30 out of the sales points located in the city of Salvador-Bahia. The mineral contents of MM showed a wide variation: K:  $917.4 \pm 166.2$  mg  $100$  g<sup>-1</sup> DM, P:  $400.0 \pm 59.8$  mg  $100$  g<sup>-1</sup> DM, Mg:  $97.9 \pm 16.4$  mg  $100$  g<sup>-1</sup> DM, Ca:  $94.0 \pm 70.4$  mg  $100$  g<sup>-1</sup> DM, Fe:  $57.3 \pm 16.9$  µg g<sup>-1</sup> DM, Zn:  $22.4 \pm 4.2$  µg g<sup>-1</sup> DM, Cu:  $6.3 \pm 3.3$  µg g<sup>-1</sup> DM, Mn:  $6.0 \pm 2.8$  µg g<sup>-1</sup> DM and V:  $4.3 \pm 2.0$  µg g<sup>-1</sup> DM. AF in MM were:  $0.60 \pm 0.77$  µmol g<sup>-1</sup> DM (InsP<sub>4</sub>);  $1.34 \pm 1.07$  µmol g<sup>-1</sup> DM (InsP<sub>5</sub>);  $4.77 \pm 3.53$  µmol g<sup>-1</sup> DM (InsP<sub>6</sub>);  $1.69 \pm 0.03$  mg eq. CE g<sup>-1</sup> DM (tannins);  $6.37 \pm 0.12$  mg g<sup>-1</sup> DM (polyphenols). Fe bioavailability might be negatively affected. Information on cowpea cultivars used for MM preparation was obtained from a structured questionnaire. 'Olho de Pombo' showed the highest concentrations for AF and minerals. MM was identified as a good source of minerals.**

**Key words:** Antinutritional factors, cowpea (*Vigna unguiculata*), crude palm oil, minerals, moin-moin.

### **INTRODUCTION**

Originally introduced from East and West African countries, moin-moin (moyi-moyi, olèl) is a steamed

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pudding made of beans, sold in the streets by typically clothed women called *baianas de acarajé*. The preparation of moin-moin (MM) starts with cowpea soaking in cold water until they can be easily dehulled. Different varieties of cowpeas such as *fradinho*, *olho de pombo* and *macáçar* or a ready-to-use cowpea paste are used to prepare moin-moin at different sales points (Dos Santos et al., 2013; Rogério et al., 2014). The dehulled cowpeas are ground to a fine paste and crude palm oil, grated onions, dry shrimps, salt and other seasonings (chestnut, peanut, ginger) are added to obtain the typical taste. The paste is formed to balls which are steamed and wrapped in banana leaves. There are some variations in the methods applied to prepare MM, such as the soaking time for the cowpeas, the amount and type of ingredients, as well as steaming time.

Cowpea (*Vigna unguiculata*), also called black-eyed pea or southern pea, is the main ingredient of MM, it is widely cultivated in the Northern and Northeastern regions of Brazil (Carvalho et al., 2012). Cowpeas contain high-quality protein (23 to 25%), carbohydrates (57%), vitamin B, minerals, dietary fiber (3.9%), and are low in fat (1.3%) (Carvalho et al., 2012). However, its nutritional value is frequently reduced by the presence of antinutrients such as phytates, trypsin inhibitors, lectins, tannins and polyphenols, affecting mineral bioavailability (Dahdouh et al., 2019; Almeida et al., 2008; Samtiya et al., 2020).

Food processing and preparation techniques may have effects on the content of minerals and antinutritional factors of the final foods (Samtiya et al., 2020). So far, there is only limited information about the content of minerals and antinutritional factors of moin-moin. Therefore, the objective of this study was to improve the knowledge of type of cowpea used in the preparation of moin-moin and its influence on mineral content and antinutritional.

## MATERIALS AND METHODS

### Study site and sampling

Moin-moin (MM) samples were collected from 30 out of the 168 MM sales points (18%) located in the city of Salvador-Bahia. The samples collected at each sales point consisted of four MM balls wrapped in banana leaves. Directly after collection, the moin-moin samples were packed in freezer storage bags and transported to the laboratory in thermal insulated boxes. After arrival in the laboratory, the samples were stored at minus 80°C for 24 h. Thereafter, the samples were freeze-dried (Freeze-dryer LS 3000 D, Terroni, Brazil) and ground in a domestic stainless-steel food processor (DCG-20 Cuisinart Coffee Grinder). The ground material was stored in amber bottles at minus 20°C until further use.

While collecting the samples, the sales women (*baianas de acarajé*) were invited to complete a structured questionnaire about the selection of ingredients and the mode of preparation. Each participant in the questionnaire gave informed consent, and it was previously approved by the Ethics Committee of the School of Nutrition of Federal University of Bahia (Protocol 730.192/2014).

