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Review

The role of nutraceuticals, functional foods and value added food products in the prevention and treatment of chronic diseases

Olaiya C. O.^{1*}, Soetan K. O.² and Esan A. M.¹

¹Nutritional and Industrial Biochemistry Research Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria.

²Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Ibadan, Nigeria.

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The interest in phytochemicals found in plant foods as bioactive components of the diet has expanded in the last few years. This is because they have been linked with the prevention or reduced progression of many chronic diseases, such as cardiovascular disease, cancer and degenerative diseases. Oxidative stress, which could bring about oxidative damage to DNA, protein and lipids has been found to be a major factor in the aetiology of these diseases. Epidemiological evidence shows that observed health benefits of these plant foods on humans, especially fruits and vegetables, are due to the presence of bioactive phytochemicals which today, play an important role in pharmaceutical applications. Research shows that these compounds modulate the risk of chronic disease development by inhibiting reactions mediated by reactive oxygen species (ROS). Consequently, strong recommendations for their ingestion through the diet have become increasingly popular to improve man's health. This article presents a review of the role of nutraceuticals, functional foods and value added food products in the prevention and treatment of chronic diseases. We also summarized the biotechnological approaches for enhancing the level of these bioactive compounds in plants, with a view to improve their nutraceutical value and phytotherapy efficiency.

Key words: Antioxidants, degenerative diseases, dietary reference intakes, disease prevention, nutraceuticals.

INTRODUCTION

Chronic diseases are generally non-communicable diseases (NCDs) of long duration characterized by slow development or progression. These diseases can be prevented and controlled, but not cured. They are

sometimes termed degenerative diseases because the structure or function of the affected tissues or organs progressively deteriorates overtime, whether due to normal bodily wear or lifestyle choices such as exercise

*Corresponding author. E-mail: coolaiya@yahoo.com.

or eating habits. They often accompany aging, a process which is synonymous with mitochondrial decay and are the consequence of poor nutrition, stress and toxic overload over a long period of time. These diseases include cancer, cataracts, immune system decline, cardiovascular disease, brain dysfunction and atherosclerosis. CVDs, especially atherosclerotic CHD and stroke, are the leading causes of disability and death in developed countries (AHA, 2007) and are projected to be the leading cause of death in the developing world by 2020 (WHO, 2005). Arterial atherosclerosis which primarily affects target organs of the heart, kidney and brain, first develops, until a major cardiovascular catastrophe occurs, leading to morbidity and sudden death. The emphasis so far has been on the functional degeneration of somatic cells during aging, but recently, oxidative damage to DNA lipid and protein has been implicated in the aetiology of these diseases. The level of oxidative DNA damage seems to be roughly related to metabolic rate in a number of mammalian species (Ames, 2001). In fact, one human cell is believed to be exposed to about 105 oxidative hits a day from hydroxyl radical and other such species of oxidants (Lopaczynski and Zeisel, 2001). ROS are normal oxidant by-products of aerobic metabolism, and under normal metabolic conditions about 2 to 5% of oxygen consumed by mitochondria is converted to ROS (Anagnostopoulou et al., 2006). The resulting oxidative stress then permanently modifies the genetic material leading to numerous degenerative or chronic diseases, such as atherosclerosis and cancer (Shanmuganayagam et al., 2007).

More than half of the human population worldwide has no access to a healthy variety of fresh food (Christou and Twyman, 2004), resulting into malnutrition which has become a significant public health issue in most nations, especially the developing world. In malnutrition, increased oxidative stress coupled with chronic inflammation often brings about an increased risk of atherosclerosis (Vinson et al., 2001). Hence, the acronyms MICS (malnutrition inflammation complex syndrome) or MIA (malnutrition inflammation and atherosclerosis syndromes) are used to indicate the combination of these two conditions in patients (Pecoits-Filho, 2002). Very recently, there is a shift towards the optimal nutrition diet because of the growing health issues and there has been an increasing awareness of the populace in most nations of the world about the interplay between nutrition and health. Much scientific evidence indicates that certain bioactive compounds in foods have various disease-fighting properties, which has made consumers worldwide become much more interested in the health benefits of foods. Consequently, dietary reference intakes (DRI) and nutrition recommendations are targeted at disease prevention (EFSA, 2010). Healthy eating guidelines have directed the general public to eat more fresh fruits, vegetables, low fat and high fiber foods throughout the world (WHO,

2003). Food manufacturers therefore tend to produce foods and food products that can satisfy consumer appetite and cravings for health promotion. Such food or food products often carry health claims portraying this attribute and include nutraceuticals, functional foods, value added food products and whole plant foods. The consumption of these foods brings about a reduction in health care costs and supports economic development, especially in rural communities (El Sohalmy, 2012). The health claims on food labels are governed by specific regulations in some countries. For instance, the Food and Drug Administration (FDA) oversees and allows such claims in the United States. We review the role of these foods in the prevention and treatment of chronic diseases. Also, we try to summarize the biotechnological approaches being employed to increase the level of bioactive compounds in food crops, so as to enhance their nutraceutical and functional values, which are important factors in nutritional therapy.

THE BURDEN OF CHRONIC DISEASES AND POSSIBLE INTERVENTION OF NUTRACEUTICALS, FUNCTIONAL FOODS AND VALUE ADDED FOOD PRODUCTS

Most countries in the world are presently going through a nutritional transition and are affected by double burden of nutritional problems. Under nutrition and specific nutrient deficiencies, especially micronutrient deficiency remain unabated, and simultaneously imbalanced diets and chronic diseases are becoming burdensome and the trend is rapidly increasing globally (Kotilainen et al., 2006). In 2001, chronic diseases were reported to contribute about 46% of the world burden of disease and this has been predicted to increase to 57% by 2020 (WHO, 2002). Most of these diseases now appear earlier in life and affect both the affluent and the poor in developing and developed nations of the world. Chronic diseases are largely preventable diseases. As a result, the promotion of healthy diets, nutrition and lifestyles to reduce the global burden of these diseases is continually being advocated, and a possible way out is the copious consumption of nutraceuticals, functional foods and value added food products, due to their ready availability and diseases preventing characteristics.

Nutraceuticals, which have gained worldwide popularity, have been defined as purified or concentrated food and food products with health-promoting and/or disease-preventing properties beyond their basic nutritional function (Gunther et al., 2004). When consumed, they enhance health and quality of life for people of all ages. Nutraceuticals range from isolated nutrients, dietary supplements, and diets, to naturally occurring substances such as herbals, vitamins, amino acids or their formulations, and processed products such as cereals, soups, and beverages (Kharb and Singh, 2004).

Table 1. FOSHU foods and Ingredients (El Sohalmy, 2012).

Carbohydrates	Proteins
Polydextrose	Casein phosphopeptide
Indigestible dextrin	Casein dodecapeptide
Galacto oligosaccharides	Soy protein
Lactulose	Minerals
Lactosucrose	Phosphorus
Isomalto oligosaccharides	Calcium as citrate malate
Maltitol	Heme iron
Palatinose	Others
Soybean oligosaccharides	Rice globulin
Fructo oligosaccharides	Eucommia leaf glycoside
Xylo oligosaccharides	Lactobacillus GG
Wheat bran	

Functional foods have been considered as foods made up of nutrients that confer physiological or medical benefit to the consumers. In fact, any healthy food confirmed to have a health promoting or disease preventing property beyond the basic functions of supplying nutrition is now regarded as a functional food (Martirosyan and Singh, 2015). Nonetheless, it is important to note that there is no universally accepted regulatory definition of the term 'functional foods' to date, despite their wide usage in marketing, and they represent one of the fastest growing segments of the food industry in most nations of the world. Therefore, in addition to providing new options for improving health and well-being, the functional foods sector offers potential for new economic opportunities. These foods often carry a special seal and have been recognized as Foods for Specified Health Use (FOSHU) in Japan (ILSI, 1999). The list of foods or ingredients that qualify for FOSHU status is shown in Table 1.

Value added food products are foods whose nutrients, shape, size or appearance has been scientifically modified from the post-harvest stage of production to improve functionality, and in some cases, reduce consumer preparation time (Feldeisen and Tucker, 2007). They are sometimes termed 'designer foods' and the enhanced phytonutrients usually make them more beneficial health wise.

