PJN
ISSN 1680-5194

# PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 9 (9): 833-843, 2010
ISSN 1680-5194


# Probiotics and Prebiotics: Unfolding Prospects for Better Human Health

C.C. Ogueke, C.I. Owuamanam, N.C. Ihediohanma and J.O. Iwouno
Department of Food Science and Technology, Federal University of Technology,
P.M.B. 1526, Owerri, Imo State, Nigeria

**Abstract:** Some bacteria have been perceived to promote good health of the host and thus are beneficial to host health. These have been called probiotics. Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. Lactic Acid Bacteria (LAB) and bifidobacteria have been identified as probiotics. These bacteria when ingested change the composition of the intestinal microflora. Various beneficial effects are attributable to the consumption of these bacteria. These include prevention of diarrhea, immune system stimulation and prevention of colon cancer. However, their presence in the gut may be transient thus requiring a permanent implantation and colonization. Thus the concept of prebiotics. Prebiotics are non digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improve host health. Prebiotics selectively stimulate the growth of probiotics resident in the gut especially bifidobacteria through the production of β-fructosidase, therefore changing the colonic microflora to a healthier composition. Prebiotics are non digestible oligosaccharides especially fructooligosaccharides. Some beneficial effects attributed to consumption of prebiotics include modulation of lipid metabolism through fermentation and increasing the absorption of minerals such as Ca and Mg from the colon. However, research data available show that the growth of lactobacilli is not selectively stimulated by the prebiotics. There is therefore need to conduct more research to determine the role of bacteriocins they produce in their ability to colonize the gut.

**Key words:** Probiotics, prebiotics, microflora, colon, beneficial effects, humans

## Probiotics

**Definition of probiotics:** The growing awareness of the relationship between diet and health has led to an increasing demand for food products that support health above and beyond providing basic nutrition (IFIC, 2006). Probiotics was derived from greek words which means "for life". The term "probiotics" was first introduced in 1953 by Kollath (Hamilton-Miller, 2003). An attempt on the definition of probiotics was made in 1989 by Roy Fuller who defined it like so; A live microbial supplement which beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1989). The definition by Fuller emphasized the requirement of viability for probiotics and introduced the aspect of a beneficial effect on the host. It can also be defined as "a preparation or product containing viable, defined microorganisms in sufficient numbers, which alter the microflora of the host intestine and by that exert beneficial health effects on the host (Schrezenmier and De Vrese, 2001).

However, according to the currently adopted definition by FAO/WHO, probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2001).

**History of probiotics:** Probiotics have been used for centuries as natural components in health promoting foods. Recognition of probiotic effects of microorganisms dates back to the 19$^{th}$ century when the French scientist Louis Pasteur postulated the importance of microorganisms in human life (IFIC, 2006). The first observation of the positive role of certain microorganisms in human health was made by the Russian scientist and Nobel laureate Eli Metchnikoff in the early 20$^{th}$ century. He suggested that it would be possible to modify the gut flora by replacing the harmful microorganisms with beneficial microorganisms (Metchnikoff, 1907). He introduced the notion that ageing process was as a result of the activities of putrefactive bacteria in the colon which produce toxic substances that include phenols, indoles, ammonia etc. from the breakdown of proteins. He said that these compounds were responsible for what he called "intestinal auto-intoxication", which caused the physical changes associated with old age. He had also observed that certain rural populations in Europe such as Bulgaria and the Russian Stepps who lived largely on milk fermented by lactic acid bacteria were exceptionally long lived. Based on these facts, he proposed that consumption of fermented milk would "seed" the intestine with harmless lactic acid bacteria and decrease the intestinal pH which would suppress the growth of putrefactive (proteolytic) bacteria. He then decided to introduce in his diet sour milk fermented with an organism which he called

**Corresponding Author:** C.C. Ogueke, Department of Food Science and Technology, Federal University of Technology, P.M.B. 1526, Owerri, Imo State, Nigeria

"Bulgarian Bacillus". His friends in Paris soon followed his example and then physicians began prescribing the sour milk diet for their patients (Vaughan, 1965).
However, in 1920 some workers (Cheplin and Rettger, 1920) demonstrated that Metchnikoff's "Bulgarian Bacillus" which was later named *Lactobacillus bulgaricus*, could not live in the human colon. This led to disputations on the theory of Metchnikoff. In 1935 some workers (Rettger *et al*., 1935) found that certain strains of *Lactobacillus acidophilus* could be very active when implanted in the human digestive tract. Trials were carried out using this bacterium and encouraging results were obtained, especially in the relief of chronic constipation. By the 1960s dairy industries began to promote the use of fermented milk products containing *Lactobacillus acidophilus* and in subsequent decades other Lactobacillus species were introduced such as *L. rhamnosus, L. casei* and *L. johnsonii* because they are intestinal species with beneficial properties (Tannock, 2003).

**Criteria for use as probiotics:** For a microorganism to be used as a probiotic certain criteria must be met (Fuller, 1989; Fuller, 1992). These include the following;

1. The probiotic must be capable of being prepared in a viable manner and on a large scale.
2. During use and under storage, the probiotic should remain viable and stable.
3. It should be able to survive in the intestinal ecosystem
4. The host animal should gain beneficially from harbouring the probiotic.

Also the organism must not be pathogenic to man or other animals.
Various microorganisms have been suggested for use as probiotics. They fall mainly within the group of Lactic Acid Bacteria (LAB) with strains of *Lactobacillus* sp. and *Bifidobacterium* sp. being the genera most widely used probiotic bacteria. These include *Lactobacillus acidophilus, L. casei, L. plantarum, L. reuteri, L. rhamnosus, L. delbrueckii, L. salivarus, L. helveticus, L. johnsonii, Lactococcus lactis, Bifidobacterium lactis, B. longum, B. infantis, B. breve, B. animalis* and *B. bifidum* (Gibson and Roberfroid, 1995; Sanders, 2007). Lactic acid bacteria have been used in the food industry for many years where they are used to convert carbohydrates in such food materials into lactic acid, responsible for the sour taste of some fermented dairy products such as yoghurt. This also lowers the pH to a level that prevents the growth of spoilage and pathogenic bacteria thus preventing various diseases transmissible through food such as gastrointestinal infections (Nicholas, 2007; Adams and Moss, 1999).
These bacteria are believed to assist the body's naturally occurring gut flora to re-establish them selves especially after antibiotic therapy. They are also believed to strengthen the immune system to combat allergies, excessive alcohol intake, stress, exposure to toxic substances and other diseases (Nicholas, 2007; Sanders, 2000). Maintenance of a healthy gut flora is, however, dependent on many factors, especially the quality of food intake. Including a significant proportion of a class of foods called prebiotics in the diet has been shown to support a healthy gut flora (Gibson and Roberfroid, 1995) and may be another means of achieving the desirable health benefits promised by probiotics.

**Beneficial effects attributable to probiotics:** There are several claims of the potential beneficial effects of probiotics. For many of the potential benefits, research is limited and only preliminary results are available. The benefits associated with probiotics are strain specific and must be shown through adequate clinical trials reflective of the dose of probiotic present in the food at the time of consumption (Gilliland and Walker, 1990; IFIC, 2006).
One area where there is good evidence for a beneficial effect is in the ability of fermented milks to alleviate the condition known as lactose intolerance (Adams and Moss, 1999; Sanders, 2000). All human infants possess the enzyme lactase (β-galactosidase). This hydrolyses lactose to glucose and galactose, thus they could be absorbed in the small intestine and metabolized. However, when the enzyme is absent the ingested lactose is not digested and will be attacked by the microbial population in the colon producing abdominal discomfort, flatulence and diarrhea. For such individuals when they take fermented milk such as yoghurt, these adverse effects are less severe or absent. This has been shown to be due to the presence of β-galactosidase in viable starter organisms (Adams and Moss, 1999).
Fermented milks (especially yoghurt) have been shown to have a strong inhibitory effect on the growth of coliforms in the stomach and duodenum of piglets and studies of human infants and adults have shown that the duration of illness (acute diarrhea and traveler's diarrhea) was shorter in those groups given yoghurt than in control groups (Adams and Moss, 1999; Reid *et al*., 2003). Also in studies of children attending day care centres, changes in severity and duration of diarrhea after consumption of specific strains, were also observed (Weizman *et al*., 2005). These days lactic acid bacteria such as *L. acidophilus* and bifidobacteria which can colonize the gut are now included in yoghurts and other fermented milks.
Probiotic bacteria have been reported to stimulate the immune system. Studies have shown that they possess the ability to activate macrophages and lymphocytes,

improve levels of IgA and production of gamma interferon (Reid *et al*., 2003; Ouwehand *et al*., 2002; Isolauri, 2001; Adams and Moss, 1999). Clinical trials have also demonstrated that probiotics may decrease the incidence of respiratory tract infections as well as dental caries in children (Hatakka *et al*., 2001; Nase *et al*., 2001). Certain probiotic strains have been shown to have a favourable effect on markers of the immune response to stress (Pujol *et al*., 2000). Also a study among the elderly found an enhancement of immune function following consumption of milk supplemented with a *Bifidobacterium lactis* strain (Gill *et al*., 2001). Some experts also believe that higher levels of bifidobacteria in the gut of breast-fed infants may be one reason why they are considered to be generally healthier than formula-fed babies (IFIC, 2006).

Probiotics have been shown to produce anti-mutagenic effects and anti tumor effects thought to be due to their ability to bind with heterocyclic amines (carcinogenic substances) formed in cooked meat (Wollowski *et al*., 2001) and the production of high levels of IgA and interferon (Adams and Moss, 1999) which may prevent cancer cell formation. Most human trials have found that for the strains tested the possible mechanism of action may be by reduction in the activity of the enzymes β-glucuronidase, azoreductase and nitroreductase (Brady *et al*., 2000; Adams and Moss, 1999). These enzymes, produced by components of the intestinal flora, can convert procarcinogens to carcinogens in the gut. Also lower rates of colon cancer among higher consumers of fermented dairy products have been observed in some population studies (Sanders, 2000; Saikali, 2004).

There are also some evidences showing that probiotics may reduce or lower serum cholesterol levels. It is believed that they bring about their hypocholestaemic action by breaking down bile in the gut thereby preventing their re-absorption into the blood as cholesterol. Some human trials have shown that dairy foods fermented with specific probiotics can produce modest reductions in total and LDL cholesterol levels in those with normal levels (St-Onge *et al*., 2000; Xiao *et al*., 2003; Sanders, 2000). This area, however, requires further study.

Consumption of probiotics has also been reported to modestly lower blood pressure. It is thought that this is due to the ACE inhibitor-like peptides which are produced during fermentation (Sanders, 2000).

There are also reports indicating that probiotics in combination with standard medical treatments could be used in the treatment of peptic ulcers caused by Helicobacter pylori (Hamilton-Miller, 2003) as well as in the treatment of antibiotic associated diarrhea (Cremonini *et al*., 2002).

Probiotic foods/supplements have also been reported to modulate inflammatory and hypersensitivity responses. These are believed to be partly due to the regulation of cytokine function (Reid *et al*., 2003). Studies have also revealed that they can prevent the re-occurrences of inflammatory bowel disease in adults, acute gastroenteritis and improve milk allergies (Reid *et al*., 2003; Isolauri *et al*., 2002; Saggiro, 2004). Consumption of such foods also decreases the risk of atopic eczema in children (Kalliomaki *et al*., 2003). It has also been suggested that probiotic lactobacilli may help in cases of malabsorption of trace minerals which occur when individuals consume foods high in phytate content (legumes, whole grains, nuts) (Famularo *et al*., 2005).

**Probiotic strains and products currently in use:** Probiotic products may come in several forms. They could be in the form of fermented milks, or they could be in the form of tablets, capsules, powders or sachets containing the bacteria in freeze dried forms. They could also be found in supplement form and as components of foods and beverages (IFIC, 2006). Today, probiotic-containing foods are commonly found and consumed in Japan and Europe (Sanders, 1999). In the USA, several probiotic-containing foods have recently been introduced into the marketplace (IFIC, 2006). Table 1 and 2 show the various probiotic strains, the producers and the proven probiotic effects observed in humans.

Table 1: Some probiotics in use and their effects on humans

| Strain | Producer | Proven effect on humans |
|---|---|---|
| *Bifidobacterium animalis* subsp. lactis BB-12 | Chr. Hansen | Immune stimulation, improves phagocytic activity, alleviates atopic eczema, prevents diarrhea in children and traveller's diarrhea. |
| *Bifidobacterium infantis* 35624 | Procter and Gamble | Irritable Bowel Syndrome (IBS). |
| *Bifidobacterium lactis* HN019 | Danisco | Immune stimulation. |
| *Lactobacillus acidophilus* NCFM | Danisco | Reduces symptoms of lactose intolerance, prevents bacterial overgrowth in small intestine. |
| *Lactobacillus johnsonii* Lal | Nestle | Immune stimulation, active against *Helicobacter pylori*. |
| *Lactobacillus reuteri* ATTC 55730. | BioGaia Biologics | Immune stimulation, against diarrhea. |
| *Lactobacillus rhamnosus* LB21 | Norrmejerier | Immune stimulation, improves digestive health, reduces antibiotic-associated diarrhea. |
| *Lactococcus lactis* L1A | Norrmejerier | Immune stimulation, improves digestive health, reduces antibiotic-associated diarrhea. |

Some are also administered as a mixture of the various probiotics. These are listed below

Table 2: Probiotic strains administered as a mixture

| Strains | Producers | Proven effects on humans |
|---|---|---|
| *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 | Chr. Hansen | Oral ingestion results in vaginal colonization and prevention of vaginitis. |
| Mixture of 8 strains of *Streptococcus thermophilus*, 4 *Lactobacillus* spp. and 3 *Bifidobacterium* spp. strains | Sigma-Tau Pharmaceuticals Inc. | Positive effects with intestinal ulcers and inflammation. |
| *Lactobacillus helveticus* R0052 and *Lactobacillus rhamnosus* R0011. | Institut Rosell. | Prevents diarrhea in children, prevents upset stomachs for patients on antibiotics, active against *Helicobacter pylori*. |

**Prebiotics:** The large intestine is by far the most heavily colonized region of the digestive tract, with up to $10^{12}$ bacteria for every gram of gut content. Through the process of fermentation, colonic bacteria are able to produce a wide range of compounds that have both positive and negative effects on gut physiology as well as other systemic influences (Gibson and Roberfroid, 1995). It is therefore important to manipulate the content of the gut flora with the view to increasing the numbers and activities of the presumed probiotics and reducing those of the pathogens. This can be brought about by the supplementation of human diet with some food ingredients that have been termed prebiotics.

A prebiotic is a non digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improves host health (Gibson and Roberfroid, 1995). Therefore intake of prebiotics can significantly modulate the colonic microflora by increasing the number of specific bacteria and thus changing the composition of the gut bacteria to favour the probiotics (IFIC, 2006).

**Criteria for food material to be a prebiotic:** As was found in the probiotics, for a food material to be used as a prebiotic certain criteria must be met (Gibson and Roberfroid, 1995; Gibson, 1999). These are as follows;

It must be neither hydrolyzed nor absorbed in the upper part of the gastrointestinal tract.

It must be a selective substrate for one or a limited number of beneficial bacteria commensal to the colon, which are stimulated to grow and/or are metabolically activated.

It must consequently, be able to alter the colonic flora in favour of a healthier composition.

It must induce luminal or systemic effects that are beneficial to the host health.

Based on these criteria listed, only a few groups of food ingredients qualify to be used as prebiotics. A good number of food materials because of their chemical structure are not absorbed in the upper part of the GIT or hydrolyzed by the digestive enzymes in humans. Such foods have been called “colonic foods” (Gibson and Roberfroid, 1995) i.e, foods entering the large intestine which also serve as food for the endogenous microorganisms.

Amongst these colonic foods are non digestible carbohydrates, some peptides and proteins. The use of peptides and proteins as prebiotics will have a major problem; their anaerobic decomposition is likely to produce potentially harmful compounds such as ammonia and amines (Macfarlane and Cummings, 1991), although they may have some beneficial effects both by facilitating the intestinal absorption of cations (mainly calcium and iron) (Scholz-Ahrens *et al*., 2001) and by stimulating the immune system (Gibson and Roberfroid, 1995; Saavendra and Tschemia, 2002; Cummings and Macfarlance, 2001).

Delzenne and Roberfroid (1994) have grouped non digestible carbohydrates to include resistant starch, non digestible oligosaccharides and non starch polysaccharides such as hemicellulose, pectins, gums and plant cell wall polysaccharides (Table 3).

Although these food materials get to the colon unabsorbed in the upper gastrointestinal tract and undigested by human digestive enzymes they could not be classified as prebiotics as they stimulate in the large intestine the growth and/or metabolic activity of several different bacterial species that could be beneficial and harmful to the host (Drasar *et al*., 1976; Salysers *et al*., 1982).

Studies on non digestible oligosaccharides have shown that the fructooligosaccharides and galactooligo-saccharides are those that have been found to selectively stimulate the growth and/or metabolic activity of the potentially beneficial bacteria (probiotics) in the colon (Roberfroid *et al*., 1998; Gibson and Wang, 1994a,b; Wang and Gibson, 1993; Rowland, 1992; Ito *et al*., 1990). Soy bean oligosaccharides have also been studied for their prebiotic potentials (Saito *et al*., 1992; Hayakawa *et al*., 1990) while the oligosaccharides of the Afican Oilbean seeds, starchyose and raffinose have been suggested as a possible prebiotic (Crittenden and Playne, 1996).

Table 3: Classification of certain carbohydrates as colonic foods and prebiotics

| Carbohydrates | Colonic food | Prebiotic |
|---|---|---|
| Resistant starch | Yes | No |
| **Non-starch polysaccharides** | | |
| Plant cell wall polysaccharides | Yes | No |
| Hemicelluloses | Yes | No |
| Pectins | Yes | No |
| Gums | Yes | No |
| **Non-digestible oligosaccharides** | | |
| Fructooligosaccharides | Yes | Yes |
| Galactooligosaccharides | Yes | ? |
| Soybean oligosaccharides | Yes | ? |
| Glucooligosaccharides | ? | No |

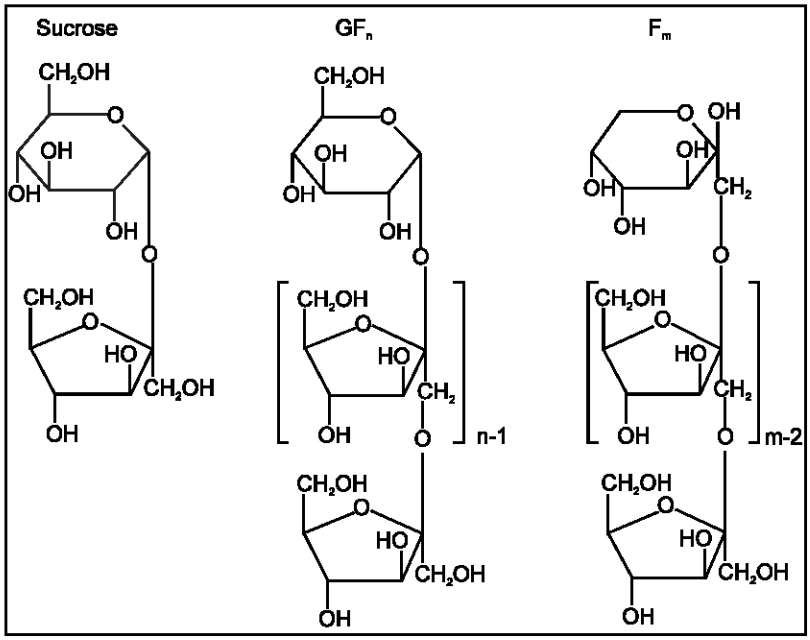


Fig. 1: Chemical structure of the various fructo oligosaccharides. G, glucose; F, fructose; n or m indicate the number of fructose moieties in the molecules

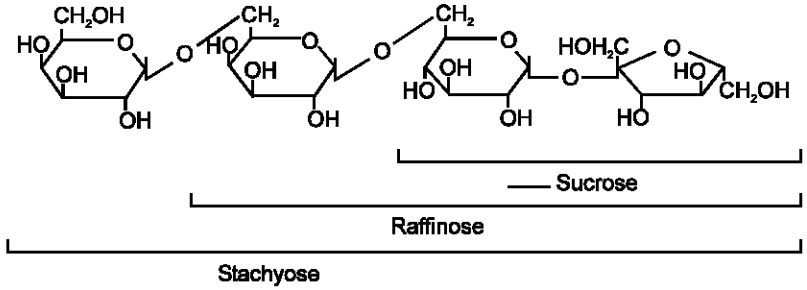


Fig. 2: Structure of some galactooligosaccharides

**Chemical structure of prebiotics:** Fructooligosaccharides are short- and medium-length chains of β-D fructans in which fructisyl units are bound by a β2-1 osidic linkage.

In some of the molecules the initial moiety is glucose. This results from the transfer of a fructosyl moiety between two sucrose molecules during synthesis in plant cells (Edelman and Dickerson, 1966). Some workers (Stone-Dorshow and Levitt, 1986; Rumessen *et al*., 1990) have shown that the β2-1 osidic including the first glucose-fructose bond is not hydrolyzed to a great extent by any mammalian digestive enzymes. Thus this accounts for its resistance in the digestive tract of humans.

Fructooligosaccharides can be classified into either oligofructose or inulin depending on the degree of polymerization. Oligofructose has a Degree of Polymerization (DP) of <9 (average DP=4.8) while inulin has DP of up to 60 (average DP=12) (Gibson and Roberfroid, 1995). Inulin is obtained industrially by hot water extraction of fresh chicory roots (Gibson *et al*., 1994) while oligofructose is produced by partial enzymatic hydrolysis of native inulin to give a product with an average DP of 4-5. Various other food materials have high content of oligofructose and inulin. These include artichoke, onion, garlic and asparagus (Van Loo *et al*., 1995).

Galactooligosaccharides have chemical structures made up of fructose, glucose and galactose molecules. These are linked together by β-fructosidic and α-galactosidic linkages. Fructose is usually the starting molecule in the oligosaccharide (Salunkhe *et al*., 1992). The most common types of galactooligisaccharides are starchyose, made up of one fructose, one glucose and two galactose molecules and raffinose made up of one fructose, one glucose and a galactose molecule (Iwe, 2003). These are common constituents of various legumes such as soy bean, African Oilbean and African Bread Fruit (Iwe, 2003).

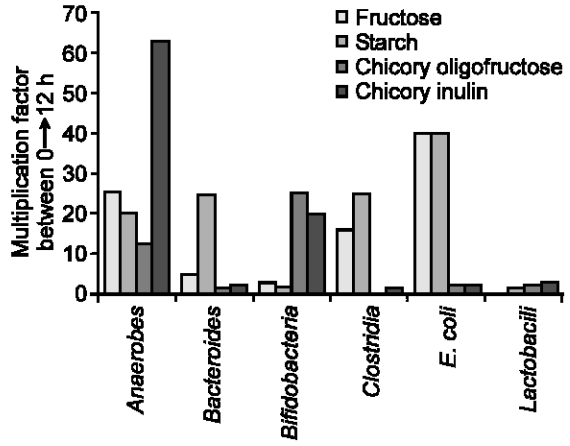


Fig. 3: Changes in the numbers of various bacteria of human faeces after the *in vitro* incubation for 12 h in the presence of 7 g/L of fructose, starch, oligofructose and inulin

**Specificity of prebiotics:** Bifidobacteria have been identified as preferred target microorganisms for prebiotics (Gibson *et al*., 1995). Experimental evidences using human faecal bacteria *in vitro* have shown that fructooligosaccharides (and galactooligosaccharides) are fermented by gut bacteria with a resultant decrease in pH of the medium during anaerobic fermentation (Gibson and Wang, 1994b; Wang and Gibson, 1993). Also studies by Wang and Gibson (1993) have shown that fructooligosaccharides in comparison with other simple and complex carbohydrates (fructose and starch) are selectively fermented by most strains of bifidobacteria (Fig. 3) and were found to increase in population while other bacteria (bacteroides, clostridia and coliforms) decreased in number. Some workers have demonstrated that the specificity of the bifidobacteria to utilize fructooligosaccharides may likely be due to the presence of β-fructosidases or 2,1 β-fructanohydrolase (Roberfroid *et al*., 1998).

Gibson and Wang (1994a) have been able to demonstrate that under anaerobic conditions obtainable in the gut chicory fructooligosaccharides, but not glucose, could selectively stimulate the growth of bifidobacteria over other species present. Using continuous chemostat cultures inoculated with 160 g/L of faecal slurries they showed that these bacteria (bifidobacteria) were almost three orders of magnitude higher than bacteroides while glucose as substrate produced two orders of magnitude higher than bifidobacteria (Table 4).

Gibson *et al*. (1995) have conducted *in vivo* studies using human volunteers. These volunteers were fed on strictly controlled diets to which chicory fructooligosaccharides were added as supplements at the rate of 15 g per day for 15 days. This they reported significantly modified the composition of the bacteria in the gut with marked increase in the number of bifidobacteria. They observed a marked decrease in the numbers of other bacteria (clostridia, bacteroides and fusobacteria) when oligofructose was administered. Kleessen *et al*. (1994) and Kleessen *et al*. (1997) also have demonstrated that supplementation of diet with 20 g per day and 40 g per day of inulin significantly ($p<0.01$) increased the population of bifidobacteria in faeces of the elderly from $10^{7.9}$ to $10^{8.8}$ and $10^{9.2}$, respectively.

The effect of ingestion of milk fermented with *Bifidobacterium* sp. on human health with or without inulin has been studied by Bouhnik *et al*. (1996). Those that contained inulin were administered at the rate of 18 g per day. They concluded that bifidobacterium fermented milk substantially increased the population of the bifidobacteria in the gut, but the concurrent administration of inulin did not enhance the effect. Interestingly two weeks after stopping the consumption of the fermented milk the volunteers who received inulin had a significant increase in the number of bifidobacteria compared with those that received the fermented milk only, although they did not study the effect on the population of other bacteria present. Roberfroid *et al*. (1998) therefore stated that the study tried to study a "symbiotic" effect rather than a prebiotic effect. The theory of synbiosis has been proposed by Gibson and Roberfroid (1995).

Other workers (Roberfroid, 1997) have also reported significant increase in faecal bifidobacteria counts with concomitant decrease in Bacteroides sp. in humans fed with controlled diets supplemented with 8g per day of chicory oligofructose. Thus this shoes that oligofructose has bifidogenic effect in the gut of humans. Some mechanisms have been proposed as to how the bifidobacteria bring about the inhibition of other bacteria.

Table 4: Composition of the microflora of human faecal slurries after six turnovers in single-stage continous fermenters containing glucose, oligofructose or inulin as the growth substrate (10 g/L)($log_{10}$ of viable bacteria per litre of medium)

| | Glucose | Chicory oligosaccharides | Chicory inulin |
|---|---|---|---|
| Anaerobes | 13.4 | 12.6 | 12.1 |
| Bacteroides | 12.0 | 9.4 | 9.3 |
| Bifidobacteria | 10.0 | 12.7 | 12.1 |
| Clostridia | 9.9 | 8.4 | 9.6 |
| Coliforms | 5.3 | 5.5 | 5.3 |
| Lactobacilli | 10.5 | 7.3 | <8 |
| Difference $Log_{10}$ Bifidobacteria-$Log_{10}$ Bacteroides | -2.0 | +3.3 | +2.8 |

These include the decrease in pH of the growth medium due to the production of various organic acids mainly acetate and lactate (Gibson and Roberfroid, 1995). Another mechanism is the production of bacteriocin-type substances which inhibit various bacteria such as Clostridium and E. coli. Some workers have also worked on synthetic fructooligosaccharides (Mitsuoka *et al*., 1987; Bouhnik *et al*., 1994; Buddington *et al*., 1996). In all they reported an increase in the bifidobacterial counts in faeces after administering their oligosaccharides at 4, 8 and 12.5 g per day, although no information was given on the effect of the supplementation on the other types of bacteria present. Therefore the results could not conclusively prove that they are selective in their action. This doubt has also been expressed by Roberfroid *et al*. (1998).

**Beneficial effects of prebiotics:** Some beneficial effects have been attributed to the consumption of these prebiotics. It is believed that their beneficial effects result from the metabolism of these compounds. Fermentation of these oligosaccharides results in the production of various organic acids and $CO_2$. Delzenne and Roberfroid (1994) have stated that the balance of such a complex process is likely to produce 40% Short Chain Fatty Acids (SCFA), 15% lactic acid and 5% $CO_2$. Therefore based on this calculation Delzenne and Roberfroid (1994) and Roberfroid *et al*. (1993) have proposed that the caloric value of fructooligosaccharides must be on the order of 4.2-6.3 KJ/g (1.0-1.5 kcal/g) or 25-40% that of a digested fructose molecule. Thus such generated SCFA and lactic acid can be absorbed from the colon of the host for generation of energy. For example, butyrate is utilized by the colonic epithelium, propionate, L-lactate and acetate (partly) by the liver and acetate (partly) by muscle and other peripheral tissues (Schumann *et al*., 1991; Demigne *et al*., 1986; Remezy and Demigne, 1983).

Some workers have also suggested that highly fermentable carbohydrates could, possibly through production of SCFA and lactate in the colon, improve the metabolic absorption of various ions such as Fe, Ca and Mg (Scharrer and Lutz, 1992; Shulz *et al*., 1993; Scholz-Ahrens *et al*., 2001), infact Delzenne and Roberfroid (1994) have been able to demonstrate that consumption of such oligosaccharides (oligofructose and inulin) can produce up to 60-65% intestinal uptake of these ions. However, this effect varies according to the individual non-digestible oligosaccharide and particular human population studied and the amount consumed as well as its specific fermentation profile (Saggiro, 2004).

Some workers have also suggested that acetate and propionate, possibly in combination with l-lactate may be involved in regulating lipid and cholesterol metabolism (Gibson and Roberfroid, 1995; Demigne *et al*., 1986). Some workers have also demonstrated in rats that consumption of feed supplemented with 10-15% fructooligosaccharides induced a significant reduction in total body carcass fat deposition, triglyceridemia (by 25%) (Delzenne *et al*.,1993; Fiordaliso *et al*., 1995). The reduction has been suggested to be due to the reduction of circulating VLDL particles. Thus the hepatic metabolism of lipids in the rats may have been modified (Gibson and Roberfroid, 1995). Thus in hyoerlipidemic subjects, when a prebiotic effect is seen, it is a reduction in cholesterol whereas in normal-lipidemic subjects, any noted effects are on serum triglycerides (Pereira and Gibson, 2002).

While some of the prebiotic beneficial effects on the function of the human gut have been established and their favourable impact on health widely supported, further scientific research is ongoing to substantiate their direct relationship to disease risk reduction (IFIC, 2006; Roberfroid, 2000).

**Conclusion:** From all the data available it could be noted that fructooligosaccharides selectively stimulate the growth of bifidobacteria especially the oligifructose. These bacteria (bifidobacteria) are able to utilize them through the process of fermentation since it has been observed that their growth is accompanied with drop in pH of faecal slurries (Gibson and Wang, 1994b). The supplementation of such bifidobacteria in fermented milk products is likely to increase their ability to outgrow other species in the gut especially when prebiotics are provided. Thus the concept of "synbiotics" which is a combination of the probiotics and prebiotics as advanced by Gibson and Roberfroid (1995) could be explored. Some products are, however, available that contain Bifidobacterium sp. (Sanders, 2007). It is also observed that in all the experiments no mention was made of the lactobacilli resident in the gut or those consumed in fermented foods. This therefore calls to question the use of these lactobacilli in fermented milks, whether they will be able to really colonize the gut upon implantation even when fructooligosaccharides are administered. What this means is that there has to be a constant consumption of such fermented milks to maintain a relatively high population of the lactobacilli for them to be able to exert their probiotic effects. Since these fructooligo-saccharides are not selectively utilized by the lactobacilli (Wang and Gibson, 1993) they will be open to the stiff competition that goes on in the large gut, unless they have other means of outwitting other competitors in the gut. Thus it may be that their ability to produce bacteriocins could help them outgrow their competitors and colonize the gut. It is therefore necessary that studies be conducted in vivo using human volunteers to determine the role of these bacteriocins in their ability to outgrow competitors and colonize the gut. Infact one of such lactobacilli, *Lactobacillus reuteri*, a natural inhabitant of the human

gut has been found to produce reuterin and reuteri-cyclin when they grow. This bacterium has been targed a special probiotic because it is an inhabitant of the gut and the substances it produces reduce or prevent the growth of many bacteria including Gram positive and Gram negative bacteria (Pszozola, 2002). The use of special delivery systems that will enable them implant and colonize the gut of the host should be developed. Already a company, Bio Gaia AB, Stockholm, Sweden has developed a special delivery system for *L. reuteri* in a product called LifeTop which has been exhibited in USA (Pszozola, 2002).

It is also necessary that the concept of synbiotics be properly further investigated as some workers (Orrhage *et al*., 2000) have demonstrated that administration of probiotics and prebiotics after antibiotic therapy helped reestablish the beneficial bacteria. Probiotic and prebiotic formulas are proving very popular and are advertised at various places (Collier, 2004) and this is because evidence that they promote good health is strong (Gibson, 2003).

Oligosaccharides are known to cause flatulence and distension in some individuals that consume them (Ruiz-Terans and Owens, 1999). Thus the use of these oligosaccharides as prebiotics can also result in the discomfort of the consumers. However, researches have not been conducted to determine the best levels of administration of the prebiotics to bring to the barest minimum the problem of flatulence and distension while still maintaining the prebiotic effects attributable to them. Such studies will try to produce an internationally acceptable dosage of the prebiotics for maintaining the good health and general wellbeing of the consumers.

Another important aspect that needs consideration is the possibility of these probiotics acquiring virulence from pathogens in the gut. This needs to be investigated and prevented so that these probiotics will remain the health promoting bacteria they are meant to be.

## REFERENCES


Adams, M.R. and M.O. Moss, 1999. Food Microbiology. The Royal Society of Chemistry, Cambridge, pp: 260-262.

Bouhnik, Y., B. Flourie, F. Ouarne, M. Riottot, N.Bisetti, F. Bornet and J.C. Rambaud, 1994. Effects of prolonged ingestion of Fructo-Oligosaccharides (FOS) on colonic bifidobacteria, faecal enzymes and bile acids in humans. Gastroenterology, 106: A598 (abs).

Bouhnik, Y., B. Flourie, C. Andrieux, N. Bisetti, F. Briet and J.C. Rambaud, 1996. Effects of *Bifidobacterium* sp. fermented milk ingestion with or without inulin on colonic bifidobacteria and enzymatic activities in healthy humans. Eur. J. Clin. Nutr., 50: 269-273.

Brady, L.J., D.D. Gallaher and F.F. Busta, 2000. The role of probiotic cultures in the prevention of colon cancer. J. Nutr., 130: 410S-414S.

Buddington, R.K., C.H. Williams, S.C. Chen and S.A. Witherly, 1996. Dietary supplement of neosugar alters the faecal flora and decreases activities of some reductive enzymes in human subjects. Am. J. Clin. Nutr., 63: 709-716.

Cheplin, H.A. and L.F. Rettger, 1920. Studies on the transformation of the intestinal flora, with special reference to the implantation of *Bacillus acidophilus*, II. Feeding experiments of man. Proc. Natl. Acad. Sci. USA., 6: 704-705.

Crittenden, R.G. and M.J. Playne, 1996. Production, properties and application of food-grade oligosaccharides. Trends Food Sci. Technol., 7: 353-361.

Collier, J., 2004. Promoting bacterial culture: The importance of probiotics and prebiotics to good health. Cellscience, 1: 1-3.

Cremonini, F., S. Di Caro, E.C. Nista, F. Bartolozzi, G. Capelli, G. Gasbarrini and A. Gasbarrini, 2002. Meta-analysis: The effect of probiotic administration on antibiotic-associated diarrhea. Aliment. Pharmacol. Ther., 16: 1461-1467.

Cummings, J.H. and G.T. Macfarlance, 2001. A study of fructo oligosaccharides in the prevention of traveler's diarrhea. Aliment. Pharmacol. Ther., 15: 1139-1145.

Delzenne, N.M., N. Kok, M.F. Fiordaliso, D.M. Deboyser, F.M. Goethals and M.B. Roberfroid, 1993. Dietary fructooligosaccharides modify lipid metabolism in rats. Am. J. Clin. Nutr., 57(suppl): 820(abs).

Delzenne, N.M. and M.B. Roberfroid, 1994. Physiological effects of non-digestible oligosaccharides. Lebensm. Wiss. Technol., 27: 1-6.

Demigne, C., C. Yacoub, C. Remezy and P. Fafournoux, 1986. Effects of absorption of large amounts of volatile fatty acids on rat liver metabolism. J. Nutr., 116: 77-86.

Drasar, B.S., D.J.A. Jenkins and J.H. Cummings, 1976. The influence of a diet rich in wheat fibre on the human faecal flora. J. Med. Microbiol., 9: 423-431.

Edelman, J. and A.G. Dickerson, 1966. The metabolism of fructose polymers in plants: Transfructosylation in tubers of *Helianthus tuberosus* L. Biochem. J., 98: 787-789.

FAO/WHO, 2001. Report of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria.

Famularo, G., C. De Simone, V. Pandey, A.R. Sahu and G. Minisola, 2005. Probiotic lactobacilli: An innovative tool to correct the malabsorption syndrome of vegetarians? Med. Hypotheses., 65: 1132-1135.

Fiordaliso, M.F., N. Kok, J.P. Desager, F. Goethals, D. Deboyser, M. Roberfroid and N. Delzenne, 1995. Oligofructose supplemented diet lowers serum and VLDL concentrations of triglycerides, phospholipids and cholesterol in rats. Lipids, 30: 163-167.

Fuller, R., 1989. Probiotics in man and animals. J. Appl. Bacteriol., 66: 365-378.

Fuller, R., 1992. Probiotics. The scientific basis. Chapman and Hall, London.

Gibson, G.R. and X. Wang, 1994a. Enrichment of bifidobacteria from human gut contents by oligofructose using continous culture. FEMS Microbiol. Lett., 118: 121-128.

Gibson, G.R. and X. Wang, 1994b. Bifidogenic properties of different types of fructose containing oligosaccharides. Food Microbiol., 11: 491-498.

Gibson, G.R., C.L. Willis and J. Van Loo, 1994. Non-digestible oligosaccharides and bifidobacteria-implications for health. Int. Sugar J., 96: 1150-1156.

Gibson, G.R. and M.B. Roberfroid, 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. J. Nutr., 125: 1401-1412.

Gibson, G.R., E.R. Beatty, X. Wang and J.H. Cummings, 1995. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastroenterology, 108: 975-982.

Gibson, G.R., 1999. Dietary modulation of the human gut microflora using the prebiotics oligofructose and inulin. J. Nutr., 129: 1438S-1441S.

Gibson, G.R., 2003. Probiotics and prebiotics and their function. Functional Nutr., 2: 11-13.

Gill, H.S., K.J. Rutherfurd, M.L. Cross and P.K. Gopal, 2001. Enhancement of immunity in the elderly by dietary supplementation with the probiotic *Bifidobacterium lactis* HNO 19. Am. J. Clin. Nutr., pp: 833-839.

Gilliland, S.E. and D.K. Walker, 1990. Factors to consider when selecting a culture of *Lactobacillus acidophilus* as a dietary adjunct to produce a hypocholesterolemic effect in humans. J. Dairy Sci., 73: 905-911.

Hamilton-Miller, J.M., 2003. The role of probiotics in the treatment of and prevention of Helicobacter pylori infection. Int. J. Antimicrob. Agents., 22: 360-366.

Hatakka, K., E. Savilahti, A. Ponka, J.H. Meurman, T. Poussa, L. Nase, M. Saxelin and R. Korpela, 2001. Effect of long term consumption of probiotic milk on infections in children attending day care centres: Double blind, randomized trial. BMJ., 322: 1327.

Hayakawa, K., J. Mitzutani, K. Wada, T. Masai, I. Yoshihara and T. Mitsuoka, 1990. Effects of soybean oligosaccharides on human faecal microflora. Microb. Ecol. Health Dis., 3: 293-303.

International Food Information Council (IFIC), 2006. Functional foods facts sheet: probiotics and prebiotics. www.ific.org/nutrition/functional.

Isolauri, E., 2001. Probiotics: Effects on immunity. Am. J. Clin. Nutr., 73(suppl): 444S-450S.

Isolauri, E., P.V. Kirjavainen and S. Salminen, 2002. Probiotics: A role in the treatment of intestinal infection and inflammation? Gut., 50(suppl. III): 54-59.

Ito, M., Y. Deguchi, A. Miyamori, K. Matsumoto, H. Kikuchi, Y. Kobayashi, T. Yajima and T. Kan, 1990. Effect of administration of galacto-oligosaccharides on the human faecal microflora, stool weight and abdominal sensation. Microb. Ecol. Health Dis., 3: 285-292.

Iwe, M.O., 2003. The science and technology of soybean. Rojoint Communication Services Ltd., Enugu, pp: 89-91.

Kalliomaki, M., S. Salminen, T. Poussa, H. Arvilommi and E. Isolauri, 2003. Probiotics and prevention of atopic disease: 4-year follow-up of a randomized placebo-controlled trial. Lancet, 361: 1869-1871.

Kleessen, B., J. Noack and H.J. Zunft, 1994. Effect of inulin on colonic bifidobacteria of elderly man. FASEB. J., 8: A185(abs).

Kleessen, B., B. Sykura, H.J. Zunft and M. Blaut, 1997. Effects of inulin and lactose on faecal microflora, microbial activity and bowel habit in elderly constipated persons. Am. J. Clin. Nutr., 65: 1397-1402.

Macfarlane, G.T. and J.H. Cummings, 1991. The colonic flora, fermentation and large bowel digestive function. In: The large Intestine: Physiology, pathophysiology and disease (Phillips, S.F., Pemberton, J.H. and Shorter, R.G. Eds.). Raven Press, New York, pp: 51-92.

Metchnikoff, E., 1907. Essais optimists. Paris. The prolongation of life. Optimistic studies. Translated and edited by P. Chalmers Mitchell. Heinemann, London.

Mitsuoka, T., H. Hidaka and T. Eida, 1987. Effect of fructo-oligosaccharides on intestinal microflora. Die. Nahrung., 31: 426-436.

Nase, L., K. Hatakka, E. Savilahti, M. Saxelin, A. Ponka, T. Poussa, R. Korpela and J.H. Meurman, 2001. Effect of long-term consumption of a probiotic bacterium, *Lactobacillus rhamnosus* GG, in milk on dental caries and caries risk in children. Caries Res., 35: 412-420.

Nicholas, A.W., 2007. Probiotics and athletic performance: A systematic review. Current Sports Medicine Rep., 6: 269-273.

Orrhage, K., S. Sjostedt and C.E. Nord, 2000. Effect of supplements with lactic acid bacteria and oligosaccharides on the intestinal microflora during administration of cefpodoxime proxetil. J. Antimicrob. Chemother., 46: 603-612.

Ouwehand, A.C., S. Salminen and E. Isolauri, 2002. Probiotics: An overview of beneficial effects. Antonie Van Leeuwenhoek, 82: 279-289.

Pereira, D.I. and G.R. Gibson, 2002. Effects of consumption of probiotics and prebiotics on serum lipid levels in humans. Crit. Rev. Biochem. Mol. Biol., 37: 259-281.

Pszozola, D.E., 2002. Antimicrobials: Setting up additional hurdles to ensure food safety. Food Technol., 56: 99-107.

Pujol, P., J. Huguet, F. Drobnic, M. Banquells, O. Ruiz, P. Galilea, N. Segarra, S. Aguilear, A. Burnat and J.A. Mateos, 2000. The effect of fermented milk containing Lactobacillus casei on the immune response to exercise. Sports Med. Training Rehab., 9: 209-223.

Reid, G., J. Jass, M.T. Sebulsky and J.K. McCormick, 2003. Potential uses of probiotics in clinical practice. Clin. Microbiol. Rev., 16: 658-672.

Remezy, C. and C. Demigne, 1983. Changes in availability of glucogenic and ketogenic substrates and liver metabolism of fed and starved rats. Ann. Nutr. Metab., 27: 57-70.

Rettger, L.F., W.N. Levy, L. Weinstein and J.E. Weiss, 1935. *Lactobacillus acidophilus* and its therapeutic application. Yale University Press, New Haven.

Roberfroid, M.B., G.R. Gibson and N. Delzenne, 1993. Biochemistry of oligosaccharides, a non-digestible dietary fibre: An approach to calculate its caloric value. Nutr. Rev., 51: 137-146.

Roberfroid, M.R., 1997. Functional foods and intestinal micro-environment: The concept of prebiotics, probiotics and synbiotics. Symposium on nutrition and gastrointestinal micro environment, Leuven, Belgium.

Roberfroid, M.B., J.A.E. Van Loo and G.R. Gibson, 1998. The bifidogenic nature of chicory inulin and its hydrolysis products. J. Nutr., 128: 11-19.

Roberfroid, M.B., 2000. Prebiotics and probiotics: Are they functional foods? Am. J. Clin. Nutr., 71(sulppl): 1682S-1687S.

Rowland, I.R., 1992. Metabolic interactions in the gut. In: Probiotics. The scientific basis. (Fuller, R. Ed). Chapman and Hall, London, pp: 29-53.

Rumessen, J.J., S. Bode, O. Hamberg and E.G. Hoyer, 1990. Fructans of Jerusalem artichokes: Intestinal transport, absorption, fermentation and influence on blood glucose, insulin and C-peptide responses in healthy subjects. Am. J. Clin. Nutr., 52: 675-681.

Ruiz-Terans, F. and J.D. Owens, 1999. Fate of oligosaccharides during production of soya bean tempe. J. Sci. Food Agric., 79: 249-252.

Sanders, M.E., 1999. Probiotics: A publication of The Institute of Food Technologists Expert Panel on Food Safety and Nutrition. Food Technol., 53: 67-77.

Sanders, M.E., 2000. Considerations for use of probiotic bacteria to modulate human health. J. Nutr., 130: 384S-390S.

Sanders, M.E., 2007. Probiotics, strain matter. Functional foods and neutraceuticals magazine, pp: 36-41.

Saavendra, J.M. and A. Tschemia, 2002. Human studies with probiotics and prebiotics: Clinical implications. Br. J. Nutr., 87: S241-S246.

Saggiro, A., 2004. Probiotics in the treatment of irritable bowel syndrome. J. Clin. Gastroenterol., 38(suppl.): S104-S106.

Saikali, J., 2004. Fermented milk, probiotic cultures and colon cancer. Nutr. Cancer, 49: 14-24.

Saito, Y., T. Takano and I. Rowland, 1992. Effects of soybean oligosaacharides on the human gut microflora in *in-vitro* culture. Microb. Ecol. Health Dis., 5: 105-110.

Salunkhe, D.K., J.C. Chavan, R.N. Adsule and S.S. Kadan, 1992. World oilseeds, chemistry, technology and utilization. An AVI book published by Van Nostrand Reinhold, New York.

Salysers, A.A., O.M. Brien and S.F. Kotarski, 1982. Utilization of chondrioitin sulfate by Bacteroides thetaitaomicron growing in carbohydrate-limited continous culture. J. Bacteriol., 150: 1008-1015.

Scharrer, E. and T. Lutz, 1992. Relationship between volatile fatty acids and magnesium absorption in mono- and polygastric species. Magnesium Res., 5: 53-60.

Scholz-Ahrens, K.E., G. Schaafsma, E. Heuvel and J. Schrezenmeir, 2001. Effects of prebiotics on mineral metabolism. Am. J. Clin. Nutr., 73(suppl): 459S-464S.

Schrezenmeir, J. and M. De Vrese, 2001. Probiotics, prebiotics and synbiotics-approaching a definition. Am. J. Clin. Nutr., 73(suppl): 361S-364S.

Shulz, A.G.M., J.M.M. Van Alelsvoort and A.C. Beynen, 1993. Dietary native resistant starch but not retrograded resistant starch raises magnesium and calcium absorption in rats. J. Nutr., 123: 1724-1731.

Schumann, W.C., I. Magnusson, V. Chandramouli, K. Rumaran, J. Wahren and B.R. Landan, 1991. Metabolism of [-$^{14}$C] acetate and its use in assessing hepatic kreb cycle activity and gluconeogenesis. J. Biol. Chem., 266: 6985-6990.

St-Onge, M.P., E.R. Farnworth and P. Jones, 2000. Consumption of fermented and nonfermented dairy products: Effects on cholesterol concentrations and metabolism. Am. J. Clin. Nutr., 71: 674-681.

Stone-Dorshow, T. and M.D. Levitt, 1986. Gaseous response to ingestion of a poorly absorbed fructooligosaccharide sweetner. Food Chem., 22: 46-61.

Tannock, G.W., 2003 Probiotics. Time for a dose realism. Curr. Intest. Microbiol., 4: 33-42.

Van Loo, J., P. Coussement, L. De Leenheer, H. Hoebregs and G. Smits, 1995. Inulin and oligofructose in the western diets. Crit. Rev. Food Sci. Nutr., 35: 525-552.

Vaughan, R.B., 1965. The romantic rationalist: A study of Elie Metchnikoff. Med. Hist., 9: 201-215.

Wang, X. and G.R. Gibson, 1993. Effect of the in-vitro fermentation of oligofructose and inulin by bacteria growing in the human large intestine. J. Appl. Bacteriol., 75: 373-380.

Weizman, Z., Asli Ghaleb and A. Alsheikh, 2005. Effect of a probiotic infant formula on infections in child care centres: Comparison of two probiotic agents. Pediatrics, 115: 5-9.

Wollowski, I., G. Rechkemmer and B.L. Pool-Zobel, 2001. Protective role of probiotics and prebiotics in colon cancer. Am. J. Clin. Nutr., 73: 451S-455S.

Xiao, J.Z., S. Kondo, N. Takahashi, K. Miyaji, K. Oshida, A.K. Hiramatsu, K. Iwatsuki, S. Kokubo and A. Hosono, 2003. Effects of milk products fermented by Bifidobacterium longum on blood lipids in rats and healthy adult male volunteers. J. Dairy Sci., 86: 2452-2461.

Pakistan Journal of Nutrition 9 (9): 844-852, 2010
ISSN 1680-5194


# Orphanage Children in Ghana: Are Their Dietary Needs Met?

A. Sadik
Department of Hotel Catering and Institutional Management, Tamale Polytechnic,
P.O. Box 3, Education Ridge, Tamale, Ghana, West Africa

**Abstract:** Nourishing the body is a basic human right. The literature argued that children are born with the potential to develop both physically and emotionally. However, socioeconomic and environmental factors affect the health and nutrition of many children in developing countries. Little research has been done on the dietary needs of children living in orphanages in Ghana. The main objective of the study was to determine the nutritional status, food consumption patterns and dietary intake of the orphanage children. A non-experimental, descriptive action research with a multi-methodological approach was used. This study was conducted in an orphanage in Tamale. Forty children, 22 boys and 18 girls, aged 2-18 years and 23 orphanage workers formed the sample. Methods included on site observation, completion of a standard demographic questionnaire, a validated quantitative food frequency questionnaire and anthropometric measurements. The nutritional status indicated that, 10% and 15% of the children were severely stunted and wasted respectively (≤-2) Z score. The dietary intake data showed energy intakes for the children aged 1-3 years as 963 kcal, 7-10 years as 1627.4 kcal and 11-14 years as 1547.53 kcal and 15-18 years as 1540.6 kcal. Protein intake for the same age groups was 33, 52.1, 50.6 and 49.3 g respectively, with fat 27 g, 33.9 g, 31.9 g, 31.9 g and carbohydrate 150 g, 284.3 g, 269.1 g and 296.1 g respectively. The top five most frequently consumed foods were coffee (232 ml) and tea (232 ml), maize meal (109 g), bread (77 g), white rice (55 g). Protein was limited with anchovies ("Keta schoolboys") and beans as the only source. Orphanage children are vulnerable and disadvantaged members of the community, especially if measures to provide adequate dietary intakes in terms of macro-and micronutrient are not in place. The findings indicated low intake of both macro-and micronutrients with the exception of protein. Nutritional status indicated that, 10% and 15% of the children were severely stunted and wasted respectively (≤-2) Z score. The results of this study formed the basis for a nutrition education and training programme that was implemented in the orphanage.

**Key words:** Orphanage, nutritional status, food consumption patterns, dietary intake

## INTRODUCTION

Adequate nutrition is a basic human right and embedded in the constitution of most developing countries (De Onis *et al*., 2002). Although nutrition is a basic human need it remains unmet for vast numbers of children, the latter who are thus unable to achieve their full genetic development potential, due to malnutrition (Rutengwe *et al*., 2001).

Poor nutritional intake has negative implication on children growth and immune-status leading to recurrent and increasing severe infectious illnesses and may ultimately threaten child's survival.

The ability to survive the first few years of life and the quality of that survival is a function of many environmental and social stresses that impinge upon the individual child, beginning during pregnancy and continuing through infancy and childhood (Kusin and Karjati, 1994). In the developed world over 97% of all children survive their pre-school years, whereas in many developing countries, 20-25% of children die before reaching their fifth birthday (Kusin and Karjati, 1994). Malnutrition is still widely prevalent among pre-school children in developing countries. The most devastating problem facing the majority of the world's poor and needy, is hunger and malnutrition, disease and poverty and these will continue to dominate the health conditions of the world's poor nations (Kusin and Karjati, 1994).

The consequences of malnutrition include death, disability, stunted physical growth and these, as a result, retard the national socio-economic development (WHO, 2000). According to the World Health Organization (WHO), (2000), 49% of 10 million deaths among children each year in the developing world, is associated with malnutrition. Henon (1998) states, that malnutrition in all its forms, exacts a heavy toll among children, in addition to causing the deaths of more than seven million children a year. It also impairs the development of millions of other young children throughout the world and continues to be an obstacle to human rights, quality and the dignity of life.

The World Summit for children held in New York during December 1990, represented by more than 150 governments, including 71 heads of states, formally endorsed the "…world declaration on survival, protection and development of children". The former president of

Ghana and his deputy were among the signatories. Among the series of goals adopted for the year 2000, was a one-third reduction in infantile deaths, halving child malnutrition, immunization levels of 90%, control of the major childhood diseases, eradication of polio, elimination of micronutrient deficiencies, halving the maternal mortality rate, primary school education for at least 80% of children, provision of clean water and safe sanitation for all communities and the universal ratification of the convention on the right of the child (Grant, 1996).

Incidences of malnutrition in Ghana orphanages are not clearly defined; due to the previous absence of national nutrition surveillance programme (GDHS, 2003). The only data available consist of a fragmented survey undertaken amongst some isolated groups (GDHS, 2003). Ghana lacks a national nutrition surveillance system; it was however recognized by the Nutrition Committee (1994), as a result of which a system was developed and implemented in clinics; these included monitoring the growth and well-being of children and giving special attention to vulnerable groups (GDHS, 2003).

Promoting children's health and nutrition in orphanages is, therefore, a priority and requires attention by all. Causes of death of children placed in orphanages are largely preventable and thousands of children can be saved if their nutritional needs are catered for (UNICEF, 1990a,b). The family has the responsibility of nurturing and protecting children from infancy to adolescence and children should be introduced to cultural values and norms of society and grow up in an environment and atmosphere of happiness, love and understanding so as to ensure them to have them to fully feel safe and develop their personalities. In this light, parents and caregivers need the support of institutions and society (UNICEF, 1990a,b).

This paper presents the findings of the study conducted in the orphanage. The objective of the study was two folds; the first part was to determine the nutritional status, food consumption and dietary intake of the orphanage children.

A need for this study was thus recognized by both the orphanage management and the researcher. An extensive literature study by synthesis, highlighting available scientific literature regarding the problem of malnutrition, the importance of the nutritional status of children and a food consumption pattern was done.

During an assessment of the food consumption patterns, kitchen facilities and staff skills that took place in October 2008 at the orphanage, the researcher identified the following problem areas:

- Insufficient funding for nutritional needs.
- Deficient knowledge of sound budgeting and cost control.
- Inappropriate methods of food procurement.
- No standardized food preparation methods.
- Absence of specific menu.
- Untrained caregivers in food service.
- Inadequate and absolute kitchen equipment.

## MATERIALS AND METHODS

The planning of the empirical study included the development of questionnaires in order to determine nutritional status and food consumption patterns, recruitment, orientation and training of field workers.

**Study design:** A non-experimental, descriptive, action research was used. The study population consisted all 40 children, 22 boys and 18 girls aged 2-18 years and 23 orphanage workers who were purposively sampled for the study and identification numbers randomly assigned to each child.

All the children were included and studied so as to determine their nutrition status and food consumption patterns, the orphanage workers who included 15 administrative staff and eight caregivers, provided information on food procurement, menu planning and children's feeding practices, food preparation and handling practices at the orphanage.

The study examined the nutritional status, food consumption patterns and dietary intakes of the orphanage children. Initial contacts were made with the management of the orphanage to approve the protocol of the study.

The study consisted of six phases that were systematically implemented. The phases are described as part of the conceptualization of the study design.