### Antinutritional factors

#### Myo-Inositol phosphates

Myo-inositol phosphates were quantified in duplicate (two independent extractions per sample) by High-performance liquid chromatography (HPLC) ion-pair chromatography, using an Ultrasep ES 100 RP18 (2 x 250 mm), as described by Greiner and Konietzny (1998). A mixture of the individual myo-inositol phosphate esters (InsP<sub>3</sub> - InsP<sub>6</sub>) was used as a standard.

#### Haemagglutinating activity

Haemagglutination assays, using trypsin-treated rabbit erythrocytes, were carried out by serial dilution, as described by Grant et al. (1983). One unit of haemagglutinating activity (HU) was defined as that contained in the amount of sample in the last dilution which caused 50% agglutination of the blood cells.

#### Condensed tannins

Condensed tannins were extracted with HCl:methanol (1:100 v/v) for 2 hours with mechanical shaking at 25°C and centrifuged at 5,000 g at 15°C for 15 min. Aliquots were immediately analyzed for tannins using the 0.5% vanillin assay (Broadhurst and Jones, 1978).

#### Polyphenols

Total phenols were extracted with water. An internal standard curve was prepared by adding 10 ml of 0 - 0.01% tannic acid to the flasks. The contents were heated for 30 min at 70°C with constant shaking. Clear supernatants were collected after centrifugation at 2500 g for 15 min, followed by filtration. Polyphenols were determined using the Folin-Denis reagent (King and Health, 1967).

#### Trypsin inhibitor

Trypsin inhibitor activity was determined as described by Kakade et al. (1974), using alpha-N-benzoyl-DL-arginine-*p*-nitroanilide hydrochloride as the substrate for trypsin. One trypsin unit was defined as the increase by 0.01 absorbance unit at 410 nm.

### Minerals

The following reagents and standard solutions were used: 65% (w/w) HNO<sub>3</sub> (Merck, Germany), 30% (v/v) H<sub>2</sub>O<sub>2</sub> (Vetec, Brazil) and 1000 mg L<sup>-1</sup> standard solutions (Specsol, Brazil) of Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Ni, P, Pb, V and Zn. All solutions were prepared with ultrapure water with a specific resistivity of 18.2 MΩ cm, provided by a Milli-Q® Purification System (Millipore, Bedford, MA, USA). All glass and plasticware was decontaminated in a 10% (v/v) HNO<sub>3</sub> bath for at least 24 hours and extensively washed with ultrapure water.

For mineral quantification, an inductively coupled plasma optical emission spectrometer, ICP OES, (OPTIMA 7300 DV - PerkinElmer, USA) was used. The ICP OES operating conditions were: measured power: 1300 W; signal integration time: 1 s; plasma gas flow: 15 L min<sup>-1</sup>; auxiliary gas flow: 1.5 L min<sup>-1</sup>; nebulization gas flow: 0.70 L min<sup>-1</sup>; sample pump flow: 0.70 ml min<sup>-1</sup>. For sample introduction, a cyclonic chamber and a GemCone™ nebulizer - Low Flow were used. The chosen nuclear (I) and ionic (II) lines (nm) were: Ca II 422.673; Cd II 228.802; Co II 238.892; Cr II 267.716, Cu

**Table 1.** Values of LOD and LOQ ( $\mu\text{g g}^{-1}$ ).

Mineral	LOD	LOQ	Mineral	LOD	LOQ
Ca	11.377	3.413	Mg	116.320	34.896
Cd	0.041	0.012	Mn	0.307	0.092
Co	3.217	0.965	Ni	8.574	2.572
Cr	1.205	0.362	P	675.404	202.621
Cu	0.550	0.165	Pb	0.408	0.123
Fe	6.536	1.961	V	1.783	0.535
K	9.007	2.702	Zn	0.309	0.098

**Table 2.** Average ( $\mu\text{g g}^{-1}$ ; n=9) and confidence intervals at 95% for certified macro and trace elements obtain by acid digestion in a microwave oven.

Mineral	Reference material (measured value)	Reference material (certified value)	Recovery (%)
<b>(g 100 g<sup>-1</sup> DM)</b>			
Ca	0.2467 ± 0.0170	0.2442 ± 0.0002	99
K	2.423 ± 0.083	2.223 ± 0.072	92
Mg	0.3005 ± 0.0082	0.2764 ± 0.0059	92
P	0.6555 ± 0.0335	0.7674 ± 0.0143	117
<b>(<math>\mu\text{g g}^{-1}</math> DM)</b>			
Cu	14.30 ± 0.46	14.8 ± 0.09	103
Fe	90.8 ± 4.0	89.8 ± 0.9	99
Mn	32.3 ± 1.1	31.0 ± 0.4	96
Zn	52.3 ± 1.3	52.0 ± 0.80	99