Whole plant foods are foods obtained solely from plants. Thus, the plant-based diet is made up of fruits, vegetables, tubers, whole grains and legumes with the exclusion or minimal intake of meat, fish, eggs and dairy products (Hu, 2003). It also does not include processed foods or sweets.

Nutraceuticals, functional foods and value added food products are therefore rich repositories of health promoting bioactive compounds. Scientific research show that a large proportion of these compounds often act as antioxidants, and have a role *in-vivo* in modulating disease development by inhibiting ROS mediated reactions, which have been associated with the initiation

and progression of a number of pathological processes (Bajpai et al., 2005). It has been suggested that the protective properties of some of these compounds may be ascribed to a novel mechanism unrelated to their conventional free-radical scavenging abilities (Cooke et al., 2002), and the susceptibility of body tissues to oxidative damage is a reflection of the balance between the extent of pro-oxidant stress and antioxidant levels (Capecka et al., 2005).

Also, from the point of view of food safety, there is growing interest in the practice of using natural antioxidants, especially from herbs and spices, as additives in the food industry. This is not unconnected with their ability to prevent lipid peroxidation (in fat - rich food products), thereby ensuring food stability and prolonged storage time (Olzcan, 2003).

The major bioactive compounds in food crops have been classified on the basis of their activities. Examples include: Carotenoids (α - and β - carotene, β - cryptoxanthin, lutein, lycopene, and zeaxanthin); Phenols (flavonoids); Cyclic phenolics (chlorogenic acid, ellagic acid, and coumarins); Glucosinolates (sulforaphane, indole-3 carbinol); Saponins; Phytosterols (campesterol, β - sitosterol, and stigmaterol); Sulfides and thiols; Phytoestrogens (isoflavones, daidzenin, genistein, and lignans)(Pradeep and Mallikarjuna, 2012). The carotenoids and flavonoids have been especially implicated in the prevention, control and management of chronic diseases.

Carotenoids comprise an extended group of natural pigments numbering about 600 of which β - carotene is the most prominent. Of this amount, about 50 are consumed in appreciable quantities in human diets (Rimm and Stampfer, 2000). They are present in vegetables, fruits, fungi, algae and bacteria, and are traditionally classified into two large structural groups: the carotenes, which are basically hydrocarbons $C_{40}H_{56}$ (α - and β - carotene and lycopene), and the xanthophylls, $C_{40}H_{56}(OH)_2$, which include different oxygenated functions in the molecule (lutein, zeaxanthin, β -cryptoxanthin and canthaxanthin). The structures of some prominent carotenoids in human plasma are shown in Figure 1. Carotenoids are responsible for the colour of foods and some occur only in specific plants e.g. lycopene in tomato, pink guava, pink grapefruit, papaya and water melon.

Many studies have shown that individuals with higher dietary intakes of carotenoids have a reduced risk of several chronic diseases (Prakash et al., 2004). The carotenes are generally tissue specific in their biological activity and the xanthophylls serve to protect other antioxidants. The mechanism by which carotenoids protect cells against ROS mediated damage depends largely on physical quenching, a process in which the energy of the excited oxygen is transferred to the carotenoid molecule (Elliott, 2005). In this regard, lycopene has been reported as the most efficient singlet

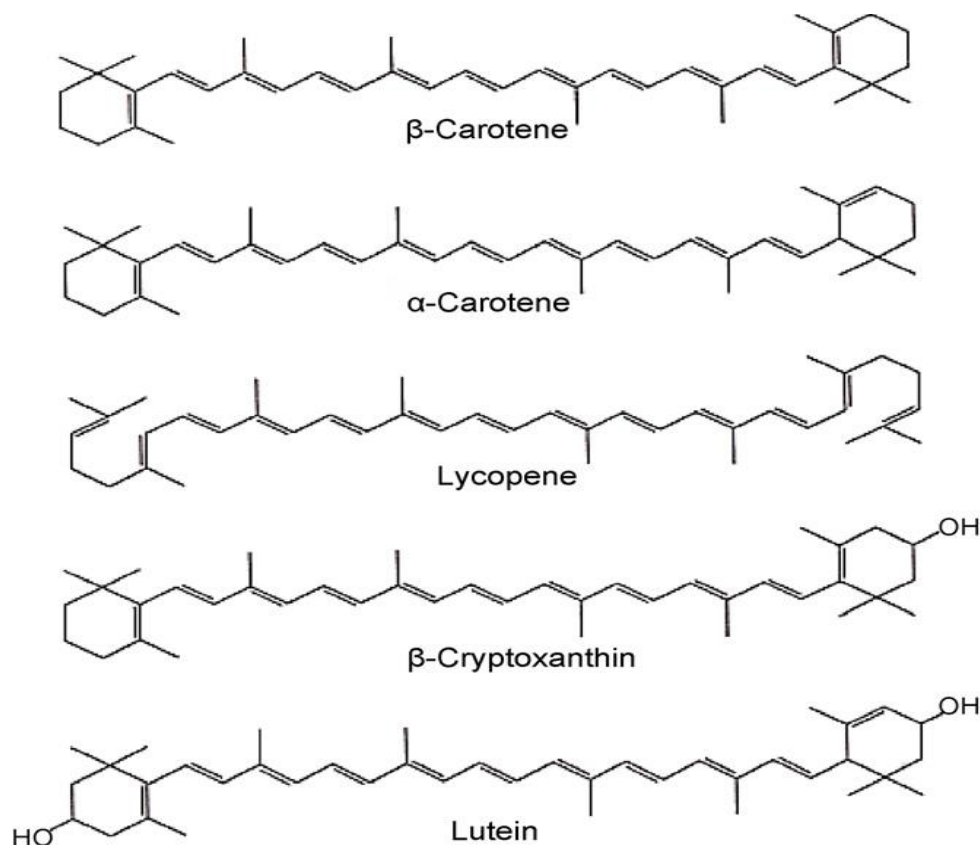


Figure 1. Chemical structures of some common carotenoids.

oxygen quencher among the naturally occurring carotenoids (Prakash and Gupta, 2009).

The major active nutraceutical ingredients in plants are flavonoids. Flavonoids are a family of low molecular weight polyphenolic compounds present in cereals, vegetables, fruits and drinks of plant origin, such as red wine, tea, cocoa and coffee. The compounds are derived from parent compounds known as flavans (Markovic, 2007). About 4000 flavonoids are known and these are classified into six major groups namely flavones, isoflavones, flavanones, flavonols, flavanols (flavan-3-ols) and flavanonols. They are potent antioxidants or free radical scavengers that offer protection against cardiovascular disease by reducing the oxidation of low density lipoproteins (Johnson, 2003). It has been reported that flavonoids may exert local anticarcinogenic effects by acting as intraluminal antioxidants in the intestine (Ren et al., 2003). They may also exert cardioprotective action by preventing or retarding damaging oxidative reactions in cells which is a predisposing factor for the development of CVDs (Di Santo et al., 2003). In particular, studies have shown that Mediterranean diets rich in resveratrol can lower the risk for the development and progression of cardiovascular disease in humans. A few of these compounds are shown in Figure 2.