**Ethical consideration:** School of Applied Science Research Committee, Tamale Polytechnic approved the study. All key ethical issues were adhered to. Informed verbal consent was obtained from the management of the orphanage prior to the commencement of the study. Neither children nor workers of the orphanage, were paid to participate in the study, participation was voluntary.

**Recruitment and orientation of field workers:** Two field workers were recruited from volunteer staff members of the Department: Hotel, Catering and Institutional Management, to assist the researcher with the implementation of fieldwork. The field workers had formal qualifications in nutrition and food service management. Both field workers were Dagomba speaking and aged 25 and 35 years respectively.

The first step of the orientation training was to explain the objectives of the project and to equip field workers with the skills required to conduct the research. The training consisted of a two-day workshop to explain the process for conducting quantitative and qualitative research by the researcher. The researcher, in an

attempt to facilitate the understanding of the fieldwork, drew up a field-training manual for field workers. The emphasis was on ensuring that field workers knew and understood the objectives and importance of the study. The field workers furthermore received detailed instructions regarding anthropometric measurements and administering of all the other questionnaires to be used in the study, by means of practical sessions with volunteer subjects.

**Study instrument**

**Demographic questionnaire:** A standard demographic questionnaire collected personal data on the 40 orphanage children, it captured data on specific variables, including age, gender, health status, educational background, ethnicity, religion and activities performed. The demographic information gathered, was important to understanding the backgrounds of the orphanage children. The dates of birth for each child were recorded twice, first during the observational visit in October 2008 and again in January 2009 in order to make age examination as reliable as possible. The birth dates of the children aged two to five years were taken from clinic health cards provided by the caregivers. The children aged six to eighteen years, provided their birth dates from birth certificates.

**Quantitative food frequency questionnaire:** The validated QFFQ (Macintyre, 1998) was used in this study to obtain qualitative and descriptive information on food consumption patterns. The QFFQ consisted of two components, namely, a list of the foods and a set of frequency-of-consumption response categories. An extensive list of defined foods was included, with the aim of estimating total food intake and thus the dietary diversity. To verify food intake, all 40 children completed the QFFQ's in individual interviews, with the assistance of field workers. For children aged two to five years old, the caregivers helped to provide the information. Food models were used simultaneously to explain to the children portion sizes and the food items.

**Pilot study:** A pilot study to test and evaluate ease of completion, suitability, clarity and value of the measuring instruments was conducted on a random sample of 10 volunteers two weeks prior to the actual fieldwork.

A simple observational survey (Brink, 1999) constituted a situation analysis at the orphanage by means of a field visit in order to observe the orphanage's feeding practices, budgeting and cost control measures and other catering needs. The purpose of this phase was to conduct a situation analysis through observations at the orphanage. The field visit was carried out in October 2008.

**Weight measurement:** The field workers took repeated measurements of weight. The weight measurements were taken before breakfast from 07H00-08H00 so as to avoid diurnal variations (Hans de Ridder, 2002). Children under two years of age were suspended in a cloth sling on a MP 25 spring scale (Weighing Equipment Ltd (Limited), London) and weighed to the nearest 100 g (Gibson, 1990; Hans de Ridder, 2002; Lindskog *et al*., 1997; WHO, 1996).

A Philips electronic scale (Model 1122; Instron corp. (Corporation), Canton Mass United States America (USA) was used for weighing the children aged two to eighteen years. The scale was placed on an even floor. Children were weighed with light underclothes without shoes. Children stood upright in the middle of the scale, facing the field worker and looking straight ahead. They stood with feet flat and slightly apart until the measurement was recorded on the Personal Information questionnaire (Demographic questionnaire). The scale was calibrated to zero reading before each weighing session by the researcher. Body weight was recorded to the nearest 100 g (Lindskog *et al*., 1997; WHO, 1996), repeated and the average of the two measurements recorded.

**Height measurement:** The length of the children under two years of age was measured to the nearest 0.1 cm while they were in a recumbent position on a wooden platform, with a stadiometer sliding head board (Hans de Ridder, 2002; Lindskog *et al*., 1997; WHO, 1996).

A modified tape measure was used to measure the height of the children aged two to eighteen years. Height was measured, with the child facing the field worker, shoulders relaxed, buttocks and heels touching the wall. The child's arms were relaxed at the sides, legs straight and knees together and head in the Frankfort's plane (Gibson, 1990; Hans de Ridder, 2002). Each child's height was taken barefooted. A direct reading of height was recorded to the nearest five millimeters (mm) and then repeated and the average of the two measurements recorded (SAVACG, 1995).

**Anthropometric measurements:** Anthropometric data, namely, weight-for-age, height-for-age, (Body mass index) BMI-for-age and weight-for-height were taken.

**Data capturing and analysis:** Data was captured and analyzed by means of a Personal Computer (PC) with Microsoft Windows 2007® software. All field-collected data were entered into appropriate computer software for statistical analysis. The details of each procedure followed to treat the data, are reported.

During April to June, the collected data of the three questionnaires were processed. The results of these questionnaires guided the researcher to plan and adjust the intervention programme that was implemented in the second part.

The demographic data were collected and captured on a Microsoft Excel® spreadsheet. The information was

then converted into the Statistical Package for Social Studies (SPSS, version 10.1).

Food consumption patterns and nutrient intake data were captured from the QFFQ and analyzed by using the software programme, Dietary Manager® 2000. The programme computed the means and standard deviations of the daily nutrient intake and the top 20 frequently consumed foods.

The anthropometric information from the Microsoft Excel® spreadsheet was then converted into the Statistical Package for Social Sciences (SPSS®, version 10.1). Descriptive statistics such as means, standard deviations and Z-scores, were calculated. The Z-scores of the children were then compared to the existing National Centre for Health Statistics (NCHS) reference values (WHO, 2000) and the Nutrition Canada National Survey, Nutrition Canada 1980, as reported by Gibson (1990).

## RESULTS AND DISCUSSION

**Demographic information:** The data in Table 1 show the age distribution of the children. The age of the children varied between two and eighteen years old.

**Home language of children:** The results summarized in Table 2, indicate that three languages were mainly spoken in the Village, with the majority (67.5%) speaking Dagbani, followed by children speaking mamprushi (27.5%) and Gonja (5%).

**Religion, education and health status of children:** All the children (n = 40) were Moslem and attended school. One-to-three-year-old children 12.5% attended a crèche and those of seven to eighteen years (87.5%) were in different grades, ranging from primary one to Junior High School. None of the children suffered from any chronic or infectious diseases and none was allergic or had any known food intolerance or was on special diet. None of the children was on medication or a vitamin supplement.

**Sports and leisure activities performed by the children:** The data in Table 3 indicate, that 100% of children watched television daily, 50% received physical training at school, 35% engaged in school sporting activities and 28% performed indoor games, for example draughts and playing-cards.

Table 4, 5, 6 are a combination of both sexes and ages; this is in accordance to the (WHO, 1995b).

Table 4 indicates that 85% (n = 34) of the children in the Tamale children Village, have a normal weight-for-age (>-2 <+2) Z-score, 10% were underweight on the (≤-2) Z-score and only 5% had weight-for-age above the (≥+2) Z-score.

Data in Table 5 show that 77.5% were of the normal height-for-age (> -2< +2) Z-score, 10% were probably severely stunted height-for-age on the (≤-2) Z-score and 5% were nourished according to the (≥+2) Z-score.

Anthropometric indices data were calculated by using SPSS® and compared, with National Health and

Table 1: Age distribution of the children

| | Male | | Female | | Both genders | |
|---|---|---|---|---|---|---|
| Age range in years | Number | % | Number | % | Number | % |
| 1-3 | 2 | 5.0 | 3 | 7.5 | 5 | 12.5 |
| 7-10 | 4 | 10.0 | 3 | 7.5 | 7 | 17.5 |
| 11-14 | 9 | 22.5 | 8 | 20.0 | 17 | 42.5 |
| 15-18 | 7 | 17.5 | 4 | 10.0 | 11 | 27.5 |
| Total | 22 | 55.0 | 18 | 45.0 | 40 | 100.0 |

Table 2: Home language of the children

| | Male | | Female | | Both genders | |
|---|---|---|---|---|---|---|
| Home language | Number | % | Number | % | Number | % |
| Dagbani | 16 | 40 | 11 | 27.5 | 27 | 67.5 |
| Mamprushi | 6 | 15 | 5 | 12.5 | 11 | 27.5 |
| Gonja | 0 | 0 | 2 | 5.0 | 2 | 5.0 |
| Total | 22 | 55 | 18 | 45.0 | 40 | 100.0 |

Table 3: Sports and leisure activities performed by the children

| | Male | | Female | | Both genders | |
|---|---|---|---|---|---|---|
| Sports and leisure activities | Number | % | Number | % | Number | % |
| Physical training | 18 | 45 | 2 | 5 | 20 | 50 |
| Running/jogging | 10 | 25 | 0 | 0 | 10 | 25 |
| Indoor games | 12 | 30 | 16 | 40 | 28 | 28 |
| Football | 20 | 50 | 0 | 0 | 20 | 20 |
| Watching television | 22 | 55 | 18 | 45 | 40 | 100 |
| School sports | 10 | 25 | 4 | 10 | 14 | 35 |
| Others; specify | 0 | 0 | 0 | 0 | 0 | 0 |

Table 4: Z-score distribution of weight-for-age for Tamale children home (n = 40)

| | Parameter | | |
|---|---|---|---|
| Z-score | Percentiles | Number | Percentage (%) |
| ≤-2 | <5% | 4 | 10 |
| >-2<+2 | 5%-95% | 34 | 85 |
| ≥+2 | >95% | 2 | 5 |
| Total | | 40 | 100 |

Table 5: Z-score distribution of height-for-age for Tamale children home (n = 40)

| | Parameter | | |
|---|---|---|---|
| Z-score | Percentiles | Number | Percentage (%) |
| ≤-2 | <5% | 4 | 10.0 |
| >-2<+2 | 5%-95% | 31 | 77.5 |
| ≥+2 | >95% | 2 | 5.0 |
| Total | | 40 | 100.0 |

Table 6: Z-score distribution of BMI-for-age for Tamale children home children (n = 40)

| | Parameter | | |
|---|---|---|---|
| Z-score | Percentiles | Number | Percentage (%) |
| ≤-2 | <5% | 6 | 15 |
| >-2<+2 | 5%-95% | 32 | 80 |
| ≥+2 | >95% | 2 | 5 |
| Total | | 40 | 100 |

Nutrition Examination survey (National Centre for Health Statistics, WHO, 2002) reference Z-score.

Data in Table 6 indicate that 80% were of normal BMI-for-age and 15% were severely wasted (≤-2) Z-score. The data further suggest that probably 5% were at risk of overweight. These interpretations indicate that although the dietary pattern of the children was not good, the children were possibly not malnourished. The Z-scores on their own, do not give a full clinical picture and although in theory, it can be said a Z-score of -0.5, is within the normal range; the individual may have clinical signs, suggesting that they are worse than they appear (Ojo *et al*., 2000).

**Food consumption and dietary intakes of the orphanage**

**Macro- and micronutrient intakes of children:** A combination of dietary deficiencies mostly is the underlying cause of malnutrition, but acute infections may be a cause. Children are the population group mostly affected, therefore also needs most attention (FAO, 1998). Various interrelated factors are usually contributory to malnutrition, such as a marginal food supply as a result of rural poverty, income and rising prices, all at the expense of child care (Den Hartog *et al*., 1995). When improving diets, nutrition education should play a major role, but the actual practices, as well as the underlying economic and socio-cultural reasons, must be fully understood before attempts are made to modify feeding practices (Den Hartog *et al*., 1995). Children globally obtain their energy, macro-and micronutrients from a variety of sources. However, identifying these sources and comparing them to age groups, present difficulties, since food are sometimes classified in different ways (Jardine and Philpott, 1997). According to Lucas (2000) and Trahms (2000), children have very high energy and nutrient needs for normal body growth development and activity.

Findings of the nutrient intake, showed that mean levels of energy for the groups within the same age, were below the RDA whereas protein intake was higher than the RDA. Intake of fat and carbohydrates was low for all the age groups of, two to eighteen years. Although the children's total protein intake was sufficient, their energy intake was low.

In Ghana the deficiency of iron, vitamin A and iodine constitute a problem of major health concern. A study by Labadarios *et al*. (1999) reported that one out of two children had an intake of less than half of the recommendation for energy and a number of nutrients (calcium, iron, zinc, vitamins A, D, C and E); other nutrients were riboflavin, niacin and vitamin $B_6$). About 21.4% of pre-school children are anaemic and 33.3% of young children had a marginal vitamin A status (Ghana Demographic Health Survey, 2003). The results of this study showed that a low mean intake of micronutrients prevailed, including iron, zinc, calcium, niacin, riboflavin, thiamin, vitamin A and vitamin C, in all the age groups.

Vitamin D is needed for calcium absorption and for deposition of calcium in the bones. Because this nutrient is available from the action of sunlight on the subcutaneous tissue, the amount required from dietary sources, depends on non-dietary factors, such as geographical location and time spent outside, therefore children in Ghana may probably need no dietary vitamin D, which could be ascribed to adequate sunlight. Results of the present study indicated, that 85% of the orphanage children were engaged in outdoor games, which is a sign that children may not have a serious problem of vitamin D deficiency.

The inclusion of meat and dairy products, poultry, nuts, liver and green vegetables which are rich sources of vitamin $B_1$, $B_2$, $B_3$ and $B_{12}$ in the eight-day cycle menu, will increase the intake, although sources of the vitamin B group were among the top 20 frequently consumed food items; the quantities consumed were too small. These results suggested that the children might probably be at risk of vitamin B complex, Iron Deficiency Anaemia (IDA) and Vitamin A Deficiency (VAD) before the intervention. A similar result by Dannhauser *et al*. (2000) showed that pre-school children younger than 72 months of age, had a low median intake of micronutrient.

This study also indicated, that intakes of calcium, magnesium, zinc, ascorbic acid, vitamin D and vitamin E, were below the RDA's. This implies that these

children might probably be at risk of developing micronutrient deficiency disorders. A possible reason for these low intakes could be that of poor food procurement, lack of a planned menu for the orphanage and inadequate nutrition knowledge of caregivers. Lucas (2000) suggested, that poorly planned menus could affect adequate nutritional intake. Frank and Klass (1996) indicated, that growth failure observed in institutionalized children, did not necessarily reflect an insufficient quantity and quality of available food, but rather too few caregivers to ensure that the available food was fed to those too young to feed themselves, a lack of tactical stimulation and care during the planning of meals for infants, children and adolescents.

These findings of nutrient intakes could probably suggest that the children were at higher risk of micronutrient deficiencies.

In general, the mean levels of daily energy intake were below the RDA, whereas protein intake was higher than the RDA for all the ages, except for the 15-18 years males, whose protein intake was below the RDA. Most of the micronutrient intake was below the RDA's for all age groups.

Results in Table 7 show, that the dietary intake of children aged one to three years old, was deficient for all nutrients, except for protein and magnesium.

The data in Table 8 show, that the dietary intake of children aged seven to ten years old, was deficient for all nutrients, except protein, when compared to RDA's.

The data in Table 9 show that with the exception of protein, iron and magnesium, the intake of all the other nutrients were below the RDA for the 11-14 year old groups.

The data in Table 10 show that, the dietary intake of children aged 15-18 years, was deficient for all nutrients, except for protein and zinc, however, the protein intake for females show an intake below the RDA.

**Food consumption patterns:** The data in Table 11 indicate, that the 20 most frequently consumed food items by weight, were coffee (232 ml), tea (232 ml), maize meal (109 g), brown bread (77 g), rice (55 g), rice porridge (50 g), white sugar (49 g), squash (28 g) and custard (17 g). The purchasing patterns confirmed that very little vegetable and fruit were bought. Protein was present in the top 20, but the average portion sizes were very small.

Procurement and preparation and could probably also be ascribed to care giver influence.

**Major limitations of the study:** Due to the limitation of available literature the greater part of the literature survey focused on the nutritional requirement of children aged two and eighteen years, as well as on nutrition education programmes.

Table 7: Mean daily macro- and micronutrient intake of children 1-3 years old (n = 5)

| Nutrient | Mean | SD | RDA |
|---|---|---|---|
| Energy (kcal) | 963 | 109 | 1300 |
| Protein (g) | 33 | 4.1 | 16 |
| Fat (g) | 27 | 5.0 | |
| Carbohydrate (g) | 150 | 32 | |
| Fibre (g/day) | 7 | 2.8 | |
| Cholesterol (mg) | 62 | 17.0 | n/a |
| Added sugar (mg) | 27 | 6.4 | n/a |
| **Micronutrients** | | | |
| Calcium (mg/day) | 112 | 25.2 | 500 |
| Iron (mg) | 4 | 1 | 10 |
| Magnesium (mg/day) | 126 | 38 | 80 |
| Zinc (mg) | 4 | 1 | 10 |
| Vitamin A (ugRE) | 237 | 61.3 | 400 |
| Vitamin $B_1$ (mg/day) | 0.4 | 0.1 | 0.5 |
| Vitamin $B_2$ (mg/day) | 0.1 | 0.3 | 0.5 |
| Vitamin $B_3$ (mg/day) | 2 | 0.2 | 8 |
| Vitamin $B_{12}$ (ug/day) | 1 | 0.2 | 0.9 |
| Vitamin D (ug/day) | 0 | 0.2 | 5 |
| Vitamin C (mg) | 9 | 1.1 | 40 |
| Vitamin E (mg) | 4 | 1.09 | 6 |

RDA = Recommended Dietary Allowance

Table 8: Mean daily macro- and micronutrient intake of children 7-10 years old (n = 7)

| Nutrient | Mean | SD | RDA |
|---|---|---|---|
| Energy (kcal) | 1627.4 | 74 | 2000 |
| Protein (g) | 52.1 | 2.5 | 28 |
| Fat (g) | 33.9 | 3 | |
| Carbohydrate (g) | 284.3 | 22 | |
| Fibre (g/day) | 14.2 | 2 | |
| Cholesterol (mg) | 74.8 | 3.4 | n/a |
| Added sugar (mg) | 56.5 | 7 | n/a |
| **Micronutrient** | | | |
| Calcium (mg/day) | 167.8 | 12 | 1300 |
| Iron (mg) | 7.7 | 1 | 10 |
| Magnesium (mg/day) | 259.6 | 31.04 | 170 |
| Zinc (mg) | 7.2 | 1 | 10 |
| Vitamin A (ug RE) | 320.7 | 101.4 | 700 |
| Vitamin $B_1$ (mg/day) | 0.9 | 0.1 | 0.6-0.9 |
| Vitamin $B_2$ (mg/day) | 0.5 | 0.1 | 1.2 |
| Vitamin $B_3$ (mg/day) | 2 | 0.2 | 8 |
| Vitamin $B_{12}$ (ug/day) | 0.1 | 0.1 | 4 |
| Vitamin D (ug/day) | 0.6 | 0.4 | 50 |
| Vitamin C (mg) | 10.5 | 3.2 | 45 |
| Vitamin E (mg) | 5.3 | 1 | 7 |

RDA = Recommended Dietary Allowance

Understanding was a problem when communicating with the caregivers, as they were dagomba's while the researcher is from Wa and could not communicate in dagomba. English was thus the language of communication.

**The major findings of the study:** The salient findings of the theoretical and empirical studies are summarized as follows:

Facilities and living conditions were good, but food preparation facilities were old, inadequate and neglected. Although hygiene was of a high standard,

Table 9: Mean daily macro- and micronutrient intake of children 11-14 years old (n = 17)

| Nutrient | Mean | SD | RDA ♀ | RDA ♂ |
|---|---|---|---|---|
| Energy (kcal) | 1547.53 | 207.04 | 2500 | 2200 |
| Protein (g) | 50.6 | 7.2 | 45 | 46 |
| Fat (g) | 31.9 | 2.5 | | |
| Carbohydrate (g) | 269.1 | 43.2 | | |
| Fibre (g/day) | 12.5 | 2.4 | | |
| Cholesterol (mg) | 76.3 | 15 | n/a | n/a |
| Added sugar (mg) | 48.9 | 11.4 | n/a | n/a |
| **Micronutrient** | | | | |
| Calcium (mg/d) | 163.2 | 21 | 1200 | 1200 |
| Iron (mg) | 7.4 | 1.1 | 12 | 12 |
| Magnesium (mg) | 241.9 | 37.01 | 240 | 240 |
| Zinc (mg) | 7.1 | 1.1 | 15 | 15 |
| Vitamin A (ug RE) | 300.1 | 78 | 1000 | 800 |
| Vitamin $B_1$ (mg/d) | 0.8 | 0.1 | 1.3 | 1.1 |
| Vitamin $B_2$ (mg/d) | 0.5 | 0.1 | 1.5 | 1.3 |
| Vitamin $B_3$ (mg/day) | 5 | 0.2 | 12-16 | 12-14 |
| Vitamin $B_{12}$ (ug/d) | 1.1 | 0.2 | 5 | 5 |
| Vitamin D (ug/d) | 0.4 | 0.1 | 10 | 10 |
| Vitamin C (mg) | 9.7 | 2 | 50 | 50 |
| Vitamin E (mg) | 4.5 | 1 | 10 | 10 |

RDA = Recommended Dietary Allowance. ♀ Male ♂ Female

Table 10: Mean daily macro- and micronutrient intake of children 15-18 years old (n = 11)

| Nutrient | Mean | SD | RDA ♀ | RDA ♂ |
|---|---|---|---|---|
| Energy (kcal) | 1540.6 | 190.2 | 3000 | 2200 |
| Protein (g) | 49.3 | 7.3 | 59 | 44 |
| Fat (g) | 31.9 | 2.5 | | |
| Carbohydrate (g) | 296.1 | 53.2 | | |
| Fibre (g/day) | 12.5 | 2.4 | | |
| Cholesterol (mg) | 75.3 | 11 | n/a | n/a |
| Added sugar (mg) | 54.1 | 13 | n/a | n/a |
| **Micronutrient** | | | | |
| Calcium (mg) | 161.8 | 30 | 1200 | 1200 |
| Iron (mg) | 7.9 | 1.2 | 12 | 15 |
| Magnesium (mg/d) | 236.7 | 47.4 | 410 | 360 |
| Zinc (mg) | 6.9 | 1.2 | 15 | 12 |
| Vitamin A (ug RE) | 290.4 | 93 | 1000 | 800 |
| Vitamin $B_1$ (mg/d) | 0.8 | 0.1 | 1.5 | 1.1 |
| Vitamin $B_2$ (mg/d) | 0.5 | 0.1 | 1.8 | 1.3 |
| Vitamin $B_3$ (mg/d) | 6 | 0.2 | 16 | 14 |
| Vitamin $B_{12}$ (ug/d) | 1.2 | 0.1 | 5 | 5 |
| Vitamin D (ug/d) | 0.5 | 0.4 | 5 | 5 |
| Vitamin C (mg) | 10.3 | 3 | 60 | 60 |
| Vitamin E ($mg_{\alpha\text{-}TE}$)[d] | 4.6 | 1 | 10 | 8 |

RDA = Recommended Dietary Allowance ♀ male ♂ female

caregivers had insufficient food preparation skills, proper food handling practices and limited understanding of nutrition.

Demographic data indicated the majority of the children in this study (70%) were adolescents. Adolescence is one of the most challenging periods in human development. Spear (2000) indicated that this is a period of sudden growth alteration, accompanied by an increase in nutritional needs. However, the adolescent has been considered nutritionally vulnerable for several reasons and these include: an increased demand for nutrients related to increase in physical growth and development and the change of life-style and food habits which affect both their nutrient intake and needs. The nutritional implications are that at this stage, the adolescent will try anything that will make him/her look better or improve the body image. This could probably be a contributing factor to the present state of nutritional status (Spear, 2000).

Table 11: Top twenty most frequently consumed foods

| Rank | Food item | Mean±SD daily intake per 40 children (gram/ml) | Mean daily intake/child (gram/ml) |
|---|---|---|---|
| 1 | Coffee brewed instant | 9288±232.2 | 232 |
| 2 | Tea brewed | 9288±232.2 | 232 |
| 3 | Maize meal (TZ) | 4376±37.1 | 109 |
| 4 | Bread brown | 3060±82.7 | 77 |
| 5 | Rice, white cooked | 2213±55.3 | 55 |
| 6 | porridge | 1963±49.1 | 50 |
| 7 | Sugar, white granular | 1643±20.5 | 49 |
| 8 | Bra soup | 1618±40.5 | 40 |
| 9 | Cold drink fanta | 1144±190.7 | 28 |
| 10 | Custard, whole milk | 667±16.7 | 17 |
| 11 | fish, boiled | 623±15.6 | 16 |
| 12 | Maize, rice | 607±26.4 | 15 |
| 13 | Fish, smoked | 575±15.1 | 14 |
| 14 | Bread white | 573±15.1 | 14 |
| 15 | Beef on the bone | 452±11.3 | 11 |
| 16 | Beef, minced | 432±10.8 | 10 |
| 17 | Anchovies ("Keta school boys" | 336±8.4 | 8.4 |
| 18 | Peanut butter | 281±7.2 | 7.03 |
| 19 | spinach, cooked | 271±7.5 | 7 |
| 20 | Creamer, non-dairy | 247±8.5 | 6.1 |

The results of the nutritional status of children indicated, that although the dietary patterns of the children were not good, the children were not malnourished 10% were probably severely stunted and 15% were severely wasted with cut-off points of (<-2) Z-score. This can be partly linked to the poor planning of menus and purchasing procedures found in the orphanage. The study demonstrated that some of these children in institutional care had poor growth and development. Otien *et al.* (1999) confirmed these findings in respect of institutionalized children.

**Conclusion:** There is a large gap in the knowledge of the nutritional status and requirements of the orphanage children. Children growing up in orphanage are most vulnerable and disadvantaged members of the community, especially if measures to provide adequate food intakes in terms of macro- and micronutrients are not in place.

The findings indicated low intake of both macro-and micronutrients with the exception of protein. Nutritional status indicated that, 10% and 15% of the children were severely stunted and wasted respectively (≤-2) Z score.

The results of this study formed the basis for a nutrition education and training programme that was implemented in the orphanage.

The establishment of the nutritional status of the children, the contributing factors and the application of appropriate interventions, were very important, taking into consideration the environment of the orphanage, the administrators, caregivers and backgrounds of the children. Furthermore, the interventions would need to focus more on the caregivers, since studies by Martin and Conklin (1999), Williams and Worthington-Roberts (1996) showed, that nutrition knowledge, motivation and behaviour could significantly improve the quality of children's health.

**Recommendations:** Based on the results of this study, the following recommendations of the orphanage children are drawn:

This study serves as a source of reference for local authorities in the Tamale in their attempt to fashion programmes to help raise the standard of living for orphanage in the north. Similarly, the authorities should take steps, so as to be able to strengthen their initiatives to alleviate poverty, which is a social menace, encouraging the establishment of orphanages.

The caregivers of the orphanage had no knowledge on the issues related to child nutrition. Since the nutritional needs of the children rely on the caregivers, it is essential that caregivers should be enlightened on how to make sound food choices to meet nutritional needs and food habits. Therefore a nutrition education and training programme was recommended.

The orphanage should establish links with qualified public health nutrition professional that can provide screening, referral and counseling for nutrition and health-related problems for both the children and caregivers.

In future, the management of the orphanage should encourage research to improve the conditions in the orphanage.

## ACKNOWLEDGEMENT

The study was funded by the Department of Hotel Catering Institutional Management (HCIM) research budget.

## REFERENCES

Brink, H.I., 1999. Fundamentals of research methodology for health care professionals. 2nd Edn., South Africa: Juta.

Dannhauser, A., C. Bester, G. Joubert, P. Badenhorst, M. Slabber, A. Badenhorst, T. Du Toit, H. Barnard, P. Botha and I. Nogabe, 2000. Nutritional status of pre-school children in informal settlement near Bloemfontein, South Africa. Public Health Nutr., 3: 303-12.

De Onis, M., C. Monteiro, J. Akré and G. Clugston, 2002. The worldwide magnitude of protein-energy malnutrition: An overview from the WHO global database on child growth. Bull. World Health Organisation, 71: 703-712.

Den Hartog, A.P., W.A. Van Staveren and I.D. Brouwer, 1995. Manual for social surveys on food habits and consumption in developing countries, Weikersheim Germany: Margraf Verlag publishers.

Food and Agriculture Organisation (FAO), 1998. Energy and protein requirements. Newsweek, 97: 47 December.

Frank, D.A. and P.E. Klass, 1996. Infants and young children in orphanages: One view from paediatrics and child psychiatry. Paediatrics, 97: 569 April.

Ghana Demographic and Health survey, 2003. Ghana Stistical Service Accra, Ghana. Noguchi Memorial Institute For Medical Research Legon, Ghana. ORC Macro Calverton, Maryland, USA.

Gibson, R.S., 1990. Principles of nutritional assessment. New York: Oxford University press.

Grant, J.P., 1996. Seeking a brighter future for children. New York, UNICEF. USA Today magazine, 124: 44 March.

Hans de Ridder, J., 2002. Anthropometric workshop 8th November 2002. (Accreditation-October 1998 in Adelaide, Australia).

Henon, P., 1998. Malnutrition: An unrecognised danger. The Courier, 16: 46 January-February.

Jardine, N. and C. Philpott, 1997. Children's nutrition: drivers for change. In: Glen Smith Children's food; Marketing and innovation. 1st Edn., United Kingdom: Chapman and Hall Suffolk.

Kusin, J.A. and S. Karjati, 1994. Maternal and child nutrition in Madura, Indonesia. Amsterdam: Royal Tropical Institute (KIT).

Labadarios, D., N. Steyn, E. Maunder, U. Macintyre, R. Swart, G. Gericke, J. Huskisson, A. Dannhauser, H. Voster and A.E. Nesamvumi, 1999. The national food consumption survey (NFCS): children aged 1-9 years. South Africa.

Lindskog, U., P. Lindskog and M. Gebre-medhin, 1997. Food and nutrition security: caring for women and children. Wageningen, The Netherlands: Margraf Verlag.

Lucas, B., 2000. Nutrition in children. In: Krause's food, nutrition and diet therapy 10th Edn., Philadelphia: W.B Saundres.

Macintyre, U.E., 1998. Dietary intake of Africans in transition in North West Province. Ph. D Thesis, Potchefstroom: PU for CHE. Republic of South Africa.

Martin, J. and M.T. Conklin, 1999. Managing child nutrition programmes: Leadership for excellence. Gauthersburg Maryland USA: Aspen.

Ojo, O., R. Deane and P. Amuna, 2000. The use of anthropometric and clinical parameters for early identification and categorization of nutritional risk in pre-school children in Benin City, Nigeria. The J. Royal Soc. Promotion Health, 120: 230-235.

Otien, P.A., R.W. Nduati, R.N. Musoke and A.O. Wasunna, 1999. Growth and development of abandoned babies in institutional care in Nairobi. East African Med. J., 76: 430-5 August.

Rutengwe, R., W. Oldewage-theron, R. Oniang and H.H. Vorster, 2001. Co-existence of over nutrition and under nutrition related diseases in low income, high-burden countries: a contribution towards the 17th IUNS congress of nutrition Vienna Austria 2001. Afr. J. Food Nutr. Sci., 1: 34-35.

Spear, B.A., 2000. Nutrition in adolescence. In: Krause's food and nutrition and diet therapy. 10th Edn., Philadelphia: W.B. Saundres.

The South African Vitamin A Consultative Group (SAVACG), 1995. Children aged 6-71 months in South Africa, 1994. Their anthropometric, vitamin A. iron and immunisation coverage status. Isando; SAVACG.

Trahms, C.M., 2000. Nutrition in infancy. In: Krause's food, nutrition and diet therapy. 10th Edn., Philadephia: W.B Saundres.

United Nations Children's Fund (UNICEF), 1990a. Children and development in 1990's, A UNICEF sourcebook. The World Summit for children. New York, 29-30 September.

United Nations Children's Fund (UNICEF), 1990b. First call for children. New York.

Williams, S.R. and B.S. Worthington-Roberts, 1996. Nutrition throughout the life cycle. 2nd Edn., St Louis Baltimore Boston Chicago: Mosby.

World Health Organisation (WHO), 1995. MDIS Working paper No 2. Global prevalence of Vitamin A deficiency. Switzerland: WHO utrition Unit.

World Health Organisation (WHO), 1996. Child malnutrition. Fact sheet No 119 (Reviewed) November 1996. [Available on internet] http://www.who.int/inf-fs/en/fact119.htm [Date of access: 15/11/02].

World Health Organisation, (WHO) Programme of Nutrition, 2000. Global database on child growth and malnutrition. [Available on internet] http://www.who.int/nutgrowthdb/intro_text.htm [Date of access: 15/11/02].

World Health Organisation 2002. The Worldwide magnitude of protein energy malnutrition:An overview from the WHO global database on child growth(available on the internet) http://www.who.int/whosis/cgrowth/bulletin.htm.Date of access; 15/11/2002.

Pakistan Journal of Nutrition 9 (9): 853-855, 2010
ISSN 1680-5194


# Physico-chemical Properties of Commercial Local Beverages in Osun State, Nigeria

R.O. Adeleke and O.A. Abiodun
Department of Food Science and Technology, Osun State Polytechnic,
Iree, P.M.B. 301, Osun State, Nigeria

**Abstract:** This research work evaluates the physico-chemical properties of local beverages form, Osun State. Nigeria. The drinks were analyzed for $P^H$, titratable acidity, specific gravity, total soluble solids, ethanol content, total solids, total sugar, reducing sugars, non reducing sugar and ascorbic acid. From the analysis, the $P^H$ value ranged from 4.2-6.3, the titratable acidity ranged from 0.8-11.7. The highest specific gravity was in fura da nunu (1.3180) and the lowest in ogogoro (0.9897). Total soluble solid ranged from 0.3-10.7%. Ogogoro (distilled palmwine) had the highest percentage of alcohol content of 37.6%, burukutu (sorghum beer) had 4.6% and palm wine had 3.1% while nunu (fermented skim milk), omi wara (cheese whey), kunnu zaki (millet food drink), Adoyo (maize drink) and fura da nunu (fermented skim milk with millet dough) had lower values. Fura da nunu had highest total solid (21.8%) and total sugars (7.5%). These values were significant different ($p \leq 0.05$) from other samples. Adoyo had highest value of 32.0 mg/100 g ascorbic acid while ogogoro had the lowest value. The $P^H$ of ogogoro was nearer to neutrality compared with the other local drinks analyzed. Titratable acidity and ascorbic acid of zobo and pito were not feasible due to the products colour. The high alcoholic content of ogogoro, burukutu and palmwine signifies that the product can cause health problem such as obesity and can damage the organs in the body. Adoyo, fura da nunu, nunu and kunnu zaki are good source of ascorbic acid but diabetic patient may take them without sugar.

**Key words:** Kunnu zaki, zobo, adoyo, burukutu, fura da nunu, pito, Ogogoro, physico-chemical propertie

## INTRODUCTION

The major Nigerian local beverages are burukutu (sorghum beer), kunnu zaki (millet food drink), pito (fermented alcoholic beverage from sorghum or maize), palmwine, adoyo (ripe pineapple juice and supernant derived form ogi), ogogoro (distilled palm wine or local gin), nunu (fermented skim milk), fura da nunu (fermented skim milk with millet dough), zobo (extracts of calyx of *Hibiscus sabdariffa*) and omi wara (cheese whey).

Kunnu zaki is a traditional non-alcoholic fermented beverage widely consumed in the northern part of Nigeria (Obadina *et al*., 2008; Adeyemi and Umar, 1994). The beverage (milky cream appearance) is a millet based food drink which is consumed within few hours of its production. Kunnu is consumed mainly by people within the low and middle income workers who cannot afford industrially produced beverages like Coca-cola, Pepsi etc. (Obadina *et al*., 2008). Zobo drink (Sorrel, zoborodo) is an aqueous extracts of calyx of rosella and is a non alcoholic local beverage made from the reddish purple, acid-succulent calyces of the flower *Hibiscus Sabdariffa*. This flower is highly cultivated in the northern part of Nigeria because of the climate (Aliyu, 2000; Osueke and Ehirim, 2004). The calyces of *Hibiscus sabdariffa* have been found to be rich in vitamin and other anti-oxidants (Wong *et al*., 2002) and mineral (Babatunde *et al*., 2000). Burukutu (sorghum beer) and pito are popular fermented alcoholic drink among the people in the Northern part of Nigeria. Both are produced mainly from the grains of guinea corn (*Sorghum vulgare* and *Sorghum bicolor*).

Palmwine or toddy is an alcoholic beverage from the sap of various species of palm tree such as palmyra and coconut palm. This is commonly called “emu” and “oguro” in western part of Nigeria. Palm wine may be distilled to produce a strong drink “ogogoro” (local gin). Adoyo is produced from ripe pineapple juice and supernant derived from ogi (ogi is a fermented product, made from sorghum or maize) (Kolawole *et al*., 2007). Adoyo is a yellowish local drink from and does not undergo fermentation. It is very high in ascorbic acid. Omi wara is one of the local drinks in the Northern part of Nigeria and it is the water obtained from cheese. It is highly nutritious and serves the same advantage with liquid milk. Nunu is a fermented skim milk (Jideani *et al*., 1999) and clear white naturally fermented thick milk product with a sour taste and is commonly taken as afternoon meal in conjunction with millet cereal “Fura” (Akinyele *et al*., 1999). Fura da nunu is the mixture of both fura, a semi solid dumpling cereal meal (Jideani and Wedzicha, 1994) and nunu. The objective of this

**Corresponding Author:** O.A. Abiodun, Department of Food Science and Technology, Osun State Polytechnic, Iree, PMB 301, Osun State, Nigeria

Table 1: Physico-chemical analysis of local beverage in Osun State, Nigeria

| Product | $P^H$ | Titratable acidity | Specific gravity | Total soluble solid % | Ethanol content % | Total solids % | Total sugar | Reducing sugars % | Non-reducing sugars | Ascorbic acid (mg/100 g) |
|---|---|---|---|---|---|---|---|---|---|---|
| Zobo | 5.6b | - | 1.0224f | 6.2c | 0.1c | 8.6c | 5.6b | 0.8c | 4.8b | - |
| Ogogoro | 6.3a | 0.8e | 0.9897g | 0.3f | 37.6a | 1.2g | 0.0e | 0.0d | 0.0d | 0.0d |
| Burukutu | 4.8bc | 11.7a | 1.0812c | 6.1c | 4.6b | 7.9cd | 0.8e | 0.2d | 0.6d | 5.6cd |
| Kunnu zaki | 5.3b | 8.4b | 1.2110a | 5.5cd | 0.2c | 9.1c | 3.7c | 0.6c | 3.1c | 15.0b |
| Pito | 5.1bc | - | 1.0634d | 4.2d | 0.3c | 5.3e | 3.4c | 1.2b | 2.2c | - |
| Palmwine | 4.3c | 8.9b | 1.0387e | 2.7e | 3.1b | 3.4f | 1.9d | 1.3b | 0.6d | 8.4c |
| Fura da nunu | 5.2b | 10.1b | 1.3180a | 10.7a | 0.3c | 21.8a | 7.5a | 1.1b | 6.4a | 17.5b |
| Nunu | 5.6b | 7.5c | 1.1653b | 7.8b | 0.0c | 11.4b | 2.2d | 1.9a | 0.3d | 5.8cd |
| Omi wara | 5.8ab | 4.5d | 1.0712c | 1.2f | 0.0c | 2.9f | 0.2e | 0.1d | 0.1d | 2.5d |
| Adoyo | 4.2c | 9.4b | 1.0546d | 5.5cd | 0.2c | 6.8d | 2.9d | 2.0a | 0.9d | 32.0a |

Value with the same letter in the column are not significantly different ($p \leq 0.05$) from each other

work was to examine the physico-chemical properties of ten local drinks produced commercially in Osun State, Nigeria.

## MATERIALS AND METHODS

Ten Nigerian local beverages were bought at different locations at Ada, Ikirun and Osogbo, Osun State, Nigeria. The beverages were zobo, kunnu zaki, ogogoro, nunu, fura da nunu, omi wara, adoyo, palmwine, burukutu and pito. $P^H$ of the products was determined with a digital pH meter at ambient temperature. The method of AOAC (1990) were used for soluble solids, specific gravity, total soluble solid, titratable acidity, alcohol content, total sugar and ascorbic acid.

## RESULTS AND DISCUSSION

The result in Table 1 revealed that Adoyo and palmwine had lower $P^H$ values of 4.2 and 4.3 respectively. This showed that they are more acidic than the other local drinks evaluated. Burukutu had $P^H$ value of 4.8 which was higher than the values 3.9 and 4.2 reported for burukutu and pito respectively (Kolawole *et al*., 2007). Also, Igyor *et al*. (2006) recorded $P^H$ value range of 3.36-4.86 for burukutu and this was within the range of value obtained. The $P^H$ obtained for zobo was lower than the value (6.58) reported by Sowonola *et al*. (2005). Ogogoro had $P^H$ of 6.3 which showed that the acid content was very close to neutrality. There was no significant difference ($p \geq 0.05$) in the $P^H$ values of ogogoro and omi wara.

Burukutu had the highest value (11.7) in titratable acidity and the lowest value (0.8) was in ogogoro. Burukutu was significant difference ($p \leq 0.05$) different from other drinks while there were no significant difference ($p \geq 0.05$) in the values for kunnu zaki, palmwine, fura da nunu and Adoyo. Higher value in titratable acidity of burukutu may be due to the traditional methods of production which are non standardized in terms of raw materials, equipment and finished products quality and handling (Wonang and Opoefe, 1999). The amount of acid present in burukutu was higher than those present in the other drinks since total titratable acidity gives a measure of the amount of acid present in a particular product. The titratable acidity of zobo and pito cannot be quantified because the colour of the two makes determination unfeasible. Fura da nunu was significantly different ($p \leq 0.05$) from other local drinks in specific gravity. It had the highest value of 1.3180 while ogogoro had the least value of 0.9897.

Fura da nunu had highest value of 10.7% in total soluble solid followed by nunu, zobo and burukutu. There was no significant difference ($p \geq 0.05$) in the values of zobo, burukutu, kunnu zaki and Adoyo. Fura da nunu had the highest suspension of soluble solid when compared with the other drinks. Out of the ten local drinks examined, ogogoro had the highest alcoholic content (37.6%) while there were no traces of alcohol in nunu and omi wara. There were traces of alcohol in zobo, kunnu, pito, fura da nunu and Adoyo. Ababio (1990) reported the percentage alcohol ranging from 2-4% in burukutu, 2-8% in palmwine and 30-60% in ogogoro. Fura da nunu had higher total solids and total sugar content. These were significant different ($p \leq 0.05$) from others. There were no traces of sugar and ascorbic acid in ogogoro. Adoyo was rich in ascorbic acid (32.0 mg/100 g) while the values for fura da nunu and kunnu zaki were 17.5 mg/100 g and 15.0 mg/100 g respectively.

**Conclusion:** This study showed the physico-chemical properties of commercial local drinks in Osun State, Nigeria. Consumption of ogogoro should be avoided because ogogoro had higher alcoholic content which can be absorbed into the blood stream and affect the nervous system. Burukutu, pito and palmwine could be taken in small quantities while adoyo, kunnu zaki, fura da nunu, nunu, zobo could be taken in large quantities but the sugar content must be reduced especially for diabetic patient.

## REFERENCES

Ababio, O.Y., 1990. Organic Chemistry: New School Chemistry, Africana-Fep, publishers Limited, 1st Edn., pp: 378-380.

Adeyemi, I.A. and S. Umar, 1994. Effects of method of manufacture on quality characteristics of kunnu zaki, a millet-based beverage. Nig. Food J., 12: 34-41.

Akinyele, B.J., M.O. Fawole and F.A. Akinyosoye, 1999. Microorganisms associated with fresh cow milk, Wara and Nono, two local milk products hawked by Fulani women in Ilorin, Kwara State, Nigeria. Nig. Food J., 17: 11-17.

Aliyu, L., 2000. Rosella (*Hibiscus sabdariffa* L.). Production as affected by pruning and sowing date. Applied Agric. Technol., 6: 16-20.

AOAC, 1990. Official Method of Analysis. 15th Edn., Association of Official Analytical Chemists, Washington DC.

Babatunde, S.O., A.O. Babalola and O.C. Aworh, 2000. Compositional attributes of the calyces of rosella (*Hibiscus sabdariffa* L.). J. Food Technol. Africa, 6: 133-134.

Igyor, M.A., C.C. Ariahu and Y. Omale, 2006. Chemical and Microbiological characteristics of burukutu- A traditional Nigerian beer. Nig. Food J., 24: 86-91.

Jideani, V.A. and B.L. Wedzicha, 1994. Reaction of sorbic acid in millet and sorghum dough: Reaction with thiols. Food Additives Contam., 11: 539-548.

Jideani, V.A., L. Nkama, E.B. Agbo and I.A. Jideani, 1999. Prediction of changes in colour during storage of millet dough. Nig. Food J., 17: 41-51.

Kolawole, O.M., R.M.O. Kayode and B. Akinduyo, 2007. Proximate and microbial analyses of burukutu and pito produced in Ilorin, Nigeria. Afr. J. Biotechnol., 6: 587-590.

Obadina, A.O., O.B. Oyewole and T.M. Awojobi, 2008. Effect of steeping time of milled grains on the quality of Kunnu-Zaki (A Nigerian beverage). Afr. J. Food Sci., 2: 033-036.

Osueke, J.C. and F.N. Ehirim, 2004. Chemical, Nutritional and sensory analysis of zobo drink *(Var Sabdariffa)* and selected soft drinks. J. Agric. Food Sci., 2: 33-37.

Sowonola, O.A., T.Y. Tunde-Akintunde and F. Adedeji, 2005. Nutritional and sensory quality of soymilk-kunnu blends. Afr. J. Food Agric. Dev., 5: 1-12.

Wonang, D.L. and W.O. Opoefe, 1999. Effect of malting period on the fungal load, mycotoxin content of malted grains and alcoholic content of burukutu produced from grains in Jos. West Afr. J. Biol. Sci., 9: 97-107.

Wong, P., Y.H.M. Salmah and Y.B. Cheman, 2002. Physico-chemical characteristics of roselle (*Hibiscus sabdariffa*). Nutr. Food Sci., 32: 68-73.

Pakistan Journal of Nutrition 9 (9): 856-857, 2010
ISSN 1680-5194


# Nutritional and Physico-chemical Properties of *Bombax glabrum* Seeds

Adeleke Rafiu Olaposi and Abiodun Olufunmilola Adunni
Department of Food Science and Technology, Osun State Polytechnic,
P.M.B. 301, Iree, Osun State, Nigeria

**Abstract:** This study evaluates the proximate composition of *Bombax glabrum* seeds and the physico-chemical properties of the seeds oil. Protein and fat content of *Bombax glabrum* seeds were 10.23% and 58.23% respectively. Carbohydrate content of the seed was low (16.60%) when compared to other oil seeds like soybeans and groundnut. The relative density of the oil was 1.125. The value was above the recommended codex standard for edible vegetable oils. The refractive index for the *Bombax glabrum* seed oil was 0.628 which was lower than the standard value obtained for cotton seed oil (1.458-1.466) and groundnut oil (1.460-1.465). Saponification and iodine value obtained were 42.93 mgKOH/g and 3.38 Wij's. The unsaponifiable matter was 4.20 g/kg and was lower than the values reported for soybeans, cotton seeds etc. Acid value was 0.71 mgKOH/g. The peroxide value (3.64 meq/kg) was lower than the codex standard for edible vegetable oils (10 meq/kg). Moisture content and free fatty acid of the oil were low. The seed is a good source of protein and fat.

**Key words:** *Bombax glabrum*, seeds, proximate, physico-chemical

## INTRODUCTION

Seeds have nutritive and calorific values which make them necessary in diets. They are good sources of edible oils and fats. The amount of energy provided by 1 g of fat and oil when fully digested is more than twice as many joules as that by carbohydrates and proteins (Odoemeclam, 2005). Other sources of oil seeds are soybeans, cotton seed groundnut, sunflower, melon, rape seed, benni seeds (Frank, 1998). French nut (*Bombax glabrum*) seeds are also high in fat content and could be classified as oil seeds. They belong to family *Bombacaceae*. *Bombax glabrum* and *Bombcopsis glabra* are synonyms to each other. It is a medium sized tree and the fruit is a smooth, green capsule, 4-8 inches in length and splits opens naturally on longitudinal sutures when ripe. The fruit is sporadically grown throughout the tropics and subtropics and used as household plant in temperate regions. The family *Bombacaceae* has 28 general and 200 species. *Bombax glabrum* is used to make hot drink similar to hot chocolate by grinding the roasted seeds. It can also be used as flavourant. The young leaves are used as soup vegetable while the fruit pulp is made into drink. This fruit is classified as underutilized crop and there are limited research work done on this seeds. This study evaluates the proximate composition of the seeds and physico-chemical composition of the seed oil.

## MATERIALS AND METHODS

Matured fruits of *Bombax glabrum* were obtained on the farm at Iree, Osun State. Nigeria. The fruits were opened and the seeds were removed and analyzed. *Bombax glabrum* seeds samples were analyzed for proximate and physico-chemical properties using the methods described by AOAC (1990).

## RESULTS AND DISCUSSION

Proximate composition is shown in Table 1. Protein content of *Bombax glabrum* was 10.23%. This value was within the range reported for chest nut (Umran Erturk *et al*., 2006). Ragone (2006) reported protein content of 13.3-19.6% for breadnut seeds flour while protein content of 15.76% (dry basis) was recorded by Nwabueze (2006) for raw flour of African breadfruit (*Treculia Africana*). These values were higher than the values determined for *Bombax glarum* seed. Fat content of *Bombax glabrum* was 58.23%. The seeds are good sources of oil and this was higher than the fat contents of 6.2-29.0% and 11.45% recorded for breadnut seeds and African breadfruit by Ragone (2006) and Nwabueze (2006). Carbohydrate content of the seed was low (16.60%) when compared to other oil seeds like soybeans and groundnut.

Table 2 showed the physico-chemical properties of the *Bombax glabrum* seeds oil. The relative density of the oil was 1.125. The value was above the recommended codex standard for edible vegetable oils. The value obtained for cotton seed oil ranged from 0.918-0.917 while they are 0.925 and 0.918-0.923 for soya oil and sunflower oil respectively. The refractive index for the *Bombax glabrum* seed oil was 0.628 which was lower than the result standard value obtained for cotton seed oil (1.458-1.466) and groundnut oil (1.460-1.465). Saponification value obtained was 42.93 mgKOH/g and this was lower than the refined oils reported by

**Corresponding Author:** Abiodun, O.A., Department of Food Science and Technology, Osun State Polytechnic, P.M.B. 301, Iree, Osun State, Nigeria

Table 1: Proximate composition of *Bombax glabrum* seeds

| Parameter (%) | Mean value |
|---|---|
| Protein | 10.23±0.2 |
| Ash | 2.84±0.5 |
| Fat | 58.23±1.2 |
| Crude fibre | 1.25±2.3 |
| Moisture | 10.85±0.8 |
| Carbohydrate | 16.60±0.2 |

Table 2: Physico-chemical properties of *Bombax glabrum* oil

| Parameter | Mean value |
|---|---|
| Relative density | 1.125±0.7 |
| Refractive Index | 0.628±0.4 |
| Saponification (mgKOH/g) | 42.93±0.2 |
| Iodine value (Wij's) | 3.38±0.6 |
| Unsaponifiable matter (g/kg) | 4.20±0.3 |
| Acid value (mgKOH/g) | 0.71±0.6 |
| Peroxide value (meq/kg) | 3.64±0.8 |
| Moisture content (%) | 4.25±0.5 |
| Free fatty acid (%) | 0.05±0.2 |

Kirk and Sawyer (1999). The iodine value obtained was 3.38 Wij's. This was lower than the values reported for soybeans, sunflower oil, cotton seeds oil and groundnut. The lower iodine value signifies low degree of unsaturation and the lesser the liability of the oil to become rancid by oxidation.

**Oil:** The unsaponifiable matter was 4.20 g/kg and was lower than the values reported for soybeans, cotton seeds etc. Acid value was 0.71 mgKOH/g. Acid value for non virgin oil, soybeans oil and cotton seed oil was 0.6. The value obtained was slightly higher than this value. Acid value is the measure of the extent to which the glycerides in oil have been decomposed by lipase or other action (Ihekoronye and Ngoddy, 1985). Peroxide value of *Bombax glabrum* oil was 3.64 meq/kg. This value was within the maximum recommended codex standard for edible vegetable oils (10 meq/kg). Peroxide value of 9.7-11.6 Meq/kg were determined for the melon seeds oils while 19.54 m mol/g was reported for melon seeds oil by Ebuehi and Avwobobe (2006). Unlike acid value, peroxide value is an indicator of deterioration of fats. Moisture content of the oil was low (4.25%) The free fatty acid of the oil was low (0.05%). This indicates the stability of the products.

**Conclusion:** *Bombax glabrum* seeds are rich in protein and fat contents. The seed could be used to enrich food products. Also the physicochemical properties showed that the oil are stable and could be used in industries for production of soap and other products.

## REFERENCES

AOAC, 1990. 15th Official methods of Analysis. Association Official Analysis Chemists, Washington DC.

Ebuehi, O.A. and O.K. Avwobobe, 2006. Physico-chemical and fatty acid composition of water melon (*Citrillus lanatus*) and melon (*Colocynthis citrillus*) seed oils. Nig. Food J., 42: 25-33.

Frank, T.O., 1998. Vegetable oil. Bailey's Industrial Oil and Fat Product, 1: 19-44.

Ihekoronye, A.I. and P.O. Ngoddy 1985. Food lipids. Integrated food science and technology for the tropics. Macmillian publishers, pp: 58-85.

Kirk, S.R. and R. Sawyer, 1999. Pearson's composition and analysis of foods. 9th Edn., Longman, UK, pp: 617-620.

Nwabueze, T.U., 2006. Effect of hydration and screw speed on the nutrient and acceptability of extruded ready-to-eat African breadfruit (*Treculia Africana*) snack. Nig. Food J., 24: 107-113.

Odoemeclam, S.A., 2005. Proximate composition and selected physicochemical properties of seeds of African oil bean (*Pentaclethra macrophylla*). Pak. J. Nutr., 4: 382-383.

Ragone, D., 2006. *Artocarpus camansi* (Breadnut), ver.2.1. In: Elevitch, C.R. (ed). Species Profiles for Pacific Island Agroforestry. Permanent Agriculture Resources (PAR). Holualoa, Haiwai'i, pp: 1-11.

Umran Erturk, Cevriye Mert and Arif Soylu, 2006. Chemical composition of fruits of some important chestnut cultivars. Brazilian Arch. Biol. Technol., 49: 183-188.

Pakistan Journal of Nutrition 9 (9): 858-860, 2010
ISSN 1680-5194


# Chemical Composition of Three Traditional Vegetables in Nigeria

Adeleke Rafiu Olaposi and Abiodun Olufunmilola Adunni
Department of Food Science and Technology, Osun State Polytechnic, Iree, Osun State, Nigeria

**Abstract:** This work was carried out to evaluate the nutritional composition of three traditional vegetables in Iree, Osun State. The leafy vegetables used were *Cnidoscolus chayamansa* (iyana ipaja), *Solanium nodiflorum* (Ogumo), and *Senecio biafrae* (worowo). The vegetables were washed in potable water to remove unwanted matters and were analyzed for proximate and mineral All analyses were carried out in three replicates and the data were evaluated for significant differences in their means with Analysis of Variance (ANOVA) ($p \leq 0.05$). *Cnidoscolus chayamansa* had higher protein content (5.91%) and carbohydrate content (8.88%) but there was no significant difference ($p \geq 0.05$) in the crude fibre value and that of *Senecio biafrae*. *Senecio biafrae* had higher moisture content (89%) while *Solanium nodiflorum* had higher ash and fat content which were significantly different ($p \leq 0.05$) from the other vegetables. *Cnidoscolus chayamansa* had higher values in all the mineral contents determined and these were significantly different ($p \leq 0.05$) from other vegetable. There were no significant difference ($p \geq 0.05$) in potassium, calcium and iron contents of *Solanium nodiflorum* and *Senecio biafrae*. The three vegetables are good sources of nutrients which could be consumed for normal growth.

