**DETERMINATION OF HEAVY METALS AND PROXIMATE ANALYSIS IN SOME SELECTED CEREALS**

**BY**

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**FACULTY OF NATURAL AND APPLIED SCIENCES**

**GODFREY OKOYE UNIVERSITY,**

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**ENUGU, ENUGU STATE**

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**GODFREY OKOYE UNIVERSITY,**

**THINKERS, CORNER**

**ENUGU, ENUGU STATE**

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**SUPERVISOR**

**PROF.I.N.E ONWURAH**

**JULY, 2016**

**CERTIFICATION**

I Okwor Meira Ebere as an undergraduate student of the Department of Chemical Sciences with registration number GOU/12/1348, hereby certify that the work embodied in this project is original and has not been submitted in part or full in any other degree programme of this university or any other university.

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EXTERNAL EXAMINER DATE

PROF. STANLEY UDEDI

DEDICATION

I dedicate this project work to all who in one way or the other contributed to the success of my academic pursuit, and thank God Almighty, who always make a way where there is no way and has seen me through in all the period of this work.

ACKNOWLEDGEMENT

Am most grateful to the Almighty God who his abundant grace and endless mercy has been upon me, his grace has found me just as I am. He has been faithful to me throughout this research work.

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ABSTRACT

This research was conducted to determine the concentrations of heavy metals and proximate composition of different cereals (maize, millet and rice). Two different samples each sold in two different markets (Ogbete and Gariki) in Enugu, were used for this work, using atomic, absorption spectrophotometer. The heavy metal screening of the cereal samples showed the presence of arsenic in the range of 0.456ppm-0.955ppm but was not detected in sample E and f which are the two different rice I purchased from Ogbete market, then mercury in the range of 0.024ppm-0.124ppm, lead in the range of 0.554ppm-1.083ppm, cadmium in the range of 0.087ppm-0.565ppm, copper in the range of 0.050ppm-0.245ppm and zinc in the range of 1.448ppm-66.954ppm which is the highest and is discussed. The result of proximate composition analysis indicated ash: 1.0%-19.0%, moisture: 8.6%-12.6%, fat: 0.2%-20.0%

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 **CHAPTER ONE**

**INTRODUCTION**

**1.0 BACKGROUND OF THE STUDY**

Cereals are enriched with niacin, iron, riboflavin and thiamine, and most cereals have abundant fiber content, especially barley, oat, and wheat. Cereals also have soluble bran that aid in lowering blood cholesterol level and keeping heart diseases at bay. Cereals consumption also means an intake of high amounts of protein; breakfast cereals are often eaten with milk that makes for a protein-rich meal. For infants, iron-fortified cereals are said to be the premium solid food.

A cereal is any grass cultivated for the edible components of its grain (botanically, a type of fruit called a caryopsis), composed of the endosperm, germ, and bran. Cereal grains are grown in greater quantities and provide more food energy worldwide than any other type of crop; they are therefore staple crop.

In their natural form (as in whole grain), they are a rich source of vitamins, minerals, carbohydrates, fats, oils, and protein. When refined by the removal of the bran and germ, the remaining endosperm is mostly carbohydrate. In some developing nations, grains in form of rice, wheat, millet, or maize constitutes a majority of daily substance. In developed nations, cereal consumption is moderate and varied but still substantial. The growth of civilizations, or development in the human diet patterns, the cultivation of cereal grains has played a significant role. The word ‘cereal’ is derived from ‘ceres’- the name of the Roman goddess of agriculture and harvest. It is said that almost 12,000 years ago, ancient farming communities dwelling in the Fertile Crescent area of southwest Asia cultivated the first cereal grains. The first Neolithic founder crops that actually initiated the development of Agriculture include einkorn wheat, emmer wheat and barley. Cereals are the important sources of many essential or beneficial components to the human diet. For example, the National Diet and Nutrition Survey of the UK showed that cereal products contributed 29/30% of the total daily energy intake of adult males/females, 22/21% of the intake of protein and 39/37% of the intake of non starch polysaccharides (the major components of dietary fiber, DF) (NDNS, 2011).

Cereals are probably the greatest source of energy for humans. Providing almost 30% of total calories in a regular diet, cereals are probably the most widely consumed caloric food in America. This percentage rises in places like rural Africa, Asia and India where cereals are reported to supply almost 70 to 80% of energy requirements (since people in these regions cannot afford to eat other food product like fruits, vegetables, meat, or milk products. Cereals are inexpensive and a widely available source of energy; this is probably the prime reason why people from all budgets prefer cereals as the major energy provider in their diet. Cereal intake tends to be quite high almost poor income families as they attain good amount of energy through mineral expenditure.

In cereal, around 95% of minerals are the sulphates and phosphates of magnesium, potassium and calcium. A good amount of phosphorous in cereals is present, called phytin. The phylates present in the cereals considerably reduce the activity of iron absorption. The unrefined cereals have more phytates as compared to refined cereals. After the cereals germinate, phytates diminish due to the breakdown of enzymes, and then the iron content is enhanced. This is the reason why molted flours of cereals are said to have more nutritional value than raw flour. Zinc, copper and manganese are also present in cereals in very small quantities. Cereals hardly have and calcium and iron, but ragi is an exception to this. Amongst cereals, rice is the poorest source of calcium and iron. Ragi, millets, jowar and bajra have high amounts of minerals and fiber.

Whole wheat products reduce the chances of breast cancer. Cereals are rich in phytosterols or plant based steroids plant estrogen that stimulates hormone estrogen. Phytosterols binds to estrogen receptors present in the tissues of the breast and blocks human oestrogen that promotes the growth of breast cancer. many studies have shown that colon cancers can be avoided by consuming whole wheat products or any fiber-rich cereals. Phytosterols increase the stool movement through the intestines, thereby constricting the re-absorption time of the estrogen into the blood through the colon wall.

Cereals have both soluble and insoluble fibers like cellulose, pectin, and hemicellulose. These fibers are present in the bran and pericarp, which often gets demolished while processing, thus it is advisable to consume whole cereals to cure extreme constipation troubles. Cereals also effectively improve peristalsis in the intestine and increase the bulk of the stools, thus keeping your internal system clean. Ragi is high in cellulose and has excellent laxative properties that relieve constipation. Brown rice is also helpful for treating this disorder.