I 324.752; Fe II 238.204; K 766.490; Mg II 279.077; Mn II 257.610; Ni II 231.604; P I 213.617; Pb II 220.000; V II 309.310 and Zn II 213.857. The samples were decomposed by acid digestion in a microwave oven with cavity (Dos Santos et al., 2013). Briefly, 9.00 ml of 65% (w/w)  $\text{HNO}_3$ , 1.00 ml of 30% (v/v)  $\text{H}_2\text{O}_2$  were added to 500 mg of the ground sample. The following heating program was applied: step 1 (6 min ramp to 90°C); step 2 (4 min at 90°C); step 3 (18 min ramp to 190°C); step 4 (7 min at 190°C). The volumes of the digested mixtures were adjusted to 15.0 ml with ultrapure water and the solutions were kept in 50 ml Falcon® tubes. For calibration, a 40.0 mg L<sup>-1</sup> multielement solution of Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Ni, P, Pb, V and Zn in 2.0 mol L<sup>-1</sup>  $\text{HNO}_3$  was used. All samples were analyzed in triplicate. The estimated limit of quantification for the method (LOQ) was calculated as 3.3-fold of the limit of detection (LOD) (Thomsen et al., 2003), based on the measurement of the analytical signal of the digest blank assays (n=10) (Table 1). The Extreme Studentized Deviate Test was used to remove outliers from the data set. INCT-SBF-4 SOYA BEAN FLOUR was used as a reference material (n = 9) to determine precision and accuracy of the method (Table 2).

#### Multivariate statistical analysis

Normalized data were uploaded to MetaboAnalyst 2.0, a web-based analytical platform for high-throughput metabolomics studies, as previously described (Ribeiro et al., 2015). ANOVA was performed to assess the overall variation in the levels of minerals, followed by post-hoc analyses (Bonferroni correction, FDR < 0.05). Partial least squares discriminant analysis (PLS-DA) was used to compare the overall variation of minerals and antinutritional factors

in moin-moin in respect to the cowpea variety used for preparation. Mineral data were also analyzed using the SPSS 13 software (SPSS Inc, Chicago, IL, USA). Each sample was analyzed in triplicate and values were given as mean ± standard patron (SP).

## RESULTS AND DISCUSSION

### Minerals

The great variation in the mineral content of MM (Table 3) could be explained by variations in the mineral content of the cowpeas used, as well as differences in the recipes and preparation methods for MM (Dos Santos et al., 2013; Rogério et al., 2014).

Potassium (K) was identified as the most abundant mineral in MM (Table 3). Due to the high potassium content of cowpeas (1121.0 100 g<sup>-1</sup> DM) (Franco, 2008), this result was expected. MM exhibited higher potassium levels than akara (545.31 - 613.01 mg 100 g<sup>-1</sup> DM), another cowpea product frequently consumed in Salvador, Bahia (Feitosa et al., 2015). This could be explained by differences in the recipe of both cowpea products. Akara is mainly made of grated onions, cowpea and salt (Feitosa et al., 2015), whereas the potassium-rich ingredients peanuts (740 mg 100 g<sup>-1</sup> DM) and cashew nuts (620 mg 100 g<sup>-1</sup> DM) (Franco, 2008) were

**Table 3.** Content of macro- and micronutrients moin-moin.

	Macronutrients (mg 100 g <sup>-1</sup> DM)		
	Minimum	Maximum	Mean ± SD
Potassium	579.3	1169.9	917.4 ± 166.2
Phosphorus	267.1	487.8	400.0 ± 59.8
Magnesium	66.9	140.5	97.9 ± 16.4
Calcium	5.0	224.9	94.0 ± 70.4
	Micronutrients (µg g <sup>-1</sup> DM)		
Iron	34.3	89.4	57.3 ± 16.9
Zinc	12.8	32.1	22.4 ± 4.2
Copper	2,5	14.6	6.3 ± 3.2
Manganese	2.8	12.5	6.2 ± 2.8
Vanadium	0	7.8	4.3 ± 2.0

Number of moin-moin samples analyzed: 30. All samples were analyzed in triplicate. SD = Standard deviation.

used in the preparation of moin-moin. Another study on minerals in abará found lower values of this element (104.32- 652.10 mg/100 g).

Phosphorus (P) was identified as the second most abundant mineral present in moin-moin (Table 3). The P content in moin-moin was higher than the P content of akara (210.55 - 353.99 mg 100 g<sup>-1</sup> DM) (Feitosa et al., 2015). This could be explained by the use of ingredients higher in P, such as peanuts (354 mg 100 g<sup>-1</sup> DM) and cashew nuts (575 mg 100 g<sup>-1</sup> DM) (Franco, 2008) for the preparation of MM and ingredients lower in P, such as grated shrimp heads (209 mg 100 g<sup>-1</sup> DM) for the preparation of akara (Franco, 2008).