**Key words:** Traditional vegetable, *Cnidoscolus chayamansa*, *Solanium nodiflorum*, Senecio biafrae

## INTRODUCTION

Leafy vegetables are important items of diet in many Nigerian homes and they are valuable sources of nutrients especially in rural areas where they contribute substantially to protein, mineral, vitamins, fiber and other nutrients which are usually in short supply in daily diets (Mosha and Gaga, 1999). They have the cheapest and most abundant sources of protein (Fasuyi, 2006) and add flavor, variety, taste, color and aesthetic appeal to diet (Mepba *et al*., 2002). In Nigeria and many Africa countries of the tropics; vegetables are very abundant immediately after the rains but becomes scarce late in rainy season and more so in dry season (Ihekoronye and Ngoddy, 1985). Among the traditional vegetables in Nigeria are *Solanium nigrum*, *Solanium nodiflorum*, *Senecio biafrae*, *Crassocephanum crepidiodes*, *Talinium triangulare Celosia orgentea* and *Vemonia amygdalia*. Most of these vegetables are consumed in the rural areas or in the communities where they are being planted. They are underutilized when compared to the introduced varieties due to the flavour and unfamiliar taste impacted on the food (Okeno *et al*., 2003; Orech *et al*., 2005; Smith and Eyzaguirre, 2007). Scarcity of vegetable in the diet is a major cause of vitamin A deficiency, which causes blindness and even death in young children throughout the Arid and Semi-Arid areas of Africa (Okigbo, 1986). African leafy vegetables play a highly significant role in food security of the underprivileged in both urban and rural setting and are also vital for income generation (Orech *et al*., 2005). The objective of this work was to evaluate the nutritional composition of three traditional vegetables in Nigeria.

## MATERIALS AND METHODS

Three traditional vegetables (*Cnidoscolus chayamansa*, *Solanium nodiflorum* and *Senecio biafrae*) were collected fresh from the farm at Iree, Osun State. The vegetables were washed in potable water to remove unwanted matters and were analyzed for proximate and mineral composition. The method of AOAC (1990) was used for the proximate analysis while the method of Novozamsky *et al*. (1983) was used for mineral determination. All analyses were carried out in three replicates and the data were evaluated for significant differences in their means with Analysis of Variance (ANOVA) ($p \leq 0.05$). Differences between the means were separated using turkey's test as packaged by SPSS 11.0 software.

## RESULTS AND DISCUSSION

The results of proximate compositions of the vegetables are shown in Table 1. Protein content ranged from 3.03-5.91%. *Cnidoscolus chayamansa* had higher protein content (5.91%) which was significantly different ($p \leq 0.05$) from the other two samples. Crude protein contents ranging from 27.17-28.93% (dry basis) was recorded by Dairo and Adanlawo (2007) for *Senecio biafrae* and *Crassophacelus crediode* vegetables. Kuti and Torres (1996) also observed protein content of 5.71% (wet basis) for *Cnidoscolus chayamansa* vegetable and 11.6-12.3% (dry basis) for two varieties of *Senecio biafrae* vegetables (Adebooye, 2000). Fasuyi (2006) reported crude protein (19.9-35.1 g/kg), crude

**Corresponding Author:** Abiodun Olufunmilola Adunni, Department of Food Science and Technology, Osun State Polytechnic, Iree, Osun State, Nigeria

Table 1: Proximate composition (wet basis) of three traditional vegetables in Nigeria

| Composition (%) | *Cnidoscolus chayamansa* | *Solanium nodiflorum* | *Senecio biafrae* |
|---|---|---|---|
| Protein | 5.91a | 3.31b | 3.03c |
| Moisture content | 82.00c | 85.00b | 89.00a |
| Ash content | 1.57c | 2.67a | 2.01b |
| Crude fibre | 0.92a | 0.78b | 0.92a |
| Fat content | 0.72b | 0.87a | 0.61c |
| Carbohydrate | 8.88a | 7.37b | 4.43c |

Mean values followed by the same letter down the column were not significantly different ($p \leq 0.05$)

Table 2: Mineral composition of three leafy vegetables in Nigeria

| Mineral content | *Cnidoscolus chayamansa* | *Solanium nodiflorum* | *Senecio biafrae* |
|---|---|---|---|
| K % | 4.02a | 0.19b | 0.18b |
| Ca % | 2.76a | 0.41b | 0.38b |
| Mg % | 1.11a | 0.33b | 0.25c |
| Na ppm | 116.26a | 28.43b | 26.08c |
| Fe ppm | 21.06a | 16.38b | 16.03b |
| Mn ppm | 19.32a | 11.63b | 11.11c |
| Zn ppm | 8.53a | 4.88b | 3.95c |
| Cu ppm | 0.78a | 0.55b | 0.45c |

Mean values followed by the same letter down the column were not significantly different ($p \leq 0.05$)

fibre (8.8-12.7 g/kg), ether extract (fat) (5.4-29.2 g/kg) and ash contents of 10.9-19.4 g/kg on dry basis for three vegetable species (*Talium triangulare, Amaranthus cruentus and Telfairia occidentalis*). Carbohydrate content ranged from 4.43-8.88%. *Cnidoscolus chayamansa* had higher value while the least value was in *Senecio biafrae* but there were no significant differences ($p \leq 0.05$) in the crude fibre value of *Cnidoscolus chayamansa* and that of *Senecio biafrae*. Crude fibre content of 1.9% was recorded for *Cnidoscolus chayamansa* (Kuti and Torres, 1996), the value obtained was lower than this value. This may be due to the location, varieties, maturity of the vegetable and the cultural practices adopted during planting. *Senecio biafrae* had higher moisture content of 89% and the least value was in *Cnidoscolus chayamansa*. Higher moisture content of *Senecio biafrae* makes the vegetable easily susceptible to deterioration. *Solanium nodiflorum* had higher ash and fat content which were significantly different ($p \leq 0.05$) from the other vegetables. The mineral compositions are shown in Table 2. In all the mineral content analyzed, *Cnidoscolus chayamansa* had higher values which were significantly different ($p \leq 0.05$) from other vegetable. This agrees with the findings of Kuti and Torres (1996) and Booth *et al.* (1992) that chaya leaf have high mineral contents. There were no significant difference ($p \leq 0.05$) in potassium, calcium and iron contents of *Solanium nodiflorum* and *Senecio biafrae*. *Senecio biafrae* had lower values in Mg, Na, Mn, Zn and Cu. The three vegetables are good sources of nutrients which could be consumed for normal growth.

**Conclusion:** This study evaluates the chemical compositions of three vegetables in Nigeria. The three vegetables examined contain appreciable amount nutrients and are good source of roughages.

## REFERENCES


Adebooye, O.C., 2000. An assessment of cultural practices for cultivating a wild but edible leaf vegetable: Crassocephalum biafrae (Asteraceae): Emphasis on propagation technique. In: Proceedings of the third Workshop on the Sustainable Use of Medicinal and Food Plants. September 15-17, University of Karachi, Pakistan, pp: 132-138.

AOAC, 1990. 15th Official methods of Analysis. Association Official Analysis Chemists, Washington DC.

Booth, S.R., R. Bressani and T. Johns, 1992. Nutrient contentn of selected indigenouu leafy vegetable consumed by Kekchi people of Alta Verapaz, Guatamela. J. Food Composition Anal., 5: 25-34.

Dairo, F.A.S. and I.G. Adanlawo, 2007. Nutritional quality of *Crassocephalum crepidiodes* and *Senecio biafrae*. Pak. J. Nutr., 6: 35-39.

Fasuyi, O.A., 2006. Nutritional potentials of some tropical vegetable leaf meals: Chemical characterization and functional properties. Afr. J. Biotechnol., 5: 49-53.

Ihekoronye, A.I. and P.O. Ngoddy, 1985. Tropical fruits and vegetables. Integrated food science and technology for the tropics. Macmillian publishers, pp: 293-311.

Kuti, J.O. and E.S. Torres, 1996. Potential nutritional and health benefits of tree spinach. In: J. Janick (Ed.) Progress in new crops. ASHS press, Arlington, VA, pp: 516-520.

Mepba, H.D., L. Eboh and D.E.B. Banigo, 2002. Effects of processing treatments on the nutritive composition and consumer acceptance of some Nigerian edible leafy vegetables.

Mosha, T.C. and H.E. Gaga, 1999. Nutritive value and effect of blanching on trypsin and chymotrypsin inhibitor activities of selected leafy vegetables. Plant Foods Human Nutr., 54: 271-283.

Novozamsky, I., V.J.G. Houba, E.C.K. Van and V.W. Van, 1983. Plant nitrogen and phosphorus in plant tissue, novel digestion technique for multi-element. Plant Anal. Commun. Soil Sci. Plant Anal., 14: 239-248.

Okigbo, B.N., 1986. Broadening the food base in Africa. The potential of traditional food plants. Food Nutr., 12: 4-17.

Orech, F.O., T. Akenga, J. Ochora, H. Friis and Aagaard-Hansen, 2005. Potential toxicity of some traditional leafy vegetables consumed in Nyang'oma Division, Western Kenya. Afr. J. Food Agric. Nutr. Dev. Online, 5(1).

Okeno, J.A., D.K. Chebet and P.W. Mathenge, 2003. Status of indigenous vegetables in Kenya. Acta Hort., pp: 621: 9.

Smith, F.I. and P. Eyzaguirre, 2007. African leafy vegetables: Their role in the world health organization's global fruit and vegetables initiatives. Afr. J. Food Agric. Nutr. Dev. Online, 7: 1-9.

Pakistan Journal of Nutrition 9 (9): 861-864, 2010
ISSN 1680-5194


# Plasma Copper and Zinc in Pregnancy Complicated with Diabetes Mellitus

Emmanuel I. Ugwuja[1], Emmanuel I. Akubugwo[2] and Boniface N. Ejikeme[3]
[1]Department of Chemical Pathology, Faculty of Clinical Medicine,
Ebonyi State University, P.M.B. 053, Abakaliki, Nigeria
[2]Department of Biochemistry, Abia State University, Uturu, Nigeria
[3]Department of Obstetrics and Gynaecology, Faculty of Clinical Medicine,
Ebonyi State University, Abakaliki, P.M.B. 053, Abakaliki, Nigeria

**Abstract:** To determine the effect of hyperglycaemia on plasma copper and zinc in pregnancy complicated with diabetes mellitus, data for 40 diabetic and 40 non-diabetic pregnant women, matched for age, gestational age, Body Mass Index (BMI), parity and socioeconomic status from a cohort of 349 pregnant women recruited at gestational age of ≤25 weeks for the assessment of impacts of trace elements on pregnancy outcomes were analyzed. In addition to plasma copper and zinc which were determined by Atomic Absorption Spectrophotometer (Buck Scientific, Model AVG 210), plasma albumin, glucose, haemoglobin concentration and Total White Blood Cell Count (TWBC) were determined using standard laboratory methods. Although diabetic and non-diabetic pregnant women had comparable ($p>0.05$) age, gestational age, BMI and plasma copper, the former had significantly ($p<0.05$) lower plasma zinc (16.49±4.74 vs. 25.31±7.07 µmol/l) with significantly higher plasma glucose concentration (13.19±1.81 vs. 6.23±1.12 mmol/l). The diabetic subjects also had significantly ($p<0.05$) higher plasma albumin and TWBC when compared to their control counterparts (3.41±0.85 vs. 2.92±0.79 g/dl and 5.72±1.75 vs. 5.10±1.33 x $10^9$/L respectively), although these were within the reference ranges. Correlation analysis showed that plasma glucose was negatively correlated with plasma zinc concentration ($r = -0.239$; $p = 0.051$). It is therefore concluded that hyperglycaemia in pregnancy complicated with diabetes mellitus impacts negatively on plasma zinc status, but lacks effect on plasma copper. This has important health implications for diabetic pregnant women and their newborns.

**Key words:** Gestational diabetes mellitus, hyperglycaemia, hypozincuria, metallothionine, antioxidant enzymes, insulin resistance

## INTRODUCTION

Both zinc and copper are essential trace elements with wide range of functions in the body including the synthesis of enzymes and nucleic acids (WHO, 1996) and are involved in the functions of several cuproenzymes that are essential for life (Goel and Misra, 1982; Raman and Leela, 1992). Studies have shown that lower consumption of dietary zinc and low serum zinc levels were associated with increased prevalence of Coronary Artery Disease (CAD), diabetes as well as other risk factors including hypertension, hypertriglyceridaemia and other factors suggestive of insulin resistance (Singh *et al*., 1998). In one study (Simon and Taylor, 2001), zinc supplementation was found to attenuate hyperglycaemia and hyperinsulinaemia in *db/db* mice, suggesting a role for zinc in pancreatic function and peripheral tissue glucose uptake. In developing countries where diet is monotonous and deficient in trace elements, pregnant women are at increased risk of copper and zinc deficiencies. This is aggravated by increased demand for these trace elements to meet both the maternal and foetal needs. Deficiencies of copper and zinc have been implicated in various reproductive events like infertility, pregnancy wastage, congenital abnormalities (Black, 2001), pregnancy induced hypertension, placental abruption, premature rupture of membranes, still birth and low birth weight (Pathak and Kapil, 2004). Studies of micronutrients (including vitamins and minerals) in diabetes have consistently documented conflicting reports (O'Connell, 2001; Bo *et al*., 2008; Hussain *et al*., 2009) and data is scarce on the study of copper and zinc metabolism in pregnancy complicated with diabetes mellitus. The aim of the present study is to determine the plasma levels of copper and zinc in pregnancy complicated with diabetes mellitus.

## MATERIALS AND METHODS

This study which was part of a larger study that investigated the impact of plasma copper and zinc

**Corresponding Author:** Emmanuel I. Ugwuja, Department of Chemical Pathology, Faculty of Clinical Medicine, Ebonyi State University, P.M.B. 053, Abakaliki, Nigeria

status on pregnancy outcomes was carried out among pregnant women attending antenatal clinic of the Department of Obstetrics and Gynaecology of the Federal Medical Centre, Abakaliki, one of the referral tertiary health institutions in the South eastern part of Nigeria. Subjects' selection and detailed methodology has been previously described (Ugwuja *et al*., 2010). Data for forty diabetic and forty non-diabetic pregnant women matched for age, gestational age, parity, anthropometrics and socioeconomic status were analysed. Plasma glucose was determined by glucose oxidase method as previously described (Barham and Trinder, 1972), plasma albumin was determined by bromocresol green as previously described (Hill, 1985), haemoglobin concentration was determined by Cyanmethaemoglobin method while total white blood cell count was estimated as in a standard haematology textbook (Dacie and Lewis, 1994).

**Data analysis:** Data were analysed for mean and standard deviation while comparison between subjects and controls were analysed using Student's t-test with statistical significance set at $p<0.05$.

## RESULTS

From Table 1, the diabetics and non-diabetics pregnant women had comparable ($p>0.05$) age, gestational age, body mass index and plasma copper. However, the diabetic pregnant women had significantly ($p<0.05$) lower plasma zinc (16.49±4.74 vs. 25.31±7.07 mmol/l) with significantly higher plasma glucose concentration (13.19±1.81 vs. 6. 23±1.12 mmol/l). The diabetic subjects also had significantly ($p<0.05$) higher plasma albumin and total white blood cell count when compared to their control counterparts (3.41±0.85 vs. 2.92±0.79 g/dl and 5.72±1.75 vs. 5.10±1.33 x $10^9$/L respectively), although these were still within the reference ranges. Correlation analysis showed that plasma glucose was negatively correlated with plasma zinc concentration ($r = -0.239$; $p = 0.051$).

Table 1: Comparison of biochemical and haematological parameters between diabetic and non-diabetic pregnant women

| Parameters | Non-diabetics (n = 40) | Diabetics (n = 40) | p-values |
|---|---|---|---|
| Age (yrs) | 27.80±4.59 | 27.18±4.22 | 0.890 |
| Gestational age (wks) | 21.60±3.79 | 21.68±3.58 | 0.112 |
| BMI (Kg/m$^2$) | 28.13±4.14 | 27.09±3.63 | 0.233 |
| HBC (g/dl) | 10.32±1.28 | 10.31±1.50 | 0.861 |
| TWBC (x$^9$/L) | 5.10±1.33 | 5.72±1.75 | 0.035* |
| Albumin (g/dl) | 2.92±0.79 | 3.41±0.85 | 0.010* |
| Glucose (mmol/L) | 6.23±1.12 | 13.19±1.81 | 0.021* |
| Copper (µmol/L) | 9.42±8.33 | 9.19±9.17 | 0.809 |
| Zinc (µmol/L) | 25.31±7.07 | 16.49±4.74 | 0.054* |

*$p<0.05$ is considered statistically significant

## DISCUSSION

This study has shown that while plasma zinc concentration was significantly lower in diabetic pregnant women in comparison to their non diabetic counterparts, the level of copper was comparable between the two groups. We have previously reported significantly higher prevalence of Diabetes Mellitus (D/M) in zinc-deficient than in zinc-adequate pregnant women (Ugwuja *et al*., 2010). Again, decreased serum zinc had been reported in type I diabetes mellitus (Isbir *et al*., 1994; Garg *et al*., 1994). Hyperglycaemia from either type 1 or 2 DM has been found to cause physiologically important loss of zinc from the body via the urine by interference with active transport of zinc into the renal tubular cells (Chausmer, 1998). Although study has suggested that tissue and plasma zinc loss via urine may be ameliorated by a compensatory increase in gastrointestinal absorption of zinc (Chausmer, 1998), down regulation of zinc transport due to increased production of metallothionine (MT), a cation binding protein with high affinity for zinc has been reported in diabetes mellitus (Escobar *et al*., 1995). However, abnormality in zinc metabolism in diabetes cannot be solely attributed to hyperzincuria (Cunningham *et al*., 1994) as zinc may be lost from cells as glucose is translocated into the muscle (Cordova, 1994). Although results from micronutrients studies in diabetes mellitus have been conflicting (O'Connel, 2001; Bo *et al*., 2008; Hussain *et al*., 2009) and data is scarce on the study of zinc metabolism during pregnancy complicated with diabetes mellitus, the present finding suggests that zinc plays an important role in the pathogenesis of diabetes mellitus in pregnant women. Studies have suggested roles for zinc in the aetiology and progression of diabetes mellitus (Sumovski *et al*., 1992; Chausmer, 1998; Spreitsma and Schuitemaker, 1994; Faure *et al*., 1995). For example, *in vitro* studies have found that zinc enhances the effectiveness of insulin in non-insulin dependent diabetes mellitus (Arquilla *et al*., 1978). Again, the development of glucose intolerance in animals deprived of zinc together with the occurrence of zinc deficiency in type 2 diabetes mellitus suggests a role for zinc deficiency in the pathogenesis of gestational DM. Zinc is required for the assemblage of insulin into structurally stable and functional hexamic structure (Brange and Langkjoer, 1993). Also, video fluorescence analysis has shown that zinc concentrated in the islet cells was related to the synthesis, storage and secretion of insulin (Zalewski *et al*., 1994). Additionally, zinc is a cofactor of antioxidant enzymes, Superoxide Dismutase (SOD) and catalase that are involved in the protection of pancreatic beta cells from reactive oxygen species (Chausmer, 1998). Hence in zinc deficiency, the synthesis, secretion and functions of insulin may be

affected. The significantly negative correlation between plasma glucose and zinc in the present study corroborates earlier studies (El-Yazigi *et al*., 1993; Williams *et al*., 1995). Decreased plasma zinc in diabetic subjects has implications for pregnant women with diabetes mellitus. Zinc is an essential trace element with diverse cellular functions, especially in rapidly dividing cells as that of the foetus (WHO, 1996). Therefore, it is speculated that pregnancy complications such as macrosomia, congenital foetal abnormalities spontaneous abortion and miscarriage associated with gestational diabetes mellitus may be mediated at least in part through zinc deficiency. Also, diabetic complications such as dehydration and susceptibility to infections may be exacerbated by zinc deficiency as evidenced by significantly ($p<0.05$) higher plasma albumin (a measure of dehydration) and total white blood cell count (index for infection), although the two parameters were still within the reference ranges in the present study. We therefore concluded that hyperglycaemia of gestational diabetes mellitus is associated with reduced plasma zinc without effect on plasma copper. This has important health implications both for the diabetic pregnant mothers and their newborns.

## REFERENCES


Arquilla, E.R., S. Packer, W. Tarmas and S. Miyamoto, 1978. The effect of zinc on insulin metabolism. Epidemiology, 103: 1440-1449.

Barham, D. and P. Trinder, 1972. An improved colour reagent for the determination of blood glucose by oxidase system. Analyst, 27: 142-145.

Black, R.E., 2001. Micronutrients in pregnancy. Br. J. Nutr., 85 (Suppl. 2): S193-7.

Bo, S., M. Durazzo, R. Gambino, C. Berutti, N. Milanesio, A. Caropreso, L. Gentile, M. Cassader, P. Cavello-Perin and G. Pagano, 2008. Associations of dietary and serum copper with inflammation, oxidative stress and metabolic variables in adults. J. Nutr., 138: 305-310.

Brange, J. and L. Langkjoer, 1993. Insulin structure and stability. Pharm Biotechnol., 5: 315-350.

Chausmer, A.B., 1998. Zinc, insulin and diabetes. J. Am. Coll. Nutr., 17: 109-115.

Cordova, A., 1994. Zn content in selected tissues in streptozotocin diabetic rats after maximal exercise. Biol. Trace Elem. Res., 42: 209-215.

Cunningham, J., A. Fu, P. Mearkle and R. Brown, 1994. Hyperzincuria in individuals with insulin dependent diabetes mellitus: concurrent zinc status and the effect of high dose zinc supplementation. Metabolism, 43: 1558-1562.

Dacie, J.V. and S.M. Lewis, 1994. Practical Haematology, 8th Edn., Churchill Livingstone, Edinburg, pp: 49-59.

El-Yazigi, A., N. Hannan and D. Raines, 1993. Effect of diabetic state and related disorders on the urinary excretion of magnesium and zinc in patients. Diabetes Res., 22: 67-75.

Escobar, O., M. Sandoval, A. Vargas and J. Hempe, 1995. Role oof metallothionine and cysteine rich intestinal protein in the regulation of Zn absorption by diabetic rats. Paed Res., 37: 321-327.

Faure, P., H.P. Ben, A. Perard, S. Halimi and A. Roussel, 1995. Lipid peroxidation in insulin dependent diabetic patients with early retinal degenerative lesions: Effects of an oral Zn supplementation. Eur. J. Clin. Nutr., 49: 282-288.

Garg, V., R. Gupta and R. Goal, 1994. Hypozincaemia in diabetes mellitus. J .A.P.I., 42: 720-721.

Goel, R. and P.K. Misra, 1982. Study of plasma zinc in neonates and their mothers. In. Paediatrics, 19: 611-614.

Hill, P.G., 1985. The measurement of albumin in serum and plasma. Ann. Clin. Biochem., 22: 565-578.

Hussain, F., M.A. Maan, M.A. Sheikh, H. Nawaz and A. Jamil, 2009. Trace elements status in type 2 diabetes. Bangladesh J. Med. Sci., Vol. 8.

Isbir, T., A. Tamer, A. Taylor and M. Isbir, 1994. Zinc, copper and magnesium status in insulin dependent diabetes. Diabetes Res., 26: 41-45.

O'Connel, B.S., 2001. Selected vitamins and minerals in the management of diabetes. Diabetes Spectrum, 14: 133-148.

Pathak, P. and U. Kapil, 2004. Role of trace elements zinc, copper and magnesium during pregnancy and its outcome. In. Paediatrics, 71: 1003-1005.

Raman, L. and J. Leela, 1992. Role of maternal nutrition. In: PIH Annual report, National Institute of Nutrition, 93: 47-50.

Simon, S.F. and C.A. Taylor, 2001. Dietary zinc supplementation attenuates hyperglycaemia in *db/db* mice. E.B.M., 226: 43-51.

Singh, R.B., M.A. Niaz, S.S. Rastogi, S. Bajaj, Z. Gaoli and Z. Shoumin, 1998. Current zinc intake and risk of diabetes and coronary artery disease and factors associated with insulin resistance in rural and urban populations of North India. J. Am. Coll. Nutr., 17: 564-570.

Spreitsma, J. and G. Schuitemaker, 1994. Diabetes can be prevented by reducing insulin production. Med. Hypotheses, 42: 15-23.

Sumovski, W., H. Baquerizo and A. Rabinovich, 1992. Oxygen free radical scavenger protect rat islet cells from damage by cytokines. Diabetologia, 32: 792-796.

Ugwuja, E.I., E.I. Akubugwo, U.A. Ibiam and O. Obidoa, 2010. Impact of maternal copper and zinc status on pregnancy outcomes in a population of pregnant Nigerians. Pak. J. Nutr., 9: 678-682.

Williams, N.R., J. Rajput-Williams, J. West, S. Nigdikar, J. Foote and A. Howard, 1995. Plasma, granulocyte and mononuclear cell copper and zinc in patients with diabetes mellitus. Analyst, 120: 887-890.

World Health Organisation, 1996. Zinc. In: Trace elements in human nutrition and health (World Health Organisation, ed.), WHO, Geneva, Switzerland, pp: 72-104.

Zalewski, P., S. Millard, I. Forbes, O. Kapaniris, S. Slavotinek, W. Betts, A. Ward, S. Lincoln and I. Mahadevan, 1994. Video analysis of labile Zn in viable pancreatic islet cells using specific fluorescent probe for Zn. J. Histochem. Cytochem., 42: 877-884.

Pakistan Journal of Nutrition 9 (9): 865-872, 2010
ISSN 1680-5194


# A Comparative Study of the Common Protozoan Parasites of *Heterobranchus longifilis* from the Wild and Cultured Environments in Benue State

S. Omeji, S.G. Solomon and R.A. Obande
Department of Fisheries and Aquaculture, University of Agriculture,
P.M.B. 2373, Makurdi, Benue State, Nigeria

**Abstract:** The aim of this study was to evaluate and compare the parasite load in populations of *Heterobranchus longifilis* obtained from a cultured environment (pond) and wild habitats. One hundred and twenty fishes were used for the study; 60 each from the cultured environment and wild which were further divided into 30 dead and 30 live fishes for each of the habitat sources. The results showed that 80% of dead *H. longifilis* from the pond were infested, as against 73.3% of the dead fishes from the wild by different protozoan parasites. Also, 83.3% of live *H. longifilis* from the pond were infested, as against 90% of the live fishes from the wild. Of the various protozoan parasites observed, *I. mutifilis* was the most abundant in both the pond (37.41%) and wild (36.40%). Among the body parts of the sampled fishes from the pond, the gills had the highest parasite load (46%). Also, the gills had the highest parasite load (44%) among the body parts of the fishes sampled from the wild. Live fishes from both sources had more protozoan parasites than the dead fishes. Bigger fishes of total length between 25-48 cm were more infected than smaller fishes of total length between 19-24 cm from both sources. Female fishes had more protozoan parasites than the male counterparts. Also, fishes between 150-750 g had more parasite load than the smaller ones of less than 150 g.

**Key words:** Protozoan parasites, *Heterobranchus longifilis*, wild environment, cultured environment, Benue State

## INTRODUCTION

Fish is important to human populace in trade and economy; it is of importance in the diet of different countries especially in the tropics and subtropics where malnutrition is a major problem (Alune and Andrew, 1996; Osuigwe and Obiekezie, 2007). As the human population inevitably increases, the demand for fish as source of protein will grow (Abolarin, 1996). In recent times, there has been tremendous increase in the development of fish farming and culture attributable to the increased need for affordable animal protein especially in the tropics(Davies *et al*., 2006) therefore, catfishes of the family clariidae are increasingly being used for freshwater aquaculture in Africa owing to several favourable cultural characteristics (Obiekezie and Ekanem, 1995). Parasitic infection and diseases are some of the factors hindering high productivity in fish farming (Doglel *et al*., 1961; Kayis *et al*., 2009).

Parasites are the most diverse and common pathogens the aqua-culturist may likely encounter and parasitic diseases are very common in fish all over the world and are of particular importance in the tropics (Roberts and Janovy, 2000) and Protozoan among other parasites cause immeasurable damage to the fishing industry (Doglel *et al*., 1961). Fish parasites are numerous and many phyla in the animal kingdom have representative that are parasitic to fish. There are by far more parasite species that infect fish than any other group of infectious disease (Blazer, 1996). Fish parasites result in huge economic losses as they increase mortality; increase farm inputs via increased treatment expenses and cause reduction in growth rate and possibly weight loss during and after the period of parasitic disease outbreak. All these militate against expansion of aquaculture. (Kayis *et al*., 2009). The most commonly encountered fish parasites are protozoa (Klinger and Francis-Floyd, 2000) and Protozoan parasites cause serious losses in fishpond and wild in Nigeria; their lesions could render the fish unmarketable. In addition, fish carrying protozoan parasites are capable of passing on the infective disease to man after its consumption. Protozoan parasites, typically, do not required intermediate hosts to reproduce (direct life cycle) and are thus capable of building up to very high numbers when fish are crowded causing loss, debilitation and mortality (Klinger and Francis-Floyd, 2000).

Most fish in the wild are likely to be infested with parasites, but in the great majority of cases, no significant harm to the host may be ensued or identified, thus, there are only few reports of parasites causing

**Corresponding Author:** S. Omeji, Department of Fisheries and Aquaculture, University of Agriculture, P.M.B. 2373, Makurdi, Benue State, Nigeria

mortality or serious damage to the fish populations, but this may be largely because such effects go unnoticed (Roberts, 2001). Fishermen or consumers often observe parasites in wild fish only when they are so obvious as to lead to rejection of fish (Roberts, 1995). In culture fish population on the other hand, parasites often cause serious outbreak of diseases (Kayis *et al*., 2009). The presence of dense populations of fish kept in particular environmental conditions may favour certain parasites so that the parasite population increases to a very high level (Rintamaki and Valtonen, 1997).

## MATERIALS AND METHODS

The study took place in Makurdi the capital of Benue State, Nigeria, located at longitude 7° 43' N and latitude 8°32' E. The town is divided into the North- and South-bank by the River Benue. River Benue exists year round though the water volume fluctuates with season. The river overflows its banks during the rainy season (May-October), but decreases drastically in volume leaving tiny island in the middle of the river during the dry season (November-April). The river contains several species of freshwater fishes of different families such as Clariidae, Mormyridae, Centrpomidae etc.

One hundred and twenty *Heterobranchus ongifilis* (60 each from the wild and a pond), comprising of 30 live and 30 dead fishes of different sizes were bought from local fishermen along the course of River Benue (The wild) and from Jab-Bella farm (pond) both in Wadata, Benue state, Nigeria. Five samples each from both the wild and pond were collected fortnightly for a period of six (6) months, June-Nov. 2008. The fishes were identified using the field guide to Nigerian freshwater fishes by Babatunde and Aminu (2004). The total and standard lengths of each fish were measured in centimeters (cm) using meter rule, while the weight of each of the fishes was taken in grams (g) using an electronic meter balance. The sexes of the fishes were also determined examination of their papillae.

External examination of each of the fish for parasites was carried out using the technique of Emere and Egbe (2006) on the gills, fins and skin. The skin, gill and fins of each of the fish were also examined for ecto parasites using hand lens. The fish samples were also felleted using scalpel blade. The tissue was placed on a Petri-dish and 3 mls of 0.9% saline solution was added and stirred using a mounted pin. Some drops of the mixed solution were collected using dropper, placed on a slide and then covered with a cover slip after which, observation on a light binocular microscope was made. Later, the gills of each of the fish were dissected using a dissecting kit, each of the gill was placed in 10 mls of normal saline in Petri- dish, later removed and then place on a slide on which 1-2 drops of saline solution was added and observed on a binocular microscope. The stomach and the intestine of each of the fish were cut opened and contents washed into the Petri-dish containing the saline solution. The lining of the gut lumen was also scrapped out and placed in the saline solution. One to two drops of the preparation was placed on slide covered with slips and observed using a light binocular microscope for endoparasites. Ecto parasitic data were collected on the gills, fins and skins of the fish while the endo parasitic data were collected on the stomach and intestine of the fish using the techniques of Emere and Egbe (2006).

The parasites were identified by making their sketches as observed on the binocular microscope and compared with the pictorial Guide on fish parasites by Pouder *et al*. (2005). The parasites observed on the binocular microscope were counted and recorded. Two-way analysis of Variance was use to determine significant differences in sex, source and status of the specimens. The ANOVA was carried out using GENSTAT Discovery Edition from Lawes Agricultural Trust Rothamsted.

## RESULTS

Results of the 30 dead and 30 live *H. longifilis* from the pond used for the study are as shown in Table 1. Out of the 30 dead *H. longifilis* used, 6 (20%) were not infested by any protozoan parasites while the remaining 24 (80%) of them were infested by different protozoan parasites and were observed to harbour a total number of 108 protozoan parasites. From the table above, *I. mutifilis* were found on the gill and skin and was the most abundant 40 (37.03%), followed by *Trichodina species* 25 (23.15%), which were found on the skin and fin, *C.iubilans* 19 (17.59%) in the stomach and intestine, *Ichthyobodo species* 17 (15.74%) on the gill, *Hexamita* 4 (3.70%), which were found in the stomach and intestine and lastly *Chilodonella species* 3 (2.78%) on the skin. It was observed that the gill has the highest number of protozoan parasites (39%) followed by the skin (30%) while the fin, intestine and stomach accounted for 16, 12 and 11% respectively. Whereas, out of the 30 live *H. longifilis* used, 5 (16.7%) were not infested by any protozoan parasites while 25 (83.3%) of them were infested by protozoan parasites and were observed to harbour a total number of 138 protozoan parasites. *I. mutifilis* were found on the gill and skin and was the most abundant 48 (37.78%), followed by *C. iubilans* 35 (25.36%), which were found in the stomach and intestine, *Trichodina species* 24 (17.39%), which were found and fin, *Ichthyobodo species* 21 (15.22%) on the gill and lastly *Chilodonella species* on the skin 10 (7.25%). It was also observed that the gill has the highest load of protozoan parasites (53%) followed by the skin (35%) while the stomach, intestine and fin accounted for 19, 16 and 15% respectively. The result shows that live *H. longifilis* from the pond has more protozoan parasites than dead *H. longifilis* from the same pond.

Table 1: Protozoa parasites and their locations in dead and live *H. longifilis* from the cultured environment (Pond)

| | Number of fish infected by each protozoa parasite | | | Percentage parasite infection per location | | Parasite load on each location | | Percentage parasite species on fish | |
|---|---|---|---|---|---|---|---|---|---|
| Protozoa parasites | Dead | Live | Location of parasites | Dead | Live | Dead | Live | Dead | Live |
| *Ichthyobodo sp* | 5 | 6 | gill | 39.00 | 53.00 | 17 | 21 | 15.74 | 15.22 |
| *I. multifilis* | 6 | 8 | gill | | | 22 | 32 | 37.03 | 37.78 |
| *I. multifilis* | 7 | 5 | skin | 30.00 | 35.00 | 18 | 16 | | |
| *Chilodonella* | 1 | 3 | skin | | | 3 | 10 | 2.78 | 7.25 |
| *Trichodina* | 3 | 2 | skin | | | 9 | 9 | 23.15 | 17.39 |
| *Trichodina* | 7 | 6 | fin | 16.00 | 15.00 | 16 | 15 | | |
| *C. iubilans* | 3 | 3 | stomach | 11.00 | 19.00 | 11 | 19 | 17.59 | 25.36 |
| *C. iubilans* | 3 | 4 | intestine | 12.00 | 16.00 | 8 | 16 | | |
| *Hexamita* | 2 | 0 | intestine | | | 4 | 0 | 3.70 | 0.00 |
| Total | 37 | 37 | | 108.00 | 138.00 | 108 | 138 | 100.00 | 100.00 |

Table 2: Protozoa parasites and their locations in dead and live *H. longifilis* from River Benue (Wild)

| | Number of fish infected by each protozoa parasite | | | Percentage parasite infection per location | | Parasite load on each location | | Percentage parasite species on fish | |
|---|---|---|---|---|---|---|---|---|---|
| Protozoa parasites | Dead | Live | Location of parasites | Dead | Live | Dead | Live | Dead | Live |
| *Ichthyobodo sp* | 5 | 6 | gill | 37.00 | 51.00 | 19 | 20 | 17.76 | 13.42 |
| *I. multifilis* | 5 | 8 | gill | | | 18 | 31 | 39.25 | 33.55 |
| *I. multifilis* | 8 | 5 | skin | 31.00 | 39.00 | 24 | 19 | | |
| *Chilodonella* | 2 | 4 | skin | | | 5 | 13 | 4.67 | 8.72 |
| *Trichodina* | 1 | 2 | skin | | | 2 | 7 | 20.56 | 10.07 |
| *Trichodina* | 7 | 5 | fin | 20.00 | 8.00 | 20 | 8 | | |
| *C. iubilans* | 3 | 7 | stomach | 13.00 | 27.00 | 13 | 21 | 17.76 | 27.52 |
| *C. iubilans* | 2 | 6 | intestine | 6.00 | 24.00 | 6 | 20 | | |
| *Hexamita* | 0 | 2 | Stomach | 0.00 | as above | 0 | 6 | 0.00 | 6.71 |
| *Hexamita* | 0 | 2 | Intestine | 0.00 | as above | 0 | 4 | | |
| Total | 33 | 47 | | 107.00 | 149 | 107 | 149 | 100.00 | 100.00 |

Results of the 30 dead and 30 live *H. longifilis* from the wild used for the study are as shown in Table 2. Out of the 30 dead *H. longifilis* used, 8 (26.7%) were not infested by any protozoan parasites while the remaining 22 (73.3%) of them were infested by different protozoan parasites and were observed to harbour a total number of 107 protozoan parasites.

From the above table, *I. mutifilis* were found on the gill and skin and was the most abundant 42 (39.25%), followed by *Trichodina species* 22 (20.56%), which were found on the skin and fin, *Ichthyobodo species* 19 (17.76%) on the gill, *C. iubilans* 19 (17.76%) in the stomach and intestine and lastly *Chilodonella species* 5 (4.67%) on the skin. It was observed that the gill has the highest load of protozoan parasites (37%) followed by the skin (31%) while the fin, stomach and intestine accounted for 20, 13 and 6% respectively. Whereas, out of the 30 live *H. longifilis* used, 3 (10%) were not infested by any form of protozoan parasites while 27 (90%) of them were infested by different protozoan parasites and were observed to harbour a total number of 149 protozoan parasites. *I. mutifilis* were found on the gill and skin and was the most abundant 50 (33.55%), followed by *C.iubilans* 41 (27.52%), which were found in the stomach and intestine, *Ichthyobodo species* 20 (13.42%) on the gill, *Trichodina species* 15 (10.07%), which were found on the skin and fin, *Chilodonella species* 13 (8.72%) on the skin and lastly *Hexamita* 10 (6.71%), which were found in the stomach and intestine. It was also observed that the gill has the highest load of protozoan parasites (51%) followed by the skin (39%) while the stomach, intestine and fin accounted for 27, 24 and 8% respectively. The results also show that live *H. longifilis* from the wild has more protozoan parasites than dead *H. longifilis* from the same wild.

Figure 1 shows the result of the size distribution and percentage parasite infection in dead and live *H. longifilis* from the pond while Fig. 2 shows the result of the size distribution and percentage parasite infection in dead and live *H. longifilis* from the wild. It was observed that bigger fishes of total length between 25-48 cm were more infected than smaller fishes (total length between 19-24 cm) from both sources.

Figure 3 shows the results of sex and percentage parasite infection in dead and live *H. longifilis* from the cultured environment (pond) and River Benue. The results also show that the dead female *C. gariepinus* from the cultured environment (pond) had a greater rate of infection (74.01%) than the dead male *C. gariepinus* (25.93%) and the live female *C. gariepinus* had a greater rate of infection (83.33%) than the live male *C. gariepinus* (16.67%). In addition, the dead female *C. gariepinus* from River Benue had a greater rate of infection (62.62%) than the dead male *C. gariepinus*

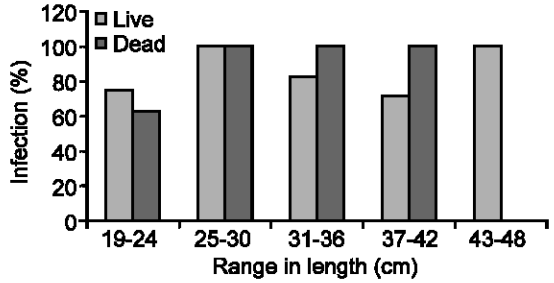


Fig. 1: Size distribution and percentage parasite infection in dead and live *H. longifilis* from the pond

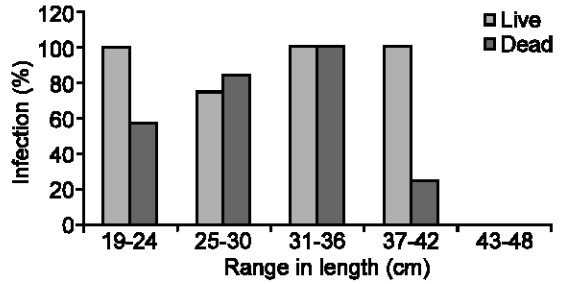


Fig. 2: Size distribution and percentage parasite infection in dead and live *H. longifilis* from the wild

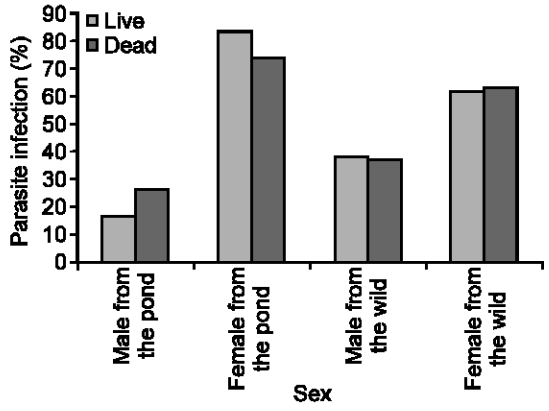


Fig. 3: Sex and percentage parasite infection in dead and live *H. longifilis* from the cultured environment (pond) and River Benue

(37.38%) and the live female *C. gariepinus* had a greater rate of infection (61.74%) than the live male *C. gariepinus* (38.26%).

Results of the percentage parasites load on the different body parts of both dead male and female and that of live male and female *H .longifilis* with respect to the weight of the fishes from the pond are as shown in Table 3 and 4 respectively. While Table 5 and 6 show the results of the percentage parasites load on the different body parts of both dead male and female and that of live male and female *H. longifilis* with respect to the weight of the fishes from the wild.

The results also show that fishes with bigger weight (150-750 g) had more parasites than smaller fishes with less than 150 g.

The mean total number of parasites for both dead male and female and live male and female *H. longifilis* from both origins is as shown in Table 7. There was a significant difference (2.91) between the live male and female *H. longifilis* from the pond but there was no significant difference between the dead male and female *H. longifilis* from the pond, live male and female *H. longifilis* from the wild and between dead male and female *H. longifilis* from River Benue. In addition, the live *H. longifilis* from both sources has higher mean number of parasites (4.03-cultured environment and 4.90 River Benue) than the dead samples (3.43-cultured environment and 3.51 River Benue) and the female samples have higher number of parasites (75 females and 45 males).

Statistical analysis of the correlation matrix for the total number of parasites found on *H. longifilis* by size, from both sources is as shown in Table 8.

From the above, there was a high correlation (0.738) between the Total Length (TL) and Total Number of Parasites (TNP) for dead *H. longifilis* collected from the culture environment (pond). In contrast, there was a low correlation (0.160) between the Total Length (TL) and Total Number of Parasites (TNP) for dead *H. longifilis* caught from River Benue. In addition, there was a high correlation (0.583) between the Total Number of Parasites (TNP) and Weight (WT) for dead *H. longifilis* collected from the culture environment (pond) but a negative correlation (-0.068) between the Total Number of Parasites (TNP) and weight for dead *H. longifilis*

Table 3: Percentage parasite load on the body parts of dead male and female *H. longifilis* from the pond

| | Percentage (%) parasite load | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Gill | | Skin | | Fin | | Stomach | | Intestine | |
| Weight of fish | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| <150 g | 54 | 16 | 15 | 37 | 15 | 26 | 15 | 11 | 0 | 11 |
| 150-250 g | 0 | 30 | 100 | 30 | 0 | 20 | 0 | 0 | 0 | 20 |
| 250-350 g | 57 | 64 | 43 | 8 | 0 | 12 | 0 | 16 | 0 | 0 |
| 350-450 g | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 50 | 0 | 30 |
| 450-550 g | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 550-650 g | 0 | 0 | 40 | 0 | 0 | 0 | 0 | 0 | 60 | 0 |
| 650-750 g | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 4: Percentage parasite load on the body parts of live male and female *H. longifilis* from the pond

| | Percentage (%) parasite load | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Gill | | Skin | | Fin | | Stomach | | Intestine | |
| Weight of fish | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| <150 | 0 | 46 | 0 | 38 | 100 | 8 | 0 | 0 | 0 | 8 |
| 150-250 g | 55 | 48 | 18 | 14 | 0 | 17 | 0 | 21 | 27 | 0 |
| 250-350 g | 0 | 45 | 57 | 27 | 43 | 27 | 0 | 0 | 0 | 0 |
| 350-450 g | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 450-550 g | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 550-650 g | 0 | 32 | 0 | 26 | 0 | 0 | 0 | 34 | 0 | 8 |
| 650-750 g | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 5: Percentage parasite load on the body parts of dead male and female *H. longifilis* from the wild

| | Percentage (%) parasite load | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Gill | | Skin | | Fin | | Stomach | | Intestine | |
| Weight of fish | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| <150 g | 29 | 29 | 71 | 36 | 0 | 0 | 0 | 36 | 0 | 0 |
| 150-250 g | 23 | 47 | 31 | 20 | 46 | 11 | 0 | 9 | 0 | 13 |
| 250-350 g | 0 | 0 | 0 | 50 | 100 | 0 | 0 | 50 | 0 | 0 |
| 350-450 g | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 450-550 g | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 550-650 g | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 650-750 g | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 6: Percentage parasite load on/in the body parts of live male and female *H. longifilis* from the wild

| | Percentage (%) parasite load | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Gill | | Skin | | Fin | | Stomach | | Intestine | |
| Weight of fish | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| <150 g | 21 | 0 | 36 | 100 | 29 | 0 | 0 | 0 | 14 | 0 |
| 150-250 g | 25 | 37 | 50 | 20 | 0 | 5 | 25 | 43 | 0 | 15 |
| 250-350 g | 0 | 63 | 0 | 15 | 0 | 4 | 29 | 0 | 71 | 19 |
| 350-450 g | 20 | 24 | 20 | 33 | 0 | 0 | 40 | 24 | 20 | 19 |
| 450-550 g | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 550-650 g | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 650-750 g | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 7: Mean total number of parasites for *Heterobranchus longifilis*

| Sex | LHP | DHP | LHW | DHW | No. |
|---|---|---|---|---|---|
| Male | 2.57[b] | 2.80[a] | 4.38[a] | 3.08[a] | 45 |
| Female | 5.48[a] | 4.05[a] | 5.41[a] | 3.94[a] | 75 |

Values on the same column with different superscripts differ significantly ($p<0.05$). Standard Error of Means = 0.715.

Note: LHP = Live *H. longifilis* from cultured environment (pond), DHP = Dead *H. longifilis* from cultured environment (pond), LHW = Live *H. longifilis* from River Benue, DHW = Dead *H. longifilis* from River Benue

caught from River Benue. Also in the culture environment (pond), a high correlation value (0.526) was recorded between the Total Length (TL) and Total Number of Parasites (TNP) for live *H. longifilis* and 0.708 for live *H. longifilis* caught from River Benue. In addition, there was a high correlation (0.686) between the Total Number of Parasites (TNP) and Weight (WT) for live *H. longifilis* collected from the culture environment (pond) and 0.676 between the Total Number of Parasites (TNP) and Weight (WT) for live *H. longifilis* caught from River Benue.

**Damaging effects of parasites on the sampled *H. longifilis*:** The following damages were observed to have been caused by the parasites found on the body parts of the sampled fishes; on the skin, *I. mutifilis* caused thickening of the epithelium. This caused restriction of the oxygen flow from the water to the blood in the gills of infected fishes. The respiratory folds of the gills, the lamellae, also become deformed, reducing the transfer of oxygen. The shear numbers of *I. mutifilis* covering the gills also could cause mechanical blockage of oxygen transfer. These conditions combine to stress the fish by hindering respiration. The epithelial layer of the gill may separate and cause loss of electrolytes, nutrients and fluids from the fish, making it difficult for the fish to regulate the water concentration in its body. Death in infected fishes resulted from asphyxiation. *Tricodina* species caused epidermal necrosis of the fin. This resulted in sluggish movement, loss of appetite, emaciation, loss of condition with larger head and darker skin than normal. Some infected fish showed

Table 8: Correlation matrix for total number of parasites found on *H. longifilis* by size

| | Dead *H. longifilis* from the cultured environment (Pond) | | | Live *H. longifilis* from the cultured environment (Pond) | | |
|---|---|---|---|---|---|---|
| | TL | TNP | WT | TL | TNP | WT |
| TL | 1.00 | | | 1.00 | | |
| TNP | 0.738 | 1.00 | | 0.526 | 1.00 | |
| WT | 0.904 | 0.583 | 1.00 | 0.913 | 0.686 | 1.00 |
| | Dead *H. longifilis* from River Benue | | | Live *H. longifilis* from River Benue | | |
| | TL | TNP | WT | TL | TNP | WT |
| TL | 1.00 | | | 1.00 | | |
| TNP | 0.160 | 1.00 | | 0.708 | 1.00 | |
| WT | 0.859 | -0.068 | 1.00 | 0.938 | 0.676 | 1.00 |

Note: TL = Total Length; TNP = Total Number of Parasites; WT = Weight

detached scales with pale skin patches and more slimy skin. *Chilodonella* species caused epidermal necrosis of the skin and excess mucus formation on the skin. This caused the skin to appear slimy and it exhibited cloudiness and showed evidence of irritation as it tried (from time to time) to "scratch" off the organisms by rubbing against the walls of the fish pond. The fish also exhibited lethargy. *Hexamita* caused erosion of the intestine and ulceration of the stomach. As a result, infected fish lose appetite and become emaciated and lethargic. *C. iubilans caused* thickening of the intestinal wall. Infected fish showed inappetance, decreased activity and stayed isolated from other fish. These fish stayed near the surface and became increasingly tachypneic.

## DISCUSSION

Several external parasites were observed and identified in the fishes used for this work. This is in agreement with the findings of Snieszko and Axelrod (1971) who reported that external parasites constitute the largest group of pathogenic organisms in freshwater fish. However, Mohamed (1999) observed that the majority of fish ecto-parasitic protozoa are commensals but some of them may produce serious diseases and mortality especially in fry, fingerlings and bigger fishes subjected to stress.

Different kinds of protozoan parasites were observed to be present in different locations in *H. longifilis*. *I. multifilis* occurred on the gill and skin where chronic infections of the fishes were observed, *Trichodina* species appeared on the skin and fin, *Ichthyobodo species* and *Chilodonella* species appeared on the skin, while *C. iubilans* and *Hexamita* appeared in the stomach and intestine. Emere and Egbe (2006), Richard (2003), Hines and Spira (1973, 1974) have reported the infection of the skin, fin and gills of fish by protozoan parasites. The present study revealed that *C. iubilans* affected only the intestine and stomach of the fish studied and in addition, these parasites were more in the intestine than the stomach but Somerville (1984) in his work reported that a large number of *Cryptobia* protozoan were found on the external surface of cultured rainbow trout in U.S.A. The occurrence of *C. iubilans* in the intestine than the stomach either might be due to the presence of digested food present there or due to the greater surface area presented by the intestine (Adebanjo, 1979). Smith (1981) reported that most protozoan parasites inhabit the intestine because of their general feeding habits. Reduced number of the protozoan parasites in the stomach might be due to the movement of the stomach muscle and acid (HCl) nature of the stomach. Adebanjo (1979) observed that the acid nature of the stomach might inhibit the parasites there. Noble and Noble (1982) reported that protozoan parasites prefer a certain pH medium.

Gills were also observed to habour the highest number of protozoan parasites. This could be because the gills are the center of filter feeding and are the sites of gaseous exchange. This observation agrees with the reported works of Emere and Egbe (2006), Nyaku *et al.* (2007), who reported highest load of protozoan parasites in the gill of *Synodontis clarias*. Investigation by Roger and Gainer (1975) and Chakroff (1976) had shown the gills to be infected by different protozoan parasites. According to Robert and Somerville (1984), the sieving ability of the gill rakers may help to trap some organisms and this could be contributed to the presence of the protozoan parasites there.

Infection in the gills caused severe degeneration, necrosis and consequent degradation of the branchial epithelium and occlusion of the capillaries. Infection also induced massive proliferation of chloride and mucus cells and also caused hyperplasia of the lining filamental epithelium. However, degradation of the epithelial layer was either limited in extent or occurred in an uneven pattern. This observation was explained in the reported work of Paperna and Van As (1991) which stated that the epithelial degradation was counteracted by the extreme process of epithelial hyperplasia.

*I. mutifilis* caused erosion of the epithelium and thickening of the gills, this could be attributed to inflammatory processes which occured during infection with this parasitic ciliate as described by Sigh *et al.* (2004). Infection with *Trichodina spp* caused removal of the epithelium and excess mucus production so that the

fin and gills of infected fishes were covered in a thick layer of mucus, in which were contained the ciliates. This agrees with the reported work of Obiekezie and Ekanem (1995).

The heavy load of parasites on the gills relative to other parts of the body impaired the gills from functioning well as an organ of respiration, hence death could result. This agrees with the reported works of Borg (1960), Omoniyi *et al.* (2002), Rahman *et al.* (2002) and Aksit and Falakali (2007) who reported mortality in fishes with heavy parasite load on the gills.

The female *H. longifilis* have the highest number of protozoan parasites than the male counterparts. This might be due to the physiological state of the females, as most gravid females could have had reduced resistance to infection by parasites. In addition, their increased rate of food intake to meet their food requirements for the development of their egg might have exposed them to more contact with the parasites, which subsequently increased their chance of being infected. Emere and Egbe (2006); Adebanjo (1979); Holden and Reed (1972) had made similar observation. Bigger *H. longifilis* were observed to have higher rate of protozoan parasites than the smaller ones. This might be because the bigger ones cover wider areas in search of food. As a result, they take in more food than the smaller ones and this exposed them more to infection by parasites. In addition, they are omnivorous and feed on any thing that comes their ways. Emere and Egbe (2006), Holden and Reed (1972), had made similar observation in *S. clarias*.

Protozoan parasite load of fish from the cultured environment (pond) did not differ significantly from those from the River Benue (wild). However, the significantly higher load of parasites in the live fish as compared to the dead could be attributed to parasite migration as a result of death of the host (fish) which occurred soon after they died, prior to the examination as described by Klinger and Francis-Floyd (2000).

Some of the fishes observed did not exhibit clinical signs associated with the parasites identified on them. This is in agreement with the findings of Mohamed (1999) that clinical signs of parasitic diseases only appear on fish with heavy infections and cases of moderate ones that are usually exposed to one or more stress factors including, rough handling during transportation from ponds, overcrowding, malnutrition, high level of free ammonia and low level of oxygen concentration.

## REFERENCES


Abolarin, M.O., 1996. A new species of *Henneguya* (myxosporida Protozoa) from West African cat fish, *Clarias lazera* (Vaal) with a review of the genus henneguya (Thelohan). The Afr. J. Trop. Hydrobiol. Fish., 1: 93-105.

Adebanjo, A.O., 1979. A survey of parasites of *Clarias lazera* in Dundaye area of Rima River, Sokoto, Project submitted to Zoology Unit of Biological Science, Usman DanfodioUniversity, Sokoto, October, 1977 (ADM NA2052).

Aksit, D. and M.B. Falakali, 2007. Gill histology of Patella L. 1758 (Molusca: Gastropoda). 38th CIESM congress proceedings, 9-13 April 2007, Istanbul/ Turkey.

Alune, E. and G. Andrew, 1996. Fishes. Cambridge University Press, London.

Babatunde, D.O. and R. Aminu, 2004. Field Guide to Nigerian Freshwater fishes, 2nd Edn., Federal College of Freshwater Fisheries Technology, New Bussa, Nigeria.

Blazer, V.S., 1996. Major Infectious Diseases of Fish (Online). htt://wwww.afip.org/vetpath/POLA/fish diseases.

Borg, I.A.F., 1960. Studies on myxobacteria associated with diseases in salmonid fishes. Wildlife Dis., 8: 1-85.

Chakroff, M., 1976. Fresh water pond culture and Management. Peace Corps volunteer in Technical Assistance. Vita publication Manual Series" No. 36.

Davies, O.A., M.B. Inko-Tariah and D. Amachree, 2006. Growth response and survival of *Heterobranchus longifilis* fingerlings fed at different feeding frequencies. Afr. J. Biotechnol., 5: 778-780.

Doglel, V.A., G.K. Petrushevski and Y.I. Polyanski, 1961. Translated by (Kabata) Parasitology of Fishes. Oliver and Boyd, Endinburgh and London, pp: 384.

Emere, M.C. and N.E.L. Egbe, 2006. Protozoan parasites of *Synodonits clarias* (A fresh water fish). Best J., 3: 58-64.

Hines, R.S. and D.T. Spira, 1973. *Ichthyophthiriasis* in the mirror carp *Cyprinus carpio* L.I. Course of infection. J. Fish Biol., 5: 385-392.

Hines, R.S. and D.T. Spira, 1974. *Ichthyophthiriasis* in the mirror carp *Cyprinus carpio* (L). 111. Pathology J. Fish Biol., 6: 189-196.

Holden, M. and W. Reed, 1972. West African fresh water fish. (Publishers Longman, London), pp: 45.