HEALTH BENEFITS

Epidemiological evidence shows that regular consumption of functional foods, value added food products and nutraceuticals is associated with a lowered risk of coronary heart disease, obesity, diabetes, cancer, osteoporosis and other chronic age-related degenerative diseases like Parkinson's and Alzheimer's diseases (Prakash and Kumar, 2011). Berry fruits and blackcurrants, for example, are employed in folk medicine for the prevention and treatment of circulatory disorders and inflammatory diseases. These foods are known to play important roles in modulating oxidative stress in disease states. Their observed health benefits has been ascribed to the presence of bioactive compounds (Priya and Santhiya, 2011) which accumulate in plasma and tissues of consumers in relation to dietary intakes and play a role in inhibiting reactions mediated by reactive oxygen species (Ames et al., 1993; Etminan et al., 2004). These compounds, either alone, or synergistically, have much therapeutic potential and exert varying biological and pharmacological effects in human health as anticarcinogenic, anti-inflammatory, anti-diabetic, antioxidants, antifungal, antipyretic, anti-apoptotic, chemopreventive, hepato-protective, hypolipidemic, analgesic, CNS stimulant and stimulation of cellular immunity

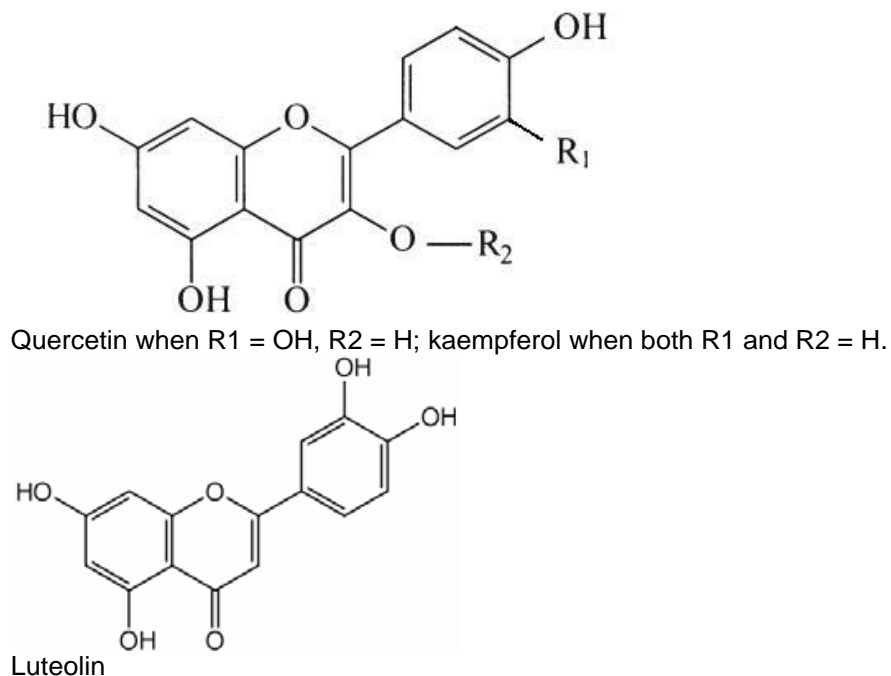


Figure 2. Chemical structures of some flavonoids.

(Hakiman and Maziah, 2009; Prakash et al., 2012). For instance, tocopherol is useful as an antioxidant therapeutic agent in attenuating the progression of heart attack (Azzi et al., 2003) and heart failure (Ghatak et al., 1996). The vitamin prevents CVDs by reducing platelet adhesion, elevating HDL level in the blood and inhibiting smooth muscle cell proliferation, a predisposing factor in atherosclerosis (Lee et al., 2005). Because they are readily available, cheaper and safer, nutraceuticals, functional foods and value added food products are becoming widely acceptable as alternatives to conventional drugs and pharmaceuticals (Chatterjee et al., 2013). Some of the pharmacological effects are briefly discussed.

Anticarcinogenic activity

Metabolic syndrome, also known as insulin resistance syndrome, has been identified as an important contributing factor to the progression of some cancers (Singh et al., 2009). Much evidence has been provided to show that a diet rich in fruits and vegetables correlates positively with reduced risk of development of cancer and chronic age-related degenerative diseases (Aberoumand and Deokule, 2010). For instance, vegetable sources like broccoli, cabbage, cauliflower and brussel sprouts with a rich repository of glucosinolates are known to exert a substantial protective support against colon cancer (Doughari, 2012).

Foods and herbs known to have high anticancer activity include ginger, garlic, cabbage, soybeans, fenugreek,

green tea, flaxseed and the umbellifous vegetables (Sakr et al., 2012). Researchers have demonstrated that blueberry anthocyanins, proanthocyanidins, resveratrol, flavonols, and tannins inhibit mechanisms of cancer cell development and inflammation *in vitro* (Arts and Hollman, 2005). Fenugreek, an aromatic herbaceous annual, has also been shown to be cytotoxic against several cancer cell lines inducing apoptosis and cell death (Shabbeer et al., 2009) while lycopene, PUFAs, Co Q10, melatonin and CLA are useful for the preventive treatment of leukaemia, lymphoma, prostate, breast, lung and colon cancers. *In-vivo* studies have shown that hesperidin, a flavonoid glycoside in orange (*Citrus sinensis* L.), and diosmin, its flavone analogue possess anticarcinogenic activity (Tanaka et al., 1997).

Antidiabetic and gastroprotective effects

The herb and spice crop, fenugreek exhibits insulinotropic and antidiabetic properties (Al-Habori and Raman, 2002) which have been associated with the amino acid, 4-hydroxyisoleucine that occur in this leguminous crop at a concentration of about 0.55%. *In vitro* studies have demonstrated that this amino acid directly stimulates pancreatic β -cells, decreases insulin resistance in muscles and liver by activating insulin receptor substrate-associated phosphoinositide 3-kinase activity. It therefore affects glucose and lipid metabolism and finds use in the control of type- II diabetes, obesity and dyslipidemia (Jette et al., 2009). The effect of aqueous or soluble gel fraction of fenugreek seeds has also been studied on

ethanol-induced gastric ulcer. The seed extract showed significant ulcer protective effect (Tadigoppula et al., 2006), showing that the soluble gel fraction was more effective than Omeprazole, a standard drug in preventing lesion formation.

Flavonoids, especially the polyphenol, quercetin (which is largely present in white wines), has also been reported to possess antidiabetic activity. Vessal et al. (2003) reported that quercetin brings about the regeneration of pancreatic islets and probably increases insulin release in streptozotocin-induced diabetic rats.

Prevention of LDL oxidation

The progression of atherosclerotic CHD is characterized by oxidative modification of LDL-C (Fillipe et al., 2004). Reducing the risk of CHD therefore entails the inhibition of LDL-C oxidation. Nutraceuticals that are beneficial in the prevention and reduction of symptoms of CHD include GSPE, lycopene, black and green tea and their flavonoids, DHA, EPA, flax lignans, soy protein and isoflavones, coenzyme Q10, pycogenol, DHEA, melatonin, carnitine, resveratrol and lutein (Kharb and Singh, 2004). Specifically, the role of lycopene in the prevention of CVDs has been extensively discussed (Rissanen, 2006). The level of oxidized LDL-C was significantly reduced in subjects ingesting tomato juice, tomato sauce and lycopene oleoresin capsules as sources of lycopene (Agarwal and Rao, 1998).

Anti-apoptotic activity

Apoptosis, also known as 'programmed cell death' is a series of regulated biochemical events (suicide mechanism) which the cell uses to kill itself. The process takes place under physiological and pathological conditions. Inappropriate apoptosis is often fatal, resulting into disorders like neurodegenerative diseases, myocardial infarction and atherosclerosis in most cases (Kumar and Jugdutt, 2003). Mounting evidence shows that flavonoids inhibit apoptosis in the myocardium (Ishikawa and Kitamura, 2000; Lopez-Lopez et al., 2004) but the mechanism of this effect is not yet fully understood. Some of the proposed mechanisms include abolition of digestion of caspase -3 substrates, free radical scavenging activity, decrease in both oxidative stress and the pro-apoptotic factors C-JUN and JNK.

Cardioprotective and hepatoprotective effects

One group of bioactive compounds, the phytosterols, found naturally in many plant foods with vegetable oils (particularly unrefined oils), nuts, seeds and grains as the major dietary sources (Piironen et al., 2000) has been

studied for their biochemical roles in chronic diseases. Their cholesterol lowering ability makes them relevant in reducing the risk of incidence of cardiovascular diseases (Xiaobo et al., 2009). The nutraceuticals β -sitosterol (BSS), β -sitosterol glucoside (BSSG) from *Vernonia amygdalina* Del. (bitter leaf) and their mixture (BSS:BSSG) has been shown to possess cardioprotective and hepatoprotective properties, with possible practical application in the management of CVDs (Olaiya et al., 2013a). Reports also indicate that fenugreek, the nutraceutical food plant has hepatoprotective effect. The polyphenolic seed extract of the plant acts as a protective agent against ethanol - induced abnormalities in the liver, demonstrating similar effects as that of silymarin, a known hepatoprotective agent (Thirunavukkarasu et al., 2003).