The calcium (Ca) content in MM varied significantly among the samples (5.0 - 224.9 mg 100 g<sup>-1</sup> DM) (Table 3). The high variation of Ca content in MM might be due to the variation already reported in Ca content for cowpeas (29 - 51 mg 100 g<sup>-1</sup> DM) (Carvalho et al., 2012). Besides cowpeas, ingredients such as dry shrimp, peanuts, cashew, ginger (164, 42, 10, 51 mg 100 g<sup>-1</sup> DM, respectively) (Franco, 2008) add to the Ca content of moin-moin. The mean magnesium (Mg) content in MM was very comparable to those observed for akara (106.98 - 125.74 mg 100 g<sup>-1</sup> DM) (Feitosa et al., 2015). With 334 ± 0.71 mg 100 g<sup>-1</sup> DM, African moin-moin was reported to contain twice the amount of magnesium (Adeyeye et al., 2012). The higher magnesium content is very likely due to the use of fish and eggs for moin-moin paste preparation in Africa.

The iron (Fe) and zinc (Zn) contents of MM were found to be low (Table 3). A lower Fe and Zn content compared to cowpeas (Fe: 51 µg g<sup>-1</sup> DM, Zn: 39 µg g<sup>-1</sup> DM) (TACO, 2011) could be explained by the removal of the mineral-rich tegument prior to preparing the paste for moin-moin. Higher values, especially for iron, might be due to the use of iron-fortified wheat flour (42 µg g<sup>-1</sup> DM Fe) (ANVISA, 2005) for the preparation of MM paste and/or the transfer of these minerals from the banana leaves used to wrap the moin-moin balls. Olayiwola et al. (2012) reported that

11.1 ± 0.12 mg kg<sup>-1</sup> DM of iron were derived from banana leaves while preparing moin-moin.

Manganese (Mn), copper (Cu) and vanadium (V) contents in MM are shown in Table 3. Manganese content was found to be lower and copper content was in the same range, compared to Nigerian moin-moin (Mn: 17.4 µg g<sup>-1</sup> DM, Cu: 0.85 and 0.98 mg 100 g<sup>-1</sup> DM) (Madukorsiri and Adoga, 2009). Values for vanadium could not be found in the scientific literature. The trace elements Cd, Co, Cr, Ni and Pb were not detected in MM. In fact, these minerals are found at very low concentrations in cowpea and other ingredients used to prepare MM. Dos Santos et al. (2013) reported that cowpeas contain 2.1 µg g<sup>-1</sup> DM Co, 0.6 -1.7 µg g<sup>-1</sup> DM Ni and 0.29 µg g<sup>-1</sup> DM Pb.

In Table 4, the contribution of one moin-moin serving (186.6 g) to the daily dietary reference intake (DRIs) for minerals in adults is given. The daily requirements for K, P, Cu, Fe and Mn could be fully covered by one MM serving, whereas the daily requirements for Mg and Zn could be covered at least by 50%. Since the content of macro-and micronutrients differed considerably between the different MM samples analyzed, the contribution of one MM serving to the daily requirements might be sometimes significantly lower. A study analyzing the data obtained in a Household Budget Surveys in 2008 and 2009 demonstrated the prevalence of inadequate intakes of Ca, Cu, K, Mg, Mn, P, Fe and Zn among men and women aged between 20 and 59 years old, mainly in the Northeast region of Brazil (Araújo et al., 2013). Therefore, moin-moin could play an important role in the supply of the population in Salvador, Bahia, with these macro-and micronutrients (Table 4).

### Content of antinutrients in moin-moin

Cowpeas have been reported to contain antinutrients such as phytates, polyphenols, condensed tannins,

**Table 4.** Contribution of a serving<sup>a</sup> of moin-moin to the Dietary Reference Intakes (DRIs)<sup>b</sup> for minerals in adults.

Minerals	Content	DRIs		DRIs	
		Male	Female	Male	Female
		Macronutrients		Micronutrients	
	(mg 100 g <sup>-1</sup> DM)			(%)	(%)
Ca	5 - 225	1,000	1,000	0.9 - 42	0.9 - 42
K	579 - 1,170	4,700	4,700	23 - 46	23 - 46
Mg	67 - 140	420	320	30 - 62	39 - 82
P	267 - 488	700	700	71 - 130	71 - 130
		Micronutrients		Micronutrients	
		(µg g <sup>-1</sup> DM)		(%)	(%)
Cu	2.5 - 14.6	900	900	51.8 - 302.7	51.8 - 302.7
Fe	34.3 - 89.4	8,000	18,000	80.0 - 208.5	35.6 - 92.7
Mn	2.8 - 13.3	2,300	1,800	22.7 - 101.4	29.0 - 129.6
Zn	12.8 - 34.1	11,000	8,000	21.7 - 57.8	29.9 - 79.5

a: an average serving size of moin-moin represents 186.6 g; b: DRIs (1997, 2001, 2005, 2011), daily requirements of adults (19 to 50 years: (IOM, 2006).