Kayis, S., T. Ozcelep, E. Capkin and I. Altinok, 2009. Protozoan and metazoan parasites of cultured fish in Turkey and their applied treatments. The Israeli J. Aquac.-Bamidgeh, 61: 93-102.

Klinger, R. and R. Francis-Floyd, 2000. Introduction to Freshwater Fish Parasites. Institute of Food and Agricultural Sciences (IFAS) University of Florida.

Mohamed, A.E.H.H., 1999. Trichodiniasis in farmed freshwater Tilapia in Eastern Saudi Arabia. J. KAU: Marine Sci., 10: 157-168.

Nyaku, R.E., R.G. Okayi, O.D. Kolndadacha and M. Abdulrahman, 2007. A survey of ectoparasites associated with three (3) species of fish; *Auchenoglanis ocidentalis, Oreochromis niloticus and Bagrus bayad* in River Benue. Annual conference proceedings, Fisheries Society of Nigeria, pp: 10-14.

Noble, E.E. and G.R. Noble, 1982. Parasitology, the Biology of Animal parasites. 5th Edn., Leand Feblyer; Philadephia USA., 522 pp: 20-31.

Obiekezie, A. and D. Ekanem, 1995. Experimental infection of Heterobranchus longifilis (Teleostei, Clariidae) with *Trichodina maritinkae* (Ciliophora, Peritrichida). Aquatic Living Resour., 8: 439-443.

Osuigwe, D.I. and A.I. Obiekezie, 2007. Assessment of the growth performance and feed utilization of fingering *Heterobranchus ongifilis* fed raw and boiled jackbean (*Canavalia ensiformis*) seed meal as fishmeal substitute. J. Fish. Int., 2: 37-41.

Omoniyi, I.A., Agbon and S.A. Sodunke, 2002. Effects of lethal and sub-lethal concentrations of Tubacco (*Nicotiana tobaccum*), leaf dustr extraction on weight and haematological changes in *Clarias gariepinus* (Burchell). J. Applied Sci. Environ. Man., 6: 37-41.

Paperna, I. and J.G. Van As, 1991. The pathology of *Chilodonella hexasticha* (Kiernik). Infections in cichlid fishes. J. Fish Biol., 23: 441-450.

Pouder, D.B., E.W. Curtis and R.P.E. Yanong, 2005. Common Freshwater Parasite Pictorial Guide Edis. (Online). http://edis.ifas.ufl.edu.

Rahman, M.Z., Z. Hossain, M.F.A. Mellah and G.U. Ahmed, 2002. Effect of diazinon 60EC on *Anabus testudineus*, *Channa punctatus* and Barbades gomonotus Naga. The ICLARM Q., 25: 8-11.

Richard, C.S., 2003. Other Ectoparasites Infecting Fish: Copepods, Branchiurans, Isopods, Mites and Bivalves: Feature Article: Aquaculture Magazine, pp: 1-6.

Rintamaki, P. and E.T. Valtonen, 1997. Epizootiology of Protozoans in Farmed Salmonids at Northern Latitudes. Int. J. Parasitol., 27: 89-99.

Roberts, L.S. and J. Janovy, 2000. Gerald D. Schmidt and Larry S. Roberts' Foundations of parasitology, 6th Edn., International Editions, Boston.

Roberts, R.J., 2001. Fish Pathology: Parasitology of Teleosts (Robert R.L.) (Online). http://www.afip.org/vetpath/POLA/99/Diseases of fish.htm.

Roberts, R.J., 1995. Parasitology of Teleosts In: Fish Pathology. 2nd Edn., 7 pp: 254-270.

Roger, W.A. and J. Gainer, 1975. Lesion of Protozoa diseases in fish in the pathology of fish. W.E. Reibellin and G. Migaki Eds., pp: 117-150.

Sigh, J., T. Lindenstrøm and K. Buchmann, 2004. Expression of pro-inflammatory cytokines in rainbow trout (*Oncorhynchus mykiss*) during an infection with Ichthyophthirius multifiliis. Fish Shellfish Immunol., 17: 75-86.

Smith, A.C., 1981. Introduction to parasitology. Willey New York, pp: 822.

Robert, R.J. and C. Somerville, 1984. Some Economic aspects of common Parasites in cultured fish: Disease and health control of farmed fish (including shelfish and crustacea) in Europe. pp: 123-130.

Snieszko, S.F. and R. Axelrod, 1971. Diseases of Fishes. T.F.H. Publications, Inc., Ltd., pp: 45.

Pakistan Journal of Nutrition 9 (9): 873-876, 2010
ISSN 1680-5194


# Growth Performance and Carcass Analysis of Broiler Chickens Fed Graded Levels of Toasted *Albizia lebbeck* Seed Meal

A.O. Olorunsanya[1], O.O. Egbewande[2], H. Ibrahim[2] and M.M. Adeyemo[1]
[1]Department of Animal Production, University of Ilorin, Ilorin, Kwara State, Nigeria
[2]Department of Animal Science, Ibrahim Badamasi Babangida University,
P.M.B. 11, Lapai, Niger State, Nigeria

**Abstract:** One hundred day old broiler chicks were used for this study. They were fed on a conventional broiler starter feed for the first seven days after which they were randomly allotted into five treatment groups of twenty birds with ten birds per replicate. A seven week trial was conducted to investigate the nutritive value of Toasted *Albizia lebbeck* Seed Meal (TASM) on the birds. The test material was included at dietary levels of 0, 5, 10, 15 and 20%. At the end of the trial, three birds were randomly selected per replicate, starved overnight, bled through jugular vein, de-feathered and eviscerated. Average feed intake, weight gain, feed conversion efficiency and mortality showed significant ($p<0.05$) difference. However, at 0 and 5% dietary levels, there was no significant ($p>0.05$) difference in the performance characteristics. The carcass parameters showed that 0 and 5% TASM dietary levels were significantly ($p<0.05$) higher than other treatments in all the parameters assessed. The above showed that the birds were able to tolerate TASM up to 5% level of inclusion, but beyond this, overall performances, carcass characteristics and mortality were affected.

**Key words:** Broilers, *Albizia lebbeck*, carcass and performance characteristics

## INTRODUCTION

The increasing demand for animal protein has aroused greater interest in the production of fast growing animals with short generation intervals (Obinne and Okorie, 2008). Apantaku *et al*. (1998) reported that expansion of poultry industry in Nigeria holds the greatest promise of bridging the animal protein gap prevailing in the country within the shortest possible time. The protein from poultry meat and eggs, according to Atteh (2004), is of such quality that it is used as a standard against which other proteins are compared. Broiler chickens are fast growing species of poultry that are commonly raised to provide tender meat for human consumption. However, the rising cost of poultry feed has continued to be a serious problem. This is because the feed alone accounts for about 70% of the total production (Olorede and Longe, 1999).

Competition for conventional feedstuffs by man and livestock has contributed immensely to the high cost of these feedstuffs in the local markets. This high cost coupled with inadequate knowledge of possible alternative and cheap ingredients have been the most important factors militating against increased commercial poultry production in Nigeria (Olorede and Ajayi, 2005). Therefore, the quest for cheap ingredients in dietary formulations to reduce production cost becomes imperative.

Some non-conventional feedstuffs have been evaluated and found to be good replacements for the expensive conventional types which have direct bearings with human beings. There exist some legumes which are underutilized but have great potentials of been developed into livestock feed. One of such is *Albizia lebbeck*, which is a tropical legume. It is one of the most widespread and common species of *Albizia* worldwide (Wikipedia, 2009). Most livestock readily eat leaves and young twigs of this promising fodder tree. Crude protein concentration is about 20% for green leaves, 13% for leaf litter, 10% for twigs and the edible material has no known toxic compounds (FACT Net, 1986). It has common names such as "woman's tongue" and "rattle pod" derived from the noise of pods shaking in the wind. It is a tree growing to a height of 18-30 m tall with a trunk 50 cm to 1 m in diameter. The leaves are bipinnate, 7.5-15 cm long, with one to four pairs of pinnae, each pinna with 6-18 leaflets. The flowers are white, with numerous long stamens 2.5-3.8 cm and very fragrant. The fruit is a pod 15-30 cm long and 2.5-5.0 cm broad, containing six to twelve seeds. Leaves are free of toxins and tannins and low in soluble phenolic compounds. Flowers contain no adverse constituents. Pods contain saponins which may limit intake but appear to have no other adverse effect. There is a claim of toxicity in the seed (Wikipedia, 2009). The objective of this study

**Corresponding Author:** O.O. Egbewande, Department of Animal Science, Ibrahim Badamasi Babangida University, P.M.B. 11, Lapai, Niger State, Nigeria

however, is to investigate the optimum level at which Toasted *Albizia lebbeck* Seed Meal (TASM) could be fed to broiler chickens for optimal performance.

## MATERIALS AND METHODS

*Albizia lebbeck* seeds used in this study were collected around Ilorin metropolis, in Kwara State, Nigeria. The seeds collected were toasted using the local groundnut processing method. Briefly, this involves putting sand and the seeds in a large frying pan over a naked flame until the colour of the seeds had changed to dark brown coupled with an appetizing aroma. The seeds were cooled and then milled before incorporated into the formulated ration.

A total of 100 day-old Anak broiler chicks were raised for this study at graded levels of 0, 5, 10, 15 and 20% (Table 1). The birds were randomly allotted to five dietary treatments replicated twice with ten (10) birds per replicate in a Completely Randomized Design (CRD) experiment. Routine and periodic management practices were carried out. The chicks were brooded on wood shavings as litter materials in a tropical type (dwarf walled and open-sided) poultry house with equal size. 100 watts electric bulbs and hurricane lanterns (during power failure), were used to provide continuous light and heat during the brooding stage (0-4 weeks of age).

The birds were fed a commercial broiler starter ration for the first seven (7) days before given the experimental diets. Feed and cool, clean water were provided *ad libitum*. The amount of feed given and the left over were recorded on daily basis. The initial body weights of the birds were determined at the onset of the experiment and subsequently at one week interval until the experiment was terminated (8 weeks old). Weekly feed intakes were also recorded. Feed conversion ratio was then calculated from weight gain and feed intake of the birds. Daily records of mortality were taken and expressed as percentage at the termination of the experiment. Data collected were subjected to Analysis of Variance (ANOVA) and significant treated means were separated using the Duncan's multiple range tests (Steel and Torrie, 1980).

## RESULTS AND DISCUSSION

**Performance of chicks on experimental diets:** Data on performance characteristics and mortality rate of the broiler chicken were summarized in Table 2. The dietary level of TASM had a significant effect on the feed intake of the birds. Birds on the control diet had the highest feed intake which differed significantly ($p<0.05$) from others except those fed 5% TASM. Feed intake of birds fed 10, 15 and 20% TASM diets were significantly ($p<0.05$) lower than those on 0 and 5% dietary levels. The above might not be unconnected with the presence of tannins and saponins in *Albezia lebbeck* which according to Liener (1989) and Bate-Smith (1973) do affect feed intake and digestion. This implies that the dietary level of TASM beyond 5% does not support feed intake of broiler chicken.

Results on body weight gain of birds fed 0 and 5% TASM showed no significant ($p>0.05$) difference but differed significantly ($p<0.05$) from those fed 10% and above. Birds on 10% TASM and above had similar weight gain but significantly ($p<0.05$) lower weight than those on 0 and 5% TASM diets. Therefore, any slight increase in TASM dietary level in broiler chicken diets above 5% may not support growth and thus reduce weight gain. This may be due to decreased feed intake and inability of the birds to utilize the diets as a result of poor digestion and absorption. This supports Wikipedia (2009) reports that the presence of anti-nutritional factors in this plant do limit feed intake.

The dietary levels of TASM had a significant ($p<0.05$) effect on feed conversion ratio of the broiler chicken. Birds fed 0 and 5% had very close feed/gain ratio which differed significantly ($p<0.05$) from those birds on 10% TASM and above. The superior feed conversion ratio exhibited by 0 and 5% dietary levels may therefore proved that birds on these dietary levels optimally utilized the feed consumed.

The inclusion level of TASM diets had significant ($p<0.05$) effect on the mortality rate of the birds. An increase in inclusion level of TASM leads to an increase in mortality rate. Birds on 10, 15 and 20% TASM diets were significantly ($p<0.05$) higher in mortality than those on 5%. There was no mortality at 0% inclusion level. This sequence may probably be due to the cumulative effects of the anti-nutritional factors in the seeds, which could be an indication that the toasting process was not appropriate enough to eliminate the anti-nutritional factors. The above findings agreed with the reports of Esonu *et al*. (1997), that most legumes have thermo-labile and thermo-stable anti-nutrients which needed more than one treatment applications.

The effect of feeding graded levels of toasted *Albizia lebbeck* seed meal on the cut-up parts of the birds is represented in Table 3. TASM diets had significant ($p<0.05$) effect on live shrunk weight of the broiler chicken. There was no significant ($p>0.05$) effect at 0 and 5% inclusion levels, but at 10% and above the live shrunk weights were depressed. This might be due to the low feed intake recorded at these levels caused by the presence of anti nutritional factors leading to reduced feed palatability, intake, digestion, utilization and growth. The dressed weight and dressing percentage were significantly ($p<0.05$) influenced by the diets. The level of TASM is inversely proportional to the weight and dressing percentage. That is, the higher the level of TASM in the diets, the lower the dressed weight and dressing percentage. At 0 and 5% dietary levels, no

Table 1: Composition of experimental diets

| | Dietary level of TASM (%) | | | | |
|---|---|---|---|---|---|
| Feed ingredient | 0 | 5 | 10 | 15 | 20 |
| Maize | 36.40 | 29.48 | 22.86 | 18.00 | 12.25 |
| Maize milling waste | 10.04 | 14.64 | 18.74 | 20.78 | 23.53 |
| Fish meal | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Wheat offal | 20.14 | 22.66 | 24.18 | 27.00 | 30.00 |
| Soybean meal | 26.67 | 22.47 | 17.47 | 12.47 | 7.47 |
| TASM | 00.00 | 5.00 | 10.00 | 15.00 | 20.00 |
| Oyster shell | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Vitamin premix | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Bone meal | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| Salt | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Methionine | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Lysine | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| **Calculated chemical composition** | | | | | |
| Crude protein (%) | 21.662 | 21.894 | 21.823 | 21.769 | 21.740 |
| Crude fibre (%) | 5.735 | 6.625 | 7.319 | 7.969 | 8.691 |
| Ether extract (%) | 3.857 | 3.867 | 3.802 | 3.845 | 3.871 |
| Ash (%) | 4.179 | 4.329 | 4.369 | 4.505 | 4.643 |
| M.E. (Kcal/kg) | 2973.46 | 2937.36 | 2908.18 | 2882.19 | 2852.83 |
| Calorie:Protein | 137.27 | 134.16 | 133.26 | 132.40 | 131.22 |

Table 2: Effect of dietary level of TASM on performance characteristics of broiler chicken

| | Dietary level of TASM | | | | |
|---|---|---|---|---|---|
| Parameter | 0 | 5 | 10 | 15 | 20 |
| Average daily feed intake (g) | 82.17$^{a}$ | 55.01$^{ac}$ | 28.63$^{bc}$ | 28.50$^{b}$ | 27.39$^{b}$ |
| Average daily weight gain (g) | 22.81$^{a}$ | 17.23$^{a}$ | 4.16$^{b}$ | 3.60$^{b}$ | 3.27$^{b}$ |
| Feed conversion ratio | 3.60$^{a}$ | 3.19$^{a}$ | 6.88$^{b}$ | 7.92$^{b}$ | 8.38$^{b}$ |
| Mortality (%) | 0.00$^{a}$ | 5.00$^{a}$ | 50.00$^{b}$ | 80.00$^{c}$ | 90.00$^{c}$ |

$^{abc}$Means with the same superscripts are not significantly (p>0.05) different.

Carcass characteristics of broiler chicken

Table 3: Effect of dietary level of TASM carcass characteristics of broiler chicken

| | Dietary level of TASM | | | | |
|---|---|---|---|---|---|
| Parameter | 0 | 5 | 10 | 15 | 20 |
| Live shrunk weight (g) | 1140.0$^{a}$ | 1073.33$^{a}$ | 304.24$^{b}$ | 274.73$^{b}$ | 277.65$^{b}$ |
| Dressed weight (g) | 898.63$^{a}$ | 781.33$^{a}$ | 233.71$^{b}$ | 161.30$^{b}$ | 158.95$^{b}$ |
| Dressing percentage (%) | 78.79$^{a}$ | 72.81$^{a}$ | 59.37$^{b}$ | 58.17$^{b}$ | 57.12$^{b}$ |
| Thigh (%) | 13.68$^{a}$ | 14.58$^{a}$ | 11.29$^{b}$ | 11.86$^{b}$ | 11.42$^{b}$ |
| Drumstick (%) | 14.45$^{a}$ | 14.19$^{a}$ | 10.82$^{b}$ | 11.38$^{b}$ | 10.63$^{b}$ |
| Breast (%) | 21.62$^{a}$ | 17.98$^{b}$ | 16.60$^{bd}$ | 14.71$^{c}$ | 14.93$^{cd}$ |
| Back (%) | 19.60$^{a}$ | 18.77$^{a}$ | 21.54$^{b}$ | 19.19$^{a}$ | 19.64$^{a}$ |
| Head (%) | 4.05$^{a}$ | 4.75$^{a}$ | 7.74$^{b}$ | 8.87$^{b}$ | 8.90$^{b}$ |
| Wing (%) | 10.90 | 10.76 | 10.35 | 11.81 | 11.66 |
| Neck (%) | 5.80 | 6.66 | 6.77 | 6.79 | 6.82 |
| Shank (%) | 6.76 | 8.39 | 6.84 | 7.23 | 7.20 |

$^{abcd}$Means with the same superscript are not significantly (p>0.05) different

significant (p>0.05) difference was observed in the two parameters. As the dietary level increases to10% and above, dressed weight and dressing percentage were significantly (p<0.05) influenced with the lowest value obtained at 20% TASM inclusion levels.

The thigh and drumstick at 0 and 5% dietary levels were not significantly (p>0.05) affected by the diets but differed greatly from others. The breast was also influenced by the amount of TASM in the diets. Birds on 0% had the highest breast value while the least value was obtained at 15% level of inclusion. The head of the birds were similar at 0 and 5% but greatly different from those obtained from other dietary levels. The higher the TASM level in the diets, the more developed were the heads relative to the dressed weights. However, the backs were not significantly (p>0.05) different across the treatments means except in 10% TASM where high back value was obtained. Wing, neck and shank were not

affected by the level of TASM in the diets. The results from the carcass evaluation relate well with those obtained in performance characteristics and it was observed that superior values were obtained for all the parameters evaluated.

**Conclusion and Recommendations:** The feeding trial revealed that the broiler chicken fed the control diet and 5% TASM were significantly ($p<0.05$) better in all parameters evaluated than those on 10% TASM and above. Therefore, it can be concluded that toasting process of detoxification of *Albizia lebbeck* alone may not be sufficient in eliminating all the anti-nutritional factors. It is recommended that TASM could be a valuable feedstuff which could be included up to 5% of the broiler diets without any deleterious effects.

## REFERENCES

Apantaku, S.O., A.M. Omotayo and A.B. Oyesola, 1998. Poultry Farmers' Willingness to Participate in NAIC Scheme in Ogun State. Oduguwa *et al.* (Eds). Proc. Of the Silver Anniv. Conf. Nig. Soc. Anim. Prod. Gateway Hotel, Abeokuta. 21st-26th March, pp: 542.

Atteh, J.O., 2004. Theory and Practice of Poultry Production. 1st Edn., Adlek Printers, Ilorin, Nigeria, pp: 1-3.

Bate-Smith, E.C., 1973. Haemanalysis of tannins, the concept of relative astringency. Phytochemistry, pp: 907-912.

Esonu, B.O., A.B.I. Udedibie and U.F. Okpudo, 1997. Effect of sprouting and boiling on the nutritive value of jack beans (*C. ensiformis*) for broiler finisher. Appl. Trop. Agric., 2: 31-55.

FACT Net, 1986. *Albizia lebbeck*-A Promising Fodder Tree for Semi-Arid Regions. Winrock International. Retrieved from: http://www.winrock.org/fnrm/factnet.htm on 8/6/2009.

Liener, I.E., 1989. Antinutritional Factors in Legume Seeds: State of the Art. In: Recent Advances of Research in Antinutritional Factors in Legume Seeds, Huisman, J., T.F.B. Van Der Poel and I.E. Liener (Eds.). Pudoc, Wageningen, The Netherlands, pp: 6-13.

Obinne, J.I. and A.U. Okorie, 2008. Effect of different crude protein and digestible energy levels on the growth performance of rabbits in the tropics. Nig. J. Anim. Prod., 35: 210-216.

Olorede, B.R. and O.G. Longe, 1999. Growth, nutrient retention, haematology and serum chemistry of pullet chicks fed sheabutter cake in the humid tropic. Arch. Zootech., 49: 441-444.

Olorede, B.R. and A.F. Ajayi, 2005. Replacement of groundnut cake and maize with *Falderbia albida* GOA in the diets of broiler chickens. Bull. Anim. Hlth. Prod. Afr., 53: 61-67.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics. A Biometrical Approach. 2nd Edn., McGraw Hill Book Co., New York.

Wikipedia, 2009. *Albizia lebbeck* from Wikipedia, the free encyclopedia. Retrieved from: http:/en.wikipedia.org/wiki/Albizia lebbeck on 8/6/2009.

Pakistan Journal of Nutrition 9 (9): 877-881, 2010
ISSN 1680-5194


# Quality Changes of Salted Kass (*Hydrocynus forskalii*) During Storage at Ambient Temperature (37±1°C)

Egbal O. Ahmed[1], Mohammed E. Ali[2] and Ali A. Hamed[2]
[1]School of Fish Sciences, AL Neelain University, P.O. Box 12702, Khartoum, Sudan
[2]Fisheries Research Centre (AL Shagara), P.O. Box 1489, Khartoum, Sudan

**Abstract:** In the present study, the chemical and microbiological quality changes in salted (25% of the fish weight) *Hydrocynus forskalii* was carried out during storage at +37±1°C. Moisture, ash, protein, lipid, fiber and pH were analyzed to determine chemical quality and total viable counts of bacteria (TVC), total Staphylococcus-Micrococcus and yeast-mould were measured to determine microbial quality during the storage period. Reduction of chemical quality was found statistically significant ($p<0.05$). No yeast and mould were detected for the period of storage. Microbial analysis demonstrated that the salted techniques reduced the microbial counts of the salted fish, whereas it retarded the microbial growth during the last two months. Based on the data, the optimal shelf life was found to be three months for salted *Hydrocynus forskalii*.

**Key words:** *Hydrocynus forskalii*, salted, quality, storage, ambient temperature

## INTRODUCTION

Salting process is considered as one of the oldest methods of fish preservation and this process is still been used in several places around the world. The effect of salt is to obstruct or destroy the growth of the microorganism, where in this end the fish meat gets it's way to durability. The preservation period of product is linked to the amount of salt added, there fore a straight proportion is present between the amount of salt used and the preservation period (Bahri *et al*., 2006). Salted fish products are popular in many countries around the globe. As these have been proven to be safe for millenniums, even in developed countries (Turan *et al*., 2007).

Through generations waters of Sudan (100.000 km$^2$ fresh water and 750 km length of coastal marine waters on the Red Sea) have been fished for centuries. Estimated that 26000-29000 tons of fish have been taken from them annually (Yousif, 1988). This represents about 29% of the estimated annual potential i.e. 104,000 tons (Henderson, 1975). More recent estimates of production were in the range of 110,000 ton/year (Federal Fisheries Administration Department, Annual Reports, 1996). In the Sudan, nearly 70% of the total fish landings are consumed in forms of fresh fish; the rest is cured either by salting, fermentation or sun-drying. Only few of the local fish supply is smoked, except in the southern Sudan where smoked and very dry fermented fish products are very popular among the local community (FAO, 1992). Salted fish is always made from *Hydrocynus* spp "Kass" which belong to the family Characidae (Idris, 1981).

The major reason given by the processors for choosing this species for salting is that this type of fish is relatively lean, Sudanese consumer prefers little fat in the salted products (Dirar, 1993). Other reasons given for the choosing of this fish for salting, include a belief that it is tastier than that from other fish types, moreover after treatment, the still preserve the original shape, colour and flesh, whereas other fish types become soft, lose firmness and fastly liquefy. The objective of this study was to investigate the effect of storage time on the nutritive value of the preserved *Hydrocynus forskalii* using dry traditional salting method.

## MATERIALS AND METHODS

**Collection of samples:** Samples of fresh fish were brought from local fishermen market, namely Kass (*Hydrocynus forskalii*). These samples were kept in polyethylene bags with crushed ice and transported to the Fisheries Research Center, where samples for chemical and microbiological analysis were immediately carried out.

**Processing:** Fresh fishes were always washed, eviscerated, washed again and transferred to baskets to dry up while covered by a thin cloth to prevent insects invade. Then fish were weighed to the nearest gram using a dial balance (KRUPS type 875), for the purpose of salting, a total weight of salt estimated 25% of the fish weight was used. The procedure used is called dry salting. In this method salt was applied by hand and brushing of the fish surface, the inner lining of eviscerated abdominal cavity and the gills chambers.

**Corresponding Author:** Egbal O. Ahmed, School of Fish Sciences, AL Neelain University, P.O. Box 12702, Khartoum, Sudan

This process was conducted by separating the fish layers by coarse salt mattresses inside a plastic container. The stack of the fish and salt are left about 7 days to let the salt penetrate the muscles. When the salt has penetrated the fish, it extracts the fish fluids through plasmolysis. The extracts fluid (pickle) was allowed to drain continuously. Used salt is removed from the fish surfaces and the fish restacked with new dry salt between the layers once during the ripening process. Salted product, was packed in polythene bags and stored at ambient temperature (37±1°C) for six months.

**Chemical analysis:** Moisture, protein, lipid, fiber and ash contents were determined according to AOAC (1996). Hydrogen ion concentration (pH) was determined by using of one gram of fish sample added to ten ml of distilled water and put into Heraeus CHRIST for digestion of the sample and then placed into a buffer tube of pH meter (JENNAY 3015) for reading.

**Microbiological examination:** Decimal dilutions (up to $10^{-6}$) of fish samples were prepared using sterile % 0.1 peptone water solution. The appropriate dilutions were pour-plated on appropriate media for enumeration of bacteria or yeast-mold (Harrigan, 1998). The microbiological media and incubation conditions used for enumeration of microorganisms were Plate Count Agar (PCA) for Total Viable Counts (TVC) (at 37±1°C, 48 h). Mannitol Salt Agar (MSA) was used for counting *Staphylococcus-Micrococcus* spp. (37±1°C 36-48 h), the numbers of *Staphylococcus aureus* were determined by applying coagulase test on bright yellow halo colonies on Mannitol Salt Agar. Potato dextrose agar (mark 0130) was used for counting mold and yeast (22±1°C 5 days).

**Sensory evaluation:** For examination purposes, end products were submitted on 20 persons test panel. From Fisheries Research Center staff, fishermen and some students of Department of Fisheries, College of Natural Resources, University of Juba and judged in comparison with salted fish. Comparison was carried out in terms of organoleptic characteristics, such as colour, flavour, taste, texture and general appearance. The panel was requested to rate each organoleptic feature of the end products according to a 10 point scale (9 = excellent; 8-9 very good; 6.5-7.9 good; 5-6.4 fair; <5 bad), using the score method as reported by (Afolbi *et al*., 1984).

**Statistical analysis:** The mean and standard deviation (mean ± SD) for the results obtained were calculated using SPSS software (Version 10).

## RESULTS AND DISCUSSION

The results of chemical composition of fresh fish used in salted fish preparation, namely *Hydrocynus forskalii* (Kass) was given in Table 1. The moisture contents have recorded high value 70.21±0.101%, similar results were obtained by various researchers, namely Abdullahi (2000) who worked on fresh water fish species: *Alestes nurse, A macrolepidotus*, *Hydrocynus brevis* and *Hepsetus odoe* and Clucas (1981) on *Hydrocynus vittatus*. The protein content was 20.20±0.368% on wet basis, this is probably due to the high moisture content. These results agreed with those obtained by other investigators for common Nile fishes, (Mahmmoud, 1977; Iskander, 1982; Ssali, 1988). Lipid content was 1.84±0.113% on wet basis .It was obvious that *Hydrocynus forskalii* belongs to the category of low fat fish classified by Ackman (1989) having fat content below 5%. Ash and fiber content were 1.9±0.368% and 1.93±0.110% respectively. These results are in accordance with those obtained by (Mahmmoud, 1977; Ssali, 1988).

Table 1: Chemical composition of fresh *Hydrocynus forskalii* (g/100 g)

| Parameters (%) | Mean±SD |
|---|---|
| Moisture | 70.21±0.101 |
| Ash | 1.90±0.368 |
| Protein | 20.20±0.368 |
| Lipid | 1.84±0.113 |
| Fiber | 1.93±0.110 |
| pH | 6.6±0.370 |

The moisture content changes were determined to be significant difference ($p<0.05$) and decreased progressively during the six months storage period (Table 2). The moisture contents of salted samples were determined least 19.31±0.101%, highest 63.41±0.103%. The reduction in moisture contents during storage can be attributed to protein denaturation and consequent loss of water-holding capacity of the protein in the used fish samples. Dry salting produced considerable loss of constituent water due to heavy uptake of salt (Martínez-Alvarez and Gomez-Guillén, 2006). The findings of present study are similar to the findings of Kucukoner and Akyuz (1992), on dry salted horse mackerel and Bahri *et al*. (2006) on salted Grey Mullet. Changes in protein content of salted *Hydrocynus forskalii* samples were observed to be significant ($p<0.05$). The protein content of samples was determined least 11.68±1.06 %, highest 18.00±0.100% (Table 2). It is evident that the protein content of processed fish has decreased after the course of salting. Loss of protein during processing is extremely variable. In our results, the losses of protein during storage period were averaged to 6.37%. Salting of fish was usually accompanied by protein losses, as water is drawn out a meal brine is formed, some protein is

Table 2: Changes in chemical composition of salted *Hydrocynus forskalii* (g/100 g) during storage (37±1°C)

| Parameters (%) | 0 (week) | Month 1 | Month 2 | Month 3 | Month 4 | Month 5 | Month 6 |
|---|---|---|---|---|---|---|---|
| Moisture | 63.41±0.103[a] | 56.82±0.108[b] | 38.42±0.103[c] | 25.71±0.10[d] | 22.900±0.10[e] | 20.00±00.100[f] | 19.31±0.100[g] |
| Ash | 11.20±0.10[a] | 12.22±0.106[b] | 13.00±0.36800[c] | 13.21±0.100[d] | 13.51±0.103[e] | 13.83±0.113[f] | 13.98±0.113[g] |
| Protein | 18.00±0.10[a] | 16.21±0.101[b] | 15.72±0.108[c] | 14.51±0.101[d] | 13.23±0.103[e] | 12.510±1.06[f] | 11.68±1.06[g] |
| Lipid | 1.41±0.101[a] | 1.200±0.368[b] | 1.00±0.100000[c] | 0.830±0.113[d] | 0.712±0.104[e] | 0.512±0.103[f] | 0.410±0.101[g] |
| Fiber | 1.31±0.103[a] | 1.200±0.101[b] | 0.927±0.110[c] | 0.832±0.113[d] | 0.523±0.108[e] | 0.340±0.716[f] | 0.367±0.104[g] |
| pH | 6.600±0.10[a] | 6.5000±0.10[a] | 6.200±0.100[a] | 6.100±0.155[a] | 5.900±0.100[a] | 5.600±0.104[a] | 5.5±0.106[g] |

Values are shown as mean ± standard deviation of triplicate measurements. Different superscript letters in the same row indicate significant differences between groups ($p<0.05$)

dissolved into the brine (Clucas, 1981). Generally the quantity of protein lost depends on the exact nature and duration of the salting process and the conditions of fish when salted (Eltom, 1989). The lipid contents changes were determined to be of significant difference ($p<0.05$), least 0.41±0.101% and highest 1.41±0.101%. It is clear from the present results that lipid content was decreased, this might due to the leaching out of some substances during processing as there were correlation between transfer rate of lipid from muscle and salt concentration in muscle. The transfer rate increased with high salt concentration. Changes of ash content of salted *Hydrocynus forskalii* samples were found significant ($p<0.05$) least 11.20±0.100%, highest 13.98±0.113%. It is known that the oozing of fish juice during salting usually is accompanied by losses of minerals thus the relatively high ash content observed in salted samples can be attributed to the salt penetration into fish flesh during the curing process. The findings of present study are lower than findings of (Salma *et al*., 1977) who reported that the ash content in salted sardine ranged between 14-18%. This difference may be attributed to the different in fish species, storage time and technological procedures. Changes of fiber content of salted *Hydrocynus forskalii* samples were found significant ($p<0.05$) least 0.367±0.104% and highest 1.313±0.103%. There were no significant differences ($p>0.05$) in mean pH levels during storage time for salted *Hydrocynus forskalii.* The pH values of samples were determined least 5.5±0.11, highest 6.6±0.10, (Table 2). Similar results were reported in salted fish by other researchers (Gokoglu *et al*., 1994; Kucukoner and Akyuz, 1992; Bahri *et al*., 2006). It has been noted that pH values in samples of trout, anchovy and mirror carp fish were found between 6.41-6.70 at the beginning and then changed depending on the storage period and varied between 5.34-6.81 (Bahri *et al*., 2006).

The total viable count of bacteria in fresh fish used as raw material (*Hydrocynus forskalii*), ranged between $10^2$ and $10^4$ cfu/g. The fish is more susceptible to microorganisms after catching. The number of bacterial counts may be possibly explained by contamination of fish during catching, handling, transportation and exposure to the surrounding environment. Shewan (1962), Liston (1980) and Gram (1989) noted that the bacterial flora on newly caught fish depends on the environment, in which it was caught rather than on the fish species. The total viable count of salted samples were varied during storage time. The total of viable counts of bacteria were found least <100 cfu/g, highest $6.5x10^3$ cfu/g (Table 3). There was a general trend of marked increase in case of total viable counts of bacteria in zero days (1st week), then after that the counts begin to decrease as salting proceeded. This could be explained on the basis that in a short processing period as that in the present case, it is hard to believe that the substrate for microbial growth comes from the degradation products of the protein. It is more likely that the microbial growth occurs as a result of attacking proteinaceous and other soluble nitrogenous compounds that exists in the fish juice. Also the early increase occurred while fish was wet and the provision of salt promoted the growth of halotolerant and halophilic bacteria in the fish. As the fish became drier, there was a decrease in water activity and this together with the accumulated salt in the flesh, resulted in suppression of bacterial growth. In this work $10^4$ cfu/g of the total of viable counts bacteria was used as the limit for the evaluation of microbial spoilage. When aerobic plate counts reach $10^6$ cfu/g, the food product was assumed to be at or near spoilage (Pascual and Calderón 2000; Arashisar *et al*., 2004; Ozogul *et al*., 2004). In this study, the microbial growth was lower in salted samples and hasn't reach $10^6$ cfu/g. However, by the end of storage period, growth was not detected. Count of *Staphylococcus-Micrococcus* were determined least <100 cfu/g, highest 3.4 x $10^3$ cfu/g. Similar result was obtained by (Bahri *et al*., 2006) from salted Grey Mullet. In present study *Staphylococcus aureus* was determined in samples. Small number of *Staphylococcus aureus* in water products do not cause any health problems. However, this microorganism can reach high levels (>5 $\log_{10}$ cfu/g) in products prepared by hand under bad conditions and can cause food poisoning (Varnam and Evans, 1991). The values of *Staphylococcus aureus* was still within the limit of $10^3$ cfu/g recommended by ICMSF (1978) in good manufacturing practices. No yeast or mould was detected in our fresh and salted samples.

The organoleptic properties of the salted *Hydrocynus forskalii* that the products were acceptable according to the panel's evaluation, though statistically there was

Table 3: Changes in microbiological quality of salted *Hydrocynus forskalii* during storage (+37±1°C)

| Microorganisms | 0 (week) | Month 1 | Month 2 | Month 3 | Month 4 | Month 5 | Month 6 |
|---|---|---|---|---|---|---|---|
| Total Viable Counts (TVC) (cfu/g) | $6.5 \times 10^3$ | $4.5 \times 10^3$ | $2 \times 10^3$ | $1.5 \times 10^3$ | <100 | • | • |
| *Staphylococcus-Micrococcus* (cfu/g) | $3.4 \times 10^3$ | $2.5 \times 10^3$ | $1.5 \times 10^3$ | <100 | • | • | • |
| *Staphylococcus aureus* (cfu/g) | $2.5 \times 10^2$ | $1.5 \times 10^2$ | <100 | • | • | • | • |
| Yeast-Moulds | • | • | • | • | • | • | • |

• Not detected

Table 4: Sensory evaluation of salted *Hydrocynus forskalii* by taste panel

| Parameters | 0 (week) | Month 1 | Month 2 | Month 3 | Month 4 | Month 5 | Month 6 |
|---|---|---|---|---|---|---|---|
| Odour | $9.1\pm0.368^a$ | $8.5\pm0.368^b$ | $8.2\pm0.368^c$ | $6.5\pm0.10^d$ | $6.0\pm0.10^e$ | $5.6\pm0.10^f$ | $4.2\pm0.10^g$ |
| Taste | $9.3\pm0.10^a$ | $8.1\pm0.368^b$ | $7.2\pm0.368^c$ | $6.6\pm0.10^d$ | $5.8\pm0.10^e$ | $5.5\pm0.10^f$ | $4.2\pm0.10^g$ |
| Colour | $9.5\pm0.368^a$ | $8.7\pm0.368^b$ | $8.2\pm0.368^c$ | $7.2\pm0.368^d$ | $6.4\pm0.10^e$ | $6.2\pm0.10^f$ | $5.7\pm0.10^g$ |
| Texure | $8.7\pm0.10^a$ | $7.2\pm0.10^b$ | $7.0\pm0.100^c$ | $6.5\pm0.368^d$ | $5.9\pm0.10^e$ | $5.3\pm0.10^f$ | $4.2\pm0.10^g$ |
| General appearance | $9.0\pm0.368^a$ | $8.4\pm0.368^b$ | $7.3\pm0.368^c$ | $6.2\pm0.10^d$ | $5.5\pm0.10^e$ | $5.2\pm0.10^f$ | $4.5\pm0.10^g$ |

Values are shown as mean ± standard deviation of triplicate measurements. Different superscript letters in the same row indicate significant differences between groups ($p<0.05$)

significant difference ($p<0.05$) in the sensory evaluation during storage period based on the panel's score (Table 4). In the present experiment, scores are the average of 20 panel taste sheets. It could be noticed that salted samples at zero (week) has received higher scores, followed by one month. Samples, has received lowest scores at three months and at six months the product was rejected. This wide range indicated the diversity in the final quality and can be largely attributed to the effect of various conditions upon the salting agents and activities. It is seen that the main factor affecting the quality is time of storage.

## REFERENCES


Abdullahi, S.A., 2000. Evaluation of the nutrient composition of some fresh water fish families in Northern Nigeria. J. Agric. Environ., 1: 141-151.

Ackman, R.G., 1989. Nutritional composition of fats in seafood. Prog. Food Nutr. Sci., 13: 161-241.

Afolbi, O.A., A.A. Omosole and L.O. Olusegun, 1984. Quality changes of Nigerian traditionally processed freshwater fish species. I. Nutritive and organoleptic changes. J. Food Technol., 19: 333-340.

AOAC, 1996. Official Methods of Analysis, 4th Edn. of Associated Official Analytical Chemists. Washington DC.

Arashisar, S., O. Hisar, M. Kaya and T. Yanik, 2004. Effects of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (*Oncorynchus mykiss*) fillets. Int. J. Food Microbiol., 97: 209-214.

Bahri, P., G.I. Ayse, O. Gulsum and I. Irfan, 2006. Microbiological and chemical qualities of salted grey mullet. Int. J. Sci. Technol., 2: 91-98.

Clucas, I.J. (compiler), 1981. Fish Handling, Preservation and Processing in the tropics: Part I. Report of the tropical products Institute. G 144. VIII-144.

Dirar, H.A., 1993. The Indigenous Fermented Foods of the Sudan. A study in African Food and Nutrition. CAB International, Wallingford.

Eltom, A.M., 1989. Microbiology and Biochemistry of Fessiekh Fermentation. M.Sc. Thesis, (Agric.) University of Khartoum, Sudan.

FAO, 1992. Fermented Fish in African Food and Nutrition. CAB International, Wallingford.

Federal Fisheries Administration, 1996. Annual Report. Ministry of Animal Resources, Khartoum, Sudan.

Gokoglu, N., H. Gün and C. Varlik, 1994. Determination of the preservation time of salted trout *Oncorhynchus mykiss* (Walbaum, 1792) (In Turkish). Istanbul University, J. Fish. Aquat. Sci., 1-2: 174-180.

Gram, L. 1989. Identification, characterization and inhibition of bacteria isolated from tilapia fish. Ph. D. Thesis. Royal Veterinary and Agriculture University, Lyngby, Denmark.

Harrigan, W.F., 1998. Laboratory Methods in Food Microbiology. 3rd Edn., London: Academic Press.

Henderson, F., 1975. The Fisheries of the Reservoirs of Central Sudan. FAO. Report to the Government of Sudan.

ICMSF, 1978. Microorganisms in Foods 2. Sampling for microbiological analysis: Principle and specific application. International Commission on microbiological specification for Foods. Toronto Canada, University of Toronto Press, pp: 92-104.

Idris, Z.O., 1981. Study on the morphmetric parameters and meristic counts of the family Characidea. Dissertation, Faculty of Science (Zoology), U of K, Sudan.

Iskander, I.M., 1982. Preliminary Evaluation of Nutritional consitutents of the Commoner Nile Fishes. B.Sc. Dissertation. Faculty of Science, University of Khartoum, Sudan.

Küçüköner, E. and N. Akyüz, 1992. Van-Ercis Yöresinde Farkli Salamura Metodlariyla Hazirlanan Inci Kefali Baliklarinin Mikrobiyolojik, Fiziksel, Kimyasal ve Duyusal Özelliklerinin Belirlenmesi. Yüzüncü Yil Üniversitesi, Fen Bilimleri Enstitüsü Dergisi, 1: 39-50.

Liston, G., 1980. Microbiology in fisheries science. In: Connell, J.J. (Ed.) Advances in fisheries science and technology, Fishing News Books Ltd., Farnham, England, pp: 138-157.

Mahmmoud, Z.N., 1977. Studies on meat quality of some common Nile Fish. M.Sc. Thesis Faculty of Science, University of Khartoum, Sudan.

Martínez-Alvarez, O. and M.C. Gomez-Guillén, 2006. Effect of brine salting at different pHs on the functional properties of cod muscle proteins after subsequent dry salting. Food Chem., 94: 123-129.

Özogul, F., A. Polat and Y. Özogul, 2004. The effects of modified atmosphere packaging and vacuum packaging on chemical, sensory and microbiological changes of sardines (*Sardina pilchardus*). J. Food Chem., 85: 49-57.

Pascual, M. and V. Calderón, 2000. Recuento de microorganismos aerobios mesófilos revivificables. In: Díaz de Santos (Ed.), Microbiología Alimentaria, Madrid, pp: 13-15.

Salama, M.E., E.K. Moustafa and M.S. Mohamoud, 1977. Chemical and quality attributes of salted sardine sold at the Alexandria market. Sudan, J. Food. Sci. Technol., 9: 47-54.

Shewan, J.M., 1962. The bacteriology of fresh and spoiling fish and some related chemical changes. Recent Adv. Food Sci., 1: 167-193.

Ssali, W.M., 1988. Chemical Composition Data for Nile Perch (*Lates niloticus*) and its application to the Utilization of the species fish technology, Laboratory, Uganda, Proceeding of FAO Experts consultation on fish tech. in Africa, Abidjan, Cote Devoire, 25-28 April 1988.

Turan, H., G. Sönmez, M.Y. Çelik and M. Yalçin, 2007. Effects of different salting process on the storage quality of Mediterranean Muscle (*Mytilus Galloprovincialis* L. 1819). J. Muscle Foods, 18: 380-390.

Varnam, A.H. and M.G. Evans, 1991. Foodborne Pathogens. The Netherlands. Wolf Science Book.

Yousif, O.M., 1988. Problems and scope of the future development of fisheries in the Sudan, Paper. presented at the international symposium on the development of animal resources in the Sudan, University of Khartoum, Faculty of Veterinary Science, 3-7 January 1988.

Pakistan Journal of Nutrition 9 (9): 882-886, 2010
ISSN 1680-5194


# The Breakfast Habits of Female University Students

Yahya Ozdogan[1], Ayse Ozfer Ozcelik[2] and Metin Saip Surucuoglu[2]
[1]Department of Nutrition Sciences, School of Home Economics, Ankara University, Ankara, Turkey
[2]Department of Nutrition and Dietetics, Faculty of Health Sciences, Ankara University, Ankara, Turkey

**Abstract:** The purpose of this study is to determine the breakfast habits of female university students. The population of the study was comprised of undergraduate students at the School of Home Economics, Ankara University. The sample consisted of 145 students who willingly agreed to participate in the study. The present study is based on state determination model. The data for the study was collected through questionnaire form and assessed via SPSS 11.5. The average age of the students was 21.87±1.44. Eighty two point seven percent of the students had normal body weight according to body mass index and 2.8% of them were married. It was observed that 49.0% of the students lived with their parents; 32.4% in a dormitory (24.8% in state-owned ones / 7.6% in private ones) and 18.6% with their friends. Fourty five point five percent of the students stated that they had three meals a day. The rate of the students who had regular breakfast was 44.8%. The leading cause for skipping breakfast was lack of time (20.7%).

**Key words:** Breakfast habits, female university students, nutrition

## INTRODUCTION

Transition from childhood to adulthood is a process characterized by a number of physiological, psychological and social changes. Observed during that period, physical growth and rapid increases in development, as well as changes in life styles and nutrition of youngsters, have an influence not only on their nutrition but also on meal patterns. Youngsters' participation in sport activities, disorders in pregnancy and eating behaviors, implementation of strict diets, drinking alcohol and smoking result in requirements for certain nutrients (Spear, 2002; Anonymous, 2006).

Regular meal pattern is an important factor in ensuring the physiological balance of the body for all age groups. Decreases in the number of meals result in less use of nitrogen, more absorption of glucose and glycogen synthesis and increases in fat storage and synthesis. This, in turn, leads to metabolic disorders. Therefore, it is suggested that one consume food in at least three meals a day by taking daily living conditions into account (Kilic and Sanlier, 2007).

The most crucial meal is breakfast. Approximately twelve hours pass between dinner and breakfast. Since the body continues to operate even during sleep, it uses all the food in the process. As a result of night hunger, a hungry person's blood sugar is at the lowest level at the time of breakfast. Therefore, not enough energy reaches the brain when it is deprived of breakfast in the morning (Anonymous, 2006; Merdol, 2001). Studies suggest that a sufficient level of blood sugar regulates a number of brain and behavioral functions including learning and recollection (Jacoby *et al*., 1998; Smith, 1993). Having a breakfast increases power and stability, leading students to be provided with a more efficient training (Matthys *et al*., 2007; Schlundt *et al*., 1992). Due to the fact that the brain cannot get enough energy when breakfast is skipped in the morning, individuals suffer from such problems as fatigue, headache, attention and perception deficit (Merdol, 2001; Pekcan and Baltaoglu 1988). Without breakfast, the body uses its own stores and loses its resistance to diseases (Duyff, 2002; Merdol, 2001). Breakfast consumption is important for nutritional balance in all population groups (Aranceta *et al*., 2001).

It was reported that an insufficient breakfast and bad food choices might have a negative effect on the remaining part of the day and lead to obesity in the long term (Ortega *et al*., 1998). A study concluded that breakfast consumption has an important impact on nutritional status; obese girls are more likely to skip breakfast than their normal peers and are at higher risk for growth deficits and health problems (AL-Oboudi, 2010). The purpose of the present study is to determine the breakfast habits of female university students.

## MATERIALS AND METHODS

The population of the study was comprised of undergraduate students at the School of Home Economics, Ankara University. The sample consisted of 145 students who willingly agreed to participate in the study. The data for the study was collected through questionnaire form and interviews. Body weight and height of the female students were measured and their Body Mass Index (BMI) was calculated. [BMI = body weight (kg)/height ($m^2$)]. Those with a BMI of <18.5 $kg/m^2$ were considered as "underweight"; those with a BMI of

**Corresponding Author:** Aras Gör Yahya Ozdogan, Ankara Üniversitesi Ev Ekonomisi Yüksekokulu, Irfan Bastug Caddesi No: 9, 06130 Aydinlikevler, Ankara, Turkey

18.5-24.9 kg/m$^2$ as "normal"; those with a BMI of "25.0-29.99 kg/m$^2$ as "slightly overweight " (Pekcan, 2008). The data was assessed through Statistical Package for the Social Sciences (SPSS 11.5). Furthermore, the data was presented in tables with absolute and percentage values. Arithmetic means and standard deviations $(\bar{X} \pm S)$ were calculated wherever necessary.

## RESULTS AND DISCUSSION

**Descriptive data:** The students who participated in the study varied in age from 20-27, making their average age 21.87±1.44. Twenty six point two percent of the students studied Child Development and Education; 24.8% Handicrafts and Nutritional Sciences and 24.2% Family and Consumer Sciences. Fourty six point two percent of the students were third grades; 43.5% fourth and 10.3% were second. The majority of the students stated that they lived at home with their parents (49.0%); 24.8% at state-owned dormitory; 18.6% with their friends and 7.6% in private dormitory. Ninety seven point two percent of the students were single (Table 1).

An evaluation of students according to BMI indicated that 82.7% of them were within normal limits; that 14.5% of them were underweight and 2.8% of them were slightly overweight. The mean of the BMI values was 20.84±2.36 kg/m$^2$. In a study on university students, it was observed that 65.4% of the students were within normal limits in terms of their body weight (Saglam and Yurukcu, 1996). Among the bad nutritional habits widely encountered nowadays, skipping a meal prevents an individual from having a adequate and balanced nutritional order. As can be concluded from Table 2, the rate of the students consuming two meals a day is 50.3% whereas that of the students consuming three meals a day is 45.5%. These groups are followed by those who consume only a meal (2.8%) and four and more (0.7%). A study reported that 51.0% of the students skipped meals (Yilmaz, 2002). Regular breakfast consumption can have a multitude of positive health benefits, yet young people are more likely to skip breakfast than any other meal (Pearson *et al*., 2009).

Fourty four point eight percent of the students stated that they had breakfast twice or three times a week; 44.1% of them every day and 11.1% of them only at weekend. Different studies suggested different rates of students having a regular breakfast. Thirty five point four percent in the study conducted by Tanaka *et al*. (2008), 60.7% in the one by Tuncay (2008), 34.8% in the one by Yaman and Yabanci (2006), 65.5% in the one by Demir *et al*. (2006), 42.0% in the one by Budak *et al*. (2005), 34.4% in the one by Mazicioglu and Ozturk (2003) and 19.1% in the one by Sahinoz *et al*. (1999) Saglam and Yurukcu (1996) found that 55.6% of the students had breakfast whereas Vanelli *et al*. (2005) reported that 22.0% of the students skipped it. Nicklas *et al*. (2000), Nicklas *et al*. (1998) and Elmacioglu (1995) discovered respectively that 19.0%, 37.0% and 32.0% of the students skipped breakfast. Similarly, Yilmaz (2002) reported that 61.0% of the female students skipped breakfast. Breakfast consumption improves school attendance and enhances the quality of the students' diets (Pollitt and Mathews, 1998).

Among the reasons specified as to why the students skipped breakfast were lack of time (20.7%), getting up late (17.2%), disliking eating early in the morning (11.1), not feeling hungry (6.2%) and inability to find appropriate food (0.7%). In their study, Sahinoz *et al*. (1999) found

Table 1: General information about the students (n = 145)

| Sociodemographic characteristics | s | % |
|---|---|---|
| **Age** | | |
| 20 | 15 | 10.3 |
| 21 | 58 | 40.0 |
| 22 | 35 | 24.1 |
| ≥23 | 37 | 25.6 |
| **Department** | | |
| Nutritional Sciences | 36 | 24.8 |
| Child Development and Education | 38 | 26.2 |
| Family and Consumer Sciences | 35 | 24.2 |
| Handicrafts | 36 | 24.8 |
| **Class** | | |
| Second | 15 | 10.3 |
| Third | 67 | 46.2 |
| Forth | 63 | 43.5 |
| **Residence** | | |
| At home with parents | 71 | 49.0 |
| State-owned dormitory | 36 | 24.8 |
| At home with friends | 27 | 18.6 |
| Private dormitory | 11 | 7.6 |
| **Marital status** | | |
| Married | 4 | 2.8 |
| Single | 141 | 97.2 |
| **BMI (kg/m$^2$)** | | |
| Underweight | 21 | 14.5 |
| Normal | 120 | 82.7 |
| Slightly overweight | 4 | 2.8 |

Table 2: Information about students' daily meals (n = 145)

| | s | % |
|---|---|---|
| **The number of meals** | | |
| One | 4 | 2.8 |
| Two | 73 | 50.3 |
| Three | 66 | 45.5 |
| Four and above | 2 | 1.4 |
| **Frequency of having a breakfast** | | |
| Everyday | 64 | 44.1 |
| Twice/three times a week | 65 | 44.8 |
| Weekend | 16 | 11.1 |
| **Causes for skipping breakfast** | | |
| Never skipped | 64 | 44.1 |
| Not feeling hungry | 9 | 6.2 |
| Waking up late | 25 | 17.2 |
| Lack of time | 30 | 20.7 |
| Disliking eating early in the morning | 16 | 11.1 |
| Inability to find appropriate food | 1 | 0.7 |
| **Whom to have breakfast with** | | |
| Alone | 22 | 15.2 |
| With certain members of the family | 41 | 28.3 |
| The whole family | 19 | 13.1 |
| With friends | 63 | 43.4 |

that the primary reason for skipping breakfast was lack of time. Another study concluded that the reasons for skipping breakfast were being late for school in the morning (45.2%), getting up late in the morning (30.7%) and having no appetite (26.3%) (Yaman and Yabanci, 2006). The present study found that 43.4% of the students had breakfast with their friends whereas 28.3% of them did so with certain members of family (Table 2). In another study, 51.0% of the males and 48.0% of the females stated that they had breakfast with certain members of their family (Shaw, 1998).

The leading place where students had breakfast during weekday and at weekend (58.6%, 71.7 %) was home. Other places were dormitory (24.1%), school (3.4%) and pastry shop (2.9%). In their study, Yaman and Yabanci (2006) found that 44.4% of the university students had breakfast at home while 24.1% of them did so at school canteen. Gulec *et al.* (2008) found that 70.3% of the students who lived in a dormitory had breakfast at dormitory canteen while 21.0% of them did so at school canteen. Furthermore, Nicklas *et al.* (1998) reported that 75.0% of the students had breakfast at home.

It was observed that weekday breakfast took place between 5.30 am and 1.00 pm whereas the students had their weekend breakfast between 8.00 am and 2.00 pm.

Table 3: The places where students have breakfast

| The places where students have breakfast | Weekday | | Weekend | |
|---|---|---|---|---|
| | s | % | s | % |
| Home | 85 | 58.6 | 104 | 71.7 |
| Dormitory | 35 | 24.1 | 39 | 26.9 |
| Pastry shop | 4 | 2.9 | 2 | 1.4 |
| School | 5 | 3.4 | - | - |
| Total | 129 | 89.0 | 145 | 100.0 |

A review of the frequency at which the students consumed food and beverages during breakfast (Table 4) suggested that the most widely consumed beverage was tea (71.0%) whereas the one never consumed was ayran (81.4%). It makes one happy to observe that cheese (86.2%), the source of calcium included in Turkey's traditional breakfast culture and vegetables, the source of vitamin C and fiber, were widely consumed during breakfast. The consumption of breakfast cereal was discovered to be at a very low level (28.3%). Nevertheless, studies concluded that cereal consumed at breakfast provided more fiber, iron, folic acid and zinc and less fat, sodium, sugar and cholesterol, compared with the nutrients in foods eaten during non-cereal breakfasts (Albertson *et al.*, 2008).