The fiber content in cereals decreases the speed of glucose secretion from food, thereby maintaining sugar levels in the blood.

Proteins are present in every tissue of the cereal grain. the concentrated protein-rich areas are scutellum, embryo and alleurone layer and moderate amount can be found in the endosperm, pericarp and testa. The concentration of proteins becomes denser in the endosperm from the center to the borderline. The cereal protein are of different types; like albumins, prolamines gliadins, globulins and glutelins. These type of proteins are called ‘’gluten’’ protein. This gluten has extraordinary elasticity and mobile properties mainly present in wheat grain, but also in some other types of cereals. Cereals usually have 6-12% protein but lack in lysine. The protein content varies in each type of cereals. For instance, rice contains less protein in comparison to other cereals. In fact, the protein percentage even varieties with different varieties of the same cereals. Although less in amount, the quality of rice protein is better than the protein of other cereals. When you consume cereals with pulses, the protein quality automatically improves, owing to the mutual supplementation. Pulses have high lysine content and are deficient in methionine; on the other hand cereals have an abundance of methionine.

Recent research suggests that greater consumption of vegetable, whole grain products and fruits may lower the risk of multimorbidity. If you are suffering from a deficiency in the vitamin B complex, add whole grain cereal to your diet. Most of the vitamins of cereals are present in the outer bran, but the refining process usually reduces the vitamin B content, and thus it is advisable to consume whole grain cereals. Cereals are usually devoid of either vitamin A or vitamin C; only maize has small amounts of carotene. The cereal grains are processed to extract oils that are rich in vitamin E. Rice bran oil has more concentrated amounts of vitamin E than other oils available on the market. Cereals grains are rich in enzymes, particularly protease, amylase, lipases and oxido-reductases. After the seed germinates, amylase actively increases. The germ encloses the protease enzymes. Cereals are undoubtedly full of nutrition, but unfortunately, the refining process degrades their quality. The degree of milling, polishing and refining to some extent decides the nutrition content of cereals. Some nutrients are lost during food preparation, especially vigorous washing, soaking and cooking methods, which results in the depletion of the nutrients on the skin of the grains. Heavy metal of cereals cannot be underestimated as these foodstuffs are important component of human diet. However, heavy metal contamination of food items is one of the most important aspects of food quality assurance (Marsshall, 2004; Radwan and Salama 2006; and Khan *et al*., 2008).

Though heavy metals are naturally present in soil, geology and anthropogenic activities increase the concentration to a harmful mount (chibuike and obiora, 2014). In many ways living plants can be compared to solar driven pumps which can extract and concentrate several elements from their environment. From the soil, all plants have the ability to accumulate heavy metals which are essential for their growth and development like magnesium, iron, manganese, zinc, copper, molybdenum and nickel (Langille and Maclean, 1976) certain plant also have the ability to accumulate heavy metals which have no biological function. These include cadmium, chromium, silver, selenium, and mercury (Hanna and Grant, 1962: Baker and Brooks, 1989). it is also well known that the growth and economic yield of plants is significantly depressed when they are raised on soils contaminated with heavy metals (Foy *et al.,* 1978; Lepp, 1981; Woolhouse,1983).

The prolonged consumption of unsafe concentrations of heavy metals through foodstuffs may lead to the chronic accumulation of heavy metals in the kidney and liver of humans causing disruption of numerous biochemical processes, leading to cardiovascular, nervous, kidney and bone diseases (WHO,1992 and Jarup, 2003).

* 1. **STATEMENT OF PROBLEM**

Metals have important and wide ranging role in biochemistry, being both essential and toxic (Guengerich 2009). Deficiency of micronutrient in soils and plant is a global nutritional problem as the major food staples are highly susceptible to such deficits (Imtiaz *et al*. 2010) for example, essentiality of Zn in the diet and its deficiency in humans was recognized in 1963 (Prasad 2012). However among all the environmental stresses, the effect of the metal accumulation has been considered one of the most disturbing factors arising in the late 19th and early 20th centuries (Azevedo *et al*. 2012) in addition to their essentiality for plant growth and human nutrition, some micronutrients may also be toxic to animals, including humans, at high concentrations (Wang *et al* 2008). For example, Cu or Zn. An important component of seed quality is its chemical composition, including the concentration of micronutrients such as Fe, Zn, and Cu (Waters and Sankaran 2011). Clearly, plants are the first step of a metal’s pathway from the soil to heterotrophic organisms such as animals and humans, so the micronutrient content in their edible parts makes a major contribution to human intake. Zhao and McGrath (2009) suggested that micronutrient in humans and environmental contamination with heavy metals or metalloids are both global and challenging problems that require concerted efforts from researchers in multiple disciplines, including plant biology, plant breeding as well as biotechnology, nutrition and environmental sciences, such as soil fertility and chemistry.

In this review, can we continue with the intake of cereals? Or is it still possible to provide an overview of data regarding micronutrients concentration in the grain of some important cereals published in the last two decades, as well as the prevailing opinions on their plant-driven entry into the food chain.

* 1. **AIM AND OBJECTIVES OF RESEARCH**

The aim of this study is to characterize some cereals for their level of some extracted heavy metal contamination.

 The specific objectives:

* To check for the level of extracted heavy metals in the selected cereals
* To determine the proximate composition of the cereals
* To compare the levels of heavy metals in different selected cereals
	1. **LIMITATIONS OF STUDY**

The study is limited to availability of very dried cereals in the market due to rain because is rainy season at the time of the research

**CHAPTER TWO**

**LITERATURE REVIEW**

2.1 COMPOSITION OF CEREALS

 In composition, grains are structurally similar as seen; however, they vary in their nutrient composition, containing varying amounts of carbohydrate, fat, protein water, vitamins and minerals.

2.1.1 CARBOHYDRATES

The main nutrient component of cereals grains is carbohydrate which makes up 79-83% of the dry matter of grain.

It exists predominantly as starch, with fiber especially cellulose and hemicelluloses, composing approximately 6% of the grain.

 Carbohydrates can be classified according to their molecular size and decree of polymerization, with each group being subdivided according to the number and composition of monosaccharide units. This is classification includes sugars (monosaccharides and disaccharides), oligosaccharides, starch (amylose and amylopectin) and non-starch polysaccharides.