**Table 5.** Content of antinutrients and calculated phytate mineral molar ratios in moin-moin.

Parameter	Minimum	Maximum	Mean ± SD
Polyphenols (mg g <sup>-1</sup> DM)	6.15	6.60	6.37 ± 0.12
Condensed tannins (mg eq. CE g <sup>-1</sup> DM)	1.65	1.78	1.69 ± 0.03
InsP <sub>6</sub> (µmol g <sup>-1</sup> DM)	0.29	12.20	4.77 ± 3.53
InsP <sub>5</sub> (µmol g <sup>-1</sup> DM)	0	4.10	1.34 ± 1.07
InsP <sub>4</sub> (µmol g <sup>-1</sup> DM)	0	3.19	0.60 ± 0.77
InsP <sub>6</sub> / Zn molar ratio	0.96	35.60	13.33 ± 9.20
InsP <sub>6</sub> / Fe molar ratio	0.28	16.93	4.91 ± 4.05
InsP <sub>6</sub> / Ca molar ratio	0.02	2.05	0.39 ± 0.55
InsP <sub>6</sub> x Ca/Zn molar ratio	6.47	1989.9	388.8 ± 500.4

Number of moin-moin samples analyzed: 30. All samples were analyzed in triplicate. SD = Standard deviation.

trypsin inhibitors and haemagglutinating activity (Almeida et al., 2008; Carvalho et al., 2012; Bolade, 2016; Rogério, et al., 2014). The processing of cowpeas, however, was reported to result in a reduction in the initial content of antinutrients. A 40-65% reduction in phytate by fermentation of cowpeas and a 50-80% reduction by germination of cowpeas was shown by Dahdouh et al. (2019). In respect to MM, Bolade (2016) revealed that every unit operation involved in its preparation contributed to varying degrees to the overall reduction in trypsin inhibitor activity, phytate and tannins. Soaking and steaming resulted in a complete absence of trypsin inhibitor activity in MM. Furthermore, Feitosa et al. (2015) reported that the cowpea paste used for the preparation of akara still contained trypsin inhibitors and haemagglutinating activity but, in the final food, none was detectable. This is in good agreement with the data on trypsin inhibitor activity obtained in this study. All moin-

moin samples analyzed were free of trypsin inhibitor and haemagglutinating activity.

The tannin content of cowpeas was reported to be highly dependent on the variety. For example, 0.34; 4.20; 3.26 and 6.88 mg eq. CE g<sup>-1</sup> DM condensed tannins were found in black-eyed, light brown, mottled and maroon-red cowpeas (Plahar et al., 1997). Dehulling reduced the content of condensed tannins to 0.23; 0.08; 0.08 and 0.46 mg CE g<sup>-1</sup> DM. Bolade (2016) reported a 96.4 - 97.5% reduction in the tannin content during the preparation of moin-moin. Dehulling was the unit operation identified to contribute the most to the reduction in tannins (39.7 - 47.6%), followed by steaming (19.6 - 24.7%), soaking (9.8 - 15.9%) and wet milling (9.5-13.1%). Feitosa et al. (2015) obtained condensed tannin contents between 1.67 and 1.69 mg eq. CE g<sup>-1</sup> DM in akara. The tannin contents in moin-moin (Table 5) were comparable to those reported for akara. While preparing



MM, other sources for condensed tannins than cowpeas might need to be considered. Megat Rusydi and Azrina (2012) identified peanuts as a source of tannins ( $32.69 \pm 2.392$  mg gallic acid equivalent/mg DM). In addition, a reduction in the amount of tannins leaching into the cooking water due to the wrapping of the MM balls in banana leaves also need to be considered when evaluating effects of processing on the tannin content of MM (Asogwa and Onweluzo, 2010). The tannin content of MM was not expected to impose a significant negative effect on their nutritional quality, once contents up to about  $4.2$  mg eg. CE  $g^{-1}$  DM were reported not to have adverse effects in humans (Plahar et al., 1997) (Table 5).

The cowpea varieties used to prepare moin-moin were reported to contain  $6.42 \pm 0.18$  mg  $kg^{-1}$  DM total polyphenols (Almeida et al., 2008). The content of polyphenols ( $6.15$  and  $6.60$  mg  $g^{-1}$  DM) in MM was very comparable to the polyphenol content of the cowpeas used for its preparation (Table 5), as well as the polyphenol content of akara ( $6.26$  to  $6.30$  mg  $g^{-1}$ ) (Feitosa et al., 2015). While preparing MM, other sources for polyphenols than cowpeas might need to be considered. Cushnie and Lamb (2005) reported a migration of polyphenols from banana leaves into the wrapped product and Megat Rusydi and Azrina (2012) found  $43.79 \pm 2.38$  mg gallic acid equivalent/mg DM of total phenolic compounds in peanuts, another MM ingredient rich in polyphenols.