Studies on university students determined that the leading beverage consumed at breakfast in the morning

Table 4: The frequency at which the students consume food and beverages during breakfast

| Food and beverages (n=145) | Often | | Sometimes | | Never | |
|---|---|---|---|---|---|---|
| | s | % | s | % | s | % |
| Black tea | 103 | 71.0 | 31 | 21.4 | 11 | 7.6 |
| Milk | 10 | 6.9 | 80 | 55.2 | 55 | 37.9 |
| Ready-to-drink juice | 10 | 6.9 | 59 | 40.7 | 76 | 52.4 |
| Coffee | 9 | 6.2 | 29 | 20.0 | 107 | 73.8 |
| Other herbal teas | 9 | 6.2 | 42 | 29.0 | 94 | 64.8 |
| Ayran (a drink made of yogurt and water) | 9 | 6.2 | 18 | 12.4 | 118 | 81.4 |
| Acidic beverages | 7 | 4.8 | 25 | 17.3 | 113 | 77.9 |
| Freshly-squeezed juice | - | - | 68 | 46.9 | 77 | 53.1 |
| Cheese | 125 | 86.2 | 20 | 13.8 | - | - |
| Bread | 115 | 79.3 | 28 | 19.3 | 2 | 1.4 |
| Vegetable (tomato, pepper, cucumber etc.) | 98 | 67.6 | 38 | 26.2 | 9 | 6.2 |
| Olive | 90 | 62.1 | 48 | 33.1 | 7 | 4.8 |
| Egg | 53 | 36.6 | 71 | 49.0 | 21 | 14.4 |
| Jam | 39 | 26.9 | 74 | 51.0 | 32 | 22.1 |
| Honey | 21 | 14.5 | 91 | 62.7 | 33 | 22.8 |
| Bagel | 14 | 9.7 | 106 | 73.1 | 25 | 17.2 |
| Toast | 14 | 9.7 | 101 | 69.6 | 30 | 20.7 |
| Soup | 13 | 9.0 | 33 | 22.8 | 99 | 68.2 |
| Savory pastry | 12 | 8.3 | 107 | 73.8 | 26 | 17.9 |
| Butter | 12 | 8.3 | 74 | 51.0 | 59 | 40.7 |
| Salami-sausage | 11 | 7.6 | 90 | 62.1 | 44 | 30.3 |
| Sesame seed paste with molasses | 10 | 6.9 | 66 | 45.5 | 69 | 47.6 |
| Pastry | 8 | 5.5 | 107 | 73.8 | 30 | 20.7 |
| Cake, etc. | 8 | 5.5 | 65 | 44.8 | 72 | 49.7 |
| Sausage | 8 | 5.5 | 95 | 65.5 | 42 | 29.0 |
| Biscuit, etc. | 6 | 4.2 | 46 | 31.7 | 93 | 64.1 |
| Margarine | 5 | 3.5 | 30 | 20.7 | 110 | 75.8 |
| Breakfast cereal | 5 | 3.5 | 36 | 24.8 | 104 | 71.7 |

Table 5: Problems experienced by the students when they skipped breakfast

| | Experienced | | Not experienced | |
|---|---|---|---|---|
| Problems (n = 81) | s | % | s | % |
| Feeling hungry | 61 | 75.3 | 20 | 24.7 |
| Weariness | 58 | 71.6 | 23 | 28.4 |
| Fatigue | 40 | 49.4 | 41 | 50.6 |
| Attention deficit | 39 | 48.1 | 42 | 51.9 |
| Headache | 29 | 35.8 | 52 | 64.2 |
| Dizziness | 26 | 32.1 | 55 | 67.9 |
| Blackout | 25 | 30.9 | 56 | 69.1 |
| Feeling cold | 22 | 27.2 | 59 | 72.8 |
| Shivering | 8 | 9.9 | 73 | 90.1 |
| Throbbing | 6 | 7.4 | 75 | 92.6 |
| Perspiration | 2 | 2.5 | 79 | 97.5 |

was tea (Demir *et al*., 2006; Mazicioglu and Ozturk, 2003; Saglam and Yurukcu, 1996) whereas the leading food was cheese Yaman and Yabanci, 2006; Mazicioglu and Ozturk, 2003; Sahinoz *et al*., 1999; Saglam and Yurukcu, 1996). These findings support those of the present study.

As can be concluded from Table 5, feeling hungry (75.3%) ranked first among the problems experienced when the breakfast was skipped, followed by weariness (71.6%), attention deficit (48.1%), fatigue (49.4%), headache (35.8%), dizziness (32.1%), blackout (30.9%) and feeling cold (27.2%). A study found that those who skipped breakfast experienced reduced performance, cold and shivering (Yilmaz, 2002). In their study, Wesnes *et al*. (2003) discovered that memory and attention were deteriorated when breakfast in the morning was skipped. Another study on university students reported that such problems as hunger, weariness, fatigue and attention deficit were experienced when breakfast was skipped (Sevindi *et al*., 2007).

**Conclusion:** It is essential that high school students have a healthy diet not only because they will be healthier but also because they will be a decent role model for the next generations. Regular breakfast make a great contribution to the food insufficiently consumed in one's daily diet and have a positive impact on success. Family members play a key role in making students get into the habit of having a breakfast. It is necessary to teach the culture of having a breakfast together with family members from early on. Specialists need to inform individuals of all ages about the importance of breakfasts and enable them to change their behaviors in a positive manner.

## REFERENCES


Albertson, A.M., D. Thompson, D.L. Franko, R.E. Kleinman, B.A. Barton and S.J. Crockett, 2008. Consumption of breakfast cereal is associated with positive health outcomes: evidence from the National Heart, Lung and Blood Institute Growth and Health Study. Nutr. Res., 28: 744-752.

AL-Oboudi, L.M., 2010. Impact of breakfast eating pattern on nutritional status, glucose level, iron status in blood and test grades among upper primary school girls in Riyadh City, Saudi Arabia. Pak. J. Nutr., 9: 106-111.

Aranceta, J., L. Serra-Majem, L. Ribas and C. Perez-Rodrigo, 2001. Breakfast consumption in Spanish children and young people. Public Health Nutr., 4(A): 1439-1444.

Anonymous, 2006. Dietary guidelines for Turkey. Onur Matbaacilik Ltd.Sti, Ankara.

Budak, N., E. Ozer, S. Kovali and N. Inceis, 2005. Kahvaltinin ögrencilerin beslenmesine katkisi ve akademik basariya etkisi. Beslenme ve Diyet Dergisi, 32: 47-54.

Demir, B., E. Karabudak, G. Kiziltan and S. Basoglu, 2006. Üniversite ögrencilerinin kahvalti aliskanliklari. V. Ulusal Beslenme ve Diyetetik Kongresi Bildiriler Kitabi, 12-15 Nisan 2006, Bayt Yayinlari, Ankara.

Duyff, R.L., 2002. American dietetic association complete food and nutrition guide. John Wiley and Sons Inc., New Jersey.

Elmacioglu, F., 1995. Klinik öncesi tip ögrencilerinin beslenme ve yemek aliskanliklarinin degerlendirilmesi. Beslenme ve Diyet Dergisi, 24: 263-271.

Gülec, M., N. Yabanci, E. Göcgeldi and B. Bakir, 2008. Ankara'da iki kiz ögrenci yurdunda kalan ögrencilerin beslenme aliskanliklari. Gülhane Tip Dergisi, 50: 102-109.

Jacoby, E., S. Cueto and E. Politt, 1998. When science and politics listen to each other: Good prospects from a new school breakfast program in Peru. Am. J. Clin. Nutr., 67 (Suppl.): 795-797.

Kilic, E. and N. Sanlier, 2007. Üç kusak kadinin beslenme aliskanliklarinin karsilastirilmasi. Kastamonu Egitim Dergisi, 15: 31-44.

Matthys, C., S. De Henauw, M. Bellemans, M. De Maeyer and G. De Backer, 2007. Breakfast habits affect overall nutrient profiles in adolescents Public Health Nutr., 10: 413-421.

Mazicioglu, M.M. and A. Ozturk, 2003. Üniversite 3 ve 4. sinif ögrencilerinde beslenme aliskanliklari ve bunu etkileyen faktörler. Erciyes Tip Dergisi, 25: 172-178.

Merdol, T.K., 2001. Kahvaltinin önemi ve kahvalti örüntümüz. Türk mutfak kültürü üzerine arastirmalar. Türk Halk Kültürünü Arastirma ve Tanitimi Vakfi Yayinlari Yayin No: 28, Takav Matbaasi, Ankara, pp: 121-137.

Nicklas, T.A., C.E. O'Neil and G.S. Berenson, 1998. Nutrient contribution of breakfast, secular trends and the role of ready-to-eat cereals: a review of the data from the Bogalusa Heart Study. Am. J. Clin. Nutr., 67 (Suppl.): 757S-63S.

Nicklas, T.A., C. Reger, L. Myers and C. O'Neil, 2000. Breakfast consumption with and without vitamin-mineral supplement use favorably impacts daily nutrient intake of ninth-grade students. J. Adolescent Health, 27: 314-321.

Ortega, R.M., A.M. Requejo, A.M. Lopez-Sobaler, M.E. Quintas, P. Andres, M.R. Redondo, B. Navia, M.D. Lopez-Bonilla and T. Rivas, 1998. Differences in the breakfast habits of overweight/obese and normal weight school children. Int. J. Vitamin Nutr. Res., 68: 125-132.

Pearson, N., S.J.H. Biddle and T. Gorely, 2009. Family correlates of breakfast consumption among children and adolescents. A Syst. Rev., Appetite, 52: 1-7.

Pekcan, G. and S. Baltaoglu, 1988. Sisman kadinlarin beslenme bilgi düzeyi ve aliskanliklarinin saptanmasi. Beslenme ve Diyet Dergisi, 17: 221-234.

Pekcan, G., 2008. Beden agirliginin denetimi. Diyet El Kitabi, Hatiboglu Yayinlari: 116, Yükseködretim Dizisi: 36, Yenilenmis 5. Baski, Ankara, pp: 67-141.

Pollitt, E. and R. Mathews, 1998. Breakfast and cognition: An integrative summary. Am. J. Clin. Nutr., 67 (Suppl.): 804S-13S.

Saglam, F. and S. Yurukcu, 1996. Ankara Üniversitesi egitim bilimleri fakültesi yüksekokul ögrencilerinin besin tüketim durumu, beslenme aliskanliklari ve beslenme bilgi düzeylerinin saptanmasi. Beslenme ve Diyet Dergisi, 25: 16-23.

Sevindi, T., G. Yilmaz, S. Ibis and B. Yilmaz, 2007. Gazi Üniversitesi beden egitimi ve spor yüksek okulu ögrencilerinin beslenme ve kahvalti aliskanliklarinin degerlendirilmesi, TSA, 11: 3.

Schlundt, H., T. Sbrocco, J. Pope, Cordle and T. Shap, 1992. The role of breakfast in the treatment of obesity. A A Randomised Clinical Trial, Am. J. Clin. Nutr., 55: 645-651.

Shaw Mary, E., 1998. Adolescent breakfast skipping: An Australian study adolescence. Winter, 33: 851-861.

Smith, A., 1993. Meals mood and mental performance. Br. Food J., 95: 16-18.

Spear, B.A., 2002. Adolescent growth and development. J. Am. Diet. Assoc., 102: S23-S29.

Sahinöz, T., S. Sahinöz, F. Cetinkaya, M. Aykut and Y. Ozturk, 1999. Yurtta Kalan üniversite ögrencilerinin beslenme aliskanliklari. Erciyes Üniversitesi Saglik Bilimleri Dergisi, 8: 16-23.

Tanaka, M., K. Mizuno, S. Fukuda, Y. Shigihara and Y. Watanabe, 2008. Relationships between dietary habits and the prevalence of fatigue in medical students. Nutrition, 24: 985-989.

Tuncay, P., 2008. Baskent Üniversitesi ögrencilerinin sabah kahvalti yapma ve beslenme aliskanliklari üzerine bir arastirma. Gazi Üniversitesi, Saglik Bilimleri Enstitüsü, Yüksek Lisans Tezi, Ankara.

Vanelli, M., B. Iovane, A. Bernardini, G. Chiari, M.K. Errico, C. Gelmetti, M. Corchia, A. Ruggerini, E. Volta and S. Rossetti, 2005. Breakfast habits of 1,202 Northern Italian children admitted to a summer sport school. Breakfast skipping is associated with overweight and obesity. Acta Biomed., 76: 79-85.

Wesnes, K.A., C. Pincock, D. Richardson, G. Helm and S. Hails, 2003. Breakfast reduces declines in attention and memory over the morning in schoolchildren. Appetite, 41: 329-331.

Yaman, M. and N. Yabanci, 2006. üniversite ögrencilerinin beslenme aliskanliklarinin degerlendirilmesi, I.Uluslararasi Ev Ekonomisi Kongresi, 22-24 Mart 2006 Kongre Kitabi, Ankara Üniversitesi Ev Ekonomisi Yüksekokulu, Olusum Yayin Dagitim Ltd. Sti., Ankara, pp: 214-223.

Yilmaz, G., 2002. Nigde Üniversitesi beden egitimi ve spor yüksek okulu ögrencilerinin beslenme ve kahvalti aliskanliklarinin degerlendirilmesi. Nigde Üniversitesi, Sosyal Bilimler Enstitüsü, Yüksek Lisans Tezi, Nigde.

Pakistan Journal of Nutrition 9 (9): 887-891, 2010
ISSN 1680-5194


# Effect of Lead Toxicity on Growth, Chlorophyll and Lead ($Pb^{+}$) Contents of Two Varieties of Maize (*Zea mays* L.)

Abdul Ghani, Ameen Ullah Shah and Umer Akhtar
Department of Biological Sciences, University of Sargodha, Sargodha, Pakistan

**Abstract:** Two varieties of maize (*Zea mays* L.) (Neelam and Desi) were exposed to different concentrations of lead [0, 10, 20 and 30 ppm $Pb(NO_3)_2 \cdot 4H_2O$] for 30 days in earthen pots. Exposure of maize varieties to excess Pb resulted in a significant root growth inhibition though shoot growth remained less affected. The results of chlorophyll analysis indicated that the highly toxic Pb level affected photochemical efficiency in Neelam, while no significant effect was observed in the Desi. This result was related to the accumulation of Pb. The results of the present study indicated that, Desi withstands excess Pb with its higher Pb accumulation capacity in roots and better upregulated protective mechanisms compared to Neelam. Therefore, Desi is more tolerant to Pb toxicity compared to Neelam which was found to be susceptible variety.

**Key words:** Maize (*Zea mays* L.), lead toxicity, Pb accumulation, Pb concentrations

## INTRODUCTION

Lead is one of the heavy metals and is considered one of the dangerous environmental pollutants. It is emitted from the industries, motor vehicles, stationary fuel, road dust composition and traffic roads. Lead is not only a toxic element but also can be accumulated in plant organs and agricultural products (Burzynski, 1987; Mahmoud and El-Beltagy, 1998), consequently enter human food chain (Wagner, 1993). As a result of consumption of food, lead accumulates in human body and it may cause renal failure, brain and liver damage and it can attack the nervous system and cause failing of sickness (Lucky and Kenugopal, 1997; Ramade, 1987). Lead is one of the most difficult pollutants to control (Salt *et al*., 1998). Environmental contamination with lead has accelerated due to its close relationship to industrialization and its wide usage in paints and gasoline.

Lead (Pb) is one of the prominent examples for anthropogenic environmental metal pollution that originates from various activities including mining and smelting of lead-ores, burning of coal, effluents from storage battery industries, automobile exhausts, metal planting and finishing operations, fertilizers, pesticides and from additives in pigments and gasoline (Sharma and Dubey, 2005). Soils contaminated with Pb cause sharp decreases in crop productivity thereby posing a serious problem for agriculture (Johnson and Eaton, 1980). Although Pb is not an essential nutrient for plants, majority of lead is easily taken up by plants from the soil and accumulated in root while only a small fraction was translocated upward to the shoots (Patra *et al*., 2004). Pb affects several metabolic activities in different cell compartments. The effect of Pb depends on concentration, type of soil, soil properties and plant species. Pb toxicity leads to decrease in germination percentage, length and dry mass of root and shoots (Munzuroglu and Geckil, 2002), disturbed mineral nutrition (Paivoke, 2002), reduction in cell division (Eun *et al*., 2000).

Pb concentrations in soil and vegetation in many countries has increased with the rapid development of agriculture and industry in recent decades. Our earlier studies demonstrated that some maize varieties were tolerant to several heavy metals such as Cu and Cd (Tanyolaç *et al*., 2007; Ekmekçi *et al*., 2008). Additionally, some authors reported that *Zea mays* L. is a Pb tolerant plant (Malkowski *et al*., 1996; Heidari *et al*., 2005). In maize crops, Pb metal ions mostly accumulate in their roots and shoots (Patra *et al*., 2004; Reddy *et al*., 2005; Kopittke *et al*., 2007) and these parts are not consumed as food. Therefore, the risk of Pb uptake by crops followed by transfer in the food chain has disappeared. Consequently, there has been considerable need in finding suitable maize varieties that are able to grow on Pb-contaminated soils for land reclamation.

The uptake, transport and accumulation of Pb by plants are strongly depended on soil type and plant species, and they differ significantly with plant species. Maize is the third most important crop following wheat and barley in Pakistan. Maize yields about 3.5 million tons per year and has a sowing area of around 800,000 ha per year (FAO, 2006). So it is very important to understand the differences between two maize varieties having different tolerance capacity in Pb uptake and translocation and distribution of Pb in maize varieties. Also, in literature, there are limited number of publications on the relationship between Pb toxicity and photochemical activity of PSII (Wu *et al*., 2008). In this study, we aimed to investigate Pb accumulation in maize varieties, to

**Corresponding Author:** Abdul Ghani, Department of Biological Sciences, University of Sargodha, Sargodha, Pakistan

evaluate the effect of Pb toxicity on chlorophyll contents and to determine the effects of lead toxicity on some growth parameters which played an important role in tolerance mechanisms of the two maize varieties.

## MATERIALS AND METHODS

**Plant materials, growth and treatment conditions:** Two maize (*Zea mays* L.) varieties, Desi and Neelam, were used in this study due to their tolerance characteristics at early seedling stage to Pb stress determined by preliminary experiment which was conducted using ten maize varieties from different origin by adding different concentrations of Pb to sand culture media, to investigate the effects of Pb on maize varieties on their germination and growth behavior (Ayhan *et al*., 2007). Seeds of the varieties were surface-sterilized with 30% (v/v) sodium hyphochloride (NaOCl) solution for 20 min. Then, they were washed and imbibed in distilled water for 24 h. The seeds of varieties were germinated under dark conditions at 23±2°C on humidified filter paper with distilled water for 6 days. The seedlings of varieties were planted on plastic pots containing sand culture and watered with Hoagland solution as and when required. Seedlings of the varieties were grown at a constant temperature regime of 23±1°C, 50±5% humidity and at 250 µmol $m^{-2}$ $s^{-1}$ light intensity for 16 photoperiod in a controlled growth cabinet.

At 30th day of the growth, lead stress treatment was initiated by applying Hoagland nutrient solution containing 10, 20 and 30 ppm $Pb(NO_3)_2 \cdot 4H_2O$ to seedlings. Control plants not treated with Pb and Pb stressed plants were grown in the earthen pots under the same conditions for another 8 days. Therefore, the tissues of 38-day-old seedlings were used in the experiment.

**Determination of growth parameters:** At the end of the experiment, biomass and length of the roots and the shoots (the distance from perlite surface to upper end of the longest leaf; cm per plant) were measured for each Pb concentration. The biomass was measured on dry weight basis after drying at 80°C for 48 h, where DW and FW stand for dry weight and fresh weight, respectively.

**Determination of lead ($Pb^{+2}$) contents:** Digested samples were analyzed for Pb of those samples of shoots, roots and leaves which were treated with different concentrations of lead under the following manufacturer's recommended conditions on a perkin Elmer 3100 EDS Atomic Absorption/Emission spectrophotometer, using a fuel which air-acetylene flame, 10 cm burner head, 357.9 nm wavelength, 0.7 nm slit and 20 ma lamp current. Some digests were diluted in order to fall into the linear calibration range of 0-5 $mgL^{-1}$ (Yoshida *et al*., 1976).

**Chlorophyll (a, b and total) analysis:** Pigment contents of 14 days old plants were extracted by using the formula of Arnon and Hoagland (1949). The leaves were chopped into small pieces that were extracted with 80% acetone. The absorbance was measured at 645 nm and 663 nm for chl a,b and total chl respectively by using spectrophotometer (Hitachi Model-U 2001 Japan). Then chlorophyll a, b and total chlorophyll were calculated according to the Litehtenhaler and Wellburn (1983) formulae:

Chl a (mg $g^{-1}$ leaf fresh weight) = [12.7(OD663) -2.69 (OD645)] x V/1000 x W
Chl b (mg $g^{-1}$ leaf fresh weight) = [22.9(OD645) -4.68 (OD663)] x V/1000 x W
Total Chl(mg $g^{-1}$ leaf fresh weight) = [20.2(OD645) -8.02(OD663)] x V/1000 x W

Where: OD = Optical Density, V = Volume of sample
W = Weight of sample

**Data analysis:** The experiment was performed in a randomized design with three replicates. Differences among the Pb concentrations and between the varieties, were tested using SPSS statistical program. Statistical analysis of the data given as percentage was performed after arcsin transformation in order to stabilize variances. After transformation, the data was acceptably normal with homogenous treatment variance. Statistical variance analysis of the all data was performed using ANOVA and compared with least significant difference (LSD) at the 5% level.

## RESULTS

**Plant growth:** Exposure of maize variety s to Pb stress resulted in a significant root growth inhibition though shoot growth remained less affected (Table 1) Highly toxic Pb level (30 ppm) exhibited substantial growth reduction yielding 76% of the root length of the control in Neelam variety, whereas this value was found in Desi variety as 85% (Table 1). Although Pb content in the roots of Desi was higher than the Neelam for all Pb treatments, Pb-induced root length (or biomass) reduction of Desi was lower than that of variety Neelam (Table 1). However, the growth of maize shoots was rather resistant to Pb stress for both varieties, significant inhibition of root growth was observed in Neelam variety compared to Desi variety (Table 1). Variation of root/shoot ratio was less affected in the Desi compared to its control.

**$Pb^{+2}$ accumulation:** The lead contents of roots, shoots and leaves of both varieties increased significantly under the Pb stress when compared to control. The increase in Pb content of both varieties was found to be dependent on different Pb concentrations. Pb content of Neelam and Desi in control roots was determined. On the other hand Pb content of both varieties in control leaves was also determined. The absorbed Pb was localized to a greater extent in roots than in leaves and shoots for both varieties. At highly toxic Pb concentration, a ten folds increase in roots and 8 folds increase in

Table 1: The effects of lead concentrations on some growth parameters of two maize varieties grown at different concentrations of lead ($Pb^{+2}$)

| Varieties | ($Pb^{+2}$) concentrations ($mgKg^{-1}$ soil) | Shoot length (cm per plant) | Shoot length (% of control) | Root length (cm per plant) | Root length (% of control) | Dry biomass of root ($gDW^{-1}$) | Root/ shoot ratio |
|---|---|---|---|---|---|---|---|
| Neelam | Control | 34.00±0.70* | 100.00 | 35.31±0.31* | 100.00 | 0.21±0.01** | 1.04 |
| | 10 | 33.75±0.58 | 99.26 | 26.18±0.48 | 74.13 | 0.18±0.01 | 0.78 |
| | 20 | 30.31±0.86 | 89.16 | 24.38±0.42 | 69.03 | 0.12±0.01 | 0.80 |
| | 30 | 27.58±0.89 | 81.61 | 23.64±0.36 | 66.96 | 0.09±0.01 | 0.86 |
| Desi | Control | 34.98±0.32 | 100.00 | 35.74±0.63 | 100.00 | 0.23±0.01 | 1.02 |
| | 10 | 31.08±0.36 | 88.90 | 29.94±0.33 | 83.77 | 0.18±0.02 | 0.96 |
| | 20 | 29.20±0.62 | 83.49 | 29.01±0.31 | 81.16 | 0.17±0.01 | 0.99 |
| | 30 | 25.67±0.51 | 73.39 | 26.66±0.99 | 74.59 | 0.11±0.01 | 1.04 |
| LSD 5% | | 1.57 | | 1.28 | | 0.03 | |

*Significant at 5% level of significance

Table 2: Contents of lead ($Pb^{+2}$) (mg $kg^{-1}$ DW) in different parts of two maize varieties exposed to different concentration of lead ($Pb^{+2}$)

| Varieties | Pb concentrations ($mgKg^{-1}$ soil) | Roots | Shoots | Leaves |
|---|---|---|---|---|
| Neelam | Control | 4.1*±1.4 | 3.4±1.4 | 2.6±1.8 |
| | 10 | 266±12.4 | 143.00±6.4 | 33.93±4.1 |
| | 20 | 330±10.9 | 165.63±8.7 | 39.9±5.6 |
| | 30 | 4.4±1.5 | 177.54±7.5 | 48.7±6.0 |
| Desi | Control | 277±16.4 | 1.15±1.3 | 2.8±1.9 |
| | 10 | 384±21.3 | 149.00±7.6 | 34.94±3.1 |
| | 20 | 396±17.2 | 168.33±4.5 | 42.7±6.4 |
| | 30 | 398±19.2 | 176.43±7.2 | 47.6±5 |

*Significant at 5% level of significance

Table 3: Chlorophyll concentrations (a, b and total) of two maize varieties grown under different concentrations of lead ($Pb^{+2}$)

| Varieties | ($Pb^{+2}$) concentrations ($mgKg^{-1}$ soil) | Chlorophyll (a) | Chlorophyll (b) | Chlorophyll (Total) |
|---|---|---|---|---|
| Neelam | Control | 0.540 | 0.285 | 0.8325 |
| | 10 | 0.386 | 0.366 | 0.8826 |
| | 20 | 0.334 | 0.378 | 0.9228 |
| | 30 | 0.225 | 0.397 | 0.9529 |
| Desi | Control | 0.640 | 0.288 | 0.8535 |
| | 10 | 0.388 | 0.376 | 0.9232 |
| | 20 | 0.337 | 0.380 | 0.9428 |
| | 30 | 0.227 | 0.389 | 0.9848 |

*Significant at 5% level of significance

leaves and 6 folds increase in shoots of Desi varietiety, whereas an 11 folds increase in roots and 9 folds increase in leaves of Neelam was observed for Pb content when compared with their corresponding controls. The results indicated that the accumulated Pb level in the roots of Desi was higher than that in the roots of Neelam for all Pb treatments but the opposite was observed in the leaves. When compared between plant parts, the Pb in roots was accounted for the majority of the total Pb (90-96%) in both varieties. On the other hand, leaves and shoots usually contained much lower Pb than that of roots.

**Chlorophyll determination parameters:** Leaf chlorophylls (chl) content revealed significant differences between the maize varieties and Pb-levels at seedling stage. Total chl contents indicated a sharp decrease in Neelam while Desi maintained a steady level. Higher levels of Pb were much more damaging to Neelam as for as this attribute was concerned.

## DISCUSSION

In this study, both maize varieties have the ability to accumulate Pb primarily in their roots and transport it to their leaves in much lesser concentrations. Similar results were reported by (Patra *et al*., 2004; Sinha *et al*., 2006). The results also indicated that the accumulated Pb level in roots of Desi was higher than the roots of Neelam in all Pb treatments, but a significantly higher percent of Pb was translocated to the leaves of Neelam compared with Desi. Most Pb remained in the roots Pb transported to the leaves of the varieties. Fritioff and Greger (2006) reported that Pb remained immobile in plants. In this study, 4% of the Pb was translocated to the leaves of Neelam while for Desi it was around 2% at the highly toxic Pb level. The limited transport of Pb from roots to the other organs might be due to the barrier of the root endodermis. Histochemical observations in barley and maize seedlings also showed that, lead can not penetrate endodermis that acts as a barrier to lead uptake to shoots and the stele (Sharma and Dubey,

2005). Tolerance to metals can either be achieved by avoiding the metal stress or by tolerating it or both (Levitt, 1980). Since the tolerant variety took up more lead than susceptible variety, it appears that tolerance depends more on detoxification than on selective absorption. An electron microscopic study of root tips from tolerant plants reveals the presence of Pb in the cell wall as well as the cytoplasm (Sharma and Dubey, 2005). It has been shown that Pb compounds bind less strongly to Phytochelatins (PCS) due to larger ion radius (Pb, octahedral) and high coordination number (Pb, 6-8) (Sharma and Dubey, 2005). Brown and Slingsby (1972) showed that the high tolerance of lead in plants results from Pb accumulation only in the cell wall without penetrating into the protoplast. These results indicate that both varieties might avoid the lead stress by binding it to their cell walls. Also, less migration of lead to the leaves of Desi meant that Desi was more tolerant than Neelam to the lead stress. On the other hand, increased concentrations of Pb induced significant growth inhibition in both varieties as observed in the reduction of the root length (Table 1). Highly toxic Pb concentration inhibited root elongation in Neelam variety whereas this value determined in Desi variety as compare to their controls. Consequently, Pb stored predominantly in roots and while shoots were less affected in both varieties. Similar results were reported by Bashmakov *et al*. (2005). They indicated that 30 ppm of Pb concentrations caused suppression of growth processes and accumulation of Pb especially in roots. It was reported that plants have developed various tolerance and resistance mechanisms in order to diminish the heavy metal stress. One of these mechanisms is to hold the heavy metal in the root and prevent the distribution to the leaves (Fernandes and Henriques, 1991). Yang *et al*. (2000) reported that when exposed to a solution containing Pb, the root biomass of the tolerant variety was higher than that of the sensitive rice variety because of the ability of the tolerant variety to develop adventitious roots. Root development in tolerant variety was associated with a mechanism that altered the Pb in the solution into a form that could no longer be taken up by a newly growing tissue. Similar response in dry biomass of roots of Desi variety was observed in this study (Table 1).

**Conclusion:** The results of this study showed that higher Pb accumulation in roots in comparison with leaves and better defense systems are the most important characteristics of maize varieties in order to tolerate excess lead. It is clear that Desi is more tolerant to Pb toxicity compared to Neelam which was found to be a particularly less tolerant variety. Desi showed lower Pb accumulation in leaves. Maize varieties were found to be tolerant to Pb treatment without damage for concentrations up to 20 ppm. We consider that *"Maize"* is an excellent crop-model to study heavy metal stress tolerances at the biochemical and growth level due to its ability to withstand certain heavy metal stress conditions. Consclusively, *Zea mays* L. varieties are Pb-tolerant plants so that they can be selected as suitable species growing on the lead contaminated soils for bioremedial purpose.

## REFERENCES


Arnon, D.I. and D.R. Hoagland, 1949. The water culture method for growing plants without soil, Cal. Agric. Exp. Sta. Cir., 3: 346-347.

Ayhan, B., Y. Ekmekçi and D. Tanyolaç, 2007. Investigation of the tolerance to heavy metal (cadmium and lead) stress of some maize variety s at early seedling stage. Anadolu Univ. J. Sci. Tech., 8: 411-422 in Turkish with English abstract.

Bashmakov, D.I., A.S. Lukatkin, V.V. Revin, P. Duchovskis, A. Brazaityte and K. Baranauskis, 2005. Growth of maize seedlings affected by different concentrations of heavy metals. Ekologija, 3: 22-27.

Brown, D.H. and D.R. Slingsby, 1972, New Phytol., 71, 297.

Burzynski, M., 1987. The uptake and transpiration of water and the accumulation of lead by plants growing on lead chloride solutions. Acta Societatis Botanicorum. Poloniae, 56: 271-280.

Ekmekçi, Y., D. Tanyolaç and B. Ayhan, 2008. Effects of cadmium on antioxidant enzyme and photosynthetic activities in leaves of two maize variety s. J. Plant Physiol., 15: 600-611.

Eun, S.O., H.S. Youn and Y. Lee, 2000. Lead distrubs microtubule organization in the root meristem of *Zea mays*. Physiol. Plant, 110: 357-365.

FAOSTAT Food and Agriculture Organization of the United Nations (FAO), 2006. Statistical databases.

Fernandes Je, and F.S. Henriques, 1991. Biochemical, physiological and structural effects of excess copper in plants. Bot. Rev., 57: 246-273.

Fritioff, A. and M. Greger, 2006. Uptake and distribution of Zn, Cu, Cd and Pb in an aquatic plant *Potamogeton natans*. Chemosphere, 63: 220-227.

Heidari, R., M. Khayami and T. Farboodnia, 2005. Effect of pH and EDTA on Pbaccumulation in *Zea mays* seedlings. J. Agron., 4: 49-54.

Johnson, M.S. and J.W. Eaton, 1980. Environmental contamination through residual trace metal dispersal from a derelict lead-zinc mine. J. Environ. Qual., 9: 175-179.

Kopittke, P.M., C.J. Asher, R.A. Kopittke and N.W. Menzies, 2007. Toxic effects of $Pb^{2+}$ on growth of cowpea (*Vigna unguiculata*). Environ. Pollut., 150: 280-287.

Levitt, J., 1980. Responses of plants to environmental stresses, vol II, 2nd Edn., Academic Press, New York, pp: 607.

Litehtenhaler, H.K. and A.R. Wellburn, 1983. Determination of total crotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Trans., 603rd Meeting Liverpool, 591-592.

Lucky, T.D. and B. Kenugopal, 1997. Metal toxicity in mammals. Plenum Press, New York and London.

Malkowski, E., J. Stolarek and W. Karcz, 1996. Toxic effect of $Pb^{2+}$ ions on extension growth of cereal plants. Pol. J. Environ. Stud., 5: 41-45.

Mahmoud, W.H. and A. El-Beltagy, 1998. Isolation, Identification and potential use of lead reduction from heavy metal polluted soil. Menufiya J. Agric. Res., 23: 1461-1473.

Munzuroglu, O. and H. Geckil, 2002. Effects of metals on seed germination, root elongation and coleoptile and hypocotyl growth in *Triticum aestivum* and *Cucumis sativus*. Arch Environ. Contam. Toxicol., 43: 203-213.

Paivoke, A.E.A., 2002. Soil lead alters phytase activity and mineral nutrient balance of *Pisum sativum*. Environ. Exp. Bot., 48: 61-73.

Patra, M., N. Bhowmik, B. Bandopadhyay and A. Sharma, 2004. Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. Environ. Exp. Bot., 52: 199-223.

Ramade, F., 1987. Ecotoxicity, John Wiley and sons, Chichester, pp: 119-135.

Reddy, A.M., S.G. Kumar, G. Jyothsnakumari, S. Thimmanaik and C. Sudhakar, 2005. Lead induced changes in antioxidant metabolism of horsegram (*Macrotyloma uniflorum* (Lam.) verdc.) and bengalgram (*Cicer arietinum* L.). Chemosphere, 60: 97-104.

Salt, D.E., R.C. Prince, I.J. Pickering and I. Raskin, 1998. Mechanism of cadmium mobility and accumulation in Indian mustard. Plant Physiol., 109: 1427-1433.

Sharma, P. and R.S. Dubey, 2004. Ascorbate peroxidase from rice seedlings: Properties of enzyme isoforms, effects of stresses and protective roles of osmolytes. Plant Sci., 167: 541-550.

Sharma, P. and R.S. Dubey, 2005. Lead toxicity in plants. Braz. J. Plant Physiol., 17: 35-52.

Sinha, P., B.K. Dube, P. Srivastava and C. Chatterjee, 2006. Alteration in uptake and translocation of essential nutrients in cabbage by excess lead. Chemosphere, 65: 651-656.

Tanyolaç, D., Y. Ekmekçi and S. Ünalan, 2007. Changes in photochemical and antioxidant enzyme activities in maize (*Zea mays* L.) leaves exposed to excess copper. Chemosphere, 67: 89-98.

Wagner, G.J., 1993. Accumulation of heavy metals in crop plants and its consequences to human health. Adv. Agron., 51: 173-177.

Wu, X., F. Hong, C. Liu, M. Su, L. Zheng, F. Gao and F. Yang, 2008. Effects of $Pb^{2+}$ on energy distribution and photochemical activity of spinach chloroplast. Spectrochim Acta A Mol. Biomol. Spectrosc., 69: 738-742.

Yang, Y.Y., J.Y. Jung, W.Y. Song, H.S. Suh and Y. Lee, 2000. Identification of rice varieties with high tolerance or sensitivity to lead and characterization of the mechanism of tolerance. Plant Physiol., 124: 1019-1026.

Yoshida, S.D.A . Farno, J.H. Cock and K.A .Gomez, 1976. Laboratory manuals of Physiological studies of rice. IRRI. Loss Bonas, Phillipines.

Pakistan Journal of Nutrition 9 (9): 892-895, 2010
ISSN 1680-5194


# Seasonal Variation in Iron in Rural Groundwater of Benue State, Middle Belt, Nigeria

Ocheri Maxwell Idoko
Department of Geography, Benue State University, P.M.B. 102119, Makurdi, Nigeria

**Abstract:** Effect of changes in season visa vise rural groundwater quality variability with respect to iron is examined in this study. Water samples were collected from 26 rural community boreholes and analyzed for iron concentrations as it affects the quality of water for drinking in line with WHO drinking water standards for both rainy and dry seasons. The analyses was carried out as prescribed according to standard method of examination of water. The result of analyses show 35% of the boreholes have elevated iron concentrations above WHO guide limit for rainy season as against that of the dry season which is only 7.7% of the boreholes. Iron concentrations in the boreholes were noted to be higher in the rainy season than in the dry season. The source of iron in groundwater may be attributed to dissolution of iron minerals from rock and soils, corrosion effect of galvanized hand pump components and land use activities. Agencies involved in rural water supply must ensure the safety of the groundwater being harnessed for use. This study demonstrate the need for groundwater quality management at the rural level because once polluted is very expensive to remedy.

**Key words:** Iron, season, variation, groundwater, borehole, water quality

## INTRODUCTION

Over one billion people lack access to clean safe water worldwide (Bresline, 2007; NAS, 2009). In sub-Saharan Africa alone, up to 300 million rural people have no access to safe water supplies. Without safe water near dwellings, the health and livelihood of families can be severely affected (United Nations, 2000; MacDonald *et al*., 2005).

Groundwater exploitation is generally considered as the only realistic option for meeting dispersed rural water demand (MacDonald *et al*., 2005). Groundwater include all water found beneath the earths surface in a saturated zone of the aquifer (Todd, 1980). They are formations that contains sufficient saturated permeable materials to yield sufficient quantities of water to wells and springs (Loham, 1972). Groundwater can be abstracted by means of hand dug wells and boreholes at various depths. A large percentage of the world population depends on groundwater as their main source of drinking water (Rajagopal, 1978; Shiklomanov, 1993; Shah, 2004). This is because it is accessible anywhere; it is less capital intensive to develop and maintain; it is less susceptible to pollution and seasonal fluctuations and of natural good quality (Bresline, 2007; Habila, 2005). However, the quality is under intense stress from increasing demand and withdrawal, significant changes in land use pattern, climate change and pollution arising from geology and geochemistry of the environment (Mackey, 1990; Edmunds and Smedley, 1996).

Benue State is predominantly a rural state with about 80% of the population residing in rural areas. This segment of the population is faced with problems of extreme water scarcity during dry season. Traditional sources of water such as streams, rivers and lakes available to this rural population are under pressure from deforestation activities like bush clearing and burning, lumbering, and various land uses. The consequence is these sources are exposed to direct effect of solar radiation leading to their drying up. Women and children who are the major drawers of water suffer untold hardships in the cause of searching for water for household use. In 1994, there an outbreak of disease that claimed several lives. This was however attributed to drinking of polluted water. In response, Benue State Rural water supply and sanitation Agency, a UNICEF Assisted and WaterAid a DFID funded British water charity organization commence activities of providing improved source of water through borehole water supply systems. A good number of rural communities now have boreholes. Although a welcome development, the quality of groundwater being exploited for rural water supply demand considerations. Water from some of the boreholes could not be used for drinking on account of colour, odour and taste. Consumers to a large extent have no means of judging the safety of water themselves, but their attitude toward drinking water and drinking water supplies will be affected to a considerable extent by the aspects of water quality they are able to perceive with their senses. It is natural for consumers to regard with suspicion water that appear dirty or coloured or has unpleasant taste or smell, even though these characteristics may not in themselves be of direct consequence to health (WHO, 2006). Polluted water isn't just dirty, it is deadly (NAS, 2009).

Studies on rural groundwater pollution has been carried out in different parts of Nigeria (Ezeigbo, 1988; Aiyesanmi *et al*., 2004; Adelunle *et al*., 2007; Olobaniyi and Owoyemi, 2008). Consistent in their findings is that groundwater is polluted from physical processes and anthropogenic activities. There are paucity of studies on the influence of season on groundwater quality variability. In this study, attempt is made to assess the influence of season on the quality of groundwater with respect to iron.

Iron in rural groundwater supplies is a common problem: its concentration level ranges from 0- 50 mg/l, while WHO recommended level is <0.3 mg/l. The iron occurs naturally in the aquifer but levels in groundwater can be increased by dissolution of ferrous borehole and handpump components (Lenntech, 2009). All natural water contained some dissolved iron in traces. Iron is present in all rocks, soil and sand. The most common is ferrious iron. Water which contains iron on exposure to air become reddish brown due to ferric hydroxide. Human beings suffer no harmful effect of water containing iron. However, long term consumption of drinking water with high concentration can lead to liver disease (Morris, 1952; Lee and Stum, 1960; Hem, 1970). High concentration of iron groundwater are widespread and sometimes underrated constraints on rural water supply. Iron can cause colour in water which may lead consumers to reject such water. This kind of water when used can cause staining of cloth, utensil and food and bitter taste. Although this has no direct health significance, problem arise if communities decide not to use this water and return to old polluted sources (MacDonald *et al*., 2005). For example, in Ghana, 20-30% of boreholes drilled water supplies contains excessive iron concentration. Water from these wells have been rejected on account of coloration effect (Peligba *et al*., 1991). Groundwater in the confined aquifer of sedimentary basin are particularly vulnerable to built-up of dissolved iron and manganese under anaerobic condition (Okagbue, 1988; Akujieze *et al*., 2003; Amadi *et al*., 1989; BGS, 2003).

**The study area:** The study area is rural communities of Benue State in the middle belt of Nigeria. The area has a population of 4, 219, 244 (NPC, 2006) and its economy is sustained principally by agricultural production. Crops produced include yam, cassava, rice, maize, oranges, mangoes etc. Because of the declining fertility application of chemical fertilizer is on the increase. The geology of the study area is predominantly a sedimentary formation comprising of sandstones, mudstones and limestones (Kogbe *et al*., 1978; Offodile, 2002). Pockets of basement complex rocks are found in Gkoko, Guma, Ushongo, Vandeikya and OjuLGAs of the study area. The soil is mainly a tropical ferrugised type with hypomorphic and lateric soils along flood plain of rivers and within hills. The study area is drained by River Benue and its tributary River Katsina-Ala. Other rivers include Aya, Guma, Konshisha, logo, Obi and Okpokwu. The climate is controlled by two air masses which is responsible for both rainy and dry seasons. Annual rainfall total ranges from 1,500-2000 mm. Temperature is generally high during the dry season leading to high rate of evaporation. The vegetation is mainly of savanna type.

## MATERIALS AND METHODS

Data for this study were obtained from water sample collected from 26 rural community boreholes across the study area. Two sets of water samples were collected in the months of October when rainfall is highest in the area and February one of the extreme months of dry season. The water samples were analyzed according to standard method of examination of water (APHA-AWWA-WPCF, 1995) and reported in WHO standards for drinking water. The concentration of iron in water sample were analyzed using Atomic Absorption Spectrophotometer (AAS) model Unicon sp 6-550. The method is based on absorption of radiation by free atoms in vapour state. The atoms of element whose lamp or flame is being absorbed at precisely the wave length as that emitted by its light source. The amount of energy at the characteristic wave length absorbed by the flame is proportional to the concentration in the sample over a limited concentration range. The results of AAS analyses is shown in Table 1.

## RESULTS AND DISCUSSION

The results of analyses as shown in Table 1 reveal BH3, BH4, BH6, BH14, BH15, BH16, BH19, BH23, BH24 and BH26 have iron concentrations above WHO prescribed limit of 0.1-1.0 for drinking water. This translate to 35% of the boreholes having elevated iron concentrations above WHO guide limit for drinking water for the rainy season. For dry season only BH4 and BH18 have iron concentrations exceeding WHO maximum limit for drinking water. This translates into 7.7% of the boreholes having iron concentrations above the WHO allowable limit for drinking water. It was also noted that water from some of the boreholes could not be used for drinking due to objectionable colour problem. However, water from some of the boreholes with objectionable colour were used for drinking in the absence of any other alternative. Prolonged consumption of this kind of water may cause health risks over time. The cause of iron in these wells may be traced to the geology of the environment, dissolution of iron minerals from rocks, corrosion emanating from the use of galvanized handpump construction and land use activities. From Table 2 iron concentration in the boreholes have a mean of 0.82 and CV 81.71% for rainy season as against a mean and CV of 0.33 and 324.24% for the dry season. Although iron concentration are higher in the rainy than in the dry season their variation in groundwater is lesser

Table 1: Atomic absorption spectrophotometer (AAS) analysis

| Rural community | Code | Iron mg/l (Rainy season) | Iron mg/l (Dry season) |
|---|---|---|---|
| Ikpayongo | BH1 | 0.85 | 0.14 |
| Tsenor | BH2 | 0.50 | 0.43 |
| Awajir | BH3 | 1.11 | 0.43 |
| Kyoor | BH4 | 2.38 | 5.02 |
| Ega | BH5 | 0.54 | 0.52 |
| Uje | BH6 | 2.20 | 0.05 |
| Obarike-Ito | BH7 | 0.08 | 0.25 |
| Ugbodom | BH8 | 0.55 | 0.38 |
| Ogi | BH9 | 0.10 | 0.61 |
| Ulayi | BH10 | 0.71 | 0.05 |
| Asaaga-Ashe | BH11 | 0.21 | 0.65 |
| Udei | BH12 | 0.59 | 0.51 |
| Fiidi | BH13 | 0.48 | 0.01 |
| Ake | BH14 | 0.24 | 0.11 |
| Uchi-Mbakor | BH15 | 1.11 | 0.46 |
| Annune | BH16 | 2.16 | 0.26 |
| Ambigir | BH17 | 0.09 | 0.09 |
| Tse Kucha | BH18 | 0.03 | 1.11 |
| Garagbohol | BH19 | 1.07 | 0.95 |
| Buruku | BH20 | 0.35 | 0.12 |
| Sati- Asema | BH21 | 0.43 | 0.20 |
| Amaafu | BH22 | 0.32 | 0.05 |
| Mbaagba | BH23 | 1.11 | 0.39 |
| Ushongo | BH24 | 1.52 | 0.16 |
| Ihugh | BH25 | 0.10 | 0.04 |
| Mbajor | BH26 | 1.38 | 0.63 |

BH - Borehole

Table 2: Descriptive characteristics of iron in groundwater in the study area

| Season | Min. | Max. | Mean | STD | CV% |
|---|---|---|---|---|---|
| Rainy | 0.03 | 2.38 | 0.82 | 0.67 | 81.7 |
| Dry | 0.01 | 5.20 | 0.33 | 1.07 | 324.24 |

STD - Standard Deviation; CV% - Coefficient of Variation

in the rainy season when compared to that of the dry season as reflected in the coefficient of variation. This probably may due to influence of rainfall infiltrating and dissolving iron mineral in rocks and soil which are leached into groundwater sources.

**Conclusion:** The study has shown the presence of iron in rural groundwater of Benue State even to objectionable level in some boreholes. The concentrations of iron are noted to vary spatially across the study area and among seasons. Although iron in water is generally perceived as aesthetic problem than health problem not withstanding some form of treatment may be required. Long consumption of this kind of water may cause health risks. Agencies involved in rural water supply must ensure quality control of the water being provided as matter of priority.

## REFERENCES


Adelunle, I.M.M., M.T. Adetunji, A.M. Gbadebo and O.B. Banjoko, 2007. Assessment of groundwater quality in a typical rural settlement in south west Nigeria, Int. J. Res. Pub. Health, 4: 307-318.

Aiyesanmi, A.F., R.O. Ipinmoroti and Adeyinwo, 2004. Baseline geochemical characteristics of groundwater within Okitipupa, S.E belt of the bituminous sand field of Nigeria. Int. J. Envir. Stu., 4: 49-57.

Akujieze, C.N., C.O. Coker and G.E. Oteze, 2003. Groundwater in Nigeria: A new millennium experience: Distribution, practice, problems and solution. Hydrogeol. J., 11: 404-413.

Amadi, P.A., C.O. Ofoegbu and T. Morrison, 1989. Hydrogeochemical assessment of groundwater in parts of Niger Delta, Nigeria. Environ. Geol. Water Sci., 14: 195-202.

APHA-AWWA-WPCF, 1995. American Public Health Association-American Waterworks Association-Water Pollution Control Federation: Standard method for Examination of water and waste waters, New York, USA., 19th Edition.

BGS., 2003. British Geological Survey, Tech. Report.

Bresline, E., 2007. Sustainable water supply in developing countries. Geol. Soc. Amer. Pap. No. 194-1.

Edmunds, W.M. and P.L. Smedley, 1996. Groundwater geochemistry and health: An overview in: Appleton, J.D., Fuge, R and MaCall, G. JH (Eds.) Environmental geochemistry and Health, BGS, Special Publ., 113.

Ezeigbo, H.H., 1988. Geological and hyrogeological influence on the Nigeria environment. J. Water Res., 1: 36-44.

Habila, O., 2005. Groundwater and the Millennium Development Goals, Proceedings Groundwater and poverty reduction in Africa. Int. Assoc. Hydrogeol. London.

Hem, J.D., 1970. Study and interpretation of the chemical characteristics of natural waters, US Geol. Sur. Water Suppl., pp: 1473.

Kogbe, C.A., A. Torkaeshi, D. Osijuk and D.E. Wozney, 1978. Geology of Makurdi sheet 257 in the middle valley, Nigeria, Occasional Publ. Dept. of Geology, Ahmadu Bello University, Zaria.

Lee, G.H. and Stum, 1960. Determination of ferrious iron in the presence of ferric iron using bathophenanthroline. J. Am. Waterworks Assoc., 52: 1567.

Lenntech, 2009. Iron in groundwater, Lenntech water treatment and purification holding B.V, Rotterdamseweg, Netherlands.

Loham, S.W., 1972. Groundwater hydraulic, U.S Geol. Surv. Prof. Pap.

MacDonald, A., J. Davies, R. Calow and J. Chilton, 2005. Developing groundwater: A guide torural water supply, ITDG publishing.

Mackey, R., 1990. Groundwater quality in Thannah and Biswas, A.K (Eds.) Environmentally sound water management, Oxford University Press.

NPC, 2006. National Population Commission, The Nigeria census, Federal Government of Nigeria.

Morris, R.L., 1952. Determination of iron in the presence of heavy metals. Analy. Chem., 25: 1376.

NAS, 2009. National Academy of Science: Overview-safe drinking water is essential.

Offodile, M.E., 2002. Groundwater study and development in Nigeria, Mecon Engineering services, Jos.

Olobaniyi, S.B. and F.B. Owoyemi, 2008. Characterisaation by factor analysis of chemical facies of groundwater in the Deltaic sand aquifer of Warri, western Niger Delta. Afr. J. Sci. Technol., 7: 73-81.

Okagbue, O.C., 1988. Hydrology and chemical characteristics of surface and groundwater resources of Okigwe area of Imo State in: Ofoegbu C.O and Vieweg S Groundwater and mineral resources of Nigeria.

Peligba, K.B., C.A. Biney and L.A. Antwi, 1991. Trace metal concentration in boreholes water of upprr region and Accra plain, Ghana, Water, Air and Soil Poll., 59: 333-345.

Rajagopal, R., 1978. Impact of landuse on groundwater quality in Grande Transverse Bay, Michigan, J. Envir. Qly., 7: 93-98.

Shiklomanov, I.A., 1993. World Fresh Water Resource In: Gleick, P.H (Ed.) Water crisis: A guide to world fresh water resources, Oxford University press.

Shah, T., 2004. Groundwater and human development: Challenges and opportunities in livelihood and environment, Proceedings Stockholm World Water Week.

Todd, D.K., 1980. Groundwater hydrology, John Wiley.

United Nations, 2000. Millennium Development Goals on water.

WHO, 2006. Guidlines for drinking water quality, first addendum to 3rd edition, Vol. 1 Recommendations, Geneva.

Pakistan Journal of Nutrition 9 (9): 896-901, 2010
ISSN 1680-5194


# Integrated Plant Nutrient Management (IPNM) on Maize under Rainfed Condition

M.D. Dilshad[1], M.I. Lone[2], Ghulam Jilani[2], M. Azim Malik[2], M. Yousaf[2],
Rizwan Khalid[1] and Fakhra Shamim[2]
[1]Soil Fertility Survey and Soil Testing Institute, Rawalpindi, Pakistan
[2]PMAS Arid Agriculture University, Rawalpindi, Pakistan

**Abstract:** Integrated use of plant nutrients aim at combined use of inorganic and organic sources of plant nutrients to improve efficiency of applied nutrients, reduce environmental hazards and improve crop productivity. A field experiment was conducted at the Research Farm of Soil Science and SWC, Arid Agriculture University, Rawalpindi, Pakistan. It was laid out according to RCBD in split-plots with three replications. The sub-plot size was 6 m x 4 m (24 $m^2$). The wheat-maize cropping system was used to record data of two summer maize crops in under rainfed environment. There were nine treatments of integrated plant nutrient management practices. These included: control (without NPK fertilizer, FYM or biofertilizer); half of recommended NPK; full dose of recommended N-$P_2O_5$-$K_2O$ (120-90-60 kg/ha); FYM @ 20 t/ha, FYM on N requirement basis + make-up dose of P/K fertilizer; ½ NPK + FYM @ 10 t/ha; ½ NPK + Biopower; ½ FYM + Biopower and ½ NPK + ½ FYM + Biopower. The significant increase in various yield attributes due to IPNM produced highest biological and grain yield of 8579 kg/ha and 3128 kg/ha in 2005; while these were recorded 8475 kg/ha and 3119 kg/ha respectively in 2006. Integrated plant nutrient treatments especially with Biopower improved NPK uptake over sole mineral/organic fertilizers. The economic analysis revealed that wheat-maize cropping system was profitable with integrated use of mineral, organic and/or biofertilizer Biopower under rainfed condition.

**Key words:** Plant nutrients, environmental hazards, crop productivity

## INTRODUCTION

The integrated use of organic and inorganic fertilizers not only increase mutual efficiency but also help in the substitution of costly chemical fertilizers (Hussain and Ahmed, 2000; Ghosh and Sharma, 1999). Maize (*Zea mays* L.) is one of the most important cereal crops of the world used as food, feed and raw materials. Globally maize is grown on 140 million hectares. Out of which 96 m ha are in the developing world. It makes 68% of the total areas but only 46% of the world maize is produced there. Average yield of maize in the industrialized countries is 8 t/ha. The major crops of rainfed areas of Pakistan are wheat, maize, sorghum, millet and mungbean. The average maize yield in Pakistan is 1.86 t/ha. Still in rainfed areas of Pakistan, grain yield of maize is 54% lower than in irrigated areas (GOP, 2008). The Pothwar zone produces more than 80% of rainfed maize. The average is very low in spite of is great yield potential. Improvement in average yield per hectare can be obtained if soil fertility is maintained through the combined use of organic and inorganic fertilizers.

Considering the economic importance of maize crop as fodder and grain, this field experiment was conducted to study the effect of IPNM on yield components and yield of maize under rainfed conditions.

## MATERIALS AND METHODS

A field experiment was conducted at the research farm of Arid Agriculture University, Rawalpindi, Pakistan to evaluate the effects of Integrated Plant Nutrient Management (IPNM) on yield and yield components of summer maize (*Zea mays* cv. Agaiti, 2002) during the Kharif seasons of 2005 and 2006. It was laid out according to RCBD in Split-plots having nine Integrated Plant Nutrient Management (IPNM) practices: control (No IPNM), ½ recommended doze of NPK, NPK, Farmer's application of FYM @ 20 t/ha, FYM (N equivalent with P make up dose, ½ NPK + biofertilizer Biopower, ½ FYM + Biopower, ½ NPK + ½ FYM, ½ NPK + ½ FYM + Biopower. All treatments were applied with three replications. The sub-plot size was 6 m x 4 m (24 $m^2$). Composite soil sample were collected from the experimental field from two depths (0-15 cm and 15-30 cm) before sowing. Soil samples were collected from each treatment from depth (0-15 cm) after the harvest of maize.