2.1.2LIPIDS

Lipid (fats and oil) makes up approximately 1-7% of kernel, epending on the grain. For example, wheat rice, corn, rye and barley contain 1-2% lipid, oats contain 4-7%. The lipid is 72-85% unsaturated fatty acids, primarily, oleic acid and linoleum acid.

2.1.3 PROTEINS

Protein composes 7-14% of the grain, depending on the grain. Cereals are low in the amino acids tryptophan and methionine, and although potential breeding may produce cereals higher in the amino acid lysine, it remains the limiting amino acid in cereals.

Grain consumption provides half of the protein consumed worldwide. However, in comparison to food such as milk, meats or eggs, grains do not include all the essential amino acid contained in animal protein.

The protein is of low biological value and therefore, less efficient in supporting body needs. Combining food sources of protein is common in cultures throughout the world. The preparation of traditional dishes combines the lower biological value grain with legumes or nuts and seeds to provide the needed amino acids to yield a complete dietary protein.

For example a combination of beans with rice, or beans with cornbread, tofu and vegetables, or tofu and cashews, chickpeas and sesame seed paste (tahini) known as hummus, peanut butter on whole wheat bread and so forth are combinations creating complete proteins.

2.1.4 VITAMINES

Vitamins present in cereals are predominantly the B vitamins-thiamin (B1), riboflavin (B2) and niacin (B3) which are among water soluble vitamins. These vitamins may be lost in the milling process and so are added back through the process of enrichment.

Whole grain products contain some fat soluble vitamins in the germ.

* Vitamin B-1 (thiamine): it is involved in breaking down of carbohydrate since it increases the production of gastric acid, it is nicknamed, the ‘’morale’’ vitamin because drops in b-1 levels can result in depression. Thiamine is also necessary for growth, fertility, lactation.
* Vitamin B-2 (riboflavin): it used in eye-formula for its ability to prevent cataract and ward off eye fatigue. Recent studies suggest that 400mg of B-2 vitamin can ward off migraine headaches.
* Vitamin B-3 (Niacin, Nicotinic acid, Niacinamide): Niacin’s fame in medicine today is its ability to at high doses lower cholesterol and high triglyceride levels.
* Vitamin B-5 (Pantothenic acids): therapeutic uses of this vitamin may include allergies, arthritis and hypoglycaemia.
* Vitamin B-6 (pyridoxine): its therapeutic uses include morning sickness, premenstrual syndrome and menopause, edema, birth control pills induced deficiencies and the reduction of homocysteine a toxic amino acid metabolite associated with heart disease.
* Vitamin B-12 (cyanocobalamin): many health professionals its therapeutic use is in muscle weakness, nerve degeneration, homoysteine control and anemia.
* Folic acid: one of the great revelations of the 90’s was the confirmation that a folic acid deficiency during pregnancy will increase the chances of having a child with spina bifida or anencephaly. Women of child bearing age who consume adequate amounts of folic acid daily (400mg) before conception will reduce their risk of having pregnancy affected by neural tube defects.
* PABA (para-Aminobenzoic acid): therapeutic applications include prevention of eczema and loss of skin pigmentation, control of burn pain and topical use in sunscreen.
* Choline: therapeutic uses include memory enhancement, liver disorders, lowering of cholesterol, nervous system disorders and hair, nail and skin enhancement.
* Inositol: it is used as a lipotropic agent for hair health.
* Biotin: therapeutic applications include hair health, eczema and Candida.

2.1.5 WATER

Water is present in cereal grains at levels of 10-14% of the grain. Of course soaking and cooking add water to cereal grains, and the grain size expands as additional water is absorbed.

If flour is high in protein content, it absorbs a lot of water compared to low protein flour.

2.1.6 MINERAL

Mineral are naturally present at higher levels in whole grains cereals than in refined grains. Zinc, calcium as well as vitamins also may be added at levels beyond not present in the original grain. Mineral in whole grain cereals are iron, magnesium, phosphorus, and zinc

2.1.6.1 IRON

Iron is an important component of hemoglobin, the substance in red blood cells that carries oxygen from your lungs to transport it through your body, if don’t have enough iron, your body can’t make enough healthy oxygen-carrying red blood cells. A lack of red blood cells leads to iron deficiency anemia.

2.1.6.2 MAGNESIUM

Magnesium ions regulate over 300 biochemical reactions in the body through their role of enzyme co-factors. They also play a vital role in the reaction that generate and use ATP, the fundamental unit of energy within the body cells.

2.1.6.3 PHOSPHORUS

Phosphorus is a mineral that makes up 1% of a person’s total body weight. It is the second most abundant mineral in the body. It is present in every cell of the body. The main function of phosphorus is in the formation of bone and teeth and the most of the phosphorus in the body is found in the bones and teeth.

2.1.6.4 ZINC

Zinc is an essential mineral that is naturally present in some foods, added to others, and available as a dietary supplement. Zinc is involved in numerous aspects of cellular metabolism. It is required for the catalytic activity of approximately 100 enzymes (Sandstead HH. Understanding zinc: recent observations and interpretation

2.1.7 FIBER

Fiber content is determined by different analysis and includes crude fiber (CF) and total dietary fiber (TDF).

These two measurements are not correlated. Crud fiber is composed of cellulose and the non-carbohydrate lignin. TDF includes cellulose and lignin, plus hemicelluloses, pectic substances, gums and mucilages.

**2.2 PROTECTIVE PHYTOCHEMICALS IN CEREALS**

Wholegrain cereals contain many different phytochemicals, which are compounds found in plant foods that have been are linked to significant health benefits. These phytochemicals include:

* Lignans - can lower the risk of coronary heart disease, and slow or turn back cancers in animals.
* Phytic acid - reduces the glycaemic index (GI) of food, which is important for people with diabetes, and helps protect against development of cancer cells in the colon
* Saponins , phytosterols, squalene, oryzanol and tocotrienols – have been found to lower blood cholesterol
* Phenolic compound – have antioxidant effects.
	1. **HEALTH BENEFITS OF CEREALS.**

 The health benefits of cereals are discussed comprehensively below:

* + 1. **Source of energy:**

Cereals are probably the greatest source of energy for humans. Providing almost 30% of total calories in a regular diet, cereals are probably the most widely consumed caloric food in America. The percentage rises in places like rural Africa, Asia and India where cereals are reported to supply almost 70 to 80% of energy requirements (since people in this region cannot afford to eat other food products like fruits, vegetables, meat or milk products. Cereals are inexpensive and a widely available source of energy; this is probably the prime reason why people from all budgets prefer cereals as the major energy provider in their diet. Cereals intake tends to be quite high amongst poor income families as they attain a good amount of energy through animal expenditure.