MOLECULAR MECHANISM OF ACTION

Disease is the end result of a series of defects in the biochemical machinery of the body. For instance, biochemical and biophysical abnormalities of cell membranes have been implicated in the pathogenesis of hypertension and CVDs. The abnormalities seem to be involved not only in vascular smooth muscle cells but also in circulating blood cells. (Elisaf, 2001).

The mechanism through which nutraceuticals prevent chronic diseases has not been fully elucidated. Many of these phytochemicals have been found to alter antioxidant defence of cells through the induction of DNA repair processes or enzymic antioxidant defence at the gene level. It has been suggested that they may also affect cell cycle progression (Hwang and Bowen, 2004), cell communication (Stahl et al., 2000), hormone and growth factor signaling (Zhang et al., 2002) and apoptosis (Zhang et al., 2003). Lycopene, a potent hypocholesterolemic agent found in tomato has been shown to prevent atherosclerosis by inhibiting LDL-C oxidation (Rissanen et al., 2002). Also, indole-3-carbinol, the most vital and important indole present in broccoli has been found to inhibit the Human Papilloma Virus (HPV) that may cause uterine cancer. This phytochemical acts by blocking the estrogen receptors specifically present in the breast cancer cells as well as down regulating CDK6, and up regulating p21 and p27 in prostate cancer cells. It brings about G1 cell-cycle arrest and apoptosis of breast and prostate cancer cells significantly and enhances the p 53 expression in cells treated with benzopyrene (Dreij et al., 2010). It also depresses Akt, NF-kappaB, MAPK, and Bel-2 signaling pathways to an appreciably good extent (Doughari, 2012).

Many research studies have shown that soy or soy phytoestrogens can inhibit the growth of some cancer cell lines. They are believed to exert actions on a range of biochemical pathways and molecular mechanisms implicated in cell growth, development and survival (Fritz

et al., 2003). This is relevant in the prevention and control of clinically important cancer.

ENGINEERING PLANTS FOR IMPROVED BIOACTIVE COMPOUNDS PRODUCTION

The prime reason for altering or elevating phytochemicals in plants is associated with their potential benefit to human health. There have been many recent assessments of technologies that abound or that can be developed for increasing plant resources for human consumption. Notably among these is the potential of biotechnology and genetic modification techniques which are being harnessed by the modern man for the production and development of functional foods, and improvement in the level and activity of bioactive compounds in many food plants (Niba, 2003). The technologies include strategies such as biosynthesis pathways engineering, usage of elicitors, large scale cultivation in bioreactor system, root culture, plant cell immobilization, high yielding cell line screening and biotransformation (Peterhansel et al., 2008). Selective breeding and metabolic engineering are also being harnessed by researchers for the development of food crops with improved nutraceutical value such as high vitamin A rice, high-iron rice, improved protein and fatty acid profiles in oil seed crops, legumes and soybeans (Niba, 2003). Currently, there is a shift from genotypes engineered to enhance single nutritional compounds to genotypes with enhanced multiple nutrients. This entails the use of bioregulators, multigene engineering or regulative genetic element with pleiotropic effects, so as to harness the synergistic interactions of these phytochemicals in food crops for their biological activities. It has been suggested that the response of a factor transferred by gene manipulation may be augmented by the use of plant bioregulators, which might act as powerful tools in enhancing the growth, productivity, quality and combating the ill effects generated by various biotic and abiotic stresses in functional food crops in the not too distant future (Olaiya et al., 2013b).

CONCLUSION

Plants provide directly, or indirectly, about 95% of the world's food supply and plant - based foods with their rich repository of phytochemicals have great beneficial effects on human health (Amrit, 2006). There is increased craving for healthier lifestyle by the populace, and this is only achievable by consuming diets rich in health - promoting compounds. Functional foods, nutraceuticals and value added food products are becoming more popular and readily available in many nations, with a potential large market in a few years to come. The fact that their consumption has been associated with healthy aging, prevention and treatment of chronic diseases

makes them more desirable. However, some food safety issues and concerns have been raised about their use, particularly the herbals (FDA, 2002). This call for effective regulations and legislation to ensure that the health claims is based on sufficiently strong scientific evidence. Efforts should be intensified to communicate their health benefits to the populace so that they can have sufficient information to make wise choices about the foods they eat and enjoy. Research into techniques of enhancing the levels of disease-fighting phytochemicals in food crops should also be sustained. Additionally, the new field of nutrigenomics, which is the application of the sciences of genomics, proteomics, transcriptomics and metabolomics to human nutrition (Kaput and Rodriguez, 2004), may allow the use of information on an individual's genetic make - up for the prediction of personalised nutraceutical and functional food supplementation to prevent or control chronic diseases in the not too distant future.

Conflict of Interest

The authors declare no conflict of interest.

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Abbreviations

BSS, β -sitosterol; **BSSG**, β -sitosterolglucoside; **CHD**, Coronary Heart Disease; **CLA**, conjugated linoleic acid; **CNS**, central nervous system; **Co Q10**, Coenzyme Q10; **CVDs**, cardiovascular diseases; **DHA**, docosahexaenoic acid; **EFSA**, European Food Safety Authority; **DHEA**, dehydroepiandrosterone; **DNA**, deoxyribonucleic acid; **DRI**, Dietary Reference Intakes; **EPA**, eicosapentaenoic acid; **FAO**, Food and Agriculture Organization of the United Nations; **FDA**, Food and Drug Administration; **FOSHU**, Foods for Specified Health Use; **GSPE**, Grape Seed Proanthocyanidin Extract; **HDL**, high density lipoprotein; **ILSI**, International Life Sciences Institute; **LDL-C**, low density lipoprotein-cholesterol; **MIA**, Malnutrition Inflammation and Atherosclerosis; **MICS**, Malnutrition Inflammation Complex Syndrome; **NCDS**, Non-Communicable Diseases; **PUFAs**, polyunsaturated fatty acids; **ROS**, reactive Oxygen Species; **WHO**, World Health Organization.

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Full Length Research Paper

Evaluation of the consumption and physicochemical characteristics of the leaves of *Salacia pynaertii* in the food practices of populations of Brazzaville (Congo)

Elenga M.^{1,2,3*}, Itoua Okouango Y. S.^{1,2,3}, Loubelo Ongnangué L. U.^{2,3} and Mananga V.^{1,2,3}

¹Multi-field Team of Research in Feeding and Nutrition (Eprancongo), Congo.

²Laboratory of Human Nutrition and Feeding (LaNAH), Congo.

³Department of Masters, Faculty of Sciences and Techniques, University Marien, NGOUABI, Congo.

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The objective of this study was to evaluate the place of *Salacia pynaertii* in the consumption of vegetables in Congo-Brazzaville, like its nutritional quality. With this intention, an investigation of consumption based on a card was carried out near 117 consumers of *S. pynaertii*. The data collected carried on the sociodemographic data of the consumers, on the knowledge of this vegetable, and on their consumption like on their frequency. In addition, the physico-chemical analysis related to the morphology of the leaves, the determination of the contents of macronutrients and some minerals. It comes out from this study that *S. pynaertii* is a vegetable known and consumed by all social categories: grooms 47%, single people 41% whose age varies between 48 and 53 years. The consumption of the leaves of *S. pynaertii* is not dependent to the educational level or on the social standing. The sheets of *S. pynaertii* are used like one alicament in the treatment of some diseases. The consumption of the leaves of *S. pynaertii* is made mainly believed (100%). This preference of consumption is acquired by practice (84.6% and for statistics: $\chi^2 = 42,818$, ddl=116, $p=0.00000$). Their consumption is frequently done with dishes made up of salted fish (37.6%), followed by fried fish (36.8%). The test of student reveals a highly significant difference $p < 0.001$. These consumers have preferences directed towards the dishes containing fish salted (51.3%), of preserve fish (25.6%), of fish fried (23.1%). As a whole, the difference is highly significant, $p < 0.001$. Concerning the morphological parameters, they arise that the leaves of *Sa. pynaertii* have one length of 2.90 ± 4.14 cm and a width of 5.91 ± 2.23 cm. These morphological parameters are close to those of *Gnetum africanum*. Concerning the contents of nutrients, *S. pynaertii* contains a significant quantity of in nutrients and contains in particular 34.25 ± 0.35 g of proteins/100 g of dry matter, 39.28 ± 0.55 g of carbohydrates/100 g of dry matter, 722.22 ± 0.03 mg of phosphorus/100 g of dry matter and 54.33 ± 0.45 mg of calcium/100 g of dry matter. The knowledge of this vegetable and especially its frequency of consumption would constitute an advantage with the nutritional balance of the populations because of her contribution in micronutrients and macronutrients.