**Chemical analysis:** Soil samples were analyzed for various physical and chemical characteristics. Soil texture was determined by hydrometer method as described by Koehler *et al.* (1984); pH in soil water suspension (1:10) with pH meter by the method outlined

**Corresponding Author:** M.D. Dilshad, Soil Fertility Survey and Soil Testing Institute, Rawalpindi, Pakistan

Table 1: Physical and chemical properties of composite soil sample before start of experiment

| Characteristics | Depth 0-15 cm | 15-30 cm |
|---|---|---|
| Clay (%) | 16.00 | 17.00 |
| Silt (%) | 39.00 | 40.00 |
| Sand (%) | 45.00 | 43.00 |
| Soil texture | ---------- Sandy loam ---------- | |
| Soil pH | 7.80 | 7.91 |
| $EC_e$ ($dSm^{-1}$) | 0.25 | 0.21 |
| Bulk density ($Mgm^{-3}$) | 1.40 | 1.53 |
| Soil moisture (g 100 $g^{-1}$) | 8.82 | 9.20 |
| Total N (µg $g^{-1}$) | 152.00 | 154.00 |
| Organic C (g100 $g^{-1}$) | 0.32 | 0.33 |
| Available P (µg $g^{-1}$) | 3.45 | 3.55 |
| Extractable K (µg $g^{-1}$) | 80.00 | 85.00 |
| Zn (µg $g^{-1}$) | 0.34 | 0.33 |
| Fe (µg $g^{-1}$) | 2.15 | 2.32 |
| Mn (µg $g^{-1}$) | 1.33 | 1.33 |
| Cu (µg $g^{-1}$) | 0.31 | 0.32 |
| Soil series | ------ Rawalpindi soil series ----- | |
| Soil order | *Inceptisol* | |
| Parent material | Loess | |

by Mc Lean (1984). Alkaline earth carbonate in soil was determined by using acid neutralization method as outlined by Richards (1954). Organic carbon was determined by the method given by Nelson and Sommers (1982). Total nitrogen was determined by Kjeldahl digestion method (AOAC, 1982). Available phosphorus was determined by Spectronic 601 (Milton Roy Co.) as described by Soltanpour and Schwab (1977). $NH_4$ acetate extractable potassium was determined by Flame photometer (PFP Jenway). The data for yield and yield components was recorded at physiological maturity. Leaf and grain samples from individual treatments were analyzed for total nitrogen by Kjeldahl digestion method; Phosphorus was determined by AB-DTPA extractable P method; Total K concentration was determined by flame photometer.

**Economic and statistical analysis:** Growth, yield and soil parameters were recorded and then analyzed statistically according to standard statistical procedures described by Sokal and Rohlf (1997). Data showing significant difference at $p \leq 0.05$ was put to comparison of treatments means by Duncan's (1961) multiple range test. All the data was processed using MSTAT software for statistical analysis. For economic analysis, after considering the cost of fertilizer N, P, K, farmyard manure and biofertilizer Biopower application, the incomes from seed yield were used for economic analysis (CIMMYT, 1988) using the formula:

Value Cost Ratio (VCR) = Value of increased yield obtained/ cost of mineral/organic/biological nutrient sources

Table 2: Effect of integrated plant nutrient management on plant height (cm) of maize

| Treatments | Years 2005 | 2006 |
|---|---|---|
| | ---------- CS ---------- | |
| $T_1$ Control | $172.2^{C}$ | $170.6^{D}$ |
| $T_2$ NPK (60-45-30) kg $ha^{-1}$ | $182.1^{B}$ | $193.5^{C}$ |
| $T_3$ NPK (120-90-60) kg $ha^{-1}$ | $206.7^{A}$ | $206.5^{AB}$ |
| $T_4$ Full FYM @ 20 t $ha^{-1}$ | $183.4^{B}$ | $201.9^{BC}$ |
| $T_5$ FYM (N Eq + P make up) | $189.1^{B}$ | $203.8^{BC}$ |
| $T_6$ ½NPK + ½ FYM | $208.3^{A}$ | $207.1^{AB}$ |
| $T_7$ ½NPK + Biopower | $185.9^{B}$ | $197.4^{BC}$ |
| $T_8$ ½FYM + Biopower | $187.9^{B}$ | $198.8^{BC}$ |
| $T_9$ ½NPK + ½ FYM + Biopower | $211.0^{A}$ | $216.0^{A}$ |
| **Analysis of variances** | | |
| P-Value | <0.001 | <0.001 |
| LSD | 9.14 | 9.85 |
| SE | 3.05 | 3.28 |
| CV (± %) | 2.75 | 2.85 |

Data are average of three replications.

• Means followed by the same letter (s) are not significantly different ($P < 0.05$; DMR Test) to each other.

CS: Cropping System. Biofertilizer Biopower (seed inoculation at sowing). NPK @ (120-90-60) kg/ha. Farmyard Manure (FYM) @ 20 t/ha. FYM * (N equivalent + P make up doze)

## RESULTS AND DISCUSSION

**Plant height:** Plant height is important parameter of yield in maize as usually taller plant bears more cobs and give more yield. Comparison of various treatment means in 2005 indicated that plant height increased significantly as compared to control (Table 2). The application of ½ NPK + ½ FYM + Biopower produced maximum plant height of 211.0 cm followed by 208.36 cm by treatment of ½ NPK + ½ FYM and 206.73 cm due to application of NPK which were statistically at par among each other. During 2006, the application of ½ NPK + ½ FYM + Biopower produced maximum plant height of 216.0 cm followed by 207.10 cm by treatment of ½ NPK + ½ FYM and 206.56 cm due to application of NPK which were statistically at par among each other. The plant height recorded in 2006 is better than plant height in 2005 due to IPNM. The treatment of ½ NPK + ½ FYM + Biopower produced maximum plant height in both years.

**Biological yield:** Biological yield represents total amount of above ground biomass accumulated by the plant. The data pertaining to the biological yield (kg/ha) of maize is given in Table 3. During first year (2005), ½ NPK + ½ FYM + Biopower ($T_9$) produced highest biological yield of 8579 kg/ha. It was followed 8292 kg/ha by treatment of ½ NPK + ½ FYM ($T_6$) with a significant difference between the two. Mineral fertilizers NPK produced 7933 kg/ha, which was significantly higher than all treatments except $T_6$ and $T_9$. FYM with P make up dose ($T_5$) and FYM @ 20 t/ha ($T_4$) gave biological yield of 7409 kg/ha

Table 3: Effect of integrated plant nutrient management on biological yield (kg/ha) of maize

| Treatments | Years | |
|---|---|---|
| | 2005 | 2006 |
| | CS | |
| $T_1$ Control | $3562^{I}$ | $3290^{H}$ |
| $T_2$ NPK (60-45-30) kg ha$^{-1}$ | $5563^{H}$ | $4191^{G}$ |
| $T_3$ NPK (120-90-60) kg ha$^{-1}$ | $7933^{C}$ | $7884^{B}$ |
| $T_4$ Full FYM @ 20 t ha$^{-1}$ | $7147^{E}$ | $6981^{D}$ |
| $T_5$ FYM (N Eq + P make up) | $7409^{D}$ | $7252^{C}$ |
| $T_6$ ½ NPK + ½ FYM | $8292^{B}$ | $8060^{B}$ |
| $T_7$ ½ NPK + Biopower | $6214^{G}$ | $6051^{F}$ |
| $T_8$ ½ FYM + Biopower | $6543^{F}$ | $6508^{E}$ |
| $T_9$ ½ NPK + ½ FYM + Biopower | $8579^{A}$ | $8475^{A}$ |
| **Analysis of variances** | | |
| P-Value | <0.001 | <0.001 |
| LSD | 51.19 | 236.2 |
| SE | 17.08 | 78.79 |
| CV (± %) | 0.43 | 2.09 |

and 7147 kg/ha respectively, which were statistically significant to each other. Both Biopower treatments with ½ NPK ($T_7$) and ½ FYM ($T_8$) produced biological yield 6214 kg/ha and 6543 kg/ha respectively, which were significantly different to each other.

During second year (2006), ½ NPK + ½ FYM + Biopower ($T_9$) produced highest biological yield of 8475 kg/ha. It was followed by 8060 kg/ha due to $T_6$ treatment with a significant difference. Mineral NPK fertilizers ($T_3$) produced 7884 kg/ha, which was significantly lower to both $T_9$ and $T_6$. FYM with P make up dose and FYM @ 20 t/ha gave biological yield of 7252 and 6981 kg/ha respectively, which were statistically significant to each other. Both Biopower treatments with ½ NPK and ½ FYM produced biological yield of 6051 and 6508 kg/ha respectively, which were significantly different to each other. The difference between highest and lowest biological yield was recorded as 5017 kg/ha and 5185 kg/ha in 2005 and 2006 respectively indicating substantial increase in biological yield due to integrated use of mineral and organic/biological nutrient sources. Biofertilizer Biopower in combination with organic and mineral fertilizers increase the microbial activity that in turn enhanced the rate of decomposition of organic matter and nutrient got available to plants for growth. The results are similar to Wu *et al*. (2005) who evaluated the effects biofertilizers on soil properties and the growth of *Zea mays* and reported not only increased the nutritional assimilation (total N, P and K) of plants, but also improved soil properties, such as organic matter content and total N in soil. However, biological yield produced in 2006 was lower than that produced in 2005. It might be due to the reason that there was lower rainfall during the maize growing season in 2006. Jadoon *et al*. (2004), Bhatti (2006), Khaliq *et al*. (2006) and Ahmad *et al*. (2008) also recorded better yield attributes of crops by integrated use of organic and mineral fertilizers.

Table 4: Effect of integrated plant nutrient management on grain yield (kg/ha) of maize

| Treatments | Years | |
|---|---|---|
| | 2005 | 2006 |
| | CS | |
| $T_1$ Control | $1310^{I}$ | $1155^{G}$ |
| $T_2$ NPK (60-45-30) kg ha$^{-1}$ | $1920^{H}$ | $1476^{F}$ |
| $T_3$ NPK (120-90-60) kg ha$^{-1}$ | $2878^{C}$ | $2840^{B}$ |
| $T_4$ Full FYM @ 20 t ha$^{-1}$ | $2584^{E}$ | $2446^{D}$ |
| $T_5$ FYM (N Eq + P make up) | $2672^{D}$ | $2570^{C}$ |
| $T_6$ ½ NPK + ½ FYM | $3045^{B}$ | $2878^{B}$ |
| $T_7$ ½ NPK + Biopower | $2251^{G}$ | $2172^{E}$ |
| $T_8$ ½ FYM + Biopower | $2375^{F}$ | $2372^{D}$ |
| $T_9$ ½ NPK + ½ FYM + Biopower | $3128^{A}$ | $3119^{A}$ |
| **Analysis of variances** | | |
| P-Value | <0.001 | <0.001 |
| LSD | 22.62 | 113.5 |
| SE | 7.545 | 37.87 |
| CV (± %) | 0.53 | 2.81 |

**Grain yield:** Data pertaining to the grain yield of maize crop is given in Table 4. During first year (2005), application of ½ NPK + ½ FYM + Biopower ($T_9$) produced highest grain yield of 3128 kg/ha. It was followed by treatment ½ NPK + ½ FYM ($T_6$) that produced 3045 kg/ha with a significant difference to preceding treatment. Mineral NPK fertilizers produced grain yield of 2878 kg/ha, which was significantly lower to both $T_9$ and $T_6$. The FYM with P make up dose ($T_5$) and FYM @ 20 t/ha ($T_4$) gave grain yield of 2672 kg/ha and 2584 kg/ha respectively, which were statistically significant to each other. Biofertlizer Biopower treatments with ½ NPK ($T_7$) and ½ FYM ($T_8$) produced grain yield of 2251 kg/ha and 2375 kg/ha respectively, which were significantly different to each other.

During second year (2006), ½ NPK+½ FYM+Biopower ($T_9$) produced maximum grain yield of 3119 kg/ha. It was followed by significantly lower grain yield of 2878 kg/ha by treatment $T_6$. Recommended dose of mineral fertilizers NPK produced grain yield of 2840 kg/ha, which was significantly lower than $T_6$. Application of FYM with P make up dose and FYM @ 20 t/ha produced grain yield of 2570 kg/ha and 2446 kg/ha respectively, which were statistically significant to each other. Biopower treatments with ½ FYM recorded a significant increase in grain yield (2372 kg/ha) over Biopower treatment with ½ NPK that produced grain yield of 2172 kg/ha. A significant increase in grain yield due to integrated nutrient management practices was observed in both years. Maximum grain yield was observed, when ½ NPK+½ FYM+ Biopower were applied. Biofertilizer Biopower in combination with organic and mineral fertilizers increased the microbial activity that might in turn enhance the rate of decomposition of organic matter and nutrients were available to plants for growth. Results of previous studies indicated that use of organic sources as FYM produced equivalent or increased plant biomass and grain yield of maize as application of inorganic fertilizers alone (Alam and Shah, 2003; Bakhtiar *et al*., 2002; Khanum *et al*., 2001).

Table 5: Effect of integrated plant nutrient management on NPK uptake (kg/ha) of maize

| | Years | | | | | |
|---|---|---|---|---|---|---|
| | 2005 | | | 2006 | | |
| Treatments | N | P | K | N | P | K |
| $T_1$ Control | 38 | 8 | 58 | 27 | 8 | 53 |
| $T_2$ NPK (60-45-30) kg $ha^{-1}$ | 70 | 12 | 106 | 48 | 13 | 80 |
| $T_3$ NPK(120-90-60) kg $ha^{-1}$ | 138 | 42 | 244 | 148 | 40 | 256 |
| $T_4$ Full FYM @ 20 t $ha^{-1}$ | 118 | 26 | 211 | 120 | 36 | 209 |
| $T_5$ FYM (N eq + P make up) | 125 | 29 | 195 | 128 | 40 | 221 |
| $T_6$ ½NPK + ½ FYM | 141 | 31 | 217 | 146 | 37 | 191 |
| $T_7$ ½NPK + Biopower | 90 | 25 | 136 | 89 | 26 | 135 |
| $T_8$ ½ FYM + Biopower | 99 | 25 | 151 | 101 | 27 | 148 |
| $T_9$ ½NPK + ½ FYM + Biopower | 143 | 48 | 228 | 156 | 46 | 220 |

The difference between highest and lowest grain yield was recorded as 1818 kg/ha and 1964 kg/ha in 2005 and in 2006 respectively, indicating substantial increase in biological yield due to integrated use of mineral and organic/biofertilizer nutrient sources. However, grain yield produced in 2006 was lower than that produced in 2005. Variation in grain yield may also be due to difference in rainfall amount and distribution and/or temperature variation during the growing season in first year and second year. Low moisture availability affects fertilizer use efficiency and yield components in rainfed areas Jadoon *et al*. (2004), Bhatti (2006), Khaliq *et al*. (2006) and Ahmad *et al*. (2008) also recorded better yield of crops by integrated use of organic and mineral fertilizers.

**NPK uptake by maize:** Data pertaining to the N, P and K uptake (kg/ha) of maize is given in Table 5. Integrated plant nutrient management practices in wheat-maize ($CS_2$) cropping system increased N, P and K uptake (kg/ha) by maize. During 2005, application of ½ NPK + ½ FYM + Biopower ($T_9$) recorded maximum N uptake of 143 kg/ha followed by N uptake of 138 kg/ha by recommended dose of NPK fertilizers ($T_3$). Maximum P uptake of 48 kg/ha was recorded in $T_9$ followed by P uptake of 42 kg/ha by mineral fertilizers ($T_3$). Maximum K uptake of 244 kg/ha was recorded by mineral fertilizers ($T_3$) followed by K uptake of 228 kg/ha in $T_9$. During 2006, application of ½ NPK + ½ FYM + Biopower ($T_9$) recorded maximum N uptake of 156 kg/ha followed by N uptake of 148 kg/ha by recommended dose of NPK fertilizers ($T_3$). Maximum P uptake of 46 kg/ha was recorded in $T_9$ followed by P uptake of 40 kg/ha by mineral fertilizers. Maximum K uptake of 256 kg/ha was recorded by mineral fertilizers ($T_3$) followed by K uptake of 220 kg/ha in $T_9$. The results showed that integrated use of organic and mineral fertilizers and/or biofertilizers showed better performance regarding grain yield of wheat. Integrated plant nutrient management had advantage over the sole application of mineral and organic and/ or biofertilizers. During 2005, application of ½ NPK + ½ FYM + Biopower ($T_9$) recorded 3.6 and 14.2% increase in uptake of N and P by maize over mineral fertililizer treatment ($T_3$) while during 2006, application of ½ NPK + ½ FYM + Biopower ($T_9$) recorded 5.4 and 15% increase in uptake of N and P by maize over mineral fertililizer treatment ($T_3$). Integrated plant nutrient treatment especially with Biopower improved NPK uptake over mineral fertilizers. This was due to the fact that Biopower contained four different species of N-fixing bacteria, which resulted into increased availability of nitrogen to the crop. Wu *et al*. (2005) indicated that half the amount of biofertilizer application had similar effects when compared with organic fertilizer or chemical fertilizer treatments. Microbial inoculum not only increased the nutritional assimilation (total N, P and K) of plants, but also improved soil properties. Combination of N fertilizer with EM also increased the concentrations of NPK in plants (Khaliq *et al*., 2006).

**The economic analysis:** The Value Cost Ratio (VCR) is the ratio between the value of the additional seed yield and the cost of the fertilizer. It is the rate of return of the money spent on fertilizer. If the VCR is greater than one, the fertilizer will be profitable. A VCR of 2 represents a 100% return on the money invested in fertilizer. At VCR lower than 2, farmer's margin of return becomes low.

Data regarding the VCR due to IPNM for maize is represented in Table 6. The VCR indicated that the IPNM showed profitable effect on maize in both years. These indicate that VCR due to IPNM is 1.91 in $T_9$ and 1.86 in $T_6$ while 1.89 in $T_3$. Better VCR in $T_9$ might be due in residual soil fertility. Similarly, VCR of 2.06 in $T_9$, 1.85 in $T_6$ while 2.04 in $T_3$ were estimated in 2006. It indicated better performance of integrated use of mineral and organic and/or biofertilizers. During 2005, highest VCR was recorded with treatment $T_7$ (½ NPK + Biopower), while lowest was recorded in control. It was followed by VCR recorded in $T_9$ (½ NPK + ½ FYM + Biopower) depicting the profitability of integrated use of inorganic and organic and/or biofertilizers for wheat production under rainfed condition. During 2006, highest VCR was recorded with treatment $T_7$ (½ NPK + Biopower), while lowest was recorded in control. It was followed by VCR

Table 6: Value cost ratio due to integrated plant nutrient management on seed yield of maize

| Treatments | Years 2005 | 2006 |
|---|---|---|
| | ---------- $CS_2$ ---------- | |
| $T_1$ Control | - | - |
| $T_2$ NPK (60-45-30) kg $ha^{-1}$ | 1.47 | 0.78 |
| $T_3$ NPK (120-90-60) kg $ha^{-1}$ | 1.89 | 2.04 |
| $T_4$ Full FYM @ 20 t $ha^{-1}$ | 1.23 | 1.24 |
| $T_5$ FYM (N Eq + P make up) | 1.27 | 1.32 |
| $T_6$ ½ NPK + ½ FYM | 1.86 | 1.85 |
| $T_7$ ½ NPK + Biopower | 2.17 | 2.34 |
| $T_8$ ½ FYM + Biopower | 1.97 | 2.25 |
| $T_9$ ½ NPK + ½ FYM + Biopower | 1.91 | 2.06 |
| **Prices 2004-06** | **Rs.** | **US $** |
| Urea (50 kg bag) | 468.0 | 7.8 |
| Diammonium phosphate (50 kg bag) | 801.0 | 13.35 |
| Triple super phosphate (50 kg bag) | 801.0 | 13.35 |
| Sulphate of potash | 996.0 | 16.6 |
| Farmyard maure | 1200.0 | 20 |
| Biofertilizer Biopower (1.0 kg packet) | 100.0 | 1.6 |

CS: Cropping System. Biofertilizer Biopower (seed inoculation at sowing). NPK @ (120-90-60) kg/ha. Farmyard manure (FYM) @ 20 t/ha. FYM * (N equivalent + P make up dose). (US $1.0 = Rupees 60 during 2004-2006)

recorded in $T_9$ (½ NPK + ½ FYM + Biopower) depicting the profitability of integrated use of inorganic and organic and/or biofertilizers for maize production under rainfed condition.

**Conclusion:** The investigation presented in this study indicated some distinct benefits of IPNM over use of mineral fertilizers. The results of this study showed that IPNM of ½ NPK + ½ FYM + Biopower in $T_9$ was most appropriate and economical for better yield of maize in rainfed areas. The results confirmed that besides increasing the crop yield, IPNM saved mineral fertilization which effected sustainable agricultural production in less fertile soils of rainfed Pothwar region of Pakistan. The higher nutrient concentration in maize demonstrated more efficient use of applied nutrients by IPNM. More intensive and systematic studies are required to provide a better understanding of the usefulness of IPNM in making crop production more profitable income generating activity for small farmers of rainfed Pothwar region of Pakistan. Integrated plant nutrient management is going to be the mainstay in the next millennium.

## REFERENCES


Ahmad, R., M. Naveed, M. Aslam, Z.A. Zahir and M. Arshad, 2008. Economizing the use of nitrogen fertilizer in wheat production through enriched compost. Renewable Agric. Food Syst., 23: 243-249.

Alam, S.M. and S.A. Shah, 2003. Effect of individual versus integrated use of phosphatic fertilizer on P uptake and yield of maize and wheat. Pak. J. Soil Sci., 22: 74-80.

AOAC, 1982. Official Methods of the Association of Official Analytical Chemists, Washington DC., 12th Edn. (Ed. Horwitz, W.), pp: 15-18.

Bakhtiar, S.M., M.J. Alam, K. Mahmood and M.H. Rehman, 2002. Integrated nutrient management under three agro-ecological zones of Bangladesh. Pak. J. Bio. Sci., 5: 390-393.

Bhatti, A.U., 2006. Restoring crop productivity of eroded lands through Integrated Plant Nutrient Management (IPNM) for sustained production. Final technical Report 2003-2006, Pakistan Science Foundation Research Project, Deptt. of Soil and Environmental Sciences, NWFP Agriculture University Peshawar, Pakistan.

CIMMYT, 1988. An Economic Training Manual: From Agronomic Data to Farmer Recommendations. International Maize and Maize Improvement Center (CIMMYT), Mexico, pp: 1-25.

Duncan, D.B., 1961. Multiple Ranges and multiple F-test. Biometrics, 11: 1-42.

Ghosh, A. and A.R. Sharma, 1999. Effect of combined use of organic manure and nitrogen fertilizer on the performance of rice under flood-prone lowland conditions. J. Agric. Sci., 132: 461-465.

Government of Pakistan, 2008. Agricultural statistics of Pakistan (2007-2008). Ministry of Food, Agriculture and Livestock (Economic Wing), Islamabad.

Hussain, T.I. and M.A. Ahmed, 2000. EM Technology-A new looks for IPNM. In: Proc. Symp., Integrated Plant Nutrient Management, NFDC, Islamabad, Pakistan.

Jadoon, M.A., A.U. Bhatti, F. Khan and Q.A. Sahabzada, 2004. Effect of farmyard manure in combination with NPK on the yield of maize and soil physical properties. Pak. J. Soil Sci., 22: 47-55.

Khaliq, A., M.K. Abbasi and T. Hussain, 2006. Effects of integrated use of organic and inorganic nutrient sources with Effective Microorganisms (EM) on seed cotton yield in Pakistan. Bioresour. Tech., 7: 967-972.

Khanum, M., M.M. Rahman and M.R. Islam, 2001. Effect of manures and fertilizers on growth and yield of BRRI Dhan 30. Pak. J. Bio. Sci., 4: 172-174.

Koehler, F.E., C.D. Moudre and B.L. McNeal, 1984. Laboratory manual for soil fertility. Washington State University, Pulman, USA.

Mc Lean, E.O., 1984. Soil pH and Lime requirement. In: Page, A.L., R.H. Miller and D.R. Keeney. (Eds). Methods of Soil Analysis Part 2. Amer. Soc. Agron. No. 9. Madison, Wisconsin, USA., pp: 199-209.

Nelson, S.W. and I.E. Sommers, 1982. Total carbon, organic carbon and organic matter. In: Page A.L (Ed.), Methods of soil analysis. Part 2. Chemical and microbiological properties. American Society of Agronomy, Madison, WI. USA., pp: 539-580.

Richards, L.A., 1954. Diagnosis and improvement of saline and alkali soils. USDA Handbook 60. Washington DC.

Sokal, R.R. and F.J. Rohlf, 1997. Biometry. The Principles and Practice of Statistics in Biological Research, W.H. Freeman, New York, USA.

Soltanpour, P.N. and A.P. Schwab, 1977. A new soil test for simultaneous extraction of macro and micronutrients in alkaline soils. Commun. Soil Plant Anal., 8: 195-207.

Wu, S.C., Z.H. Cao, Z.G. Li, K.C. Cheung and M.H. Wong, 2005. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: A greenhouse trial. Geoderma, 125: 155-166.

Pakistan Journal of Nutrition 9 (9): 902-904, 2010
ISSN 1680-5194


# Detoxification of *Jatropha curcas* Seeds for Use in Nutrition of Monogastric Livestock as Alternative Feedstuff

A.A. Annongu[1], J.K. Joseph[3], D.F. Apata, A.O. Adeyina[2], M.B. Yousuf and K.B. Ogunjimi
[1]Nutritional Biochemistry and Toxicology Division, Faculty of Agriculture,
P.O. Box 10004, University of Ilorin Post Office, Ilorin, Nigeria, West Africa
[2]Animal Physiology Unit, University of Ilorin, Ilorin, Nigeria
[3]Department of Food Science and Home Economics, University of Ilorin, Iorin, Nigeria

**Abstract:** An experiment was conducted to investigate the utilization of Jatropha seed cake by Albino rats. Jatropha Seed Cake (JSC) treated by boiling, fermentation followed by extraction with equal volumes of hexane and ethanol was included in diets at graded levels of 5, 10, 15, 20 and 25%. Data obtained on performance and body organ indices showed that rats tolerated up to 15% dietary JSC without adverse effects on the measured parameters in relation to the corn-soy reference diet ($p>0.05$). However, 20 and 25% inclusion levels elicited mortality in all the animal subjects receiving the diets within one week in the course of the experimental trial.

**Key words:** Jatropha seed cake, Albino rats, performance, organ weights indices

## INTRODUCTION

As the world human population increases averaging seven billions, there is concomitant increase in demand and competition for conventional food/feedstuffs used both by man and monogastric livestock. This is especially true in underdeveloped and developing countries where food production cannot keep pace with the high growth in population. There is also an increase in the prices of these orthodox feedstuffs as a result of competition between feed industries and man. This has caused developing countries to embark on researches focused on novel feedstuffs, that are not staple for human consumption to alleviate the problems of shortage and competition for the available traditional feedstuffs. It is for these reasons that *Jatropha curcas* seeds are considered as alternative feedstuff in this experiment.

*Jatropha curcas* is regarded as a wonder plant because of its numerous attributes; the seeds contain up to 60% oil with a fatty acid pattern similar to that of edible oil, the percentage of essential amino acids and mineral content can be compared to those of other seeds (Makkar and Becker, 1999a). The use of Jatropha in animal nutrition is however faced with several problems of anti-nutritional factors such as lectins, saponins, tannins, phytic acid, trypsin inhibitors, hydrocyanides and phorbolesters (Makkar and Becker, 1999b). Due to these phytotoxins, the seeds, cake or its oil cannot be used for human or animal consumption. Nevertheless, in order to search for alternative feeds, this experiment attempted to investigate the detoxication of Jatropha seeds to improve its nutritional value so that it could be used in monogastric nutrition.

## MATERIALS AND METHODS

10 kg of Jatropha seeds were collected from Ilorin, Nigeria and sun-dried to constant weight. The seeds were boiled, fermented as described by Annongu *et al.* (1996) and soaked in equal volumes of hexane and ethanol for 24 h to remove some of the lipo-soluble toxins. After the chemical extraction, the seeds were milled to flour before inclusion in diet mixtures. Six diets of equal energy and protein content were formulated as given in Table 1 on diets composition. Treated Jatropha Seed Cake (JSC) meal was included in diets at 5, 10, 15, 20 and 25%. 72-Albino rats (at 3-weeks old) of equal sexes were randomly allotted to six dietary treatments made of a corn-soy reference diet and the other diets containing the processed JSC at the percentages given above. A treatment was made of two replicates with six rats per replicate and the experiment followed a completely randomized design, CRD.

Rats were fed to appetite for 21-days during which data was collected on performance and carcass characteristics. For carcass studies, one rat per replicate was sacrificed and the organs, heart, lungs, intestines and liver were removed for determination of absolute and relative organ weights.

**Chemical analyses:** Quantification of residual toxins in dietary JSC was carried out following the appropriate methods for tannins (Joslyn, 1970), phytic acid (Wheeler and Ferrel, 1971), cyanides (AOAC, 1990) while saponins were determined as outlined by Hudson and El-Difrawi (1979). The proximate chemical composition of the samples was carried out according to AOAC (1990).

**Corresponding Author:** A.O. Adeyina, Animal Physiology Unit, University of Ilorin, Ilorin, Nigeria

Table 1: Composition of the experimental diets on as fed basis

| | Diets | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Ingredients (g) | Control | 5% JSM | 10% JSM | 15% JSM | 20% JSM | 25% JSM |
| Corn starch | 516.00 | 516.00 | 516.00 | 516.00 | 516.00 | 516.00 |
| Soybean meal | 250.00 | 200.00 | 150.00 | 100.00 | 50.00 | 0.00 |
| JSM | 0.00 | 50.00 | 100.00 | 150.00 | 200.00 | 250.00 |
| Sucrose | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Cellulose | 40.00 | 40.00 | 40.00 | 40.00 | 40.00 | 40.00 |
| DL-methionine | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 |
| Vitamin-premix | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 |
| Total | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |

**Statistical analysis:** Analysis on performance and organ weights data were made using the analysis of variance, ANOVA following the complete randomized design (Steel and Torrie, 1990).

## RESULTS AND DISCUSSION

Table 2 presents data on performance characteristics of Albino rats fed graded levels of processed Jatropha Seed Meal Cake (JSM) in diets. There was no statistical significant difference in feed intake by the surviving groups of rats offered the test diets 2, 3 and 4 relative to the group fed the conventional diet ($p>0.05$). Body weight gain significantly increased as the inclusion level of treated JSM increased from 5-15% ($p<0.05$) in comparison with the control diet. There was however, no significant difference in feed to gain ratio among the treatments ($p>0.05$). Albino rats given diets 2, 3 and 4 containing 5, 10 and 15% processed dietary JSM gave 100% survival rate compared with the reference diet while all the rats offered diets 5 and 6 containing 20 and 25% JSM died within one week in the course of the experiment.

Feed intake on diets containing the test feedstuff at 5, 10 and 15% was comparable with the control diet since no significant difference was observed suggesting that Jatropha seeds boiled, fermented and extracted with hexane and ethanol could be included up to 15% in monogastric animal diets without adverse effects on feed consumption. Rats receiving diets with 10 and 15% dietary treated JSM were heavier in weight than those on the standard and 5% dietary JSM probably due to the high protein content of Jatropha seed cake meal. Previous works (Makkar and Becker, 1999a) showed that Jatropha seed cake or meal contains between 56-60% crude protein. In this study, JSM was included at the expense of soybean cake usually containing 44% protein. The higher protein content of JSM might have aided the increased weight gain on the diets in question. Efficiency of feed utilization on diets 2-4 was similar to the conventional diet indicating equality in nutritional composition. Similarly, no mortality was recorded on diets 2-4 containing substituted treated JSM at 5, 10 and 15% relative to the reference diet. However, 20 and 25% dietary JSM elicited death of all the animal subjects with in one week of the experiment. Mortality recorded on these diets suggested that inclusion of treated JSM at levels above 15% is not tolerated by the monogastric rats. The intolerance might be as result of cumulative effect of Jatropha seed phytotoxins which residual influence persisted in the processed test feedstuff in diets.

Analysis of diets containing the treated JSM for residual toxins of tannins, saponins, cyanides, phytic acid showed that the diets still contained residues of these toxic chemicals from Jatropha seed cake. This might also be true for lectins, trypsin inhibitors and phorbolesters undetermined in this experiment. The residues of these phytotoxins lend support to explain that even though dietary JSM was treated in this study, inclusion levels above 15% proved fatal due to the residual effects of the toxins.

Table 3 presents data on Absolute Organ Weights (AOW) in rats fed graded levels of JSM in diets. There were significant differences in absolute weights of the heart, lungs, liver and intestines compared with the organs of rats on the control diets ($p<0.05$). Weight of organs increased concomitant with increase in dietary treated JSM from 5-15%. Relative Organ Weights (ROW) data (Table 4) followed the trend similar to that on absolute weights. Results on AOW and ROW showed that intestines, liver, lungs and hearts of rats on the treated test feedstuff increased in weight as the inclusion level of the JSM increased up to the acceptable level of 15% by the rats. The increment in weight of the organs on these diets agreed with the increase in weight and could also be explained on the basis of higher protein content of JSM compared with soybean in the control diet.

Results of analyzed residual Jatropha seed toxins in diets and the proximate chemical composition in native JSM given in Table 5 and 6 presents residual cyanides, saponins, phytic acid and tannins besides haemagglutinins, trypsin inhibitors and phorbolesters in the virgin JSM respectively. The residues of these toxicants in the treated test feedstuff seemed to have deleterious effects on the nutrition of monogastric

Table 2: Performance characteristics of Albino rats fed graded levels of processed dietary JSM

| | Diets | | | | | | |
|---|---|---|---|---|---|---|---|
| Parameters | 1 | 2 | 3 | 4 | 5 | 6 | SEM |
| Avg. feed intake (g/r/d) | 21.53 | 24.43 | 21.11 | 21.10 | - | NS | 1.6 |
| Weight gain (g/r/d) | 15.00[a] | 15.40[a] | 17.10[b] | 17.30[b] | - | * | 1.17 |
| Feed efficiency (f/g) | 1.40 | 1.58 | 1.20 | 1.22 | - | NS | 0.17 |
| Survival rate (%) | 100.00 | 100.00 | 100.00 | 100.00 | - | - | - |

[a-b]Mean values in rows not sharing common superscripts are significantly different ($p<0.05$).
NS, No significant difference ($p>0.05$)

Table 3: Absolute organ weights of Albino rats given graded levels of dietary treated JSM

| | Diets | | | | | | |
|---|---|---|---|---|---|---|---|
| Organs (g) | 1 | 2 | 3 | 4 | 5 | 6 | SEM |
| Heart | 0.50[a] | 0.85[b] | 0.82[b] | 0.83[b] | - | - | 0.16 |
| Lungs | 1.40[a] | 2.65[b] | 2.60[b] | 2.39[b] | - | - | 0.58 |
| Intestines | 2.70[a] | 4.76[b] | 4.82[b] | 4.69[b] | - | - | 1.02 |
| Liver | 0.55[a] | 1.01[b] | 0.92[b] | 0.92[b] | - | - | 0.20 |

Table 4: Relative Organ Weights (ROW) of Albino rats fed graded levels of processed dietary JSM

| | Diets | | | | | | |
|---|---|---|---|---|---|---|---|
| ROW (g) | 1 | 2 | 3 | 4 | 5 | 6 | SEM |
| Heart | 0.80[a] | 0.85[c] | 0.82[b] | 0.84[c] | - | - | 0.02 |
| Lungs | 2.47[a] | 2.63[b] | 2.55[b] | 2.40[a] | - | - | 0.09 |
| Intestines | 4.78[b] | 4.76[b] | 4.82[c] | 4.69[a] | - | - | 0.05 |
| Liver | 0.92[a] | 0.92[a] | 1.00[b] | 1.01[b] | - | - | 0.04 |

Table 5: Analyzed residual phytotoxins concentration in treated dietary JSM

| | Diets | | | | | |
|---|---|---|---|---|---|---|
| Toxins | 1 | 2 | 3 | 4 | 5 | 6 |
| Cyanides (ppm) | - | 1.01 | 1.09 | 1.12 | 1.15 | 1.20 |
| Saponins (ppm) | - | 0.70 | 0.75 | 0.82 | 0.85 | 0.90 |
| Phytic acid (%) | - | 5.15 | 5.36 | 6.18 | 6.80 | 7.83 |
| Tannins (g/dm$^3$) | - | 0.05 | 0.05 | 0.05 | 0.06 | 0.06 |

Table 6: Analyzed phytotoxins content in native JSM

| Phytotoxin | Concentration |
|---|---|
| Phorbolesters | 2.79 mg/g |
| Total phenols | 0.36% |
| Tannins | 0.04% |
| Phytates | 9.40% |
| Saponins | 2.60% |
| Trypsin inhibitors | 21.3 mg/g |
| Lectins (1/mg meal/ml assay) | 102 mg/g |

animals like rats when included at levels above 15% in diets as observed in this study. Untreated cake or meal or oil from Jatropha seeds is not suitable for monogastric nutrition since it is extremely poisonous.

**Conclusion:** It is submitted that treating JSM by methods used in this experiment and including in diet at 15% has no deleterious effects on monogastric animals like Albino rats. Methods of detoxification to enable higher inclusion levels above 15% are receiving attention.

## REFERENCES

Annongu, A.A., U. Ter Meulen, J.O. Atteh and D.F. Apata, 1996. Toxikologische beurteilung von nativem und industriell fermentiertem Schibutter-Oelkuchen fuer die Futterung von Broilern. Arch. Gefluegelk., 60: 221-226.

AOAC, 1990. Official Methods of Analysis. 15th Edn., Association of Official Analytical Chemists, Washington DC., USA.

Hudson and El-Difrawi, 1979. The method for sapogenins determination in seeds of *Lupine* spp. and Jatropha plant. Food Chem., 3: 181-186.

Joslyn, M.A., 1970. In: Methods in Food Analysis. Academic Press, London, pp: 845.

Makkar, H.P.S. and K. Becker, 1999a. Edible provenances of *Jatropha curcas* from Quinta RPP state of Mexico and effect of roasting on anti-nutrients and toxic Factors in seeds. J. Agric. Food Chem., 45: 3152-3157.

Makkar, H.P.S. and K. Becker, 1999b. Plant toxins and detoxification methods to improve feed quality of tropical seeds. Asian Aust. J. Anim. Sci., 12: 467-480.

Steel, R.G.D. and J.H. Torrie, 1990. Principles and Procedures of Statistics. A Biometrical Approach, 2nd Edn., McGraw-Hill Book Company, New York.

Wheeler, E.L. and R.E. Ferrel, 1971. A method for phytic acid determination in wheat fractions. Cereal Chem., 48: 312-320.

Pakistan Journal of Nutrition 9 (9): 905-908, 2010
ISSN 1680-5194


# Comparative Studies on Nutritional Composition of Four Melon Seeds Varieties

O.A. Abiodun and R.O. Adeleke
Department of Food Science and Technology, Osun State Polytechnic, Iree,
P.M.B. 301, Osun State, Nigeria

**Abstract:** This study evaluates four Cucurbit species in Iree, Osun state, Nigeria. The species used were *Cucumeropsis manni* (Naudin), *Cucumis sativus*, *Leganaria siceraria* and *Cucumeropsis edulis* (Hook). Proximate and mineral analyses were carried out on the shelled Cucurbit species. Protein, fat, ash, crude fibre, moisture and carbohydrate content ranged from 33.80-39.96%, 40.26-45.21%, 3.35-4.89%, 1.66-2.16%, 4.78-5.21 and 7.08-14.15% respectively. There were significant difference ($p \leq 0.05$) in the values obtained in protein, fat, ash moisture content and carbohydrate. But there was no significant difference ($p \leq 0.05$) in crude fibre values for *Cucumeropsis edulis* and *Cucumis sativus*. *Cucumis sativus* had higher calcium content (2.03%) while *Cucumeropsis manni* had higher values in Mg (8.87%), Na (162.76 ppm), Mn (107.72 ppm) and Fe (39.71 ppm) contents. *Leganaria siceraria* also had higher values in K (5.43%), Cu (5.09 ppm) and Zn (19.75 ppm). Acid value ranged from 3.13-4.22 mgKOH/g, free fatty acid ranged from 3.4-3.9%. Saponification and peroxide values of 188-193 mg KOH/g and 9.7-11.6 Meq/kg were obtained for the melon seeds oils. Also, iodine values for the melon seeds ranged from 95.5-98.2 Wijs. All the seeds serve as good sources of protein, fat and minerals.

**Key words:** Cucurbit species, saponification, peroxide, nutritional composition, proximate

## INTRODUCTION

Melon is a cucurbit crop that belongs to the *Cucurbitaceae* family with fibrous and shallow root system. It is a tendril climber or crawling annual crop, mostly grown as a subsidiary crop interplanted with early maize and yam in some savannah belt of Nigeria. Melons are major food crops with several varieties which serves as a major food sources (Mabalaha *et al*., 2007). *Cucurbit* sp. are among the economically most important vegetable crops worldwide and are grown in both temperate and tropical regions (Pitrat *et al*., 1999; Paris, 2001). Melon seed kernels are major soup ingredients and they are used as a thickener and flavour component of soups. Melon seeds are less expensive and widely distributed. They can contribute substantially towards obtaining a balanced diet (Fokou, *et al*., 2004). Melon seeds are generally a rich source of oil. Oil seeds are generally processed to yield condiments such as 'ogiri'. *Cucurbita* spp comprises of overlapping groups of cultivars that yield seed or edible fruit. In West Africa, they are called egusi derived from Yoruba language; the seeds are considered a delicacy. Despite extensive research on melon seeds in many part of West Africa (Loukou *et al*., 2007; Fokou *et al*., 2004; Onyeike and Acheru, 2002; Badifu and Ogunsua, 1991), there is a dearth of studies in our locality. Therefore, the research work evaluates the nutritional composition of four melon seeds in Iree, Osun State, Nigeria.

## MATERIALS AND METHODS

Melon seeds (*Cucumeropsis manni* (Naudin), *Cucumis sativus, Leganaria siceraria and Cucumeropsis edulis* (Hook) were bought at the local market in Iree, Osun State, Nigeria. The melon seeds (except *Leganaria siceraria*) were shelled and kept in airtight container for analysis.

Proximate analysis were carried out with the method of AOAC (1990) while the mineral contents were determined using the method of Novozamsky *et al*. (1983). The oils were extracted using Soxhlet extractor and the physico-chemical properties (saponification value, acid value, peroxide value, smoke point, iodine value and free fatty acid) of the oil were carried out using the method described by AOAC (1990). All analyses were carried out in triplicates and the data were evaluated for significant differences in their means with Analysis of Variance (ANOVA) ($p \leq 0.05$). Differences between the means were separated using turkey's test as packaged by SPSS 11.0 software.

## RESULTS AND DISCUSSION

The proximate composition of the four melon seeds are shown in Table 1. The protein content ranged from 33.80-39.96% with *Leganaria siceraria* having the highest value which was significantly different ($p \leq 0.05$) from other samples. The values obtained for these seeds were in agreement with the finding of Fokou *et al*. (2004) who reported a range of 24.3-41.6% for protein content in five melon seeds. The values were higher than the values (23.7-30.68%) reported by (Olaofe *et al*., 1994) for melon, pumpkin and gourd seeds. The samples are rich in crude protein content and could be used to enrich food products.

**Corresponding Author:** O.A. Abiodun, Department of Food Science and Technology, Osun State Polytechnic, Iree, P.M.B. 301, Osun State, Nigeria

Table 1: Proximate composition of melon seeds varieties

| Composition | *Cucumeropsis manni* (Naudin) | *Cucumeropsis edulis* (Hook) | *Leganaria siceraria* | *Cucumis sativus* |
|---|---|---|---|---|
| Protein | 34.86c | 35.31b | 39.96a | 33.80d |
| Fat | 42.29b | 40.26d | 40.86c | 45.21a |
| Moisture | 5.13b | 4.78d | 5.21a | 4.88c |
| Ash | 3.51c | 3.35d | 4.89a | 3.75b |
| Crude fibre | 1.66c | 2.00b | 2.16a | 2.03b |
| Carbohydrate | 12.55b | 14.15a | 7.08d | 10.33c |

Value with the same letter along the row are not significantly different ($p \leq 0.05$) from each other

The fat content ranged from 40.26-45.21%. *Cucumis sativus* had higher fat value which was significantly different ($p \leq 0.05$) from the other three samples. These values were higher than the value (23.1%) reported by Kamel *et al*. (1985) for water melon seed oil. The fat values reported by Fokou *et al*. (2004) for *C. manni* (42.9%) and *Cucumis sativus* (57.3%) were higher than the values obtained. This could be due to the cultivars used. Mabalaha *et al*. (2007) also reported oil yields of seeds ranging from 24.8-30.0% in *Citrillus lanatus* and *C. colocynth* species respectively while Madaan and Lai (1984) recorded oil content values of 41.0-56.6% in melon seeds. The values of lipid obtained for the four varieties of melon seeds were within these values. Melon seeds have high fat contents, thus the seeds are classified as excellent sources of dietary oil (Nwokolo and Sim, 1987).

Moisture contents of the melon seeds ranged from 4.78-5.21%. *Leganaria siceraria* had higher value while *Cucumeropsis edulis* had the lowest value (4.78%). The samples used had lower moisture content values. Moisture content of 4.33% in *Cucumis sativus* and 7.26 (*Cucurbita moschata*) were reported by Fokou *et al*. (2004). The ash content ranged from 3.35-4.89%. *Leganaria s*iceraria seeds had higher value in ash content and were significantly different ($p \leq 0.05$) from other samples. Fokou *et al*. (2004) also reported a range of 2.82-5.0% in the melon seeds. The results showed that the samples have significant amount of ash which are important sources of minerals.

Crude fibre ranged from 1.66-2.16%. *Leganaria siceraria* was significant different ($p \leq 0.05$) from other samples due to the high value in crude fibre. But there were no significant difference ($p \leq 0.05$) in the crude fibre values of *Cucumeropsis edulis* and *Cucumis sativus*. Crude fibre contents of 0.90-1.63% were reported by Fokou *et al*. (2004) while Madaan and Lai (1984) and Loukou *et al*. (2007) reported crude fibre of 1.25-2.60% and 2.30-2.94% respectively. The crude fibre contains indigestible materials which can reduce constipation by increasing bowel movement. Carbohydrate content ranged from 7.08-14.15%. The highest value was in *Cucumeropsis edulis* while the least was in *Leganaria siceraria*. These values were higher than the values recorded by Fokou *et al*. (2004) but were within the range of values recorded for *C. lanatus* (9.87%), *C. manni* (13.86%) *and C. melo* (23.18%) by Loukou *et al*. (2007).

Table 2 showed the mineral composition of the four melon seeds. *Cucumis sativus* had higher Ca content (2.03%) than the other samples while *Cucumeropsis manni* had higher values in Mg (8.87%), Na (162.76 ppm), Mn (107.72 ppm) and Fe (39.71 ppm). These values were significantly different ($p = 0.05$) from other melon seeds analyzed. *Leganaria siceraria* also had higher values in K (5.43%), Cu (5.09 ppm) and Zn (19.75 ppm). Calcium level of 129.7-269.7 mg/100 g d.w was recorded by Fokou *et al*. (2004). The samples were rich in mineral contents which aid in digestion, formation of strong bone and teeth and hemoglobin formation. The variation in mineral composition could be due to the climate, species, soil type, water and the cultural practices adopted during planting (Steven *et al*., 1985).

Table 3 showed the physico-chemical properties of melon seeds oil. Acid value ranged from 3.13-4.22 mgKOH/g. The acid values of *Cucumeropsis edulis* and *Cucumis sativus* were slightly higher than the Codex standard value for virgin vegetable oils. These values were within the range reported for water melon (3.41 mg/g) and melon seed (4.26 mg/g) (Ebuehi and Avwobobe, 2006). Free fatty acid ranged from 3.4-3.9%. Low free fatty acids indicate the stability of the oil. Saponification values of 188-193 mgKOH/g were obtained for the melon seeds oils. These were higher than water melon value (175.98 mg/g) and lower than that for melon seeds oil (201.15 mg/g) reported by Ebuehi and Avwobobe (2006). They were within the range (182.1-193.8 mgKOH/g) reported for *Curcubitatacea* seeds oil by Mabalaha *et al*. (2007).

Peroxide value of 9.7-11.6 Meq/kg were determined for the melon seeds oils while 19.54 m mol/g was reported for melon seeds oil by Ebuehi and Avwobobe (2006). The values were higher than the codex standard value (10 Meq/kg) for refined vegetable oil and lower than the maximum value (20 Meq/kg) allowed for unrefined olive oil (FAO/WHO, 1993). This implies that the melon seeds oil have lower degree of rancidity. This finding agrees with the report of Ebuehi and Avwobobe (2006).

Also, iodine values for the melon seeds ranged from 95.5-98.2 Wijs. Mabalaha *et al*. (2007) reported iodine values of 95.8 Wijs in Tsama melon to 124.0 Wijs in Desert melon. The values obtained were within the codex standard value for groundnut oil (80-106 Wijs). The lower iodine value signifies low degree of

Table 2: Mineral composition of melon seeds varieties

| Composition | *Cucumeropsis manni* (Naudin) | *Cucumeropsis edulis* (Hook) | *Leganaria siceraria* | *Cucumis sativus* |
|---|---|---|---|---|
| Ca (%) | 1.49c | 1.19d | 1.79b | 2.03a |
| Mg (%) | 8.87a | 7.64d | 8.13c | 8.57b |
| K (%) | 5.28b | 4.83d | 5.43a | 5.14c |
| Na (ppm) | 162.76a | 62.26d | 88.25b | 79.24c |
| Mn (ppm) | 107.72a | 54.83d | 80.67b | 66.25c |
| Fe (ppm) | 39.71a | 20.16d | 26.45b | 21.43c |
| Cu (ppm) | 3.37b | 2.81d | 5.09a | 3.28c |
| Zn (ppm) | 13.46c | 16.54b | 19.75a | 11.66d |

Value with the same letter along the row are not significantly different ($p \leq 0.05$) from each other

Table 3: Physico-chemical properties of melon seeds oil

| Composition | *Cucumeropsis manni* (Naudin) | *Cucumeropsis edulis* (Hook) | *Leganaria siceraria* | *Cucumis sativus* |
|---|---|---|---|---|
| Acid value (mgKOH/g) | 3.13c | 4.22a | 3.67b | 4.1a |
| Free fatty acid % (AVx100/wt) | 3.4c | 3.9a | 3.5bc | 3.6b |
| Saponification value | 188.0bc | 193.0a | 190.0ab | 185.0c |
| Peroxide value (Meq/kg) | 10.2b | 11.6a | 9.7c | 10.4b |
| Iodine value (Wij's) | 95.5b | 98.0a | 98.2a | 97.6a |
| Moisture content (%) | 1.4b | 1.2c | 1.9a | 1.1c |
| Smoke point | 225.0c | 235.0a | 228.0bc | 230.0b |

Value with the same letter along the row are not significantly different ($p \leq 0.05$) from each other

unsaturation and the lesser the liability of the oil to become rancid by oxidation.

**Conclusion:** From the results obtained, the melon seeds are good source of protein and fat. The seeds from the four varieties could be used to enrich soup and fortify other products in order to improve the protein content. *Cucumeropsis manni* (Naudin) and *Leganaria siceraria* were richer in mineral contents than other samples. The oil is very useful due to the stability of the oil and the physico-chemical properties could be improved by refining the oil.

## REFERENCES


AOAC, 1990. 15th Official methods of Analysis. Association Official Analysis Chemists, Washington DC.

Badifu, G.L.O. and A.O. Ogunsua, 1991. Chemical composition of kernels from some species of Cucurbitaceae grown in Nigeria. Plant Food Human Nutr., 41: 35-44.

Ebuehi, O.A. and O.K. Avwobobe, 2006. Physico-chemical and fatty acid composition of water melon (*Citrillus lanatus*) and melon (*Colocynthis citrillus*) seed oils. Nig. Food J., 42: 25-33.

FAO/WWHO, 1993. Fats, oils and related product. Food standard program. Codex Alimentarius Commission. Food and Agriculture Organization of the United Nations. World Health Organization, Rome, 8: 33-35.

Fokou, B., M.B. Achu and T.M. Chaungues, 2004. Preliminary nutritional evaluation of five species of egusi seeds in Cameroon. Afr. J. Nutr. Agric. Dev., 4: 8.

Kamel, S.B., H. Dawson and Y. Kakuda, 1985. Characteristics and composition of melon and grape seed oils and cakes. J. Am. Oil Chem. Soc., 62: 881-883.

Loukou, A.L., D. Gnakri, Y. Dje, A.V. Kippre, M. Malice, J.P. Baudoin and I.A. Zoro Bi, 2007. Macronutrient composition of three cucurbit species cultivated for seed consumption in Cote d'Ivoire. Afr. J. Biotechnol., 6: 529-533.

Mabalaha, M.B., Y.C. Mitei and S.O. Yoboah, 2007. A comparative study of the properties of selected melon seeds oils as potential candidates for development into commercial edible vegetable oil. J. Am. Oil Chem. Soc., 84: 31-34.

Madaan, T.R. and B.M. Lai, 1984. Some studies on the chemical composition of Cucurbit kernels and their seed coats. Plant Foods Human Nutr., 34: 81-86.

Novozamsky, I., V.J.G. Houba, E.C.K. Van and V.W. Van, 1983. Plant nitrogen and phosphorus in plant tissue, novel digestion technique for multi-element. Plant Anal. Commun. Soil Sci. Plant Anal., 14: 239-248.

Nwokolo, E. and J.S. Sim, 1987. Nutritional assessment of defatted oil meals of melon (*Colocynthis citrillus L*) and fluted pumpkin (*Telfaria occidentalis* Hook) by chick assay. J. Sci. Food Agric., 3: 237-246.

Olaofe, O., F.O. Adeyemi and G.O. Adediran, 1994. Amino acid and nutritional composition and functional properties of some oil seeds. J. Agric. Food. Chem., 42: 878-881.

Onyeike, E.N. and G.N. Acheru, 2002. Chemical composition of selected Nigeria oil seeds and physicochemical properties of the oil extracts. Food Chem., 77: 431-437.

Paris, H.S., 2001. Characterization of the Cucurbita pepo collection at the new year research center, Israel. Plant Genet. Res. News, 162: 41-46.

Pitrat, M., M. Chauvet and C. Foury, 1999. Diversity history and production of cultivated Cucurbits. Acta Hort., 492: 21-28.

Steven, R.T., R.Y. Vernon and C.A. Michael, 1985. Vitamins and Minerals. In: Fennema, O. (Ed). Food Chemistry. 2nd Edn., Marcel Dekker, New York, pp: 523.

Pakistan Journal of Nutrition 9 (9): 909-911, 2010
ISSN 1680-5194


# Some Physicochemical Characteristics of Defatted Flours Derived from African Walnut (*Tetracarpidium conoforum*): An Underutilized Legume

E.C. Ndie[1], C.V. Nnamani[2] and H.O. Oselebe[3]
[1]Department of Nursing Science, [2]Department of Applied Biology,
[3]Department of Crop Production and Landscape Management,
Ebonyi State University, Abakaliki, Nigeria

**Abstract:** The nuts of African Walnut were processed into flour and a portion defatted and the samples were analyzed for proximate composition, water absorption capacity, solubility, bulk density and rapid visco characteristics. Results showed that the flour is rich in protein and fat (21.6 and 47.7%) respectively. The defatted samples have higher solubility, water absorption capacity, peak viscosity breakdown values, final viscosity and set back values when compared with undefatted sample. This result indicates that defatting of African Walnut flour improves the pasting characteristics of the flour whose high protein content makes a good protein supplement.

**Key words:** Defatted flour, undefatted flour, legumes, *Tetracarpidium conophorum*, protein deficiency

## INTRODUCTION

In the developing countries legumes have high acceptability and utilization due to their importance as sources of dietary protein. African Walnut (*Tretracarpidim conoforum*) has a long history as food plant and is grown by peasant farmers across West African rain forest. The climber bears capsules which are greenish in colour when young and greenish-yellow when fully ripe. They contain four shelled seeds (Willis, 1966). The seeds take 4-6 months to mature and are found in the local markets between the months of June and September. In Nigeria, it is traditionally eaten as nut after boiling (Akpuaka and Nwankwor, 2000). African Walnut is included in the list of lesser known food stuff (Achievement, 1998), while Ogunsma and Ddeboma (1983) and Osogie *et al.* (1986) reported that it is rich in protein (22.8-23.5%) and Fat (41.5-50). In the light of the nutritional values of Africa Walnut, (*Tretracarpidium Conoforum*) the flour derived from the nut could serve as protein supplement in food formulation.

The present study aims at studying the physicochemical and pasting characteristics of defatted and undefatted flours derived from African Walnut.

## MATERIALS AND METHODS

**Sample preparation:** Mature nuts of African Walnut were purchased from Oye Awgu market of Enugu State, Nigeria. The shells were removed and seeds milled in an attrition mill and dried in an electric oven at 40°C. One half of the sample was defatted using hexane while the other half remained undefatted.

**Chemical analysis:** Fat, carbohydrate, ash and moisture were determined using AOAC (1990) and protein determined using Kjeldohl Method (AOAC 1990). Solubility was determined by the method of Leach *et al.* (1959), while water absorption capacity and bulk density were determined by the method of Okaka and Porter (1977).

The pasting characteristics were determined using rapid Visco Analyzer (RVA) model RVA-3D at International Institute of Tropical Agriculture (II TA) Ibadan, Nigeria.

## RESULTS AND DISCUSSION

**Proximate composition:** The result of proximate analysis of African Walnut (*Tetracarpidium conoforum*) is shown in Table 1. Results indicate that moisture, protein, carbohydrate, fat, fibre and ash content of *T. conoforum* are 9.5, 21.6, 16.9, 47.7, 2.9 and 2.4%, respectively. The results are within the range reported by Ogunsma and Ddeboma (1983) and Osogie *et al.* (1986). The main nutritional values of legumes lie on their supply of cheap dietary protein and calories in the developing countries of the world. Survey had shown that very few people in tropical countries suffer from simple protein deficiency. Most prevalent deficiency is protein-energy, in which an overall energy deficiency forces the metabolism to utilize the limited intake of protein as a source of energy (US NAs, 1980). In this regard, African Walnut could play a role considering the high protein content of the flour (21.6%).