**2.3.2** **High Mineral Content:**

 In cereals, around 95% of minerals are the sulphate and phosphate of magnesium, potassium and calcium. A good amount of phosphorus is present, called phytin. The phylates present in the cereals considerably reduce the activity of iron absorption. The unrefined cereals have more phytates compared to refined cereals. After the cereals germinate, phytates diminish due to the breakdown of enzymes, and then the iron content is enhanced. This is the reason why malted flours of cereals are said to have more nutritional value than raw flour. Zinc, copper and manganese are also present is cereals in very small quantities. Cereals hardly have and calcium and iron, but ragi is an exception to this. Amongst cereals, rice is the poorest source of iron and calcium. Ragi, millet, jower and bajra have high amount of mineral and fiber.

* + 1. **Prevent cancer:**

Whole wheat products reduce the chances of breast cancer. Cereals are rich in phytosterols or plant based steroids and plant estrogen that stimulate the hormone estrogen. Phytosterols bind to estrogen receptors present in the tissue of the breast and block human oestrogen that promotes the growth of breast cancer. Many studies have shown that colon cancers can be avoided by consuming whole wheat product or any fiber rich cereals. Phytosterols increase the stool movement through the intestines, thereby constricting the re-absorption time of estrogen into the blood through the colon wall.

**2.3.4 Cereals help protect against heart disease:**

 Eating cereals foods (especially wholegrains and those with fiber from oats or barley is associated with protective effects against heart disease in adults. It has been studies have shown that a high intake of wholegrain (at least 2.5 serves per day) is associated with 21 per cent lower risk of cardiovascular events. Also, a study of postmenopausal women found that six or more servings of wholegrain foods per week protected against the effects of cardiovascular diseases.

Heart disease is often caused by high blood cholesterol levels. Regularly eating cereals that are rich in soluble fiber, such as oats (containing beta-glucans) and psyllium, has been found to significantly reduce the amount of cholesterol in the bloodstream.

* + 1. **Cereals and type 2 diabetes:**

Results from the nurse’s health studies I and II showed that two serves of wholegrain foods daily can reduce the risk of developing type 2 diabetes by 21 percent. Cereals fiber in particular protects against this condition. People with diabetes also benefit from eating wholegrain cereals, which have been linked to improvements in insulin sensitivity.

* + 1. **Cereals and weight management:**

People who are obese tend to have energy-dense diets. High –fiber foods, such as wholegrain breads and cereals, can be an effective part of any weight loss program as they tend to have a lower energy density, which means they provide fewer kilojoules per gram of food. High-fiber foods they take longer to digest and create a feeling of fullness, which discourages overeating. Whole grains are also naturally low in saturated fat and contain good polyunsaturated fatty acids.

**2.3.7** **Whole grains, cereals and bowel health:**

High-fiber foods such as wholegrain cereal products increase movement of food through the digestive tract. The result is increased stool (faeces or poo) bulk, softer and larger stools, and more frequent bowel action. This provides a good environment for beneficial bacteria, while decreasing levels of destructive bacteria and build-up of carcinogenic compound. Wheat fiber can bind certain toxins and remove them from the large bowel.

A high-fiber diet, especially one high in insoluble fiber, has been associated with decreased risk of developing colon cancer and diverticular disease (a condition where `pouches` form in the wall of the intestine).

* 1. **Other benefits of wholegrain cereals**

Wholegrain cereals are a rich source of many essential vitamins, minerals and phytochemicals. The typical wholegrain cereal food is:

* Low in saturated fat, but is a source of polyunsaturated fats, including omega-3 linolenic acid
* Cholesterol-free
* High in both soluble and insoluble fiber, and resistant starch
* An excellent source of carbohydrate
* A significant source of protein
* A good source of Bb group vitamins, including folate
* A good source of many minerals, such as iron, magnesium, copper, phosphorus and zinc
* A good source of antioxidants and phytochemicals that can help lower blood cholesterol levels

**2.5** **Cereals recommendation for health:**

 Wholegrain cereals are recommended as part of a healthy diet. The Australian guide to healthy eating recommends adult aged 19-50 years eat least six serves approximately 4 to 6 serves of cereals foods respectively. In adult aged 70years and over, men and women should consume at least 4.5 serves and three serves of cereal foods respectively. Amounts recommended for children and adolescents depend on their age and sex.

 **2.6 Nutritional content of wholegrain cereals:**

Grains consist of three major parts, including:

* Bran-the outer layer of the grain (fiber, omega-3 fatty acids, vitamins and dietary minerals)
* Endosperm – the main part of the grain (mainly starch)
* Germ- the smallest part of the grain (vitamin Ee, folate, thiamine, phosphorus, magnesium)

Whole grains contain all three layers of the grain

**2.7 Refined wholegrain**

When grains are refined (for example, to produce white flour), the bran and germ layers are generally removed, leaving only the endosperm. This process can cause significant losses of fiber, vitamins, minerals, antioxidants and phytochemicals from the grains.

Some fiber, vitamins and minerals may be added back into refined cereals products (such as white bread), which compensates for losses, but it is impossible to add the mix of photochemical that is lost in the processing. In Australia, it is mandatory for wheat flour used in bread making to be fortified with folic acid and thiamine, and for the salt to be iodized.

Refined cereals often have high levels of added sugar, fat or salt, and generally have a higher GI than their wholegrain equivalents. Eating refined cereals causes a sharp rise in blood sugars and a strong response from the pancreas, which is not good.

**2.8 Proximate composition**

Proximate are used in the analysis of biological materials as a decomposition of a human-consumable good into its major constituents. They are a good approximation of the contents of packaged comestible goods and serve as cheap and easy verification panels i.e. testing can be used to verify lots, but cannot b e used to validate a food processor or food processing facilities; a nutritional assay must be conducted on the product to qualify said producer. Nutritional panels on the unite states are regulated by the FDA and must undergo rigorous testing to ensure the exact and precise content of nutrient in order to prevent a food processor from making unfounded claims to the public.