According to Rogério et al. (2014), akara paste contained  $12.63$   $\mu\text{mol } g^{-1}$  DM phytate (InsP<sub>6</sub>),  $2.21$   $\mu\text{mol } g^{-1}$  DM *myo*-inositol pentakisphosphate (InsP<sub>5</sub>) and  $0.14$   $\mu\text{mol } g^{-1}$  DM *myo*-inositol tetrakisphosphate (InsP<sub>4</sub>). The corresponding values for akara were reported to be  $7.72$ ,  $1.95$  and  $0.27$   $\mu\text{mol } g^{-1}$  DM for InsP<sub>6</sub>, InsP<sub>5</sub> and InsP<sub>4</sub>, respectively. The *myo*-inositol phosphate contents of moin-moin (Table 5) were found to be comparable to those of akara. Besides cowpeas, peanuts and cashew nuts may contribute to the *myo*-inositol phosphate content of moin moin. Schlemmer et al. (2009) reported cashew nuts to contain  $0.19$  -  $4.9\%$  (w/w DM) phytate and peanuts  $0.17$  -  $4.47\%$  (w/w DM). Once the mean content of InsP<sub>6</sub> and InsP<sub>5</sub> were lower compared to akara, steam cooking can be assumed to have a greater efficiency than deep frying in the reduction in *myo*-inositol phosphates.

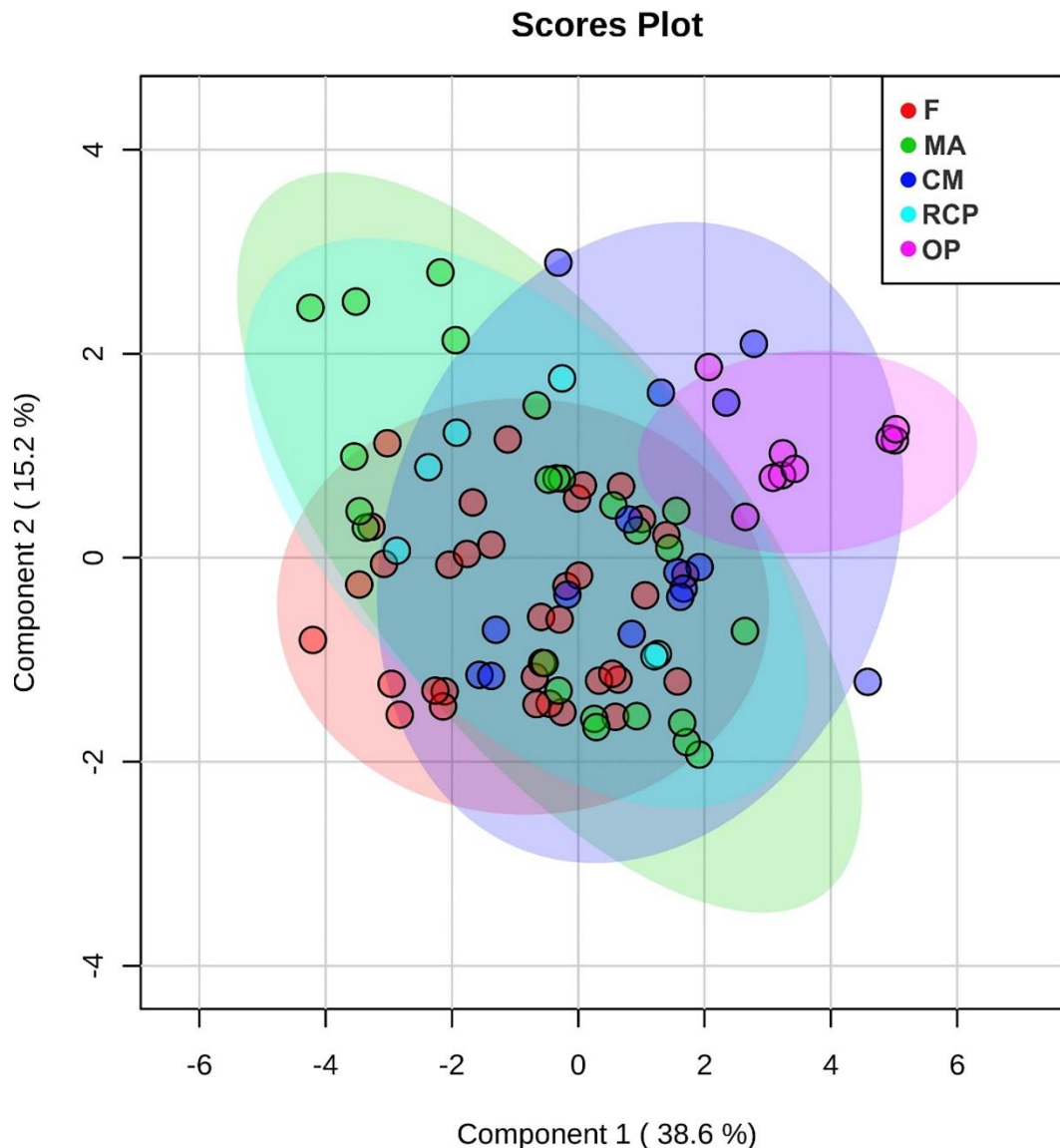
To evaluate the effect of phytate on the bioavailability of Ca, Fe and Zn, the phytate: mineral molar ratio was calculated (Table 5). According to Gibson et al. (2006), iron bioavailability is reduced at phytate:Fe molar ratios above 1, whereas phytate: Zn molar higher than 15 is associated with low estimated bioavailability, and Phy:Zn between 5 and 15 and below 5 is associated with moderate and high estimated bioavailability, with a zinc absorption corresponding to 15, 30 and 50%, respectively. In only 10% of MM samples analyzed, the phytate:Fe molar ratio was calculated to be 1. Thus, a low Fe bioavailability from MM is expected. In respect to Zn, 16.7, 56.7 and 26.7% of the