The fat content of the flour of *T. conoforum* was also very high (47.7%) indicating that the seed of the plant

**Corresponding Author:** H.O. Oselebe, Department of Crop Production and Landscape Management, Ebonyi State University, Abakaliki, Nigeria

Table 1: Proximate composition of *Tetracarpidium conophorum* (African Walnut)

| Parameter | Composition (%) |
|---|---|
| Moisture | 9.5 |
| Protein | 21.6 |
| Carbohydrate | 16.9 |
| Fat | 47.7 |
| Crude fibre | 2.9 |
| Ash | 2.4 |

Values are means of three replicates

should be exploited as an oil seed. In this regard, it has been used for the generation of dry oil (Akpuaka and Nwankwor, 2000; Tchiegang, 2001).

**Effect of defatting on some functional properties of flour derived from *Tetracarpidium conophorum* (African Walnut):** The result of defatting on the solubility, water absorption capacity and bulk density of flour derived from African Walnut is shown on Table 2. The results indicated that solubility of the undefatted was 18.4±1.6%, while that of defatted flour was 25.3±1.5%. The water absorption capacity of defatted and undefeated flour was 108±40% and 103±3.4%, respectively. The bulk density of defatted and undefatted flour was 563±0.11 and 0.31±0.13 wt/vol). This results show that defatted flour had increased solubility and water absorption capacity when compared to undefatted flour. The use of any flour as food ingredients is depended on the water-flour interaction, which determines the rehydration of flour. The higher solubility percentage and water absorption capacity of defatted flour of *T. conophorum* may be attributed to the removal of the non-polar groups that interfere with the flour-water interaction (Nputong and Weldran, 2002).

**Effect of defatting on the pasting characteristics of flour derived from African Walnut:** Results of the Rapid Visco Analysis (RVA) indicated that defatted flour had a peak viscosity of 183±5.2, a breakdown value of 136.83±4.0 and a final viscosity of 531.25±6.4 (Fig. 1). Lower RVA values were obtained for undefatted flour, 32.25±3.5, 29.92±3.2 and 42.33±3.4, for peak viscosity, breakdown value and final viscosity, respectively (Fig. 2). The set back value, pasting time and pasting temperature for defatted and undefatted flour are shown in Table 3. Again defatted flour had higher set back value 394.42±6.1, compared to undefatted flour 12.42±3.4. The pasting time was 5.6 and 6 min, while the pasting temperature was 77.3°C and 80 °C for defatted and undefatted flour, respectively.

The pasting characteristics of flour determine their best use in food processing which in most cases are dependent on the botanic species of plants (Okoli, 1998). The viscosity of starch paste after heating and stirring at a maximum temperature for 15 min define the stability of the starch granules and the ability of the swollen starch to resist deformation and busting during constant heating and stirring (Morris, 1990). On the other

Table 2: Effect of defatting on the solubility (%), water absorption capacity (%) and bulk density (wt/vol) of flour derived from African Walnut

| Flour | Water absorption capacity (%) | Solubility (%) | Bulk density |
|---|---|---|---|
| Defatted | 130±3.4 | 25.3±1.5 | 0.31±0.13 |
| Undefatted | 108±4 | 18.4±1.6 | 0.56±0.15 |

Values are means of three replicates

Table 3: Effect of defatting on the rapid visco characteristic of flour of *Tetracorpidium conophorum* (African Walnut)

| Flour | Set back value | Pasting time | Pasting temp |
|---|---|---|---|
| Defatted | 394.42±6.1 | 5.6 | 77.3 |
| Undefatted | 12.42±3.4 | 6.0 | 80.0 |

Values are means of three replicates

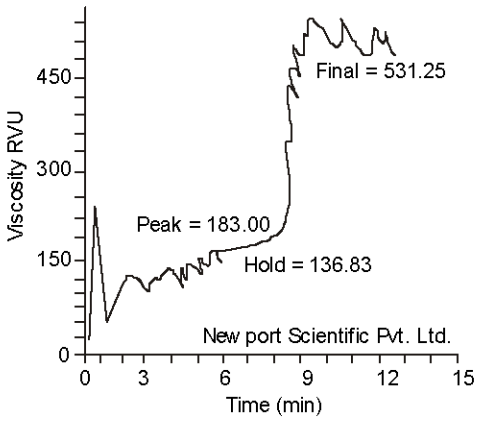


Fig. 1: Peak viscosity, breakdown value and final viscosity of defatted flour of African Walnut (*Tetracarpidium conoforum*)

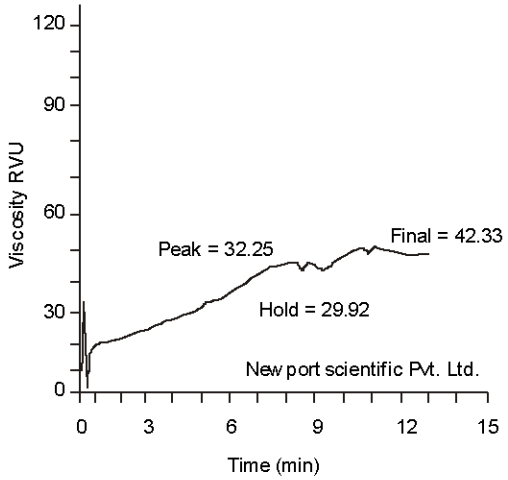


Fig. 2: Peak viscosity, breakdown value and final viscosity of undefatted flour of African Walnut (*Tetracarpidium conoforum*)

hand, the set back value is a measure of recrystalization of gelatinizing flour (retrogradation) and is a function of amylose and amylopectin configuration (Okoli, 1998). The low peak viscosity, breakdown value and set back value of the undefatted flour indicate that the flour would be more stable when compared with defatted flour. This may be due to the fact that the granules of defatted flour may have been made weaker by the removal of fat. The high set back value of defatted flour has an implication for the use of the flour in preparation of foods when a gel of high rigidity is needed as in bread making. In such instances, undefatted flour of *T. conophorum* may be preferred.

**Conclusion:** It may be concluded from this study that defatted flour derived from African Walnut with high protein content, high water absorption capacity, high solubility and good pasting characteristic could be used as composite flour in preparation of bread and other confectionaries. The African Walnut could also be exploited as an oil seed.

## REFERENCES


Achievement, S.C., 1998. Nuts and seeds: In Nutritional Quality of 7 plant foods (Ogogie, A.U. and O.U. Eka, Eds.). Post Harvest Unit, University of Benin, Nigeria, pp: 153.

Akpuaka, M.U. and E. Nwankwor, 2000. Extraction, analysis and utilization of a drying-oil from *Tetracarpidium conophorum*. Bioresour. Technol., 73: 195-196.

AOAC, 1990. Official method 7 Analysis. 5th Edn., Association of Anolytical chemist, Washington DC. 105.

Leach, H.W., L.D. Mcowen and T.T. Schoch, 1959. Structure of the starch granule, swelling and solubility pattern of various starch. Level Chem., 36: 534-538.

Morris, V.J., 1990. Starch gelation and retrogradation. In: Trends Food Sci. Technol. London, 1: 2-6.

Nputong, Y.N. and K.W. Weldran, 2002. Functional properties of cowpea-soy-dry red bean composite flour paste and sensory characteristics. J. Food Eng., 54: 3-15.

Ogunsma, A.O. and M.B. Ddeboma, 1983. Chemical composition of *Tetracorpidium Conophorrum* (Conophurint). Food Chem., 10: 173-177.

Osogie, A.U., W.L. Okoye, B.O. Oluwagose and O.A. Dowodu, 1986. Chemical quality parameters and fatty acid composition of oils of some under-exploited tropical seeds. Nig. J. Applied Sci., 4: 151-162.

Okaka, J.C. and N.N. Porter, 1977. Functional and Storage properties of cowpea provider/wheat flour blend in bread making. J. Food Sci., 42: 826-833.

Okoli, E.C., 1998. Effect of grammar irradiation on biochemical molting and keeping quality of sorghum grain. Ph.D. Thesis, Obafami Awolowo University Ile-Ife, Nigeria.

Tchiegang, C., 2001. Chemical composition of oil from *Tetracarpidium conophorum* ( (m L. ARG.) Hutch. and Dalz.) nuts. J. Food Lipids, 8: 95-102.

US NAs, 1980. Dietory allowance: US National Academy of Science, Washington DC.

Willis, J.C., 1966. A Dictionary of the Flowering Plants and Ferns, seventh ed. Cambridge University Press, Cambridge, p: 1108.

Pakistan Journal of Nutrition 9 (9): 912-914, 2010
ISSN 1680-5194


# Studies on the Oil and Nutritive Value of Seeds of *Crotalaria retusa L.* (Fabaceae)

S.C. Umerie[1], I.F. Okonkwo[2], N.A. Nwadialor[3] and J.C. Okonkwo[2]
[1]Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Nigeria
[2]Department of Agricultural and Bioresource Engineering,
Nnamdi Azikiwe University, Awka, Anambra, Nigeria
[3]Department of Production Technology, Nnamdi Azikiwe University, Awka, Anambra, Nigeria

**Abstract:** The proximate composition of *Crotalaria retusa L.* seeds and the physico-chemical characteristics of the oil fraction were studied using standard techniques. The proximate value was 15.00, 37.50, 15.00, 4.37, 15.00 and 13.13% for moisture, fibre, ash, crude protein, oil and carbohydrate, respectively. Characterization of the oil showed that it was non-drying and of low unsaturation. The saponification value implicated the oil as non-edible oil but could be used in the production of hair shampoos, skin cream and shoe polish. The fairly high acid value suggested that oil required little purification to improve its shelf-life. Mineral elements determined by Atomic Absorption Spectrophotometry revealed the presence of essential minerals. The seed could serve as source of fibre and some minerals for poultry and livestock.

**Key words:** *Crotalaria retusa L.* seed, proximate composition oil extraction, characterization

## INTRODUCTION

*Crotalaria retusa L.* (English: rattle seed box, wedge-leaf crotalaria or wedge-leaf rattle-pod) belongs to the family fabaceae/Leguminosae (Kar, 2007), order fabales and genus Crotalaria (Anon, 2009a). Crotalaria is a genus of herbaceous plants and woody shrubs commonly known as rattle-pods. About 550 to 600 or more species of Crotalaria are described worldwide (Evans, 2002; Dutta, 1995) mostly from the tropics; at least 500 species are known to originate from Africa. Some species of Crotalaria are grown as ornamentals. The common name rattle pod or rattle box is derived from the fact that the seeds become loose in the pod as they mature and rattle when the pod is shaken. Asia or coastal Eastern Africa is its native range. In New Guinea and other places it occurs as a weed of roadside dump sites, stream banks and grazed grassland. It is also found at low altitudes mainly in areas with low seasonal rainfall (Anon, 2009a).

The *C. retusa L.* plant is an annual herb with ridged erect stem which is up to 13 dm long. The leaves are simple, oblanceolate and have veins on each side of the midveins. The flowers have yellow petals with fine purple lines near the base. The pods are dark brown to black in colour at maturity, 3-4 cm long, nonstipitate and globrous and contain about 23 seeds per pod. The seeds are smooth and brown coloured and measure up to 4.5 mm in length (Anon, 2009b).

Crotalaria species are used as food plants by the larvae of some Lepidoptera species including *Endoclita sericeus*, *Etiella zinckenella* and *Utetheisa ornatrix*. The toxic alkaloids produced by some members of this genus are known to be incorporated by *Utetheis* larvae and used to secure their defense from predators (Anon, 2009a). *C. spectabilis* Roth supports nitrogen-fixing bacteria and considered a "soil builder", however it is poisonous to cattle due to the presence of the toxic alkaloid monocotaline, a pyrrolizidine alkaloid (Anon 2009a; Kar, 2007).

The seeds of *C. retusa* are used in ethno-medicine for treatment of fever and as a vermifuge and possibly as an antispasmodic (uterus and intestine) agent (Oliver, 1959). However, much is not known about the chemical composition of the seed of *C. retusa*. The present communication is on the proximate and mineral composition of *C. retusa* seeds and the physico-chemical characteristics of the oil derived from the seed.

## MATERIALS AND METHODS

**Samples of *C. retusa* seeds:** The matured dried fruits were plucked from the plants growing wild in the premises of Nnamdi Azikwie University, Awka and identified by Prof. J.C. Okafor (consultant plant taxonomist), Fame Consultancy Plant Research Centre, Enugu, Enugu State, Nigeria. The seeds were removed from the pods, dried in a solar drier for three days and then ground into a fine meal using a manual grinding machine.

**Proximate analysis of *C. retusa* seeds:** The standard procedures described by Egan *et al.* (1981) and the AOAC methods (1975) were used for the determination of moisture, ash, fiber, fat and crude protein contents. The gross energy was obtained by multiplying the values

**Corresponding Author:** S.C. Umerie, Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Nigeria

of protein, fat and carbohydrate by the Atwater factors of 4, 9 and 4, respectively and expressing of the products in kilocalories per 100 g (Davidson *et al*., 1975; Osborne and Voogt, 1978). The mineral composition was determined by use of the Atomic Absorption Spectrophotometric method.

**Oil extraction:** Oil was extracted from the ground seeds of *C. retusa* with petroleum ether the (60-80). The solvent was distilled off at about 80°C and the oil content calculated from the weight of oil and weight of the ground seeds from which the oil was extracted.

**Physico-chemical properties of the oil:** The iodine saponification and acid values of the oil were determined by standard procedures described by Plummer (1987), AOCS (1960) and Glasser (2008). The free fatty acid was calculated from the relationship given by Norris (1965): 1 unit of Acid value = 0.503% x FFA (calculated as oleic acid). The mean molecular weight of the oil was estimated by the method of Glasser (2008) and the heat of combustion from the Bertam's formula given by Norris (1965): heat of combustion = 11380-iodine value - 9.15 (saponification value). The ester value was obtained by subtracting the acid value from the saponification value (Baltes, 1964).

## RESULTS AND DISCUSSION

The oil of *C. retusa* had a yellow colour, remained liquid at room temperature and had some unpleasant odour reminiscent of the plant. The proximate composition of the plant is given in Table 1, while Table 2 shows the characteristics of the oil. Table 3 shows the mineral composition of the plant seed.

The moisture content of *C. retusa* seeds after drying was fairly good. However, they could be dried further to extend their shelf-life (Table 1). The fibre content was found to be high suggesting that they could, like other matured leguminous seeds, serve as a source of dietary fibre (Davidson *et al*., 1975). Crude fibre helps in the production of semi-solid colonic contents and thus the maintenance of normal peristaltic movement of the intestinal tract. Hence, diets containing high fibre would discourage constipation that will lead to colon diseases and excessive use of purgatives (Davidson *et al*., 1975; Omosuli *et al*., 2009). The ash content was also found to be high indicative of a high mineral content. However, the protein content was low (4.37 g/100 g) relative to the average value (17-25 g/100 g) given for proteins in legumes (Davidson *et al*., 1975).

The amount of oil in the *C. retusa* seeds (15.0%) fell within the value for soya bean, 11-18% (Norris, 1965), okra seed (15-22%) and passion fruit (Kamel and Kakuda, 1994) and was higher than the range (1-5 g/100 g) given for legumes/pulses. Thus the seed could serve as moderate source of energy. The *C. retusa* oil had a low iodine value (125.75 g/100 g) (Table 2) and can be classed as a non-drying oil of low unsaturation, since the value came within the range of 100-140 g/100 g (Glasser, 2008).

Table 1: Proximate composition of *Crotalaria retusa* seeds

| Parameter | Value (%) |
|---|---|
| Moisture | 15.00 |
| Fibre | 37.50 |
| Ash | 15.00 |
| Crude protein | 4.37 |
| Oil | 15.00 |
| Carbohydrate (by difference) | 13.13 |
| Gross energy (kcal/100 g) | 205.00 |

Table 2: Characteristics of *Crotalaria retusa* seed oil

| Parameter | Value |
|---|---|
| Acid value (mg KOH/g) | 10.36 |
| Iodine value (g/100 g) | 125.73 |
| Saponification value (mg/KOH/g) | 112.00 |
| Free fatty acid (% as oleic acid) | 5.21 |
| Ester value (mg, KOH/g) | 101.64 |
| Heat of combustion (g/cal/g) | 10229.47 |
| Mean molecular weight | 500.00 |

Table 3: Mineral composition of *Crotalaria retusa* seeds

| Element | Amount (mg/g) |
|---|---|
| Iron | 3.000 |
| Lead | 0.075 |
| Zinc | 3.025 |
| Copper | 12.845 |
| Sodium | 0.475 |
| Arsenic | 0.100 |
| Potassium | 1.005 |
| Cadmium | 0.005 |
| Chromium | 2.550 |
| Bismuth | 0.450 |
| Mercury | 1.200 |
| Selenium | 0.010 |

The oil is also regarded as non-edible oil since the saponification value (112.00 mg KOH/g) lies outside the range (180-200 g/100 g) for most edible oils and fats (Glasser, 2008). Moreso, the heat of combustion (10,209.47 gcal/g) was found to be greater than the approximate value for edible oils 9500 g/g, (Norris, 1965). The acid value is appreciable suggesting some *in vivo* hydrolytic activities in the oil seeds (Eromosele *et al*., 1988). This indicates that the plant might be poisonous for livestock and explains the why cattle, sheep and goats do not browse the leaves, stems and seeds. However, the oil may be suitable for soap, shampoo and alkyd resins production. This is in keeping with the fact that oils with high acid values served better for soap making (Ajiwe *et al*., 2007). The FFA can be reduced or removed by alkaline refining to increase the shelf-life of the oil.

The mineral content of *C. retusa* seeds (Table 3) showed that the concentration of the essential elements sodium and potassium, are low. The quotient of Na:K ratio is 0.47 and falls within the recommended range, <1

(Nieman *et al*., 1992), an indication that the consumption of the seed would propably not induce high blood pressure disease. The concentrations of nonessential trace elements cadmium, arsenic, lead and mercury are also low to be of any toxicological significance (Donaldson, 1982). The observed values for the essential trace elements iron, zinc and chromium are low. However, the fairly high content of copper implicates the seed as a source of copper which is an essential constituent of some naturally occurring pigments and a cofactor for certain enzymes including amine oxidase, cytochrome oxidase, tyrosinase and copper-dependent superoxide dismutase (Murray, 2006). Copper is also essential for hemoglobin synthesis, normal bone formation and the maintenance of myelin within the nervous system (Tyler, 1977).

**Conclusion:** The seeds and seed oil of *C. retusa* contain oil and elemental mineral which could be useful to man. The oil could be commercialized for diverse applications, while the defatted seeds can also serve as a source of dietary fibre and minerals for poultry and livestock.

## REFERENCES


Ajiwe, V.I.E., L. Chinedu, S.O. Umeh, A.C. Ajiwe and G.I. Ndukwe, 2007. Analysis of *Hura cropitans* seed, seed coat, seed oil and application of its oil. Anachem J., 1: 64-68.

Anon, 2009a. Crotalaria http://en.,wikipaedia.org/wiki/crotalaria (09/09/2009).

Anon, 2009b. *Crotalaria retusa L* (Fabaceae). www.google.com (09/09/2009).

AOAC, 1975. Association of Official Analytical Chemists, Official Methods of Analysis, twelth edition, Washington DC.

AOCS Official Methods, 1960. Sampling and Analysis of Commercial Fats and Oils, American Oil Chemists Society, Chicago, IL, pp: 801-805.

Baltes, J., 1964. Classical Methods in fat analysis. In: Bockenoogen, H.A. (Ed). Analysis and Characterization of Oils, Fats and fat products. Interscience Publishers London, pp: 1-58.

Davidson, S., R. Passmore, J.F. Brock and A.S. Truswell, 1975. Human Nutrition and Detetics, sixth edition. The English Language Book Society and Churchill Livingstone, Edinburgh, U.K.

Donaldson, W.E., 1982. Trace element toxicity. In: Introduction to Biochemical Toxicology, eds, Ernest Hodgson and Frank E. Guthrie, Elsevier Science Publishing Co. Inc. New York, pp: 330-340.

Dutta, A.C., 1995. Botany for Degree Students, sixth edition, Oxford University Press, Calcutta, pp: 550-560.

Egan, H., R.S. Kirk and R. Sawyer, 1981. Pearson's Chemical Analysis of Foods, eight edition. Churchill Livingston, New York.

Eromosele, I.C., C.O. Eromosele, Innazo and P. Njerim, 1988. Studies on some seeds and seed oils. Bioresour. Technol., 64: 285-247.

Evans, W.C., 2002. A taxonomic approach to the study of medicinal plants and animal-derived drugs. In: Trease and Evans Pharmacognosy, fifteenth edition. W.B. Saunders Company Ltd., Edinburgh, UK, pp: 15-40.

Glasser, A.C., 2008. Analysis of fixed oils, fats and waxes. In: Pharmaceutical Chemistry, Theory and Applications, Vol 1, Leslie G. Chattern (editor). CBS Publishers and Distributor. New Delhi, India, pp: 405-437.

Kamel, B.S. and Y. Kakuda, 1994. Tropical fruits: A source of lipids. In: Technological Advances in Improved and Alternate sources of lipids. B.S. Kamel and V. Kakuda Editors Blackie Academic and Professional, London, U.K., pp: 116-149.

Kar, A., 2007. Pharmcognosy and Pharmaco-biotechnology (revised-expanded second edition), New Age International (P) Limited Publishers, New Delhi, India.

Murray, R.K., 2006. Plasma proteins and Immunoglobulin. In: Harper's Illustrated Biochemistry, twenty seventh edition. Robert K. Murray, Daryl K. Granner and Victor W. Rodwell (Editors), McGraw-Hill Education (Asia), Singapore, pp: 588-605.

Nieman, D.C., D.E. Herworth and C.N. Nieman, 1992. Nutritions: Wm C. Brown Publisher Dubugie, U.S.A., pp: 276-282.

Norris, F.A., 1965. Fats and Fatty Acids, In: Kirk-Othmer Encyclopedia of Chemical Technology, Vol. 8; John Wiley, New York, pp: 170-886.

Oliver, E.W.H.M., 1959. Medicinal plants of Nigeria, Part II. Technical Memorandum No. 7, Federal Institute of Industrial Research, Published by the Federal Ministry of Commerce and Industry, Lagos, Nigeria.

Omosuli, S.V., I.T. Adwale and O. Dare, 2009. Proximate and mineral composition of roasted and defatted cashew nut (*Anarcadium occidentale*) Flour. Pak. J. Nutr., 8: 16490-1651.

Osborne, D.R. and P. Voogt, 1978. Calculation of Calorific Value. In: The Analysis of Nutrients in Foods. Academic Press, New York, pp: 239-240.

Plummer, D.T., 1987. Quantitative analysis of lipids. In: An Introduction to Practical Biochemistry, third edition McGraw. Hill Book Company (UK) Ltd. England, pp: 195-197.

Tyler, D.D., 1977. Water and mineral metabolism, In: Review of Physiological Chemistry, sixteenth edition. H.A. Harper, V.W. Rodwell and P.A. Mayer (Editors) Lange Medical Publications, California, pp: 516-541.

Pakistan Journal of Nutrition 9 (9): 915-919, 2010
ISSN 1680-5194


# Nigerian Cocoa and Cocoa By-products: Quality Parameters, Specification and the Roles of Stakeholders in Quality Maintenance

J.A. Folayan
Department of Agricultural Economics and Extension Services,
Joseph Ayo Babalola University, Ikeji Arakeji, Nigeria

**Abstract:** The Study analyses the issue of cocoa and cocoa by-products; the quality parameters and specification, the quality and quality measures adopted by farmers, Licensed Buying Agents (LBAs), warehousing operators, exporters and processors as stakeholders in Nigerian Cocoa economy; all in attempt to ensure that cocoa and cocoa by-products meet acceptable international quality standard. The study employed descriptive statistics to determine the response of stakeholders on the various quality parameters and specification of cocoa and by-products as well as the measures adopted to ensure good quality cocoa. The study revealed that quality specification of cocoa and cocoa by-products are set by different terminal or future markets as bases for acceptability at the international market and that stakeholders in cocoa play paramount, roles at ensuring good quality maintenance of the commodity.

**Key words:** Cocoa and cocoa by-products, quality, parameters, specification

## INTRODUCTION

Cocoa (Theobroma Cacao) is said to have determined both the economic and political fate of many countries of the world of which Trinidad, Ghana, Cote D'Ivoire, Brazil, Costarica and Fernandopo are prominent. About 92% of the world's output of cocoa beans is produced in eight countries namely Cote D'Ivoire, Ghana, Indonesia, Brazil, Nigeria, Cameroon, Malaysia and Ecuador, Cocoa Producers Alliance (2002). These are countries in Africa, South America and Asia. The total world output of cocoa in 2002/2003 was 3,114,000 metric tones while the Nigeria share of the output was 165,000 metric tones, about 5.3% (Folayan, 2005). However the largest cocoa consumers in terms of products (Cake, powders and butter) are United States of America, Federal Republic of Germany, Netherlands, Brazil and United Kingdom (Folayan, 2003).

Before the discovery of Oil as foreign exchange earner in Nigeria, cocoa was the largest foreign exchange earner, even the arrival of petroleum to the central stage merely relegated the crop to the second place in the Nigeria economy. Inspite of its declining contribution to Nigerian total export in the recent past; cocoa still contributes the lion share of non-oil export in Nigeria. That was why ICCO (1999) intunned that "Although its contribution to the total national export earning during the past two decades dropped considerably due to the enormity of foreign exchange earnings of crude petroleum, yet cocoa remains Nigeria's biggest agricultural export". In view of this, it is logical to submit that any mishandling of the cocoa or its by-products in terms of quality will deal a mortal blow on non-oil export in Nigeria. The need for stakeholders in cocoa therefore to ensure that their cocoa and cocoa by-products meet approved international quality standard cannot be over-emphasized. Poor quality cocoa may lead to arbitration cases and loss of revenue apart from tarnishing the image and loss of market by the exporting countries. Assured quality standard will also forestall complaints from some overseas buyers about the quality and purity of Nigerian cocoa at the out turn.

The root causes of quality anomalies in cocoa could be traced to poor farm management, infestation and other diseases, poor handling, bad fermentation, inadequate drying and hence high moisture content, capable of making the produce vulnerable to mould and bacterial growth, poor and longtime storage, leading to fat degradation and pest infestation, without providing for fumigation and other forms of quality maintaining measures. Cocoa farmers, exporters, buying agents, warehouse operators, government agents, including produce officers at the states and federal levels and other pre-shipment inspection agents are in one way or the other collaborators in cocoa quality maintenance Folayan (2005).

**Quality:** Various experts have defined quality as "fitness for use" "conformitance to requirement" "freedom from variation" and so on. According to the American Society for Quality Control, Kotler (2003) also corroborated by International Standards Organization or International Organization for Standardization (ISO) founded in 1947 in Geneva at Switzerland; Oladipo (2007), quality is defined as the totality of features and characteristics of a product or service that bear on its ability to satisfy stated or implied needs". This is clearly a customer-oriented definition.

As quality is the basis of transaction of any commodity; quality is sought to ascertain the overall fitness of the produce for the purpose for which it is needed in cocoa and its by-products. This quality is adjudged through flavour, purity, consistency, yield, physical aspect such as been size, percentage shell and fat content all of which influence the choice of cocoa beans can be related to the cocoa products in the sense that the physical characteristics of the beans and the flavour of the cocoa are major determinants of cocoa quality.

The critical quality issues in cocoa and cocoa products market include mould, slaty, bean count, insect damaged and other defects in cocoa bean (Folayan, 1993). Free fatty acid, acid value, saponification value and unsaponifiable matter and other parameters in cocoa butter, fat content, moisture content and pH value and other parameter in cocoa cake and cocoa power and fat, fines, flavor, shell content and extraneous matter in cocoa liquor, RMRDC (1993). The quality issue may either be stated in the trade contract or in quality certifications and the benefit of certifications is usually better prices and premium prices Koekoek (2003).

## MATERIALS AND METHODS

**Study area:** The study was carried out through Cocoa Farmers, Cocoa Licensed Buying Agents (LBAs) and cocoa warehousing quality operators in Ondo State, Cocoa exporters and cocoa processors in Ondo, Ogun and Lagos states of Nigeria.

**Data sources and type:** Data used for this study were collected from both primary and secondary sources. Primary data were obtained from the responses of fifty cocoa farmers, thirty five cocoa LBAs, fourteen cocoa warehousing/quality operators, twenty cocoa exporters and seven cocoa processors in the study area. The data were collected with the aid of a set of structured questionnaire. Secondary data were collected from relevant text books, published and unpublished materials, including seminar and conference papers. For reliable data to be collected, the set of questionnaire was first pre-tested and was later reviewed. Data were collected on the following information relating to cocoa and cocoa by-products, quality parameters and quality specification and roles of stakeholders at maintaining good quality.

**Sampling technique:** The study made use of both the random and purposive sampling technique. Cocoa farmers, Cocoa LBAs and Cocoa exporters were selected by random sampling technique from sampling frame constructed from the information provided by produce departments while quality/warehousing operators and cocoa processing industries were purposively selected. A total of 126 respondents including 50 cocoa farmers, 35 LBAs, 20 exporters, 14 quality control and warehousing operators and 7 cocoa processing industries were selected from administered questionnaire.

**Method of data analysis:** The study used simple descriptive statistics of frequency distribution and percentage to analyze the data collected.

## RESULTS AND DISCUSSION

**Number of currently functioning Cocoa Processing Factories in Nigeria:** Findings from the study revealed that the presence of cocoa and the need to add value to and enhance the quality of cocoa and its by-products in Nigeria had led to the establishment of seventeen cocoa processing industries in some part of the cocoa producing states of Nigeria between 1964 and 2006. However, of the seventeen cocoa processing factories as contained in Table 1, only seven of them are currently operating. The rest have either not been completed, closed down or did not come on board at all. The processing companies have many problems such as inadequate working capital, irregular power supply, high cost of cocoa beans, inefficient and sometimes obstructive government policies (Salami, 2000).

**International standard quality parameters for cocoa and cocoa by-products:** The basis upon which cocoa and its by-products are graded/analyzed according to the bean count as well as quality and standards as set by different terminals or future markets as a basis for deciding whether a particular parcel is suitable for tendering on the market at the contract price, premium or discount is stipulated by two international bodies namely Alliance Fracais in Commerciate Dles Cocoa (AFCC) Cocoa Association of London (CAL) (Folayan, 2005).

AFCC is a Trade Association based in Paris which issues contract terms used by French speaking origin of cocoa not destined to the united States of America (or Canada). The association defines various cocoa qualities and has arbitration procedure in the event of disputes contracts are subject to the law of France.

CAL, Cocoa Association of London based in London. CAL contract are used by English speaking origins of cocoa not designed to the United States of America (or Canada). The Association defines various cocoa qualities and has arbitration procedures in the event of dispute between buyers and sellers. Contracts are subject to the law of England and Wales.

As stipulated by CAL and AFCC contracts; there are two (2) grades of cocoa viz (I and II) with the quality parameter specification as contained in Table 2 and 3, while the quality parameter specification of cocoa by-products of butter, cake, powder and liquor are contained in Table 4, 5, 6 and 7.

Table 1: Functioning and non-functioning cocoa processing factories in Nigeria

| Name of factories | Date Established | Date operation commenced | Initial Capacity | Status |
|---|---|---|---|---|
| Cocoa product industries Ikeja, Lagos State. | Na | 1967 | 30,000 | Operating |
| Ile-Oluji Cocoa mill, Ile-Oluji, Ondo State. | 1981 | 1984 | 30,000 | Operating |
| Coop. Cocoa Product currently being managed by Olam Nig. Ltd. Akure, Ondo State. | 1990 | 1997 | 10,000 | Operating |
| Cocoa product industry Nig. Ltd., Ede, Osun State. | 1964 | Na | 30,000 | Defunct |
| Ebun Industry Ltd, Lagos | na | Na | Na | Moribund |
| Stamark holdings Ltd, Ondo, Ondo State | 1991 | 1993 | 10,000 | Operating |
| Tulip Cocoa Factory Processing (formerly of Temple and Golder) Ijebu Mushin, Ogun State. | na | Na | 10,000 | Operating |
| Oregun Cocoa Mills, Lagos, Lagos State. | na | Na | 10,000 | Moribund |
| Lad Group Ltd, Isolo, Lagos State | Na | Uncompleted | 30,000 | Moribund |
| Fedma Cocoa Processing Industry Uruala, Umuahia Abia State | na | Uncompleted | na | Moribund |
| Romod Industry Ipoti Ekiti, Ekiti State | na | Uncompleted | na | Moribund |
| Idanre Processing Mill, Idanre, Ondo State | na | Uncompleted | na | Moribund |
| Owena Mill Akure, Ondo State | na | Na | 4,000 | Moribund |
| Carry Fast Nig Ltd, Akure, Ondo State. | na | Na | na | Moribund |
| Multitrex Cocoa Processing, Ibafo, Ogun State. | 2005 | 2006 | 30,000 | Operating |
| Standard Organization Cocoa Processing Industry, Akure, Ondo State | 2007 | 2009 | 10,000 | Operating |
| Agro Traders Cocoa Processing Ltd., Akure, Ondo State. | 2007 | Work in progress | Na | Installation work in progress |

Source: Computed from Field Survey, 2008

Table 2: Cocoa beans quality parameter specification

| | Main crop | | Light crop | |
|---|---|---|---|---|
| Parameters | Grade I | Grade II | Grade I | Grade II |
| Total mould | 3% max | 4% max | 3% max | 4% max |
| Slatey beans | 3% max | 6% max | 3% max | 6% max |
| Other defect | 3% max | 8% max | 3% max | 8% max |
| Wt of 300 beans | ≥310 g | ≥310 g | ≤310 g | ≤310 g |

Source: Effect of quality in Agricultural Produce Business, Folayan (1995)

Table 3: Parameter specification of fermented cocoa bean

| Parameters | Good fermented | Fair fermented |
|---|---|---|
| Other effects | 5% max | 10% max |
| Slatey | 5% max | 10% max |

Source: Effect of quality in Agricultural Produce Business, Folayan (1995)

Table 4: Quality specification of cocoa butter

| Parameters | Specification |
|---|---|
| Free fatty acid | 1.75 max |
| Acid value | 3.5 max |
| Saponifiable value | 188-198 |
| Insaponifiable matter | 35 max (with pe-ether) |
| Iodine value | 35-39 (wijs method) |
| Refractive index | 1.4565 = 1.4575 at 40°C |
| **Melting characteristics** | |
| (I) Incipient fusion (fusion point) | 31-32°C |
| (ii) Slip point | 32-33°C |
| (iii) Clear point | 33-34°C |

Source: Raw Materials Research and Development Council (1993)

**Quality control measure adopted by cocoa stakeholders:** As shown in Table 8, 100% of the Cocoa farmers in the study area indicated adequate farm management practices including weeding and use of appropriate chemicals and timely harvesting of ripped Cocoa pods, while 86% and 72% of the respondents indicated adequate fermentation of cocoa and good storage of well dried cocoa beans as devices to ensure good quality cocoa.

Table 9 indicated that 100 per cent of the LBAs in the study area indicated education of cocoa farmers, 80% indicated outright rejection of bad quality cocoa; while 60% indicated payment of differential prices as method adopted for influencing maintenance of good quality cocoa beans through the cocoa farmers.

The result of findings in respect of quality and warehousing agents is as contained in Table 10. From the findings, 100% of cocoa warehousing/quality control operators performed quality inspection services of weighing and weight determination, quality determination, advisory services, issuance of reports and certificates and routine maintenance all in an attempt to ensure good quality cocoa. 100% of the warehousing agents, as shown in Table 11 indicated that percentage presence of mouldy beans, slatey beans, insect damaged, bean count (size) and moisture content are usually sought for as the quality parameters by international bodies.

Table 5: Quality specification of cocoa cake

| Parameters | Specification |
|---|---|
| Fat content | 11-13% |
| Moisture content | 45 |
| pH value | 7% max |
| Ash content | <10% |
| Total plate count max | 20,000/gm |
| Mould | 50/gm max |
| Test | 50/gm max |
| Coliform | 19/gm Max |
| *E-coli* | Negative |
| *Semonella* | Negative |

(I) Maximum of 75 microscopic insect fragment per 50 gm when 5,50 m samples are examined.
(ii) Max of 2 rodent hairs per 50 gm when 650 gm sub-samples are examined. Or maximum of 4 rodent hairs on any sample.
Source: Raw Materials Research and Development Council (1993)

Table 6: Quality specification of alkanised cocoa powder

| Parameters | Specification |
|---|---|
| Appearance | Fine smooth powder |
| Colour | Reddish or sandy Brown |
| Odour and taste | Typical of cocoa, no flavour additive |
| Moisture | 11-13% |
| pH of 10% solution | 6.8 + 0.20 |
| Sedimentation | Max 0.25 ml over 5 min in 1 ml |
| 1 ml off glass of mesh | Max 2% above 200 175 micron sieve |
| **Microbial quality** | |
| Total aerobic count | Max 10,000/gm |
| Moulds and yeast | Max 50/gm |
| Coliform | Less than 10/gm |
| *E-coli* | Negative |
| *Somalia* | Negative |

Source: Raw Materials Research and Development Council (1993)

Table 7: Quality specification of cocoa liquor

| Parameters | Specification |
|---|---|
| Fat content | 52% max |
| finess | 2% Residue on 200 mesh sieve |
| Moisture content | 1.5 max |
| pH value | 5.2-5.8% |
| Flavour | Foreign off flavour |
| Shell content | Max 1.0 |
| Free fatty acid | 1.65 |
| Total plate count | Max 5,000/gm |
| Coliform | Max 10/gm |
| *E-coli* | Negative |
| *Samolina* | Negative |
| Yeast and mould | Max 50/gm |
| Yeast | Max 50/gm |
| Extraneous matter | Negative |

Source: The success story of cocoa processing and chocolate manufacture in Ghana

Table 8: Cocoa quality control measure adopted by cocoa farmers in study area

| Control measure | Freq. | Percent |
|---|---|---|
| Adequate farm management practices | 50 | 100 |
| Timely harvesting of ripped cocoa pods | 50 | 100 |
| Adequate fermentation of cocoa beans | 43 | 86 |
| Adequate drying of cocoa | 50 | 100 |
| Good storage of well dried cocoa beans | 36 | 72 |

Freq. = Frequency; Source: Computed from field survey, 2008

Table 9: Quality control measure of cocoa beans adopted by LBAs in study area

| Control measure | Freq. | Percent |
|---|---|---|
| Payment of differential prices | 21 | 60 |
| Outright rejection of poor quality cocoa | 28 | 80 |
| Educating the cocoa farmers | 35 | 100 |

Freq. = Frequency; Source: Computed from field survey, 2008

Table 10: Roles of cocoa warehousing agents in cocoa quality control in study area

| Roles | Freq. | Percent |
|---|---|---|
| Weighing and weight determination | 14 | 100 |
| Quality analysis | 14 | 100 |
| Quality control advisory services | 14 | 100 |
| Issuance of quality reports and certificates | 14 | 100 |
| Routine warehouse maintenance | 14 | 100 |

Freq. = Frequency; Source: Computed from field survey, 2008

Table 11: Quality parameters usually sought for in cocoa beans by quality inspection/warehousing agents in study area

| Quantity parameters | Freq. | Percent |
|---|---|---|
| Mouldy beans | 14 | 100 |
| Slatey beans | 14 | 100 |
| Insect damaged | 14 | 100 |
| Bean count | 14 | 100 |
| Moisture content | 14 | 100 |

Freq. = Frequency; Source: Computed from field Survey, 2008

Table 12: Cocoa quality control measure adopted by cocoa exporters in study area

| Quality parameters | Freq. | Percent |
|---|---|---|
| Ordering redrying of wet cocoa beans | 20 | 100 |
| Discounted prices for poor quality cocoa | 12 | 60 |
| Premium price for good quality cocoa | 14 | 70 |
| Outright rejection of poor quality cocoa | 20 | 100 |
| Advance payment for suppliers of good quality cocoa | 14 | 70 |

Freq. = Frequency; Source: Computed from field Survey 2008

Table 12 showed that 100% of the cocoa exporters indicated ordering of redrying of wet cocoa beans and outright rejection of bad quality cocoa while 70% each indicated premium price for good cocoa and advanced payment for suppliers of good quality cocoa while 60% revealed that discounted prices are paid for bad quality cocoa as devices for the cocoa LBAs to improve on the quality of cocoa supplied.

All the cocoa processors, 100% indicated the use of quality control agents, purchase on seeing and buying basis, avoidance of unduly prolonged storage of by-products of cocoa and fumigation of stored cocoa against pests, routine and proper maintenance of processing machines and equipments as the quality control measure, while 85.71% of the cocoa processors adopted outright rejection of bad quality cocoa and proper storage of stock pilled cocoa and 78.57% indicated premium price for good quality cocoa as quality control measures for their products (Table 13).

As shown in Table 14, 100% of the seven functioning cocoa processors indicated cocoa cake, cocoa powder

Table 13: Quality control measure adopted by cocoa processing factories in study area

| Control measures | Freq. | Percent |
|---|---|---|
| Use of quality control agent | 14 | 100.00 |
| Outright rejection of bad quality cocoa | 12 | 85.71 |
| Premium price for good quality cocoa | 11 | 78.57 |
| Purchases on seeing and buying basis | 14 | 100.00 |
| Proper storage of stock pilled cocoa bean | 12 | 85.70 |
| Avoidance of unduely prolonged storage of cocoa and by-products of cocoa | 14 | 100.00 |
| Routine and proper maintenance of processing machines and equipments | 14 | 100.00 |
| Fumigation of stored cocoa beans against pests and rodents | 14 | 100.00 |

Freq. = Frequency; Source: Computed from field Survey, 2008

Table 14: By Products of cocoa processing factories in study area

| By products | Freq. | Percent |
|---|---|---|
| Cocoa Butter | 7 | 100 |
| Cocoa Cake | 7 | 100 |
| Cocoa Powder | 7 | 100 |
| Cocoa liquor | 7 | 100 |

Freq. = Frequency; Source: Computed from filed work 2008

and cocoa butter, as their cocoa by-products while only 42.80% indicated cocoa liquor as one of the by-products. They also revealed that the quality of their products are done in accordance with the laid down international standard.

**Conclusion:** The study revealed that cocoa, butter, cake, powder and liquor are by products of processed cocoa beans and that there are international quality standard specification set by different terminals or future markets as a basis for acceptability of cocoa and its by-products. The result of findings showed that appropriate and adequate farm management practices by cocoa farmers; education of cocoa farmers, outright rejection of quality cocoa and payment of differential prices by cocoa LBAs; Quality inspection and quality maintenance advisory services by cocoa warehousing agents; use of quality control agents and ordering of redrying of cocoa beans by exporters and avoidance of unduely prolonged storage, fumigation of stored cocoa against pests, routine and proper maintenance of processing machines and equipments by cocoa processors are devices by stakeholders to ensure good quality maintenance of cocoa and its by-products in the study area.

**Recommendations:** Based on the facts emanating from the findings that good quality cocoa and its by-products are premised on control measures adopted by stakeholders in the commodity; the outcome of which are better prices and premium rather than arbitration cases and discounting in the case of poor quality cocoa. It is therefore recommended that all stakeholders in cocoa and its by-products should strive hard at contributing to improve the quality at all level by embracing the culture of excellence, bearing in mind that there is no alternative to producing good quality cocoa; so as to keep reaping the benefit of exporting good quality cocoa towards sustaining good image at the international market scene.

Government should encourage policies and programmes such as adequate funding of research, improved extension services cocoa rehabilitation and financial assistance that would facilitate increased capacity utilization by processing factories and export trade. In addition the cocoa processing factories should be funded or encouraged to establish plantations directly or through the use of out-growers.

## REFERENCES

CPA, 2002. Cocoa Producers Alliance Extra Ordinary General Meeting, Assembly Report June 5-7, 2002.

Folayan, J.A., 1993. Effect of quality on marketing and acceptability of cocoa on International Market. Paper presented at the Nigerian Institute of food and Technology Workshop, Ondo State Polytechnic, Owo, Ondo State, Nigeria.

Folayan, J.A., 1995. Effect of Quality in Agricultural Business: Published in the Ondo State Commodities Handbook, Akure, Ondo State.

Folayan, J.A., 2003. Challenges and options of cocoa quality assurance in Nigeria. Paper presented at the workshop organized by cocoa Association of Nigeria at Premier Hotel Ibadan, Nigeria.

Folayan, 2005. Economic Analysis of Cocoa Marketing in Ondo and Ekiti States of Nigeria, unpublished Ph.D. Thesis. Department of Agricultural Economics and Extension Federal University of Technology, Akure, Nigeria.

ICCO, 1999. International Cocoa organization, London 1999.

Koekoek, F.J., 2003. The organic cocoa market in Europe - Summary of a market study Export Promotion of organic products from Africa (EPOPA).

Kotler, P., 2003. Marketing Management, 11th Edn., published by Asoke K. Ghosin, Printice-Hall of India Limited.

Oladipo, B., 2007. Modern Material Management Published by Panaf Press Limited, 2007.

RMRDC, 1993. Raw Materials Research and Development Council in Nigeria, 2nd Edn.

Salami, A.O., 2000. Cocoa marketing under regulated and deregulated regimes in Nigeria: Unpublished Ph.D. Thesis in the Department of Agricultural Economics, University of Ibadan, Nigeria.

Pakistan Journal of Nutrition 9 (9): 920-925, 2010
ISSN 1680-5194


# Proximate Analysis and Microbiological Quality of Cheese Produced from Raw Cow Milk Obtained from Fulani Settlement in Ogun State Nigeria, Using Lactic Acid Bacteria and Extract from Sodom Apple Leaf (*Calotropis procera*)

P.O. Uaboi-Egbenni[1], P.N. Okolie[2], T.I. Akintunde[3], O. Bisi-Johnson[4], L. Enwe[2] and P.O. Bessong[5]
[1]Department of Microbiology, School of Mathematics and Natural Science,
P.M.B. 5050, Thohoyandou, Limpopo Province, South Africa
[2]Department of Food Technology, Yaba College of Technology, P.M.B. 2011, Yaba Lagos
[3]Department of Public Health, University of Venda,
P.M.B. 5050, Thohoyandou, Limpopo Province, South Africa
[4]Department of Medical Microbiology, Walter Sisulu University, P.B. X1,
Mthatha 5117, Eastern Cape Province, South Africa
[5]Department of Microbiology, School of Mathematics and Natural Science,
University of Venda, P.M.B. 5050, Thohoyandou, Limpopo Province, South Africa

**Abstract:** Raw cow milk obtained from Fulani settlement in Ogun State, Nigeria was inoculated with pure cultures of *Lactobacillus bulgaricus* and *Streptococcus thermopilus* with extract from Sodom apple leaf (*Calotropis procera*) as coagulant in the absence of rennin. Fermentation was done for four (4) days for the development of necessary aroma and coagulation. Physiochemical analysis of the fermenting sample showed a gradual drop in pH from 5.8-3.20 and an increase in total titratable acidity from 0.049-0.137%. Proximate analysis of the cheese sample showed a moisture, ash, fat, protein and carbohydrate (by difference) of 64, 0.60, 13.4, 12.86 and 9.14% respectively. Microbiological analysis of the cheese product revealed that the sample was completely free of coliforms, mould and yeasts and hence safe for consumption. This Sodom Apple produced cheese is hereby recommended for both growing children and adult due to the retention of a high percentage of protein after fermentation and its expected ability to correct protein deficiencies. The microbial production and nutritional analysis of the cheese sample is discussed.

**Key words:** *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Calotropis procera*, coagulation, cheese,

## INTRODUCTION

Cheese is the curd or hard substance formed by the coagulation of milk of certain mammals by rennet or similar enzymes in the presence of lactic acid produced by added or adventitious microorganisms from which part of the moisture has been removed by cutting, warming and/or pressing, which has been shaped in a mould and then ripened by holding for sometime at suitable temperatures and humidity. The conventional method for the production of cheese has been discussed extensively by Frazier and Westhoff (1988). Standard cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* have been employed as starter cultures for cheese production (Frazier and Westhoff, 1988). For the production of high quality cheese rennet enzyme is added for effective curdling and in its absence it is possible to use extract from leaves of Sodom Apple (*Calotropis procera*) as alternative. The need arise to develop an appropriate formulation for the production of cheese due to inconsistency in the uniformity of cheese being produced locally.

From the writings or submission of Helen and Elisabeth (1990), there is no real origin of cheese or cheese making but in the earliest records of human activities refers to cows and milk. These may be found in Sanskrit writings of the Sumerians 4000BC, in Babylonian records, 2000BC. It is also found in Vedic hymns.

The preparation of cheese probably dates back many centuries to the time when nomadic tribes of Eastern Mediterranean countries carried milk of domesticated mammals in sacks made from animal skins or gourds or in vessels such as stomachs or bladders (Helen and Elisabeth, 1990). When the milk is kept warm, it rapidly became sour and separated into curds and whey. In the absence of liquid milk, the curd is supplied as supplement as much of the milk value is retained.

According to Helen and Elisabeth (1990) cheese is classified into three categories-soft, blue veined and hard-pressed cheese. This vary in moisture content and therefore in keeping quality and method of ripening. Soft cheese retains a high proportion of moisture (whey) 55-80% and these varieties are eaten fresh (Cambridge, Coulommier Bondon, etc.), whilst others are ripened,

**Corresponding Author:** P.O. Uaboi-Egbenni, Department of Microbiology, School of Mathematics and Natural Science, P.M.B. 5050, Thohoyandou, Limpopo Province, South Africa

usually by growth of surface moulds (Brie, Camembert, Pont (Eveque, etc.). Semi-soft cheeses (Limburger, Tilst, Brie) are made from slightly firm curds 45-55% moisture and are ripened by surface growth of micro organisms particularly by *Brevibacterium linens*. These are the smear-ripened cheese (Helen and Elisabeth, 1990). Blue-veined cheeses such as Stilton, Roquefort and Gorgonzola are made from semi-soft/semi-hard curd 42-52% moisture and are ripened by species of *Penicillium*, which grow with the cheese. Semi-hard cheeses such as Edam and Gouda are made from firmer curd with moisture content within ranges of 45-50%. These are ripened by bacteria and consumed within 2-3 months.

The hard-pressed cheeses are made from relatively dry curd 35-45% moisture, ripened by bacteria and mature slowly within 3-12 months. The very hard, grating cheeses such as Parmesan, Romano and Asiago are made from curd below moisture 26-34%, made partly from skimmed milk and are ripened by bacteria slowly over period of one to two years (Specialist Cheese Makers Association, 2002).

Prentice and Neaves (1986) have argued that the variety of cheese to be produced in any class is strictly determined by the type of milk used, preparation of the young curds and inclusion in the milk or curds of certain microorganisms responsible for the development of acidity during manufacture and development of characteristic features and flavours during ripening.

Cheese is generally made from cow milk, but in some countries and for making certain varieties of cheese, milk of other mammals is used (Helen and Elisabeth, 1990). For example, Ewe's milk is used for making Roquefort cheese and varieties such as Feta, Ricotta, Pecorino, etc.; goats' milk for making varieties of cheese in Italy and Greece and Buffalo's milk in India and Egypt. Ewe and goat's milk are increasingly used for cheese making in UK (Prentice and Brown, 1983).

Milk intended for use in cheese production must be stored at 40°C and transported to factory where it is stored in insulated silos until it is used (FAO, 2008). Prentice and Neaves (1986) observed that raw milk on arrival at the creamery will have total counts of $10^3$-$10^7$/ml depending on the levels of hygiene at the farms. They also observed that organisms present consist of psychrotrophs mostly *Pseudomonas*, *Aeromonas*, *Alcaligens*, small number of lactic acid bacteria, spore-forming gram-positive rods, coryneform bacteria, *Micrococcus* and coli forms. Of these, only the psychrotrophs will multiply during transport and storage, particularly if temperature in insulated tanks and milk silos is allowed to rise.

The temperature employed could also determine the type of starter to be employed. For example, a temperature of 38-40°C will attract the use of a thermophilic starter, whereas, a temperature of 32-45°C may attract the use of a mesophilic starter (http://www, ys,f403,2004). Starter culture used for cheese fermentation have been back slopping (Prentice and Brown, 1983).

Sharp (1979) stressed that species of lactic acid bacteria use as starters in cheese making belong to the genera *Streptococcus*, *Leuconostoc* and *Lacbacillus*. It is possible to use single strain starter as in the case of *Streptococcus lactis* or a combination of both (Billie *et al*., 1985). It is also possible to employ multi-strain starters (Timson *et al*., 1982) or mixed strain starters involving mixture of strains of *Streptococcus cremoris*, *Streptococcus lactis*, *Streptococcus diacetylactis* and *Leuconostoc* (Timson *et al*., 1982).

Starter cultures in cheese making is a medium of harmless, active microorganisms, which by growing in cheese milk and curd assist the development of mature cheese with desirable characteristics of flavour, aroma, pH, texture and body (Scott *et al*., 1998). Billie *et al*. (1992) observed that the rate of acid production is crucial/critical in the manufacture of certain product like cheddar cheese. Mullan (1986) observed that in addition, antibiotic substances now referred to as bacteriocins, produced by starters e.g., nisin may also have a role in preservation.

## MATERIALS AND METHODS

**Sample collection:** Fresh milk was obtained from the shorthorn of the Fulani at Abeokuta, Ogun State, Nigeria in a sterile 4 litres keg container. The sample after collection was kept in an ice-frozen container and immediately transported to the laboratory and kept in the refrigerator at 4°C until it was ready for use. Sodom apple leaves (*Calotropis procera*) were obtained from the premises of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.

**Starter cultures:** *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were obtained from culture collection unit in the Department of Biotechnology, Federal Institute of Industrial Research, Oshodi. The isolates were maintained on MRS Agar slant for *Lactobacillus bulgaricus* and Nutrient Agar slant for *Streptococcus thermophilus*. The slants were kept in the refrigerator at 4°C until they were used.

**Preparation of the inoculum (Seeding culture):** Starter cultures of the selected organisms were grown in separate flasks. About 50 ml of MRS broth was used for the growth of *Lactobacillus bulgaricus* and 50 ml of nutrient broth for the cultivation of *Streptococcus thermophilus*. Both flasks were agitated in a shaker incubator at 100 rpm for 18 h after which they were centrifuged at 5000 rpm for 30 min. The supernatant liquid was decanted and used.

**Fermentation of milk sample:** Freshly collected cow milk previously refrigerated was pasteurized at 68°C for 30 min, cooled to 45°C and thereafter inoculated with the microorganisms. The culture slurry was aseptically inoculated into the pasteurized milk sample at 45°C. Fermentation was allowed to proceed for 24 h at 43°C. After fermentation, the fermented milk sample was heated to boiling point with extract from Sodom apple leaves for the formation of curd at 85°C for 10 min. The fermented curd was then allowed to remain in the whey for the development of necessary yoghurt cheese-like aroma.

**Analysis of fermenting sample:** The fermenting samples were analyzed after 24 h for the following parameters:

**pH determination:** About 10 ml of the fermenting milk sample was dispensed into conical flask and its pH determined using the pH (Unicam 9450 Model). The pH meter was standardized using standard buffer of pH 4.0 and 7.0.

**Determination of the total titrable acidity:** This was done by dispensing 10 ml of the fermenting milk into conical flasks and adding 3 drops of phenolphthalein indicator. Thereafter, 0.1 N NaOH was used for titration to a noticeable pink colour for endpoint determination. The acidity was calculated as lactic acid using the relationship:

$$\text{Lactic acid (\%)} = \frac{\text{Titre value x Normality of Alkali x 9}}{\text{Volume of sample}}$$

Normality of alkali = 0.1, Volume of sample = 25 ml

**Proximate analysis of cheese sample**

**Determination of moisture content of cheese:** The procedure employed was that of AOAC (1990). About 5 g of the cheese sample was weighed into pre-weighed aluminum dry dishes and the sample was leveled carefully in the dish. The dish and its content were then transferred into the oven at a temperature of 105°C and were dried for 3 h. This was then allowed to cool in a desiccator and weighed. The dish was returned into the oven for another half hour and again cooled and reweighed. The process was repeated until a constant weight was reached.

**Determination of ash content:** About 5 g of the cheese sample was weighed into porcelain crucible previously ignited and weighed. The material was ignited in the fume cupboard until no fume was seen charred of organic matter. This was then transferred into muffle furnace at 550°C using a pair of tongs and was ignited for 3 h, cooled in a desiccator and weighed immediately.

**Determination of fat content by soxhlet extraction method:** About 5 g of cheese sample was weighed and put in thimbles using a dry paper and plugged with cotton wool. The thimbles were dried and inserted into a soxtec system HT2. The extraction cups were dried and weighed and then 50 ml solvent (petroleum ether) was added in each cup. The cups were inserted into the soxtec. The samples were extracted for 15 min in boiling position. The extraction was carried out continuously for 3 h. This was cooled and reweighed.