From an industry standard proximate includes five constituents:

* Ash
* Moisture
* Protein
* Fibre
* Fat
* Carbohydrate(calculation)

Analytically, four of the five constituents are obtained via chemical reactions and experiments. The fifth constituent, carbohydrates, is a calculation based on the determination of the four others. Proximate should nearly always add up to 100%, any deviation from 100% displays the resolution of the chemical test i.e small variation in the way each test is performed chemist to chemist will accumulate or overlap the composition make-up.

2.8.1 **MOISTURE CONTENT**

Water content or moisture content is the quantity of water contained in a material, such as soil (called soil moisture), rock, leaves, ceramics, fruits, or wood. Water content is used in a wide range of scientific and technical areas and is expressed as a ratio, which can range from 0 (completely dry) to the value of the materias` porosity at saturation. It can be given on a volumetric or mass (gravimetric) basis.

From the Annual Book of ASTM (American Society for Testing and Materials) standards, the total evaporable moisture content in Aggregate (C 566) can be calculated with the formula:

 P= W - D

 W

Where p is the fraction of total evaporable moisture content of sample, W is the mass of the original sample, and D is mass of dried sample.

2.8.2 **CRUDE PROTEIN**

The classic assays for protein concentration in food are the Kjeldahi method and the Dumas method. These tests determine the total nitrogen in a sample. The only major component of most food which contains nitrogen is protein (fat, carbohydrate and dietary fiber do not contain nitrogen). If the amount of nitrogen is multiplied by a factor depending on the kinds of protein expected in the food the total protein can be determined. This value is known as the “crude protein” content. On food labels the protein is given by the nitrogen multiplied by 6.25, because the average nitrogen content of protein is about 16%. The Kjeldahl test is typically used because it is the method the AOAC International has adopted and is therefore used by many food standards agencies around the world, though the Dumas method is also approved by some standards organizations.

2.8.3 **ASH CONTENT**

Ash in analytical chemistry, the compound that remains after a scientific sample is burned. The ash content shows just how much of the leaf sample is ash when burned at extreme temperature. The ash content is then calculated by dividing the weight of the ash sample by the weight of the sample before ashing. The weight is represented in grams.

2.8.4 **FAT CONTENT**

Fats, like protein and carbohydrates, are a nutrient energy to the body. Fats are organic compounds that come in liquid or solid form. They are composed of saturated and unsaturated fatty acids. Saturated fats cause high levels of cholesterol, known as LDL, and can be found in animal products and vegetable oils. Unsaturated fats help to lower bad cholesterol, but still must be consumed in moderation because of their caloric content. Unsaturated fats are found in most liquid vegetable oils. (Kiki M, 2013) this shows what percentage of the sample is fat.

**2.9 HEAVY METAL CONTAMINATION**

Heavy metals are chemical elements with a specific gravity that is at least five times that of water. The specific gravity of cadmium is 8.65, lead 11.34, arsenic 5.73, mercury 13.633, zinc 7.2, copper 8.89 (Lide, 1992) .heavy metals like copper and manganese occur naturally in plants. They could serve as plant nutrients depending on their concentrations. However, lead and many other heavy metals are directly distributed as a result of human activities which could be toxic even at low concentrations.

Plant parts take up these heavy metals absorbing them from airborne deposits in the part exposed to the air from polluted environment as well as contaminated soils through root systems (Elbagemi *et al.*, 2012). These heavy metals can be accumulated in the shoots, fruits and roots of plants at low, medium and high levels. Heavy metals contamination of cereals may also occur due to their irrigation with contaminated water (AL jassir *et al.*, 2005).

Additional sources of heavy metals for plants are rainfall in atmospheric polluted areas, traffic density, use of oil fossil fuels for heating, atmospheric dusts, plants protection agents and fertilizers which could be absorbed through leaves blades (Sobukola, 2008).

**CHAPTER THREE**

**Materials and Method**

 3.1 MATERIALS

The following materials were employed in the study

3.1.1 Equipment/Apparatus

* Beaker
* Electric blender
* Distilled water
* High density polyethylene sieve
* Whatman No.42 Filter paper
* 100ml reflux flask
* Electronic blender
* Heating mantle
* Volumetric flask
* Conical flask
* Platinum crucible
* Bunsen burner
* Atomic Absorption Spectrophotometer Machine

3.1.2 Chemicals/Reagents

* Concentrated Nitric acid (NHO3)
* Concentrated Hydrogen Peroxide ( H2O2)
* Perchloric Acid (HClO4)
* De-ionized (DI) water and
* Sodium hydroxide ( NaOH )
* Distilled water
* Tetraoxosulphate(VI) Acid ( H2SO4)
* N-Hexane

**3.2 METHODS**

**3.2.1 Collection of samples**

A total number of twelve cereals were purchased. Six were purchased from ogbete main market and the other six from garriki market in Enugu state

Table 1: samples and their source of production

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| S/N | SAMPLE  | IDENTIFICATION | PLACE OF PURCHASE | SOURCE OF PRODUCTION |
| 1 | A | Millet | Ogbete market | North western NIG. Kano state |
| 2 | B | Millet | Ogbete market | North western NIG. Kano state |
| 3 | C | Millet | Garriki market | North western NIG. Sokoto state |
| 4 | D | Millet | Garriki market | North western NIG. Sokoto state |
| 5 | E | Rice | Ogbete market | North western NIG. Kano state |
| 6 | F | Rice | Ogbete market | North western NIG. Kano state |
| 7 | G | Rice | Garriki market | South eastern NIG. Abakaliki Eboyi state  |
| 8 | H | Rice | Garriki market | South eastern NIG. Abakaliki Eboyi state |
| 9 | I | Corn | Ogbete market | Mangu Local. GOV Plateau State |
| 10 | J | Corn | Ogbete market | Middle belt of NIG. Jos |
| 11 | K | Corn | Garriki market | Mangu Local. GOV Plateau State |
| 12 | L | Corn | Garriki market | Middle belt of NIG. Jos |

**3.3 ANALYSIS OF PROXIMATE COMPOSITION IN DIFFERENT CEREALS**

Proximate composition was determined by the method of (AOAC, 1984)

Moisture Content:

Moisture content determination involved drying a known weight of sample to a constant weight at 60oC in an oven (universal hot air oven)

Crude Ash:

Determination of ash content involved incineration in an oven (universal hot air oven) at 250o C for 3days

**Crude Fat:**

Crude fat determination involved extraction of a known weight from sample with petroleum ether and calculated as a percentage of the weight before the solvent is evaporated.