Key words: Vegetable-leaves, *Salacia pynaertii*, investigation of consumption, Brazzaville, nutritional quality.

INTRODUCTION

The local leaves vegetables are cheap and easily accessible at many communities, in the rural, periurban

and urban zones. They are also rich in micronutrients and can be crucial for the food and nutritional safety of the

poor families (Jansen et al., 2004).

In Africa, the populations often have recourse to the vegetable species (Ambé, 2001). Among the vegetable species, forest products not ligneous family, represents the most significant share of the edible products (FAO, 2002; Canadian Food Inspection Agency, 2001). According to experts' in nutrition of the French-speaking countries of sub-Saharan Africa, the vegetables constitute a source of micronutrients in general (FAO/OMS, 2007). They can also constitute from their composition an appreciable rock salt complement and proteins in the food (Ern, 1979).

The consumption of vegetables plays a capital role in the nutritional balance of the populations and this because of their strong content of micronutrients and some macronutrients (Batawila, 2007).

In Congo, there is an abundance remarkable consumable vegetables, that is to say, 166 food vegetable species (Profizi et al., 1993) which contribute to the daily food of the population. Among the forest products not ligneous, Celastraceae occupy a significant place in the cover of the food needs in Republic for Congo. Celastraceae set out again in 50 kinds and 800 species of which *Salacia pynaertii* vegetables sheets which lead to the wild state in the forests. They form a unit made up of the Chlorophyllian parts to the example of the fruits, leaves, stems and inflorescences (Bressani et al., 1980). This vegetable-leafy generally returns in the food consumption of the populations of Brazzaville. So, the objective of this study was to evaluate the place of *S. pynaertii* in the food consumption of the populations of Brazzaville, like its nutritional quality.

MATERIALS AND METHODS

Target population

The choice of the household considered as statistical unit, made it possible to collect desired information (information on the consumers of the leaves of *Salacia pynaertii*). Each surveyed consumer was identified by a code. The questioned people must agree to take part in the study, to be old of less than 18 years and to be consumers.

Vegetable material

The investigation was directed into *S. pynaertii*, vegetable-sheet consumed with Brazzaville because of its importance in the food practices of the population.

Didactic material

The didactic material is summarized in a card of investigation being

presented in the form of questionnaires posed at the surveyed consumers of *S. pynaertii* and whose answers are noted or notched on the card by the investigator. This card is made up of the open questions and closed questions. The great points of this card are the statute sociodemographic, the age and the educational level of the consumers, the knowledge of *S. pynaertii*, the consumption of the sheets of *S. pynaertii*, the preference of consumption of vegetable-leaves, the preference of consumption of the dishes with the leaves of *S. pynaertii*, the interdictions to consume the leaves of *S. pynaertii*, the advantages of the consumption of the leaves of *S. pynaertii*, the intention and the spending patterns and its frequency of consumption.

Inquire in the households

The investigation was carried out in the urban district of Brazzaville from May to June of the year 2015. It is about an investigation of consumption of the sheets of *S. pynaertii* into a population of 117 households in the nine districts of Brazzaville. The investigation is of transversal type, with a survey with three degrees for the drawing of lots of the zones of enumeration, the blocks and the statistical units on the level of the pieces retained in the zone of enumeration. The investigation was held so that an investigator was charged to work near all the households concerned with the geographical area gathering the unit with zones of enumeration. For each listed household, the investigator had as a task to evaluate the characteristics of the household and the consumption of these leaves.

The questionnaires relating to the consumers were submitted to the people who consume usually the leaves of *S. pynaertii* in their household. A household where the principal housewife missed or who refused to answer the interview was replaced at once by the household nearest, by geographical proximity, in the direction of the step of progression of the investigation.

In each household, only one consumer was questioned. The questioned consumer was appointed by the person who usually deals with the meals. In the case of a building, each apartment is regarded as a household. The interviews were possibly carried out in official language (French) or national languages (Lingala and Kutuba). The starting point of each zone to inquire was the intersection of the streets nearest to the definite point of fall. The first piece to begin the interview was determined by the method crushes or face (with a coin). The direction of progression of the investigation was defined according to the direction growing of the numbers of pieces until obtaining ten households. The houses were surveyed gradually according to a step of five houses for the pieces with an even number and three houses for the pieces with an odd number.

Preparation of the sample for the chemical analysis

The leaves of *S. pynaertii* were weighed then dried with the drying oven at the temperature of 70°C until stabilization of the mass. With the resulting one from this drying, the sheets were crushed. The powder obtained was used in this form for the chemical analysis.

Determination of the water content

The water content was determined by a drying of the leaves of *S.*

*Corresponding author. E-mail: elengamichel@yahoo.fr. Tel: 00 (242)06-654-38-56.

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pynaertii to the drying oven of mark thermosi SR3000. A mass M_f of fresh sheets was weighed and placed at the drying oven at the temperature of 70°C. Drying was stopped after obtaining constant mass M_s and the difference in weight gives the water content reported to 100 g of fresh matter.

Determination of the content of lipids

The content of lipids of the sample was determined by extraction according to the method with the soxhlet by using cyclohexane like solvent of extraction according to the protocol hereafter: 50 g of the powder resulting from the crushing of the leaves of *S. pynaertii* was placed in a cartridge, which in its turn is placed in the soxhlet. In an empty balloon of 250 ml weighed beforehand (M_0), one pours 150 ml solvent. The balloon is heated for 4 h, and then cooled. The solvent is evaporated by rotovapor. After evaporation, the balloon containing of lipids is weighed (M_1). The difference in mass between the balloon containing of lipids and the empty balloon gives the mass of lipids reported at 100 g of vegetable matter.

Determination of the content of proteins

The total nitrogen content was determined by the method of kjeldahl (AOAC, 1990) which consists of the mineralization of the organic matter by the concentrated sulphuric acid, in the presence of a catalyst.

The contents of proteins were determined by the method of Kjeldahl by using a coefficient of conversion of nitrogen in protein of 6.25.

Determination of the content of carbohydrates

The carbohydrates were extracted by their solubility in ethanol after delipidation of the broyat of the sheets of *S. pynaertii*.

Determination of the content total rock salt (ashes)

The contents of total rock salt were determined by incineration with the muffle furnace at a temperature of 550°C for 8 h. Once the 8 h passed, the furnace was extinct and the ashes obtained was allowed to cool until the ambient temperature. Ashes were left in the furnace, and then weighed with a balance of precision.

Determination of the contents rock salt: Ca, Fe, P

The minerals (Ca, Fe, and P) were analyzed starting from the solution of ashes by atomic absorption spectrometry (S.A.A) or of emission of flame. For the determination of these minerals, the solution of lanthanum with 1% and one range standard which differ compared to each mineral were used.

Determination of the energy value

The corresponding energy value was calculated by using the specific coefficient of Merrill and Watt (1955) for proteins, lipids, and carbohydrates.

Treatment and analyzed data

Processing the data collected as well as the seizure and the

production of the rough tables were carried out using the software Excel 2003, EPI-INFO.6.04d Fr-2001. The variables were expressed in the form of percentages. The significant differences perceived between two percentages were checked according to traditional tests' of the differential statistics. The comparison of more than two percentages is carried out using the Student test.

For this, the compare value $\chi^2 (h-1)$ is given by the tables of χ^2 to $(h-1)$ degree of freedom, with a threshold of significant of 5%.

RESULTS

Statute sociodemography of the population

Table 1 presents the statute sociodemography of the surveyed population. Three parameters are observed: the matrimonial situation, the household structure and the number of participants cooking in the household.