**Determination of protein content of fermented cheese:** Kjedahl nitrogen method was employed for the determination of protein content of the fermented cheese. About 1.0 g of the cheese sample was weighed into the digestion flask. Kjedahl catalyst (5 selenium tablets) was added to the sample. About 20 ml of concentrated tetraoxosulphate VI acid was added to sample and then fixed for 8 h in the digestion unit (450°C) of the Kjedahl apparatus in fume cupboard. The digest, pure yellow after cooling changed into a colourless liquid that was transferred into 100 ml volumetric flask and made up to mark with distilled water. About 20 ml of 4% boric acid solution was pipetted into conical flask. A drop of methyl red was added to the flask as indicator. The sample was thereafter diluted with 75 ml of distilled water. About 10 ml of the digest was made alkaline with 20 ml of NoaH (20%) and distilled. The steam exit of the distillatory was closed and the change of colour of boric acid solution to green was timed. The mixture was distilled for 15 min (AOAC, 1990). The filtrate was then titrated against 0.1 N HCl. The protein content was calculated from the relationship:

$$\text{Total protein (\%)} \frac{\text{Titre x Normality of acid x 0.014}}{\text{Sample weight}} \text{ x 100}$$

Protein conversion factor = 6.38 for Milk
Protein (%) = % Nitrogen x 6.38
Normality of acid (HCl) = 0.1 N
Sample weight = 1.0 g

**Determination of the carbohydrate content of fermented cheese:** This was determined by subtracting from 100 the sum of the percentage moisture, ash, protein and fat. The remainder value gives the carbohydrate content of the sample.

Carbohydrate (%) = 100-(sum of moisture, protein, ash and fat)

**Microbiological analysis of fermented cheese sample:** The standard method of Harrigan and McCance (1976) was employed. About 1 g of the cheese sample was aseptically weighed using a weighing balance and carefully introduced into 9 ml of sterile distilled water. This was shaken manually in order to have a

homogeneous suspension. About 1 ml of this was taken and introduced into the second tube, followed with series of dilutions up to $10^{-10}$ dilution. One ml was taken from $10^{-4}$ dilution and introduced into sterile plates and molten agar (50°C) added by pour plate method using the following agar and incubation periods:

**Nutrient agar:** This was used for the determination of total viable bacteria in the sample. The plates were incubated at 37°C for 24-48 h.

**MacConkey agar:** This was used for the enumeration of total coliform organisms in the sample. The plates were incubated at 35°C for 24-48 h.

**Sabouraud dextrose agar:** This was used for the enumeration of mould and yeast in the sample. The plates were incubated at 30°C for 24 h for yeasts and 3-5 days for mould.

## RESULTS

The results of the various analysis done on fermented cheese sample are as displayed in the tables below. All results are average values of four (4) determinations.

Table 1 shows the gradual decrease in the pH of fermenting milk during cheese production over a 72-h period from 5.80-3.20. This final value is the mean value over four (4) determinations. This value is far lower than those obtained in previous studies. The total titratable acidity of fermenting sample during cheese production increased from an initial value of 0.049-0.137 over 72 h (Table 2). The mean moisture content of the cheese product was 64.0% (Table 3). The mean ash content of the fermented cheese was 0.6% (Table 4). The mean % fat content of cheese was 13.4%. The mean protein content of the fermented cheese was 12.8% (Table 6). The mean carbohydrate content was 9.14% (Table 7). The microbiological level as determined from this study (Table 8) shows the total plate count was $1.1 \times 10^{1}$. There were no coliforms isolated from the cheese, neither were moulds or yeasts isolated from the final cheese product. This finding shows the high quality of the laboratory-made cheese.

## DISCUSSION

The production of fermented products with special reference to cheese and yoghurt has been discussed by several workers (Frazier and Westhoff, 1988; Helen and Elisabeth, 1990; Prentice and Neaves, 1986; Specialist Cheese Makers Association, 2002; Prentice and Brown, 1983; Billie *et al*., 1992). The use of starter cultures have also been mentioned by several workers; *Streptococcus thermopilus* (Mullan, 1986; Helen and Elisabeth, 1990); *Lactobacillus delbrueckii subspecies bulgaricus* (Timson *et al*., 1982); *Lactobacillus acidophilus* (Rogosa *et al*., 1951); *Lactococcus* (Mullan, 1986); *Streptococcus cremoris / Leuconostoc* (Billie *et al*., 1985; Timson *et al*., 1982).

Table 1: pH of fermenting milk sample during cheese production

| Time (h) | pH values |
|---|---|
| 0 | 5.80 |
| 24 | 4.2 |
| 48 | 3.70 |
| 72 | 3.20 |

Table 2: Total titrable acidity of fermenting sample during cheese production (samples per h)

| Time | 0 | 24 | 48 | 72 |
|---|---|---|---|---|
| 1st titre (ml) | 1.30 | 3.30 | 3.50 | 3.80 |
| 2nd titre (ml) | 1.40 | 3.40 | 3.70 | 3.80 |
| Average titre (ml) | 1.35 | 3.35 | 3.60 | 3.80 |
| Lactic acid (%) | 0.049 | 0.121 | 0.129 | 0.137 |

Table 1 is the pH of the fermenting milk sample, which shows a gradual drop in pH value from 5.80-3.20. The low acid pH value obtained in this study is similar to the observation made by Seo *et al*. (2009) in their studies of yoghurt samples in which they obtained a pH value of 3.82. This difference in the acid level may have stemmed from the fact that yoghurt is more of liquid than cheese. The acid level of this Sodom Apple leaves extract coagulated cheese is far lower than Rennet coagulated cheese (pH 5.8-6.5). The decrease in acid level of leaf extract of *Calotropis procera* fermented cheese may be attributed to presence of acid in the extract that enhance a fall in the acidic content of the ferment or that the cultures had higher potential at producing high level of lactic acid. This needs to be seriously investigated. Egan *et al*. (1988) in their analysis explained that pH is the measure of the acidity and alkalinity of the fermenting medium.

The total titrable acidity of the fermenting milk sample (Table 2) reveals that there is a gradual increase in the titrable acid from 0.049-0.137%. The enhanced titrable acidity is due to the presence of lactic acid produced by lactic acid culture during fermentation. The titrable acidity obtained in this study is in line with those reported by other workers in which they obtained a value of 0.127% (Davies and Wilkinson, 1973; Davis *et al*., 1993; Aworh and Akinniyi, 1989). This attribute enhances acidity of fermenting sample to the milk as well as to the buffering capacity of the product.

Proximate analysis revealed that the moisture content of the cheese sample (Table 3) was 64.0%. The result obtained is similar to those reported by Aworh and Akinniyi (1989) and Fasakin and Unokiwedi (1992) who obtained 61.3% and 60.8% respectively. Egan *et al*. (1988) and Frazier and Westhoff (1988) had stressed that the moisture content is a measure of the water content and accounts for the texture of cheese. Analysis showed that the ash content of the cheese sample (Table 4) is 0.6%. This result is also similar to those obtained by Aworh and Akinniyi (1989) in which they obtained a value of 0.8%. The fat content of the cheese (Table 5) was 13.4%. Similar observation was made by Wong *et al*. (1988) where they obtained a value

Table 3: Moisture content of fermented cheese sample

| Sample | Wt of sample (g) | Wt of sample+dish before drying (M1g) | Wt of dish + sample after drying M2 (g) | Moisture (%) |
|---|---|---|---|---|
| Cheese sample | 5.0 | 47.22 | 44.22 | 64.0 |

Table 4: Ash content of fermented cheese sample

| Sample | Wt of sample (g) | Wt of empty crucible | Wt of crucible + Ash | Ash (%) |
|---|---|---|---|---|
| Cheese | 5.00 | 65.00 | 65.03 | 0.6 |

Table 5: Fat content of cheese sample

| Sample | Wt of sample (g) | Wt of empty cup | Wt of cup + extracted oil (g) | Fat (%) |
|---|---|---|---|---|
| Cheese | 5.00 | 30.00 | 30.67 | 13.4 |

Table 6: Protein content of fermented cheese sample

| Sample | Wt of sample (g) | 1st titre | 2nd titre | Average titre | $N_2$ (%) | Protein (%) = %$N_2$ x 6.38 |
|---|---|---|---|---|---|---|
| Cheese | 1.00 | 14.3 | 14.5 | 14.4 | 2.016 | 12.86 |

Table 7: Carbohydrate content of cheese sample

| Sample | Moisture (%) | Ash (%) | Fat (%) | Protein (%) | Carbohydrate (by difference) (%) |
|---|---|---|---|---|---|
| Cheese | 64.0 | 0.6 | 13.4 | 12.86 | 9.14 |

Table 8: Microbiological analysis of the cheese sample

| Type of analyses | Colony forming Unit/g |
|---|---|
| Total plate count | $1.1 \times 10^1$ |
| Coliform count | Nil |
| Mould and yeast count | Nil |

Above readings were average values of four (4) determinations

of 12.7% for heat-acid coagulated cheese. However, the result of Fasakin and Unokiwedi (1992) (47.50%) were at variance with those obtained in this study and previous studies. This may be due to the fact that their product was not heat-treated. Significantly, fat is important as a source of energy to the body (Hannon *et al*., 2006). The study of the protein content of cheese gave a mean value of 12.86%. This value is higher than those reported by earlier workers on cheese (Frazier and Westhoff, 1988, 5.33%) and lower than the value reported by Fasakin and Unokiwedi (1992) (44.5%). The high protein content of this product shows that its consumption will help eliminate protein deficiencies that have become the bane of poor nations, Nigeria inclusive. The carbohydrate content of the fermented product (Table 7)

shows it contain 9.14%. Similar observation was reported by Fasakin and Unokiwedi (1992) during the chemical analysis of cheese from milk and melon milk. Microbiological data of the cheese sample (Table 8) revealed that the total plate count was $1.1 \times 10^1$ cfu/g with the absence of coliforms, moulds and yeasts. The total aerobic count of $1.1 \times 10^1$ cfu/g is within the acceptable limit and hence the product is safe for human consumption. The high quality of this product was possible because good laboratory practice was maintained throughout the study process culminating in the production of an acceptable product of low microbial quality.

This report has shown that in the absence of rennet, extract from *Calotropis procera* (Sodom Apple) leaves can be used to coagulate milk product without necessarily destroying the nutritive value of milk but rather improve its quality. It also shows that such extracts are adequate replacement for rennet as the plant are available everywhere. It is hoped that the chemical characteristics of the Sodom Apple leaves will be analyzed in subsequent studies to ascertain its constituent.

## REFERENCES

AOAC, 1990. Association of Official Analytical Chemists, Washington DC., USA.

Aworh, S. and A. Akinniyi, 1989. Proximate analysis and nutritional component of locally produced cheese. Nig. J. Food. Sci. and Tech., 9: 220-227.

Billie, P.G., W.E. Espie and W.M.A. Mullan, 1992. Evaluation of media for the isolation of Leuconostocs from fermented products. Milchwissenschaft, 47: 640.

Billie, P.G., W.M.A. Mullan and W.E. Espie, 1985. Observation on the effectiveness of agar media for the enumeration of Leuconostocs from cheese and starter cultures. Irish J. Food Sci. Technol., 9: 78.

Davis, P.A., J.F. Platon, M.F. Gershwin, G.M. Halpern, C.L. Keen, D. Dipaolo, J. Alexander and V.A. Ziboh, 1993. A Linoleate-enriched cheese reduce low-density lipoprotein in moderately hypercholesterolemic adults. Ann. Intern. Med., 119: 555-559.

Davies, F.L. and Wilkinson, 1973. *Bacillus cereus* in milk and dairy products. In; B.C. Hobbs and J.H.B. Christian. The microbial safety of food. Proceedings of the Eighth Internation Symposium on food microbiology (pp: 57-67). London, Academic Press.

Egan, H., R.S. Kirk and R. Sawyer, 1988. Pearson's Chemical Analysis of Foods. Bath. Aron., pp: 15-20.

FAO, 2008. Milk for Health and Wealth. FAO, Rome, Italy.

Fasakin, A. and C. Unokiwedi, 1992. Chemical analysis of fermented cheese obtained from Cow milk and melon. Nig. J. Microbiol., 5: 559-566.

Frazier, W.C. and D.C. Westhoff, 1988. Food Microbiology. Tata-McGraw-Hill Publishers Co., New Delhi, pp: 410.

Harrigan, W.F. and M.E. McCance, 1976. Laboratory Methods in Food and Dairy Microbiology, Academic Press, London, pp: 400.

Helen, R. and M. Elisabeth, 1990. Microbiology of Cheese. Institute of Food Research Shirfield Berkshire, UK., pp: 409.

Mullan, W.M.A., 1986. Bacteriophage induced starter problems. Dairy Industries Int., 51: 40-43.

Prentice, G.A. and J.V. Brown, 1983. The microbiology of cheddar cheese manufacture. Dairy Industries Int., 48: 23-26.

Prentice, G.A. and J. Neaves, 1986. J. Appl. Bacteriol., Symp., 8: 435-575.

Rogosa, M., J.A. Mitchell and R.F. Wiseman, 1951. Media for the isolation of Lactic Acid Bacteria. J. Bact., 62: 132-133.

Scott, R., R.K. Robinson and R.A. Wilbey, 1998. Cheese Making Practice. 3rd Edn., Applied Science Publication, Ltd., London.

Seo, M.H., S.Y. Lee, Y.H. Chang and H.S. Kwak, 2009. Physicochemical, microbial, and Sensory properties of yogurt supplemented with nanopowdered chitosan during storage. J. Dairy Sci., 92: 5907-5916.

Sharp, M.E., 1979. Identification of the Lactic Acid Bacteria: In: Identification methods for Microbiologists. 2nd edn. (Eds. F.A. Skinner and D.W. Loverlock) pp: 233-259, London, Academic Press.

Specialist Cheese Makers Association, 2002. The specialist cheesemakers code of best practice. Available from http://www. specialistcheesemakers. co.uk/about.html

Timson, W., M.C. Broome, A.J. Hillier and G.R. Jago, 1982. Metabolism of *Streptococcus thermopiles*. Aust. J. Dairy Technol., 37: 14-16.

Wong, H.C, L.Y. Chen and C.L. Chen, 1988. Growth germination and toxigenic activity of *Bacillus cereus* in milk productions. J. Food Protection, 51: 707-710.

Pakistan Journal of Nutrition 9 (9): 926-934, 2010
ISSN 1680-5194


# Potential Production and Application of Biofertilizers in Sudan

Gadalla A. Elhassan[1], Migdam E. Abdelgani[2], Awad G. Osman[2],
Somaya S. Mohamed[2] and Belgees S. Abdelgadir[2]
[1]Sudan University of Science and Technology, Sudan
[2]National Research Centre, Sudan

## INTRODUCTION

During the last decades, the increased costs of fertilizer production coupled with the progressively increasing use of chemical fertilizers are adding to the cost of crop cultivation. In addition, chemical fertilizers are harmful when they persist in the soil and enter the food chain. Instead, an approach is adopted to introduce into the soil potential microorganism, a practice known as inoculation. The inoculants are also known as biofertilizers. Several microorganisms and their association with crop plants are being exploited in the production of biofertilizers. The microorganisms which are potential biofertilizers are symbiotic and non-symbiotic nitrogen fixing microorganisms, phosphorous solubilizing microorganisms and silicate bacteria. The potential uses of biofertilizers in agriculture play an important role of providing an economically viable level for achieving the ultimate goal to enhance productivity.

On the other hand, the value of organic materials as a source of plant nutrients is greatly enhanced by composting. Composted materials are also more stable and pleasant to handle.

In this paper the discussion is restricted to a review of the main groups of microbial biofertilizers in addition to fertilizers from the organic origin.

### Symbiotic nitrogen fixation

**Rhizobium inoculation:** In a study of Rhizobial cross inoculation groups of *Faidherbia albida* and *Acacia nilotica*, *Acacia senegal*, *A. tortilis*, *A. seyal* and *A. melifera*, it was found that the frequency of nodulation and total nitrogen content were maximized when each individual plant species was inoculated with its own isolate of *Rhizobium*.In addition of NPK fertilizer benefited *Acacia spp*, as it resulted in more root nodules, when it was combined with inoculation with their own *Rhizobium* isolates (ElAtta and Osman, 1993). The impact of soil moisture, temperature and soil reaction (pH) on nodulation of six *Acacia spp*, *A. nilotica*, *A. senegal*, *A. seyal*, *A. tortilis*, *A. melifera* and *A. albida* (*Faidherbia albida*) was studied. Increasing the moisture content from 15-35% doubled the frequency of nodulation. The frequency of nodulation increased from 6.5 nodules in winter to 15.3 nodules in summer. Absolutely no nodules were produced by any plant in acidic soil (Osman and ElAtt, 1995).

Alfalfa (*Medicago sativa* L.) was found to respond positively to *Rhizobium* inoculation in three different locations in Khartoum state. Yield of fresh and dry fodder were significantly higher in inoculated plants as compared to uninoculated control plants (Mohamed and Osman, 1996).

The inoculation of faba bean with *rhizobium* strain TAL 1400 constantly resulted in severe increments in the fresh and dry weights of shoot, root, nodules, number of nodules, nodule dry weight, grain yield and $N_2$ fixation. However, inoculation with *Rhizobium leguminosarum* significantly increased all these parameters (Osman and Mohamed, 1996).

Cultivation of groundnut in Western Sudan is still lacking nitrogen fertilizers. Hence three imported strains were compared to local strains. The results indicate that, the imported *Rhizobium* strain has no benefits for groundnut production in Western Sudan. Hence, the future research on nitrogen fixation by groundnut in this area should be directed to selection and identification of the most effective rhizobia strains from the adapted local population (Ali, 2003). However, inoculation with a compatible strain of rhizobia was found to enhance nodulation, dry weight of nodules, nitrogen fixation and yield of alfalfa (Medicago *sativa*), Fenugreek (*Trigonella foenugraecum*), cluster bean (*Cyamopsis tetragonoloba*), field pea (*Pisum sativum*) and common bean (*Phaseolus vulgaris*) grown in dry land. It was concluded that, the productivity of leguminous crops in dry land could be improved by *Rhizobium* inoculation (Abdelgani *et al*., 2003b).

*Bradyrhizobium* inoculation to guar significantly improved nodulation and dry matter production particularly by locally isolated bradyrhizobia. Nitrogen fertilization improved dry matter production but depressed nodulation. Phosphate mitigated the depressive effect of nitrogen on nodulation and further enhanced its stimulatory effect on dry matter production (Mahdi and Mustafa, 2005).

Investigation on nodulation of guar and five other species of legumes (*Cajanus cajan*, *Vigna unguiculata*, *Crotalaria saltiana* and *cassia occidentalis*) in the Sudan indicated that although these legumes were naturally nodulated, inoculation by introduced or locally-isolated bacterial strains improved nodulation and dry matter production (Mahdi and Mustafa, 2005).

**Corresponding Author:** Gadalla A. Elhassan, Sudan University of Science And Technology, Sudan

*Rhizobium* multi strain inoculation significantly increased dry weight of shoots, roots and nodules and number of nodules of chickpea. Single *Rhizobium* inoculation of *Phaseolus vulgaris* showed significant difference for the studied parameters (Mohamed Ahmed *et al*., 2009).

$N^{15}$ methodology was used to determine the amount of nitrogen fixed by summer legumes guar, pigeon pea and mung bean compared to groundnut. The result showed that pigeon pea and guar could compete well with groundnut as N2-fixers. Levels of fixation were 79, 77, 80 and 12% of the total crops N need for guar, groundnut, pigeon pea and mung bean, respectively (Adlan and Mukhtar, 2004).

**Rhizobium inoculation and chicken manure:** Inoculation of *Sinorhizobium* and application of chicken manure to alfalfa significantly increased plant density, forage fresh yield and protein content and significantly decreases crude fibre content (Elsheikh *et al*., 2006).

**Rhizobium inoculation and phosphorus:** Phosphorus application increased plant density of alfalfa, whereas *Rhizobium* inoculation seemed to have a negative effect on plant density. Both treatments (Phosphorus application and *Rhizobium* inoculation) led to increase in seed yield components and seed yield and significantly increased growth parameters and improved nodulation. The introduced cultivar Pioneer 5929 was superior to the local cultivar Hegazi in most studies growth parameters (Abuswar and Mohamed, 1997a,b). The effect of four doses of phosphorus (0, 100, 200, 300 kg $p_2O_5$/ha) on the growth and forage yield of clitoria (*Clitoria ternatae*), lablab (*Lablab purpureus*) and phillipesara (*Vigna trilobata*) were studied. Addition of phosphorus significantly increased plant height, number of fruting branches/plant and fresh weight of leaves (Ibrahim *et al*., 1996).

***Rhizobium* inoculation and virus:** Viral infections of faba bean with Broad Bean Mottle Bromovirus (BBMV) and Bean Yellow Mosaic Virus (BYMV) significantly decreased shoot and root dry weight, number of nodules, nodule dry weight, grain yield and $N_2$ fixation. However, inoculation with *Rhizobium leguminosarum* significantly increased all these parameters (Elsheikh and Osman, 1995). Viral infection had an adverse effect on yield, protein content and IVPD (Babiker *et al*., 1995). Bean Yellow Mosaic Virus (BYMV) inoculum significantly decreased shoot, root, nodule dry weight, nodule number, number of flowers and pods per plant, total plant nitrogen and nitrogen fixation of faba bean, whereas inoculation with *Rhizobium* has significantly increased these parameters. The results also indicated that *Rhizobium* strain TAL 1397 is effective in fixing nitrogen in normal and in virus infected faba bean plants (Osman and Elsheikh, 1994).

***Rhizobium* co-inoculation:** Inoculation of groundnut with *Bradyrhizobium* and *Azospirillum* could be a promising technology for improving groundnut production giving 8% increase in yield which was almost similar to the 90% increase caused by 86 kg N/ha. The nitrogen derived from the air was calculated to be about 79-80% indicating that groundnut fixed more than 70% of its N need from the air (Ahmed *et al*., 2005).

Inoculation with the PSB *Bacillus megatherium* var. *phosphatcum* (Osman *et al*., 2007) increased nodulation, nodule dry weight, nitrogen and phosphorus content in shoot and fresh and dry fodder. Co-inoculation with *Rhizobium* and PSB had a synergetic effect manifested in increased nodulation, nodule dry weight, shoot dry weight and shoot nitrogen and phosphorus content, compared to uninoculated control (Hassan and Abdelgani, 2009).

Rugheim and Abdelgani (2009) assessed the effect of inoculation by different *Rhizobium* and phosphate solubilizing bacterial strains and their interaction on yield and seed quality of faba bean at EL-Hudeiba Research Station farm in north Sudan. *Rhizobium* inoculation individually significantly increased yield, seed moisture, ash, crude fiber and crude protein. Phosphate solubilizing bacteria individually significantly increased yield, seed moisture, ash and fat in faba bean. A synergetic effect was observed when the two types of microorganisms were combined, they significantly increased yield and seed quality (moisture, crude protein, fat, crude fiber and ash content) of faba bean plants.

***Rhizobium* inoculation and micronutrients:** Studies were conducted to investigate the effects of *Bradyrhizobium* inoculation, molybdenum and zinc application on growth and yield of groundnut. Leaf N content was positively related to rhizobia effectiveness, and leaf MO content was negatively related to rhizobia effectiveness. Increase in applied MO and Zn levels increased their contents in leaves. The highest pod and seed yields were obtained from the interaction of the lowest soil Zn application and the indigenous strains-subjected plants (Hassan *et al*., 2006 a,b).

Micronutrient fertilizers consistently gave a considerable increase in the number of pods and consequently higher yield. *Rhizobium* inoculation had no significant effect on the parameters measured. A significant interaction was found between cultivars and fertilizers only in leaf nitrogen content (Elballa *et al*., 2004).

***Rhizobium* inoculation and salinity:** Elsheikh (1998a) reported that *Rhizobium* inoculation has a great potential for improving fertility in saline soils.

The effect of chemical (nitrogen and phosphorus) and biological fertilizers (*Rhizobium* and Vesicular Arbuscular Mycorrhizae (VAM) (*Glomus sp*) on growth

and symbiotic properties of faba bean under saline conditions were investigated. salinity significantly reduced the shoot fresh and dry weight, number of nodules and percentage of mycorrhizal infection. Both VAM inoculation and phosphorus fertilization significantly increased the shoot and root fresh weight and number and dry weight of nodules. Inoculation with *Rhizobium* significantly increased shoot and root fresh weight and number and dry weight of nodule under saline and non-saline conditions (Ahmed and Elsheikh, 1998).

According to Abdelgani *et al*. (2003a,b), Salinity of > 4 $dsm^{-1}$ significantly reduced growth of TAL 169 and two local isolates: ENRRI 16A and ENRRI 16C. However, average number of viable cells per ml tended to higher in TAL 169 up to EC8 than in the total isolates In laboratory experiments. However, the salinity level of 6$dsm^{-1}$ significantly reduced nodulation, nodule dry weight and shoot nitrogen content of guar plant. The locally isolates strain ENRRI 16A was found to be more tolerant to salinity compare to stain TAL169.

Eight Fenugreek, (*Trigonella foenumgraecum*) cultivars and four *Rhizobium* strains were screened for their salt tolerance in three types of soils. *Rhizobium* meliloti strains were more salt-tolerant than fenugreek cultivars. Several pot experiments were designed to study the effect of salinity, chicken manure and nitrogen on nodulation. Application of chicken manure significantly increased all measured parameters. Salinity significantly reduced all measured parameters (Elsheikh and Forawi, 1995).

***Rhizobium* inoculation and fungicides:** Laboratory experiments were conducted to study the effect of different concentrations (0, 10, 20, 50, 100, 200, 500 and 1000 µg/l) of the fungicides captan, thiram, luxan, fernasan-d and milcurb on inhibition of growth and colony size of seven *Rhizobium* strains (4 introduced and 3 locally isolated). The effects were determined by measuring the colony size and the diameter of the zone of inhibited growth. Fungicides differed in their effects on *Rhizobium* and *Bradyrhizobium* strains. Captan at the concentration of 100 and 1000 µg/l was the most toxic. All strains tolerated low fungicide concentrations ($\leq$100 µg/l) but they were sensitive to high concentrations ($\geq$500 µg/l) with varying degrees of sensitivity. *Rhizobium* strains were more tolerant than *Bradyrhizobium* strains and no clear differences were observed between the introduced and locally isolated strains (Mohamed Ahmed *et al*., 2009).

A study was conducted to investigate the effect of fungicide Bayleton in some nitrogen fixing bacteria (*Rhizobium, Pseudomonas, Flavobacteria*). *Pseudomonas putida* was the most resistant to the fungicide in comparison to the other species. $LD_{50}$ of these microbes were greater than the field dose therefore these microbes can be used as biofertilizers with bayleton (Osman and Abdelgani, 2005).

**Rhizobium inoculation and seed composition:** Elshiekh (2001) reported that positive results were found on plysical and chemical properties of legume seeds.

Afield experiment was conducted to study the response of four faba bean (*Vicia faba* L.) genotypes to *Rhizobium* inoculation, nitrogen and chicken manure fertilization. With the exception of one genotype, all treatments significantly increased protein content and the *in vitro* protein digestibility. The *Rhizobium* inoculation and chicken manure fertilization significantly increased the crude fibre and significantly decreased the carbohydrate content (Elsheikh, 1998b).

*Rhizobium* inoculation and/or intercropping treatments significantly increased the ash, crude fibre, fat, protein content, moisture and tannin contents compared to the uninoculated monocrop control (Elsheikh and Ahmed, 2000).

On the other hand, study was conducted to investigate the effect of *Bradyrhizobium* inoculation and chicken manure and sulphur fertilization on minerals composition of soybean (*Glycine max* L). The results showed that inoculation, chicken manure, sulphur and their interactions significantly improved both major and trace minerals composition of the seeds. The highest value of each mineral was observed with either 10 ton/fed chicken manure or 100 kg/fed sulphur with or without *Bradyrhizobium* inoculation (Ibrahim *et al*., 2008a). In addition to that, a study was carried out to investigate the effect of two *Bradyrhizobium* strains (local and imported), chicken manure fertilization (7 t/ha) and intercropping with sorghum on the chemical composition and physical characteristics of soybean seed. For both monocropping and intercropping systems, moisture, protein, tannin, 100 seeds weight, hydration coefficient, cookability and mineral composition of the seeds were increased for all treatments while ash, fibre and carbohydrate contents were fluctuated for both systems and treatments (Elsheikh *et al*., 2009).

More over, a study was conducted to investigate the effect of *Bradyrhizobium* inoculation, chicken manure or sulphur fertilization on physical characteristics and chemical composition of hyacinth bean seeds. The results indicated that hydration coefficient, cookability, moisture, ash, fat, fiber, protein, carbohydrates, major and trace elements were increased with increasing level of amendments (manure or sulphur) in the presence or absence of *Bradyrhizobium* and the highest value of each parameter was observed with either 10 t/fed chicken manure or 100 kg/fed sulphur (Ibrahim *et al*., 2008b).

**Financial studies:** Results from two faba bean cultivars showed that *Rhizobium* inoculation and nitrogen fertilization significantly increased yield. However, *Rhizobium* inoculation gave significantly higher yield

than nitrogen fertilization. The financial analysis showed that both treatments were financially feasible, but the net returns obtained from *Rizobium* inoculation were by far greater than those of nitrogen fertilization for the two cultivars, Silaim and Agabat (Osman *et al*., 1996).

**Rhizobium carrier materials:** A study was carried out to assess the suitability of locally available materials as carriers for rhizobia. It also aimed at evaluating the effect of carrier sterilization on the shelf life of inoculants. Sterilization of the carrier material enhanced growth of rhizobia and prolonged the shelf life of the inoculum. Charcoal powder was found to be the best among the tested materials with a storage ability of 60 days at room temperature. Sterilized groundnut shells were better than sterilized Nile silt and bagasse mixture (1:1 by weight) for storage for 40 days. The combination of Nile silt with bagasse (1:1) was better than the mixture of (3:1). Sterilized bagasse carrier could be used for storage for 40 days while sterilized rice residues could be used for storage for 20 days. Nile silt alone was found to be the least suitable material to be used as carrier for rhizobia (Abdelrahim *et al*., 2005).
In another study (Elsharif and Abdelgani, 2007), filter mud from 2 local sources was tested for its efficiency as a carrier for rhizobia, compared to charcoal. The results showed that filter mud from ElGenaid Sugar Factory maintained the highest numbers of rhizobia and was the best to be used as a carrier. Sterilization of carrier material showed better results compared to the non-sterilized carrier. The shelf life of the inoculants was 7 weeks when kept at room temperature (with maximum limit of 32°C).

**Commercial production of elokadin biofertilzer:** The commercial production of Elokadin biofertilizer was started in 1992 by the Biofertilization Department of the environment and natural resources research institute of the National Center for Research (NCR) (Table 1 and 2 and Fig. 1)

**Cyanobacteria:** Very little work has been devoted to investigate the biofertilizer role of cyanobacteria in Sudanese agriculture (Mahdi, 1993). Eco-physiological studies, it was found that all cyanobacterial orders including nitrogen fixers were represented in soil and water samples taken from Khartoum and North Kordofan states. Cyanobacterial plasticity enables them to live in a wide range of temperature, light intensity.pH and salinity. In addition, they have considerable crusting potentials (Ali, 2010).

**Mycorrhizal association:** Mycorrhizae are a group of fungi that include a number of types based on the different structures formed inside or outside the root. These fungi grow on the roots of these plants. Seedlings that have mycorrhizal fungi growing on their roots survive

Table 1: Area and major crops in Sudan that fertilized by *Rhizobium* inoculant (Elokadin)

| Area | Major Crops |
|---|---|
| Khartoum State | Alfalfa, Broad bean, Lubia, Bean, Garden pea |
| Northern State | Broad bean, Chickpea |
| River Nile State | Lentils, Broad bean, Alfalfa |
| Blue Nile State | Guar |
| ElGadaref State | Guar, Groundnut |
| Northern Kordofan | Guar, Groundnut |
| Northern Darfur | Alfalfa, Broad bean |

Table 2: Production area from 1992-2009

| Year | Area (Feddan) |
|---|---|
| 1992 | 240 |
| 1994 | 355 |
| 1995 | 1750 |
| 1996 | 2805 |
| 1997 | 1183 |
| 1998 | 890 |
| 2000 | 2249 |
| 2001 | 1484 |
| 2002 | 400 |
| 2003 | 1953 |
| 2004 | 518 |
| 2005 | 2000 |
| 2006 | 736 |
| 2007 | 2220 |
| 2008 | 2498 |
| 2009 | 1361 |

Source: ENRRI annual scientific reports

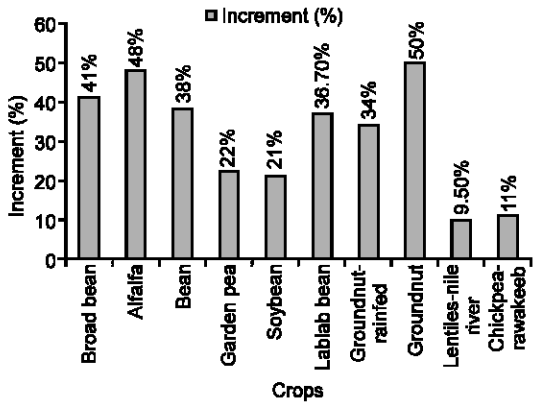


Fig. 1: Increase in production in some corps fertilized by Elokadin biofartilizer

better after transplantation and grow faster. The fungal symbiont gets shelter and food from the plant which, in turn, acquires an array of benefits such as better uptake of phosphorus, salinity and drought tolerance, maintenance of water balance, and overall increase in plant growth and development.

**Mycorrhizal research in the Sudan:** Atabani (1988) studied the effect of four mycorrhizal fungi, namely *Glomus mossae, Gigaspora margarita, Gigasporea calospora and Acaulospora sp* on hyacinth bean (*Lablab*

*purpureus*) and soybean *(Glycine max*). Inoculation with any of the four mycorrhizal fungi established successful symbiotic association between these fungi and the crops and resulted in improved plant performance which was further enhanced in the presence of phosphate fertilization. According to Galal (1993), inoculation of cowpea with local and introduced *Glomus sp* VAM fungi significantly enhanced plant nodulation, dry matter yield and tissue N&P contents in silt and sandy soils. No significant differences were reported between the efficiency of the introduced and the local VAM fungi. Mahdi (1993) reviewed the use of VAM fungi as a biofertilizer in Sudan. He suggested that VAM fungi have a great potentiality for using as a biofertilizer. Shoots and roots dry weight and phosphorus content of Dolichos bean plants increased with *Glomus sp* inoculation. *Glomus sp* significantly reduced the number of galls induced by the root-knot nematode *M. incognita* and hence reduced the infestation effect of the nematodes (Ahmed *et al*., 2009).

A study was conducted to investigate the effects of mycorrhizal inoculation and phosphorus application on infection, symbiotic activity and yield of faba bean. Mycorrhizal infection occurred with low P concentration. Mycorrhizal inoculation significantly increased nodule number, nodule dry weight, flower set, pod production and seed yield compared to non-mycorrhizal plants. The stimulation of faba bean symbiotic activity and seed yield by mycorrhizal inoculation was suppressed by the application of phosphorus (Ahmed *et al*., 2000).

Mycorrhizal inoculation and/or superphosphate significantly increased both oil and protein content of groundnut seeds. *Bradyrhizobium* and/or mycorrhizal inoculation significantly increased the ash, crude fibre, IVPD and tannin content (Elsheikh and Mohamedzein, 1998).

Nodulation and plant growth of soybean were significantly enhanced by mycorrhization and P fertilization, but the effect was greater in presence of both treatments. Increase in mycorrhizal colonization was associated with a corresponding increase in plant N and P contents (Mahdi *et al*., 2004).

A study was conducted to evaluate the efficiency of *Tricoderma viride*, VA mycorrhiza and dry yeast, separately and in combination as an integrated strategy of Rhizctonia disease management in potato crop. VA mycorrhiza enhanced both the growth and yield of measurements of *Rhizoctonia* inoculated potato plants and significantly reduced the efficacious biocontrol agent to *Rhizoctonia* compared to the other two organisms, yet it also significantly improved the situation of the infected plants (Mohamed *et al*., 2008).

**Non-symbiotic nitrogen fixers:** Different micro-organisms were isolated characterized and identified from six peat based inoculants (Biogen, microbin, Bioplant-K pseudomin, flavobacterin and mobilin) that are world wide used. The results revealed that Biogin inoculant contain *Azotobacter vienlandi*, Bioplant-K contained *Kelbsiella planticola* and Microbin contains four bacterial species *-Azospirillum brasiliense Azotobacter vienlandi, Bacillus megatherium var phosphaticum* and *Pseudomonas aurantiaca*. (Osman *et al*., 2007), mobilin contain *Azomonas, flavobacterin* contain *flavobacterium*, Pseudomin contain *Pseudomons putida* (Osman, 2003).

Suitability of charcoal, groundnut shells, nilesilt filtermud and bagasse as carries for *flavobacterium* and *Azomonas* was studied by Osman and Mohamed Ahmed (2005). The results revealed that the best carrier for *flavobacterium* was Nile silt and for *Azomans* charcoal was the best. Nitrogen fertilization at 60 and 90 kg/ha and the combined inoculation of *Azospirillum brazilense* and *Bacillus polymyxa* +30 kg N/ha significantly increased plant height, yield per plant, number of seeds/plant and seed yield. It is recommended to use the composite inoculum +30 kg N/ha since 60 kg N/ha can be spared off (Mohammed *et al*., 2010).

IAEA funded a programme to increase the productivity of new varities of sugarcane, tomatoes, wheat and banana through introduction of new production packages. Non-symbiotic nitrogen fixing bacteria for banana and tomatoes were isolated by ARC, Medani. The biofertilization department started now the study of some local meterials as carries for these bacteria, in addition to the shelf life of the inoculants for mass production.

**Phosphorous solubilizing bacteria:** Thirty nine local isolates of phoshorus solubilizing bacteria were isolated from different Sudanese soil types. The isolates were characterized and identified as *Bacillus megatherim var phosphaticum*. The efficiency of those isolates under laboratory condition revealed that all of them can solubilize different quantities of $Ca_3 (PO_4)_2$ in 48 h. The maximum solubilizing quantity was 66.6 ppm.

Charcoal, filter mud, Nile silt, Bagass and groundnut shells were used as carries for two phosphorus solubilizing bacteria, *streptomyces albus* and *Bacillus megatherium var phosphaticum*. The results showed that the four materials can be used as carriers. However, Charcoal was found to be the most efficient carrier (Osman and Mohamed, 2006).

**Silicate bacteria:** Biological potassium fertilizer is efficient and non polluting biological fertilizer that is manufactured by advanced production technology under strict quality control. It can activate Mg, Fe. Mo, P etc, releasing balanced nutrients from soil, increase fertility of soil and also facilitates secretion of gibberellins, IAA, cytokinin and other hormones. It can improve the structure of soil, enhance rooting of seeds and enlarge

the shoot and branches. It is widely used in many plants, food crops, economic crop, fruits and vegetables with varied impact on yield between 10-50% over the untreated.

Some microscopical and biochemical characteristics of two isolates of silicate bacteria isolated from peat-based silicate bacteria inoculant were carried out. The results revealed that the inoculants contained two strains of silicate bacteria. These isolates were found to be close to *Bacillus circulans* and *Bacillus mucilaginous* (Osman, 2009).

**Compost production in Sudan:** There are 2 factories which produce compost in Sudan: Elkhaseeb factory with production capacity of 8000 ton/year and Elkhierat factory with production capacity of 6000 ton/year.

**Elkhierat compost production**

**Production rate:** In 2007 the factory started producing small quantities (1 t/month) applied in nurseries and house gardens. In 2008 the factory was established in an area of 5.5 feddans at Elselate scheme and the average production was 5t/month. The average production rate in 2009 reached 150 t/month.

**Elkhaseeb organic fertilizer:** Elkhaseeb organic fertilizer is manufactured locally in El Bagair industrial area. It is a mixture of sheep manure, farmyord manure and chicken in 1:2:1 ratio. It is heated up to 70°C for 30 min and then beletted (2.5, 4 millimeter), it is free from plastic materials, weeds seeds, insects, warms, nematode and pathogenic microorganisms (*shigella*, *salmonella* and *E. coli*). The analysis of this fertilizer proved that, heavy metals, micronutrients, sodium, potassium chlorine, total nitrogen, C.N ratio, EC, pH, bulk density, strange materials were within the range compared to finished compost. However, calcium carbonate, phosphorus lignin, cellulose, hemicellulose and fiber were all over the standard range compared to finished compost. CEC and magnesium were lower than the standard range. The preliminary results of the effect of this fertilizer on growth, yield and quality of onion, tomatoes, potatoes and wheat showed increments in crop yields and qualities in Khartoum state (Osman *et al*., 2009).

**Imported biofertilizers:** Although there are some restrictions to import microorganisms in any formulation, there are some imported biofertilizers that contain (foreign) microorganisms are found in Sudan.

**a) Effective microorganisms (EM):** It is a liquid microbial consortium based on diluted molass. The analyses of the EM were conducted at Biofertilization Department .ENRRI, NCR it contains yeast: *saccharomyces cerevisiae*, bacteria: lactic acid bacteria, photosynthetic bacteria (*Rhodopseudomonas palustris*, and *Rhodobacter sphaeroides*). It is free from pathogenic bacteria, *E. coli*, *shigella* and *salmonella*.

**b) Zander mycohorriza:** It contains six strains of VAM, nitrogen fixing bacteria and other benefical microorganisms in pelleted form with different additives e.g. organic fertilizer, humic acids, cortenoides and Gibberellin. Those strains were isolated from aquatic environment in Eastern Europe. It is packed in 10 kg to fertilize one feddan. The shelf life is 8 month at 50°C. The efficiency is for 15 year. It was manufactured in Arab Emirates licensed by British Company. The above informations are mentioned in Zander Company brochure.

**c) Granular rhizobia inoculant:** It was imported from Australia. It contains $3.9 \times 10^7$ cfu/g. The moisure content was 5%, Ash 73.20%, pH 8.74, total carbon 26.88% P 2.2 ppm and N 0.37&.

The analysis of the inoculant was condcuted by biofertiliztion department, ENRRI, NCR.

**Future research in biofertilizers**

Future Research Areas in biofertilizers in Sudan should include the following:

**Rhizobium inoculation:** Further researches are required to study the response of *Cicer aritenum, Cajanus cajan, Arachis hypogaea and Phaseolus vulgaris* in Gazira scheme and rainfed areas, to Rhizobium inoculation.

**Non-symbiotic nitrogen fixers:** Researches are required to investigate the effect of non-symbiotic N fixing bacteria with different non-leguminous crops, in addition to co-inoculation with Rhizobium, Mycorrhiza, Phosphorus Solubilizing Bacteria (PSB), Mineral fertilizers and Organic fertilizers.

Table 3: Elkhierat compost use in Sudan

| | | | | | Average yield | |
|---|---|---|---|---|---|---|
| Crop | Location | Area | Season | Rate of application | Control plots | Compost fertilized |
| Okra | Selate scheme | 4 fed | 2008/2009 | 1t/fed | 2t/fed | 3t/fed |
| Wheat | Selate scheme | 6 fed | 2008/2009 | 1t/fed | 4 sac/fed | 12 sac/fed |
| Cotton | Gezira scheme | 351 fed | 2009/2010 | ½ t/fed | 4-5 kontar/fed | 15-20 kontar/fed |

**PSB, silicate bacteria and mycorrhiza:** Researches are required to investigate the effects of PSB, silicate bacteria and mycorrhiza on cereals and vegetables growth and yield.

**ElKhaseeb and ElKhierat:** More studies are required to investigate the effects of those organic fertilizers on different crops.

**Cyanobacteria:** Researches are required to study the response of rice grown in White Nile schemes to inoculation with nitrogen fixing cyanobacteria.

**Effect of inoculation:** The effect of inoculation with the above mentioned microorganisms on seed quality of different crops must be studied.

**Suggestions:** To promote adoption of biofertilizer production and use in Sudan the following suggestions should be taken into consideration:

- Support of applied research in the field of biofertilizer through financial support, training of personnel and providing logistics.
- Continuous improvement of skills and capacity building of the agricultural extensionists in the areas of biofertilizer use.
- Strengthen collaboration between the different research institutions, the fedral and state ministries of agriculture.
- Establishment of technical committees for development of production and use of biofertilizers technology.
- Adoption of international quality control standards for production of microbial inoculants.
- Establishment of extension programmes among farmers and agricultural companies for dissemination of biofertilizers.
- Encouragement of bioferilizers commercial production in Sudan.
- Close supervision from the government authorities to the imported biofertilizers is highly needed.

## REFERENCES


Abdelgani, M.E., G.A. ElHassan and A.M. Rugheim, 2003a. Impact of salinity on growth and symbiotic properties of some guar rhizobia. J. Sci. Technol., 4: 7-18.

Abdelgani, M.E., A.G. Osman and S.S. Mohamed, 2003b. Restoring soil fertility of desertified lands through biological nitrogen fixation In: Desertification in the Third Millennium (eds. A.S. Sharhan, W.W. Wood, A.S. Goudie, A. Fowler and E.M. Abdellatif). A.A. Balkima Publishers. Lisse, the Netherlands, pp: 335-338.

Abdelrahim, Y.M., M.E. Abdelgani and E.E. Abdelrahman, 2005. Testing the suitability of locally available materials as carriers for rhizobia. Gezira. J. Agric. Sci., 3: 123-136.

Abuswar, A.O. and A.S. Mohamed, 1997a. Effect of phosphorus application and Rhizobium inoculation on two cultivars of alfalfa plant density and seed ptoduction. U.K. J. Agric. Sci., 5: 1-11.

Abuswar, A.O. and A.S. Mohamed, 1997b. Effect of phosphorus application and Rhizobium inoculation on two cultivars of alfalfa II Growth and nodulation. U.K. J. Agric. Sci., 5: 12-23.

Adlan, M.A.M. and N.O. Mukhtar, 2004. Quantifying $N_2$ fixed by groundnut (*Arachis Is hypogaea* L.) as comared to some sumer legumes using $N^{15}$ methodology with different reference crops. U.K. J. Agric. Sci., 12: 357-369.

Ahmed, S.H., M.E. Abdelgani and A.M. Yassin, 2009. Effects of interaction between vesicular-arbuscular mycorrhizal (VAM) fungi and root-knot nematodes on dolichos bean (Lablab niger Medik.) plants. American-Eurasian J. Sustainable Agriculture (In press).

Ahmed, A.E., M.A.M. Adlan and N.O. Hukhtar, 2005. Effect of plant growth promoting bacterium (*Azospirillum brasilense*) and *Bradyrhizobium* on growth, yield and nitrogen fixation of groundnut (*Arachis hypogaea* L.). Gezira. J. Agric. Sci., 3: 137-147.

Ahmed, A.E. and E.A.E. Elsheikh, 1998. Effects of biological and chemical fertilizers on growth and symbiotic properties of faba bean (*Vicia faba* L.) under salt stress. U.K. J. Agric. Sci., 6: 150-166.

Ahmed, F.E., S.O. Yagoub and E.A.E. Elsheikh, 2000. Effects of mycorrhizal inoculation on the nodulation, mycorrhizal infection and yield components of faba bean grown ynder two different watering regimes. U.K. J. Agric. Sci., 8: 107-117.

Ali, E.H.S., 2010. Eco-physiological studies on cyanobacteria in Sudan. M.Sc. Thesis. Faculty of Agriculture. University of Khartoum.

Ali, E.H., 2003. Effect of Rhizobium inoculation on the yield of groundnut (*Arachis hypogaea* L.) in the sandy soils of the Kordofan region western Sudan. Arab gulf J. Scientific Res., 21: 232-236.

Atabani, E.M., 1988. Interaction between *Bradyrhizobium*-inoculated hyacinth bean and soybean and endo mycorrhizal fungi. M.Sc. Thesis, University of Khartoum.

Babiker, E.E., E.A.E. Elsheikh, A.G. Osman and A.H. Eltinay, 1995. Effect of nitrogen fixation, nitrogen fertilization and viral infection on yield, tannin and protein contents and in vitro protein digestibility of faba bean. Plant Foods Human Nutr., 47: 257-263.

ElAtta, H.A. and U.E. Osman, 1993. Rhizobial cross inoculation groups of *Faidherbia albida* and *Acacia* spp. U.K. J. Agric. Sci., 1: 127-136.

Elsharif, E.A. and M.E. Abdelgani, 2007. Assessing the effeciency of filter mud as a carrier for Rhizobium inoculants. Albuhuth, 11: 77-87.

Elsheikh, E.A.E., 2001. Effect of inoculation with Rhizobium on the seed chemical and physical properties of legumes. Aspects Applied Biol., 63: 151-163.

Elsheikh, E.A.E., 1998a. A note on the effect of fertilization on the seed quality of faba bean. U.K. J. Agric. Sci., 6: 167-172.

Elsheikh, E.A.E., 1998b. Response of legume-Rhizobium symbiosis to salinity in the Sudan: A Review. U.K. J. Agric. Sci., 6: 142-156.

Elsheikh, E.A.E. and E.I.A. Ahmed, 2000. A note on the effect of intercropping and Rhizobium inoculation on the seed quality of faba bean (Vicia faba L.). U.K. J. Agric. Sci., 8: 157-163.

Elsheikh, E.A.E., N.N.B. Elnesairy and A.A. Mahdi, 2006. Effect of sinorhizobium inoculation and chicken manure on nodulation and forage yield of alfalfa (*Medicago sativa* L.) under irrigation in a Semi-arid environment. U.K. J. Agric. Sci., 14: 182-197.

Elsheikh, E.A.E. and H.A.S. Forawi, 1995. Response of fenugreek to inoculation as influenced by salinity, soil texture, chicken manure and nitrogen. U.K. J. Agric. Sci., 3: 77-90.

Elsheikh, E.A.E. and E.M.M. Mohamedzein, 1998. Effect of Bradyrhizobium, VA mycorrhiza and fertilizers on seed composition of groundnut. Ann. Applied Biol., 132: 325-330.

Elsheikh, E.A.E., S.S. Mohamed, A.A. Elhussein and E.E. Babiker, 2009. Effects of intercropping, *Bradyrhizobium* inoculation and chicken manure fertilization on the chemical composition and physical characteristics of soybean seed. Food Chem., 112: 690-694.

Elsheikh, E.A.E. and A.G. Osman, 1995. *Rhizobium leguminosarum* inoculation decreases damage to faba bean (*Vicia faba* L.) caused by broad bean mottle bromovirus and bean yellow mosaic potyvirus. World J. Microbiol. Biotechnol., 11: 223-227.

Elballa, M.M.A., A.H.B. Elamin, E.A. Elamin and E.A.E. Elsheikh, 2004. Interactive effects of cultivars foliar application of microelements and *Rhizobium* inoculatiom on snap Bean (*Phaseolus vulgaris* L.) performance. U.K. J. Agric. Sci., 12: 321-332.

Hassan, M.A. and M.E. Abdelgani, 2009. Effect of microbial biofertilization on nodulation, nitrogen and phosphorus content and forage yield of lablab bean (*Lablab purpureus* L.). American-Eurasian J. Sustainable Agriculture (In press).

Hassan, A.E., M.D. Dennett and T.M. Hago, 2006a. Effects of molybdenum and zinc application and Bradyrhizobium inoculation on growth and yield of groundnut (*Arachis hypogaea* L.) 1. nodulation, leaf nutrient content and biomass production. U.K. J. Agric. Sci., 14: 32-49.

Hassan, A.E., M.D. Dennett and T.M. Hago, 2006b. Effects of Molybdenum and zinc application and *Bradyrhizobium* inoculation on growth and yield of groundnut (*Arachis Is hypogaea* L.) 11. Pod yield and its coponents. U.K. J. Agric. Sci., 14: 50-63.

Galal, I.M., 1993. Effects of inoculation with va-mycorrhizal fungi on nodulation and dry matter yield of *Bradyrhizobium* inoculated cowpea. M.Sc. Thesis. University of Khartoum.

Ibrahim, K.A., E.A.E. Elsheikh and E.E. Babiker, 2008a. Minerals composition of Hyacinth Bean (*Dolichos hyacinth* L.) seed as influenced by Bradyrhizobium inoculation and/or chicken manure or sulphur fertilization. Pak. J. Nutr., 7: 785-792.

Ibrahim, K.A., E.A.E. Elsheikh and E.E. Babiker, 2008b. Bradyrhizobium inoculation and chicken manure or sulphur fertilization of Hyacinth Bean (*Dolichos hyacinth* L.) changes in physical characteristics and chemical composition of the seeds. Austr. J. Basic Applied Sci., 2: 605-616.

Ibrahim, A.S., E.K. Osman and M.A.M. Khair, 1996. Effect of phosphorus fertilizer on growth and forage yield of *Clitoria ternatae, Lablab purpureus and Vigna trilobata*. U.K. J. Agric. Sci., 4: 18-30.

Mahdi, A.A., 1993. Biofertilizer research in Sudan a review. U.K. J. Agric. Sci., 1: 137-153.

Mahdi, A.A. and E.M.A. Mustafa, 2005. Response of guar to Bradyrhizobium inoculation and to nitrogen and phosphorus fertilization. U.K. J. Agric. Sci., 13: 97-110.

Mahdi, A.A., A.B. Nur Eldaim and A.A. Arbab, 2004. Influence of mycorrhizal inoculation form of phosphate fertilization and watering regime on nodulation and yield of soybean. U.K. J. Agric. Sci., 12: 345-356.

Mohamed, A.S., S.M. Elhassan, M.M.A. Elballa and E.A.E. Elsheikh, 2008. The role of Trichoderma, VA mycorrhiza and dry yeast in the control of Rhizoctonia disease of potato (*Solanum tuberosum* L.). U.K. J. Agric. Sci., 16: 284-300.

Mohamed Ahmed, T.H., E.A.E. Elsheikh and A.A. Mahadi, 2009. The *in vitro* compatibility of some *Rhizobium* and *Bradyrhizobium* strains with fungicides. Afr. Crop Sci. Conf. Proc., B: 1171-1178.

Mohamed, S.S. and A.G. Osman, 1996. The Biofertilizer use of *Rhizobium* strain TAL380 for increasing Alfalfa production in Khartoum State. Al Buhuth J., 5: 115-125.

Mohammed, S.S., A.G. Osman, A.S. Abdalla and A.M. Rugheim, 2010. Response of wheat to biological and nitrogen fertilization. U.K. J. Agric. Sci., 18: 31-42.

Osman, A.G., 2009. Study of some characteristics of silicate bacteria. J. Sci. Technol., 10: 24-31.

Osman, A.G., 2003. Isolation and partial characterization of some non-symbiotic nitrogen fixing bacteria isolated from peat inoculants. J. Sci. Technol., 4: 106-113.

Osman, A.G. and M.E. Abdelgani, 2005. Effects of the fungicide Bayleton on different nitrogen-fixing bacteria. Shendi Univ. J., 2: 121-134.

Osman, A.G., H.A. Abdalla, A.S. Abdalla, S.A. Saad and M.A. Hassan, 2007. Characteristics of some bacterial species isolated from peat based inoculants. J. Sci. Technol., 7: 1-17.

Osman, U.E. and H.A. ElAtt, 1995. The impact of some factors on nodulation of *Acacia* spp. U.K. J. Agric. Sci., 3: 98-107.

Osman, A.G. and T.H. Mohamed Ahmed, 2005. Suitability of local materials as carriers for non-symbiotic nitrogen fixing bacteria. Shendi Univ. J., 4: 105-120.

Osman, A.G. and S.S. Mohamed, 1996. Effect of inoculation with *Rhizobium* strain TAL 1400 on the symbiotic properties of faba bean in Khartoum State. AlBuhuth J., 5: 126-139.

Osman, A.G. and S.S. Mohamed, 2006. Testing the suitability of some materials as carriers for phosphorus solubilizing bacteria. J. Sci. Technol., 7: 52-61.

Osman, A.G. and E.A.E. Elsheikh, 1994. Possibility of controlling bean yellow mosaic virus on faba bean (*Vicia faba* L.) by *Rhizobium* inoculation. U.K. J. Agric. Sci., 2: 93-107.

Osman, A.G., N.A. Hag Elamin and E.A.E. Elsheikh, 1996. Effects of *Rhizobium* inoculation and N-fertilization on the productivity of two faba bean cultivars. AlBuhuth J., 5: 127-137.

Osman, A.G., S.A. Saad, A.A. Satti, B.M. ElAmin, M.H. Elnasikh, M. Abdelrahman, N.A. Gaili and I.A. Elsheikh, 2009. Evaluation of the production of Elkhaseeb organic fertilizer. Environment and Natural Resources Research Institute. Annual Scientific Report, pp: 37-50.

Rugheim, A.M. and M.E. Abdelgani, 2009. Effects of microbial and chemical fertilization on yield and seed quality of faba bean. 9th Conference of the African Crop Science Society: Science and Technology Supporting Food Security in Africa. Cape Town, South Africa 28 September-1 October 2009.