**Crude Protein:**

Determination of crude protein was done using the micro Kjeldahl nitrogen method which involves the digestion of a given weight of a sample with concentrated H2SO4 and catalyst to convert any organic nitrogen to ammonium sulphate, (NH4) 2 SO4 in solution followed by the decomposition of ammonium sulphate with NaOH the ammonia liberated was distilled in to 5% boric. The nitrogen from ammonia was deduced from titration of the trapped ammonia with 0.05M HCl using methylene red and methylene (double indicator solution) indicators. The value of nitrogen obtained was multiplied by the general factor (6.25) to give the percentage crude protein.

**Crude Fiber:**

Crude fiber was obtained from the loss in weight on ignition of dried residue remaining after digestion of fat free sample with 1.25% each of sulphuric acid and sodium hydroxide solutions under specified condition i.e

 Loss of weight of ignition

% crude fiber= ----------------------------------------- x 100

 Wight of sample used

Carbohydrate:

Carbohydrate content was determined by subtracting the total ash content, crude fat lipid, crude protein and crude fiber from the total dry matter.

Carbohydrate content =100- (fiber + ash + fat + protein content)%.

**3.4 HEAVY METAL DETERMINATION IN CEREALS**

**Sampling:**

Samples were collected, analyzed and washed with distilled water because of dust and other unwanted particles in it. They were the seed of three cereal crops including rice (*Oryza sativa*), millet (*panicum millaceum*), and maize (*zea maize*), they were already dried at the time of purchase. An electric blender was employed in the milling of the sample. A suitable sieve made of high density polyethylene (HDPE) was used for sieving the samples.

**Procedure:**

**Digestion:** 2.5 g of the sample, previously dried to constant weight was placed in 100 ml reflux flask. 15 ml of concentrated HNO3 and 5 ml of concentrated H2O2 were mixed with the sample. The mixture was allowed to stand for about 48 h at room temperature. It was then refluxed on a heating mantle at 90oC until brown fumes ceased to evolve, 4-6 h and allowed to cool. 5 ml of 60% (v/v) HClO4 was added to the mixture and further refluxed for 30 min. the digest was allowed to cool at room temperature. It was filtered in to a 100 ml volumetric flask with a Whatman NO. 42 filter paper and made to the mark with de-ionized (DI) water. This was repeated for all the samples, blank was also prepared similary and then ready for AA analysis (Wallace, 2001).

**Analysis:** The digests were analyzed for their content of Pb, Cu, Zn, Cd, Hg, Ar with the aid of an Atomic Absorption Spectrophotometer, (Model: PG990), manufactured by PG instruments limited, Beijing, china. The acetylene gas pressure and flow rate as well as burner positioning were automated. The instrument was allowed to warm for 30 minute before analysis. Determinations were made at the following.

**CHAPTER FOUR**

4.1 **RESULTS AND ANALYSIS**

4.1.1 **PROXIMATE COMPOSITION**

The nutritional analysis of proximate composition of dry cereals of corn, millet and rice.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Samples | Identification | Crude protein(%) | Crude fat (%) | Crude fiber (%) | Crude ash(%) | Carbohydrate content (%) | Moisture content (%) |
| A | Millet | 2.4 | 1.8 | 4.2 | 19 | 60 | 12.6 |
| B | Millet | 3.5 | 0.2 | 8.5 | 5.0 | 70.2 | 12.6 |
| C | Millet | 6.5 | 20 | 0.5 | 5.0 | 55 | 13 |
| D | Millet | 4.8 | 20 | 5.4 | 3.0 | 55 | 11.8 |
| E | Rice | 3.3 | 0.2 | 4.6 | 15.0 | 65.3 | 11.6 |
| F | Rice | 5.3 | 0.2 | 5.5 | 5.0 | 78.8 | 10.2 |
| G | Rice | 10.2 | 0.2 | 7.2 | 1.0 | 69.9 | 11.68 |
| H | Rice | 0.2 | 19.8 | 3.4 | 0.0 | 65.0 | 11.6 |
| I | Corn | 2.3 | 2.2 | 6.8 | 1.0 | 75.9 | 11.8 |
| J | Corn | 4.0 | 20 | 5.1 | 4.0 | 55.3 | 11.6 |
| K | Corn | 4.2 | 1.8 | 6.6 | 3.0 | 75.8 | 8.6 |
| L | Corn | 4.5 | 1.6 | 4.3 | 1.0 | 78.6 | 11.0 |

4.1.2 Table1: proximate Nutritive Composition of Corn, Millet and Rice

**4.2: TABLE 2: HEAVY METALS IN CEREALS SAMPLE**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Serialnumber | Samplecode | Cereals | Source | Arsenic(ppm) | Mercury(ppm) | Lead(ppm) | Cadmium(ppm) | Zinc(ppm) | Copper(ppm) |
| 1 | A | Millet  | Ogbete | 0.601 | 0.084 | 0.564 | 0.148 | 38.312 | 0.245 |
| 2 | B | Millet | Ogbete | 0.545 | 0.085 | 0.680 | 0.111 | 4.323 | 0.080 |
| 3 | C | Millet | Garikki | 0.787 | 0.080 | 0.653 | 0.098 | 16.509 | 0.159 |
| 4 | D | Millet | Garikki | 0.767 | 0.103 | 0.791 | 0.099 | 15.517 | 0.090 |
| 5 | E | Rice | Ogbete | 0.00 | 0.024 | 1.083 | 0.565 | 1.448 | 0.142 |
| 6 | F | Rice | Ogbete | 0.00 | 0.073 | 0.599 | 0.116 | 5.024 | 0.152 |
| 7 | G | Rice | Garikki | 0.846 | 0.080 | 0.750 | 0.125 | 66.954 | 0.161 |
| 8 | H | Rice | Garikki | 0.955 | 0.083 | 0.970 | 0.114 | 48.735 | 0.117 |
| 9 | I | Corn | Ogbete | 0.456 | 0.085 | 0.554 | 0.134 | 9.898 | 0.061 |
| 10 | J | Corn | Ogbete | 0.854 | 0.093 | 0.639 | 0.092 | 9.415 | 0.068 |
| 11 | K | Corn | Garikki | 0.580 | 0.056 | 0.668 | 0.087 | 6.700 | 0.050 |
| 12 | l | Corn | Garikki | 0.813 | 0.124 | 0.871 | 0.113 | 16.831 | 0.115 |