Concerning the matrimonial situation, the most significant consumers are the grooms with a proportion of 47.0%; followed by single people with 41.0%; then widowers with 6.8%. The statistical test reveals a highly significant difference with $p < 0.001$.

Concerning the size of the household, it arises that the households having 3 and 4 people consume more sheets of *S. pynaertii* with a percentage of 20.5%; followed by households having 5 people with a percentage of 17.1%. Then the households come having 6 people with 16.2%; then those having 2 people with a percentage of 12%. The statistical test gives a value of 25,505. The difference is highly significant, $p < 0.001$.

With regard to the cooking, the households having two people consume more leaves of *S. pynaertii* with a percentage of 51.3%; followed by households having a person with a percentage of 43.6%. The participation in the cooking appears as an activity which returns exclusively to the only head of household and to the joint one. Among these households, 2.6% contribute to a cooking from 3 to 4 people. The statistical test gives a value of 26,790. The difference is highly significant, $p < 0.001$.

Age and educational level of the consumers

The age and the educational level of the consumers of the leaves of *S. pynaertii* are shown in Table 2.

The results show that the age bracket surveyed represented the consumption of leaves of *S. pynaertii* ranging between 48 and 53 years with a percentage of 20.5%, followed that whose age is higher than 65 years with a proportion of 19.7%. The statistical test reveals a highly significant difference, $p < 0.001$.

The results also show that the educational level of the consumers of the leaves of *S. pynaertii* more represented secondary education I (first cycle) with a percentage of 43.6%, followed primary education with percentage of 29.1%. The statistical test gives a highly significant difference ($p < 0.001$).

Table 1. Statute sociodemographic.

Parameter	Variable	Frequency	Percentage	Confidence interval at 95%	Value of the student test
Matrimonial situation	Married	55	47.0	37.7-56.5	$\chi^2=18,944$; ddl=116; p= 0.00000
	Free union	3	2.6	0.5-7.3	
	Single person	48	41.0	32.0-50.5	
	Divorced	3	2.6	0.5-7.3	
	Widower	8	6.8	3.0-13.0	
Household structure	1 People	7	6.0	2.4-11.9	$\chi^2=25,508$; ddl=116; p= 0.00000
	2 People	14	12	6.7-19.3	
	3 People	24	20.5	13.6-29.0	
	4 People	24	20.5	13.6-29.0	
	5 People	20	17.1	10.8-25.2	
	6 People	9	7.7	3.6-14.1	
	≥7 People	19	16.2	10.1-24.2	
Number of people taking part in the cooking	1 People	51	43.6	34.4-53.1	$\chi^2=26,790$; ddl=116; p= 0.00000
	2 People	60	51.3	41.9-60.9	
	3 People	3	2.6	0.5-7.3	
	4 People	3	2.6	0.5-7.3	

Table 2. Age and educational level of consumers.

Parameter	Variable	Frequency	Percentage	Confidence interval at 95%	Value of the student test
Age of the consumers (years)	18-23	1	0.9	0.0-4.7	$\chi^2=32,773$; ddl=116; p= 0.00000
	24-29	4	3.4	0.9-8.5	
	30-35	5	3.4	1.4-9.4	
	36-41	18	15.4	9.4-23.2	
	42-47	14	12.0	6.7-19.3	
	48-53	24	20.5	13.6-29.0	
	54-59	17	14.5	8.7-22.2	
	60-65	11	9.4	4.8-16.2	
	≥65	23	19.7	12.9-28.0	
Educational level of the consumers	Primary education	34	29.1	21.0-38.2	$\chi^2 = 23,698$; ddl=116; p=0.00000
	Secondary education I	51	43.6	34.4-53.1	
	Secondary education II	20	17.1	10.8-25	
	Tertiary education	11	9.4	4.8-16.2	
	None	1	0.9	0.0-4.7	

Characteristics related to the sheets of *S. pynaertii*

Knowledge of *S. pynaertii*, modality of consumption and preference food of *S. pynaertii*

Table 3 presents the results on knowledge, parts used, the mode of consumption of the leaves of *S. pynaertii* and the others types of consumed vegetables. It gets clear that on the whole of the surveyed people, 100% know leaves of *S. pynaertii* and use all the part of the sheet for consumption.

These sheets are often consumed raw with a percentage of 100%. This preference of consumption is acquired by practice with a percentage of 84.6% and for statistics: $\chi^2 = 42,818$, ddl=116, p=0.00000

Among the other types of vegetables consumed by the population in this study, *Gnetum africanum* occupies the first place with a percentage of 64.1%, followed by *Trilepisium madagascariense* with a percentage of 11.1%. This consumption is generally justified either by the availability on the market (60.7%), or by their good taste (33.3%), with a highly significant difference (p < 0.001).

Table 3. Knowledge, mode of consumption of the leaves of *Salacia pynaertii* and Other types of consumed vegetables.

Parameter	Variable	Frequency	Percentage	Confidence interval at 95%	Value of the Student test
Knowledge of the leaves of <i>Salacia pynaertii</i>	Yes	117	100.0	96.9-0.0	-
Parts used of this plant	Leaves	117	100.0	96.9-0.0	-
Mode of consumption more used	Vintage	117	100.0	96.9-0.0	-
Reasons	Good taste	15	12.8	7.4-20.3	$\chi^2=42,818$; ddl=116; p=0.00000
	practice	99	84.6	76.8-90.6	
	Does not know	3	2.6	0.5-7.3	
Consumption of other types of vegetables	Yes	116	99.1	95.3-100.0	$\chi^2=118,000$; ddl=116; p= 0.00000
	Not	1	0.9	0.0-4.7	
Types of vegetable-leaves often consumed	Gnetum a.	75	64.1	54.7-72.8	$\chi^2=12,855$; ddl=116; p= 0.00000
	luméricié	6	5.1	1.9-10.8	
	Trilepisium	13	11.1	6.1-18.3	
Reasons	others	23	19.7	12.9-28.0	$\chi^2=15,958$ ddl=116 p= 0.00000
	Available	71	60.7	51.2-69.6	
	Quantity	5	4.3	1.4-9.7	
	Good taste	39	33.3	24.9-42.6	
	Others	2	1.7	0.2-6.0	

Dishes consumed with the leaves of *S. pynaertii*

Table 4 presents the various dishes consumed with the sheets of *S. pynaertii*. The results show that the surveyed people frequently consume the dishes with the leaves of *S. pynaertii*. These dishes composed of salted fish (37.6%), followed by fried fish (36.8%). The test of the student reveals a highly significant difference ($p < 0.001$). These consumers have preferences directed towards the dishes: salted fish (51.3%), preserve fish (25.6%), fried fish (23.1%). As a whole, the difference is highly significant ($p < 0.001$).

Advantages, prohibited and other utilities of the consumption of the leaves of *S. pynaertii*

The advantages, prohibited and other utilities of the consumption of the leaves of *S. pynaertii* are shown in Table 5. Concerning the advantages, the results show that the leaves of *S. pynaertii* are mainly a source of the micronutrients (64.1%). Being the interdicts, the results show that the leaves of *S. pynaertii* almost do not present interdicts at consumption (96.6%; $p < 0.001$). Concerning the other utility of the leaves of *S. pynaertii*, the results show that apart from the food aspect, the leaves of *S. pynaertii* are used with a medicinal aim. So, *S. pynaertii* is considered like one alicament.

Recall of 24 h, frequency of consumption and perspective of culinary improvement of the leaves of *S. pynaertii*

Consumption the day before, the frequency of consumption and the perspective for culinary improvement of the leaves of *S. pynaertii* are shown in Table 6. The results show that low consumption has the sheet day before *S. pynaertii*. (25.6%) and the frequency of the most significant consumption is twice in the month (32.5%); although 11.1% of the households consume this vegetable 7 times per month. This frequency of consumption is varied.

Concerning the perspectives of culinary improvement, 82.1% of the households are favorable to the cooking of the sheets of *S. pynaertii* as it is the case of the majority of the consumed vegetables. These households would wish to prepare these leaves of *S. pynaertii* in the groundnut dough (46.9%), in the nut juice of palm (32.3%).