MM samples exhibited phytate:Zn molar ratios below 5, between 5 and 15 and above 15 (Gibson and Ferguson, 1998). A significant reduction in Zn bioavailability from MM due to phytate is therefore very likely. The dietary phytate x Ca:Zn molar ratio has been reported to be a more useful parameter to assess Zn bioavailability compared to the phytate:Zn molar ratio. Molar ratios above 200 have been reported to result in a significant reduction in Zn bioavailability; 36.7% of the MM samples showed values below 200, the threshold value for a significant negative effect on Zn bioavailability (Fordyce et al., 1987). Furthermore, the phytate:Ca molar ratio may be used as an indicator for calcium bioavailability in humans (Fordyce et al., 1987). Values above 0.24 point to a reduction in Ca bioavailability. The phytate:Ca molar ratio was shown to be below 0.24 for 76.7% of the MM samples. Therefore, Ca bioavailability should not be affected by phytate for the majority of the MM samples.

#### Multivariate statistical analysis of the compositional data obtained for the different moin-moin samples

The information on the cowpea cultivars used by the saleswomen (*baianas de acarajé*) to prepare moin-moin was obtained from a structured questionnaire. Principal component 1 (PC1) accounted for 38.6% of total variance, whereas 15.2% were explained by principal component 2 (PC2) (Figure 1). A clear separation of moin-moin samples prepared with different cowpea cultivars was observed along principal component 1. This result is not very surprising, since cowpeas are the major ingredient used for moin-moin preparation and studies aiming at assessing the mineral composition of cowpea seeds have shown a wide variation in mineral content among different cultivars (Carvalho et al., 2012). The MM samples prepared with the 'Olho de Pombo' (OP) cowpea exhibited clear differences in respect to their content of minerals and antinutritional factors compared to moin-moin samples prepared with 'fradinho' (F) and 'maçácar' (MA) cowpeas. Curiously, moin-moin samples prepared with F and MA cluster together, indicating that both cultivars were characterized by very similar mineral and antinutrient profiles. Additionally, important insights into the origin of the cowpea mixture (CM) or ready-to-use cowpea paste (RCP) used for MM preparation can be obtained from the PLS-DA plot. It was obvious that the mineral and antinutrient composition of MM prepared with RCP was very comparable to the mineral and antinutrient composition of moin-moin prepared with cultivars F and MA, whereas CM might also include OP cowpeas.

To obtain a possible contribution of each individual mineral and antinutritional factor to the observed differences in the cowpea cultivars used for moin-moin preparation, the variable importance in projection (VIP) threshold was set to 1 (Xia and Wishart, 2011). Zinc potassium, polyphenols, tannins, iron, phosphorus and