**CHAPTER FIVE**

**5.0 DISCUSS AND CONCLUSION**

**5.1 Discussion**

The rice samples contained high quantities of carbohydrates ranging from 55.00% to 78.80%. Although these values are higher than the values obtained by Eggum (1982), they are a bit lower than the values (75.37 to 76.37%) reported by Edeogu et al. (2007) who analysed the proximate compositions of staple food crops in Ebonyi State. Sample H variety had the lowest carbohydrate content. This low carbohydrate content may be attributed to its high crude fibre content which also affects the digestion activities (Online USA Rice Federation, 2002) and other environmental factors. The high percentage carbohydrate contents of the rice varieties show that rice is a good source of energy. The ash content of a food sample gives an idea of the mineral elements present in the food sample. Among the five rice varieties, sample G rice had the highest protein content (10.20%). This is in agreement with the findings of Ebuehi and Oyewole (2007) who analyzed the effect of cooking and soaking on physical characteristics, nutrient composition and sensory evaluation of indigenous and foreign rice varieties in Nigeria. The percentage fiber contents among the five rice samples were in the range of 0.5 to 8.5%. Although this range is a bit lowerthan obtained by Edeogu et al. (2007), it is similar to the mean value obtained by Sotelo et al. (1990). Milling of rice generally decreases the fibre contents of rice. Sotelo et al. (1990) who analyzed the chemical compositions of different fractions of 12 Mexican varieties of rice obtained during milling. Sample A and B variety contained the highest percentage moisture content while sample K variety contained the lowest percentage moisture content. The high percentage moisture content may be attributed to low drying temperature (Xheng and Lan, 2006) and prolonged parboiling. Such high percentage moisture content affects the milling characteristics and the taste of cooked rice (Xheng and Lan, 2006). Ebuehi and Oyewole (2007) reported that the moisture content of rice also affects its storage. It follows that sample K variety may have a longer shelf life compared to the other samples due to the lower moisture content. The values for percentage crude protein content are in the range of 0.2 to 10.20%. This range is lower than the range obtained by Edeogu et al. (2007) who analyzed the proximate composition of cereals in Ebonyi Sate. This may be attributed to prolonged parboiling which lowers the protein content of the samples and some other environmental and edaphic factors. However, the range is comparable with the range obtained by Ibukun (2008). The percentage fat content of the rice is in the range of 0.2 to 20.0%. The results of this study are in agreement with earlier results reported by Willis et al. (1982) and Juliano (1985) who also gave the fat range 0.9 to 19.97% in different milling fractions. However, this range is lower than the range obtained by Edeogu et al. (2007). This difference may be attributed to the degree of milling.

While their heavy metal detection varies at minute quantities, this could be as a result of environmental factors, expect zinc that recorded higher value in all the samples

**5.2 CONCLUSION**

The result of this study can be exploited by cereal consumers in their choices regarding mineral and proximate compositions. All samples were observed to have good percentage values. Therefore, all the samples should get more attentions in terms of proximate and mineral compositions. While their heavy metal detection varies at minute quantities, this could be as a result of environmental factors, expect zinc that recorded higher value in all the samples

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**APPENDIX**

**TABLE FOR NUTRITIVE COMPOSITION OF CEREALS**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Samples | Identification | Crude protein(%) | Crude fat (%) | Crude fiber (%) | Crude ash(%) | Carbohydrate content (%) | Moisture content (%) |
| A | Millet |  | 1.8 |  | 19 |  | 12.6 |
| B | Millet |  | 0.2 |  | 5 |  | 12.6 |
| C | Millet |  | 20 |  | 5 |  | 13 |
| D | Millet |  | 20 |  | 3 |  | 11.8 |
| E | Rice |  | 0.2 |  | 15 |  | 11.6 |
| F | Rice |  | 0.2 |  | 5 |  | 10.2 |
| G | Rice |  | 0.2 |  | 1 |  | 11.68 |
| H | Rice |  | 19.8 |  | 0 |  | 11.6 |
| I | Corn |  | 2.2 |  | 1 |  | 11.8 |
| J | Corn |  | 20 |  | 4 |  | 11.6 |
| K | Corn |  | 1.8 |  | 3 |  | 8.6 |
| L | Corn |  | 1.6 |  | 1 |  | 11 |