Morphological characteristics of the leaves of *S. pynaertii*

Table 7 presents the morphological characteristics of the large leaves of *S. pynaertii*. The results show that the sheets of *S. pynaertii* show the following characteristics:

Table 4. Dishes consumed with the leaves of *Salacia pynaertii*.

Parameter	Variable	Frequency	Percentage	Confidence interval at 95%	Value of the student test
Existence of dishes frequently consumed with the leaves of <i>Salacia pynaertii</i>	Yes	117	100.0	96.9-0.0	
If so, which	Fried fish	43	36.8	28.0-46.2	$\chi^2=22,223$; ddl=116; p=0.00000
	Salted fish	44	37.6	28.8-47.0	
	Preserve fish	18	15.4	9.4-23.2	
	Smoked fish	12	10.3	5.4-17.24	
Existence of dishes consumed preferably with the leaves of <i>Salacia pynaertii</i>	Yes	117	100.0	96.9-0.0	
Existence of preferred dishes consumed with the leaves of <i>Salacia pynaertii</i>	Yes	117	100.0	96.9-0.0	
If so, which	Fried fish	27	23.1	17.2-32.1	$\chi^2=23,073$; ddl=116; p=0.00000
	Salted fish	60	51.3	44.2-60.6	
	Preserve fish	30	25.6	18.0-34.5	
Reasons of this preference	Good taste	107	91.5	84.8-95.8	$\chi^2=23,480$; ddl=116; p=0.00000
	Bad taste	1	0.9	0.0-4.7	
	Practice	8	6.8	3.0-13.0	
	Does not know	1	0.9	0.0-4.7	

Table 5. Advantages, prohibited and other utilities of the consumption of the leaves of *Salacia pynaertii*.

Parameter	Variable	Frequency	Percentage	Confidence interval at 95%	Value of the student test
Advantages of the consumption of the leaves of <i>Salacia pynaertii</i>	Good taste	-	0.9	0.0-4.7	$\chi^2=30,320$; ddl=116; p=0.00000
	Source of minerals and vitamins	-	64.1	54.7-72.8	
	Stop the hunger	-	1.7	0.2-6.0	
	Does not know	-	33.3	24.9-42.69	
Existence of prohibited on the consumption of the leaves of <i>Salacia pynaertii</i>	Yes	0	0	0	$\chi^2=57,245$; ddl=116; p=0.00000
	Not	113	96.6	91.5-88.1	
	Does not know	4	3.4	0.9-8.5	
Utilities of the leaves of <i>Salacia pynaertii</i>	Alicament	117	100.0	96.9-0.0	-
	Does not know	0	0		

length 12.90 ± 4.14 cm, width 5.91 ± 2.23 cm, length of the petiole; 0.59 ± 0.13 and number of ribs 9.50 ± 2.22 .

Nutritional values of the leaves of *S. pynaertii*

The nutritional values in macronutrients and some micronutrients of the leaves of *S. pynaertii* are presented

in Table 8. It is deduced from this table, that the leaves of *S. pynaertii* show the water content of 57.97%, content of proteins 34.25 ± 0.37 g/100 g dry matter, content of carbohydrates 26.18 ± 0.55 g/100 g of dry matter and in lipids of 0.70 g/100 g of dry matter. The leaves of *S. pynaertii* present an energy value of 301.02 kcal is 1258.26 kj.

This vegetable presents an ash content of 5.05 ± 0.86

Table 6. Recall of 24 H, frequency of consumption and Perspectives of culinary improvement of the leaves *Salacia pynaertii*.

Parameter	Variable	Frequency	Percentage	Confidence interval at 95%	Value of the Student test
Yesterday, you consumed dishes accompanied by the leaves of <i>Salacia pynaertii</i>	Yes	30	25.6	18.0-34.5	$\chi^2=43,007$; ddl=116; p=0.00000
	Not	87	74.4	65.5-82.0	
How much time you consumed the leaves of <i>Salacia pynaertii</i> during the last month	1 time	22	18.8	12.2-27.1	$\chi^2=13,580$; ddl=116; p=0.00000
	2 times	38	32.5	24.1-41.8	
	4 times	13	11.1	6.1-18.3	
	7 times	13	11.1	6.1-18.3	
	Does not know	31	26.5	18.0-34.5	
Does the frequency of consumption remain about constant all the month?	Yes	12	10.3	5.4-17.2	$\chi^2=36,381$; ddl=116; p=0.00000
	Not	44	37.7	28.0-46.2	
	Does not know	61	52.1	42.7-61.5	
Which are the shapes of culinary consumption of the leaves of <i>Salacia pynaertii</i> which you would recommend?	Cooked with water	96	82.1	73.9-88.5	$\chi^2=4,845$; ddl=116; p=0.00000
	Does not know	21	17.9	9.9-30.53	
	Others	18	15.4	9.4-23.2	
How you prepare them?	Groundnut paste	45	46.9	27.7- 64.7	$\chi^2=5,289$; ddl=116; p=0.00000
	Juice of nut of palm	31	32.3	24.5-57.3	
	Others	20	20.8	10.8-25.2	
In which quantity?	Heap	70	72.9	59.1-96.7	$\chi^2=5,289$; ddl=116; p=0.00000
	Paquet	26	27.1	23.3-40.9	

Table 7. Morphological characteristics of *Salacia pynaertii*.

Physical characteristics	<i>Salacia pynaertii</i>
Length (cm)	12.90±4.14
Width (cm)	5.91±2.27
Length of the petiole (cm)	0.59±0.13
Number of ribs	9.50±2.21

Table 8. Food values in macronutriments, ashes (g/100 g) and in some biogenic salts (mg/100 g) of the leaves of *Salacia pynaertii*.

Moisture and macronutriments		Ash and micronutriments	
Moisture (%)	57.97	Ashes (g)	5.05 ± 0.86
Proteins (g/100 g)	34.25± 0.35	Phosphorus, P (Mg)	722.2 ±0.03
Lipids (g/100 g)	0.70± 0.23	Iron, Fe (Mg)	4.075 ±0.00
Carbohydrates (g/100 g)	39.28±0.55	Calcium,Ca (Mg)	543.3 ± 0.45
Energy in Kcal	301.02	-	-

g/100 g of dry matter, a phosphorus content of 722.2±0.03 mg/100 g of dry matter, a content of iron of 4.075 mg/100 g of dry matter and a calcium content of 543.3±0.4 mg/100 g of dry matter.

DISCUSSION

The objective of this study was to describe the consumption of the leaves of *S. pynaertii*. To this end, the

surveys of consumption of the leaves of *S. pynaertii* was carried out and to evaluate the nutritional characteristics of this vegetable.

Our study showed the importance of *S. pynaertii* in the feeding of the population of Brazzaville. This importance of vegetables in the feeding of the populations was confirmed by several authors (Ambé, 2001; Malaisse, 1997).

The results of our study show that *S. pynaertii* is consumed by all the social categories. This observation is in agreement with the studies undertaken on other vegetables by Soro et al. (2012) and Mensah et al. (2008). The consumers of *S. pynaertii* in general have the age bracket ranging between 48 and 53 years, a secondary level and are in the households having 4 to 5 people. The consumption of the leaves of *S. pynaertii* is not related to the social standing of the hearth nor on the educational level of the subject. Thus, we accept the assertion according to which "in Africa, the populations often have resorts to the vegetable species for which vegetables meet their food needs and to ensure the food safety" evoked by unquestionable people who consume the vegetables (Jansen et al., 2004; Ambé, 2001).

The consumers affirm that apart from the food aspect, *S. pynaertii* is used in the medicinal aspect. So *S. pynaertii* is regarded as one alicament. Thus, our results coincide with the assertions according to which "the consumption of the fruits and vegetables can help to prevent several no transmissible diseases related to the feeding (FAO/OMS, 2007); the vegetables sheets are a source of fibers, intervening on the level of the digestive tract and prevent the absorption of an excess of cholesterol "evoked by Soro et al. (2012) and Mensah et al. (2008).

The leaves of *S. pynaertii* are often consumed raw (100%). This form of consumption could avoid the disappearance of some micronutrients which are sensitive to the temperature or their reduction by diffusion of the water-soluble components in the water of cooking as reported by Causeret (1986).