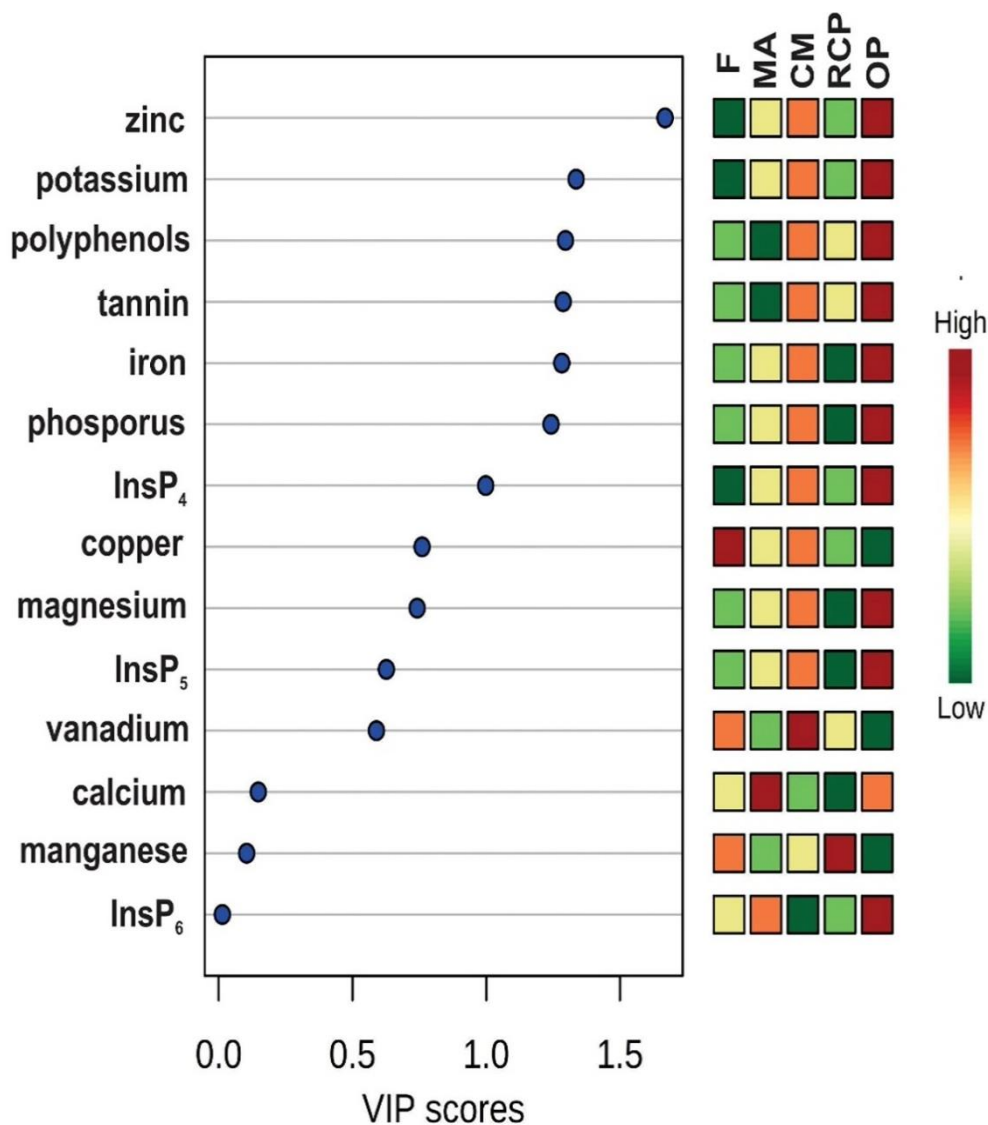
**Figure 1.** Partial least squares discriminant analysis (PLS-DA) based on the composition (minerals and antinutritional factors) of moin-moin. The cowpea cultivars used by the saleswomen (*baianas de acarajé*) to prepare moin-moin was obtained from a structured questionnaire: F= fradinho; MA= maçácar; CM = use of different cowpea cultivars simultaneously, RCP = ready-to-use cowpea paste, OP = olho de pombo

InsP<sub>4</sub> are the moin-moin constituents identified to contribute the most to the differentiation of cowpea cultivars used for MM preparation (Figure 2). Moin-moin samples prepared with the OP cultivar showed the highest concentrations for all these constituents. The lowest concentrations for these constituents were observed for MM samples prepared with RCP and the F cultivar. Moin-moin samples prepared with CM were found to have intermediate concentrations for these constituents, supporting the hypothesis that CM might also include cowpeas of the OP cultivar. Therefore, multivariate statistical analysis was shown to be a tool to identify the cowpea cultivars used for the preparation of

moin-moin.

### Conclusion

The content of minerals and antinutritional factors varied considerably between the different moin-moin samples. The cowpea cultivar used for moin-moin preparation had the most significant effect on their mineral and antinutrient composition. The daily requirements for K, P, Cu, Fe and Mn could be fully covered by one moin-moin serving, whereas the daily requirements for Mg and Zn could be covered at least by 50%. Thus, moin-moin could



**Figure 2.** Summary plot of moin-moin constituents based on the variable importance in projection (VIP) score. The mini heatmap on the right indicates differences in the concentration of minerals and antinutrients among the different cowpea cultivars used to prepared moin moin: F= fradinho; MA= maçãcar; CM = use of different cowpea cultivars simultaneously, RCP = ready-to-use cowpea paste, OP = olho de pombo

play an important role in the supply of the population of Salvador, Bahia, with macro-and micronutrients.

#### CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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