 **RESULTS ON PROXIMATE ANALYSIS**

**ASH CONTENT**

Weight of empty platinum crucible (PC) = W1

Wt of PC + sample after burning = W2

Ash content =W2-W1

% Ash = W2-W1 × 100

 1g 1

Therefore,

Using the formular above

To solve for sample A

 Weight of empty platinum crucible = 40.31g

Weight of platinum crucible + sample after burning = 40.50g

Ash content = 40.50– 40.31g = 0.19g

% Ash = 0.19g × 100% = 19% 1g

To solve for sample B

 Weight of empty platinum crucible = 39.29g

Weight of platinum crucible + sample after burning = 39.34g

Ash content = 39.34 - 39.29 =0.05g

% Ash = 0.05 × 100% =5 %

 1g

To solve for sample C

 Weight of empty platinum crucible =44.75g

Weight of platinum crucible + sample after burning = 44.80g

Ash content =44.80 – 44.75 =0.05g

% Ash = 0.05 × 100% =5%

 1g

To solve for sample D

 Weight of empty platinum crucible =39.74g

Weight of platinum crucible + sample after burning =39.71g

Ash content =39.74 – 39.71g

% Ash = 0.03 × 100% = 3%

 1g

To solve for sample E

 Weight of empty platinum crucible =39.34g

Weight of platinum crucible + sample after burning =39.49g

Ash content =39.49 – 39.34 = 0.15g

% Ash = 0.15 × 100% =15%

 1g

To solve for sample F

 Weight of empty platinum crucible = 43.60g

Weight of platinum crucible + sample after burning =43.65g

Ash content =43.65 – 43.60 = 0.05g

% Ash = 0.05 × 100% =5%

 1g

To solve for sample G

 Weight of empty platinum crucible =43.50g

Weight of platinum crucible + sample after burning =43.51g

Ash content =43.51 – 43.50 = 0.01g

% Ash = 0.01 × 100% =1%

 1g

To solve for sample H

 Weight of empty platinum crucible =46.43g

Weight of platinum crucible + sample after burning =46.43g

Ash content =46.43 – 46.43 =0.00g

 %Ash = 0.00 × 100% =0%

 1g

 To solve for sample I

 Weight of empty platinum crucible = 40.95g

Weight of platinum crucible + sample after burning = 41.00g

Ash content =41.00 – 40.95 = 0.01g

% Ash = 0.01 × 100% =1%

 1g

To solve for sample J

Weight of empty platinum crucible = 40.50g

Weight of platinum crucible + sample after burning =40.54g

Ash content =40.54 – 40.50= 0.04g

% Ash = 0.04 × 100% =4%

 1g

To solve for sample K

 Weight of empty platinum crucible =36.86g

Weight of platinum crucible + sample after burning = 36.89g

Ash content =36.89 – 36.86 = 0.03g

% Ash = 0.03 × 100% =3%

 1g

To solve for sample L

 Weight of empty platinum crucible = 40.58g

Weight of platinum crucible + sample after burning =40.59g

Ash content =40.59 –40.58 =0.01 g

% Ash = 0.01 × 100% =1%

 1g

MOISTURE CONTENT

Using automated machine moisture content, of the cereals was analyzed

Mn = (Mw - Md) × 100

 Mw

Where; Mn = % moisture content of the material

 Mw =wet weight of the sample

 Md = weight of the sample after drying. (provided by the machine)

 Mn = moisture content of the material

 Mw = 5g

 Md = 4.35g

To solve for sample A

Mn = (5 – 4.37)g × 100 =12.6%

 5g

Using the same formular above

For sample B

Mn = (5 – 4.37)g × 100 =12.6%

 5g

 For sample C

Mn = (5 – 4.35)g × 100 = 13%

 5g

For sample D

Mn = (5 – 4.41)g × 100 =11.8 %

 5g

For sample E

Mn = (5 – 4.42)g × 100 =11.6 %

 5g

For sample F

Mn = (5 – 4.49)g × 100 =10.2 %

 5g

For sample G

Mn = (5 – 4.416)g × 100 =11.68%

 5g

For sample H

Mn = (5 – 4.42)g × 100 =11.6%

 5g

For sample I

Mn = (5 – 4.41)g × 100 =11.8%

 5g

For sample J

Mn = (5 – 4.42)g × 100 =11.6%

 5g

For sample K

Mn = (5 – 4.57)g × 100 =8.6%

 5g

For sample L

Mn = (5 – 4.45)g × 100 =11%

 5g

**CRUDE FAT:**

 B – A

Crude Fat (%) = 100 ----------

 C

Where

A = weight of clean dry beaker (g)

B = weight of beaker with fat (g)

C = weight of sample (g)

For sample A

 32.32 –32.23

Crude Fat (%) = 100 -------------------- =1.8%

 5

For sample B

 33.54 –33.53

Crude lipid (%) = 100 ------------------ =0.2%

 5

For sample C

 28.65 – 27.65

Crude lipid (%) = 100 ------------------- = 20%

 5

For sample D

 33.38– 32.38

Crude lipid (%) = 100 ------------------ = 20%

 5

For sample E

 34.94 –34.93

Crude lipid (%) = 100 ----------------- = 0.2%

 5

For sample F

 33.43 –33.42

Crude lipid (%) = 100 ------------------- = 0.2%

 5

For sample G

 32.79 – 32.78

Crude lipid (%) = 100 ------------------- = 0.2%

 5

For sample H

 26.96 – 25.97

Crude lipid (%) = 100 ----------------- = 19.8%

 5

For sample I

 45.84 – 45.73

Crude lipid (%) = 100 ------------------- = 2.2%

 5

For sample J

 35.86 – 34.86

Crude lipid (%) = 100 ----------------- = 20%

 5

For sample k

 40.63 – 40.54

Crude lipid (%) = 100 ------------------- =1.8%

 5

For sample L

 42.03 – 41.95

Crude lipid (%) = 100 ------------------ = 1.6%

 5

TABLE 2: HEAVY METALS IN CEREALS SAMPLE

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| S/NO | SampleCode | Cereals | Source | Arsenic(ppm) | Mercury(ppm) | Lead(ppm) | Cadmium(ppm) | Zinc(ppm) | Copper(ppm) |
| 1 | A | Millet  | Ogbete | 0.601 | 0.084 | 0.564 | 0.148 | 38.312 | 0.245 |
| 2 | B | Millet | Ogbete | 0.545 | 0.085 | 0.680 | 0.111 | 4.323 | 0.080 |
| 3 | C | Millet | Garikki | 0.787 | 0.080 | 0.653 | 0.098 | 16.509 | 0.159 |
| 4 | D | Millet | Garikki | 0.767 | 0.103 | 0.791 | 0.099 | 15.517 | 0.090 |
| 5 | E | Rice | Ogbete | 0.00 | 0.024 | 1.083 | 0.565 | 1.448 | 0.142 |
| 6 | F | Rice | Ogbete | 0.00 | 0.073 | 0.599 | 0.116 | 5.024 | 0.152 |
| 7 | G | Rice | Garikki | 0.846 | 0.080 | 0.750 | 0.125 | 66.954 | 0.161 |
| 8 | H | Rice | Garikki | 0.955 | 0.083 | 0.970 | 0.114 | 48.735 | 0.117 |
| 9 | I | Corn | Ogbete | 0.456 | 0.085 | 0.554 | 0.134 | 9.898 | 0.061 |
| 10 | J | Corn | Ogbete | 0.854 | 0.093 | 0.639 | 0.092 | 9.415 | 0.068 |
| 11 | K | Corn | Garikki | 0.580 | 0.056 | 0.668 | 0.087 | 6.700 | 0.050 |
| 12 | L | Corn | Garikki | 0.813 | 0.124 | 0.871 | 0.113 | 16.831 | 0.115 |