The food interdicts have a negligible influence on the rate of consumption of the vegetable-leaves. The leafy of *S. pynaertii* does not have prohibitions of consumption. Several authors showed that there are interdicts of consumption of vegetables. Thus, Batawila (2005) reported that at Mossi and Togo, the ritual one precede the consumption of the leaves of *Adansonia digitata*; Dansi et al. (2008) reported that in the north of Benin, *Cerototheca sesamoides* is interdict with the men who have supernatural capacities.

The frequency of the most significant vegetable consumption is twice in the month (32.5%). This matter is encouraged by certain authors (Tirilly and Bourgeois, 1999; FAO, 2002) who evoked that "the vegetables leaves, that they are wild or are cultivated, resulting from lianas, of the tuber or of trees, also bring to the populations which have at their disposal only of very little

meat or fish, of essential proteins, especially with the women pregnant or nursing and or to the period infants of growth. The vegetable-sheets especially play a significant role in the maintenance of the food balance of the underprivileged populations".

The results on the morphological aspects show that the leaves of *S. pynaertii* have a length of 12.90 ± 4.14 cm, a width of 5.91 ± 2.23 cm, a length of the petiole of 0.59 ± 0.13 cm and a number of ribs of 9.50 ± 2.22) found in our study are close to those of *G. africanum* (length: 12.92 ± 0.94 cm; width: 06.87 ± 0.49 cm a number of ribs: 10.50 ± 0.86) announced by Mbemba et al. (2013) and those of domestic *G. africanum* (length: 8.50 ± 0.70 cm, width: 3.35 ± 0.25 cm, a number of ribs: 3.00 ± 00.00) announced by Elenga et al. (2016).

The water content of the leaves of *S. pynaertii* is 57%. This content is lower than that of certain vegetable sheets studied by Dorosz (1999) having found variations of the water content between 70 and 90%.

The leaves of *S. pynaertii* constitute a significant source of plant proteins. Indeed, their content of proteins is 34.25 ± 0.35 g/100 g of dry matter. But, this value is lower than that of *Vigna unguiculata* (26.48 g/100 g of dry matter dry) observed by Tchiégang and Kitikil (2004) and near to some vegetables studied by Adu-Dupaah (1999) for which the content of proteins varies between 28 to 34 g/100 g of dry matter.

The quantities of the carbohydrates (39.28 ± 0.55 g/100 g of dry matter) obtained in the leaves of *S. pynaertii* are lower with those of sheets of *Moringa oleifera* (49.52 ± 0.00 g/100 g) found by Tchiegang and Kitikil (2004).

Phosphorus is the only mineral quantitatively most significant after calcium. It plays a plastic role because it constitutes a combination with calcium and the mineral screen of the bones. The needs for phosphorus are about 800 mg per day in the adult (Dorosz, 1999). *S. pynaertii* presents the phosphorus content of about 722.2 ± 0.03 mg/100 g of dry matter. The values found in our study are close to these recommendations.

The calcium content (543.3 mg/100 g) found in *S. pynaertii* is close to that of *M. oléifera* (531 mg/100 g) observed by Toury et al. (1963).

Our study shows that the content of iron in the vegetable-sheets of *S. pynaertii* is of 4.075 ± 0.0 mg/100 g of dry matter. Iron prevents ferriprive anemia (Dansi et al., 2008). It plays a very significant role in the constitution of hemoglobin and it constitutes myoglobin of the muscles. In the sub-Saharan countries where the populations suffer from frequent anemia caused by paludism, the contribution of the iron is very significant and recommended.

Conclusion

This study made it possible to show that the leaves of

S. pynaertii are vegetable-leafy green, known and consumed or cooked in the households of the population of Brazzaville. The results of the investigation of consumption showed their importance in the food of the population of Brazzaville and it does not matter the social standing and instruction. The share taken by this vegetable-sheet is in the diets; in fact the frequency of consumption can be significant for the balance of the nutritional needs for the poor populations and could play a role with food safety. The sheets of *S. pynaertii* are considered by certain people as alicament used in traditional medicine in the palliative curative treatment: gastritis (epigastric pains) like a gastric bandage; diabetes (hyperglycemia) like anti hyper glycemie; constipations like a laxative.

The sheets of *S. pynaertii* studied are rich in proteins, in carbohydrates and in some minerals elements, particularly, phosphorus and calcium. The high percentage of these minerals, the leaves of *S. pynaertii*, is of great interest on the nutritional level especially for the fight against the deficiencies in micronutrients.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Dietary pattern and nutritional status of primary school pupils in a South Western Nigerian state: A rural urban Comparison

Bamidele Bello¹, Oyenike Ekekezie² and Olusegun Temitope Afolabi^{3*}

¹Population and Reproductive Health Programme, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

²National Postgraduate Medical College, Ijanikin, Lagos, Nigeria.

³Department of Community Health, Obafemi Awolowo University, Ile-Ife, Nigeria.

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Nutrition may be defined as the process, act, or study of how living things use nutrients for energy, growth, and maintenance. Contrary to widely held notions, malnutrition may be caused by people choosing to eat the wrong types of food, rather than a lack of what to eat. The objectives of the study were to describe the dietary pattern of primary school pupils in an urban and rural local government area of Lagos State, Nigeria and to examine the relationship between dietary pattern and prevalence of malnutrition. This cross-sectional comparative study adopted a multistage random sampling which included all children aged between six and twelve years in primary schools in randomly selected urban and rural local government areas (LGAs) in Lagos State. Using interviewer-administered questionnaire, information on socio-demographics, dietary history/pattern, food frequency and anthropometric measurements of selected pupils was obtained. Obtained data were analysed with standardized nutritional indices, and treatment means were compared with bivariate analysis at 95% level of significance. While underweight (49.6% v 15%) and wasting (24.2% v 13.6%) pupils were more prevalent in the rural areas, overweight (15.1% v 13.2%) pupils were more prevalent in the urban areas. Eating patterns among pupils living in the rural areas were significantly different from those living in urban areas, especially with respect to vegetable, snack and fizzy drink consumption. Comprehensive nutritional education programme for pupils, their parents and teachers should be introduced to promote nutritional health.

Key words: Nutrition, nutritional status, rural, urban, nutritional pattern.

INTRODUCTION

Nutrition may be defined as the science of food and its relationship to health. It is concerned primarily with the

part played by nutrients in body growth, development and maintenance (WHO, 1971). Undernutrition (nutrient

*Corresponding author. E-mail: temitopesegun@yahoo.com. Tel: +234-803-388-5447.

deficiency) is the prevalent type in tropical developing countries. Obesity (nutrient excess) and its co-morbidities are less widespread in developing countries but rates are increasing (Lucas and Giles, 2003; Afolabi et al, 2015). Community beliefs and family dietary practices have also been identified as important predisposing factor to malnutrition (Abubakar et al, 2011; Wong, 2014). It is believed that almost one third of children in developing countries are malnourished (FAO, 2015). Contrary to widely held notions that malnutrition is due to poverty, anecdotal evidence suggest that this may be caused by people choosing to eat the wrong types of food, rather than a lack of what to eat (Torpy, 2004). Infants and young children bear the brunt of undernutrition and suffer the highest risk of disability and death associated with it. Young children who are undernourished are more susceptible to diseases. Even feeding them later in life is too little, too expensive and too late to improve nutrition or future productivity (World Bank, 1998; Burgess and Danga, 2008). About 60% of children who die from common diseases like malaria and diarrhoea would not have died if they were not undernourished in the first place (WHO, 2016). In 2001, 54% of all childhood mortality was attributable, directly or indirectly, to malnutrition. The children who die represent only a small part of the total disease burden due to malnutrition (Salem and Hamza, 2005). Worldwide, more than 80% of deaths associated with childhood undernutrition involve mild or moderate undernutrition, though immediate cause of death may be other conditions (Ogbonna et al, 2003; Levinson and Bassett, 2007; Weisstaub et al 2014). Children living under conditions of poverty and deprivation are always at risk of becoming undernourished. This has been noticed to be true even if the overall image of the society is one of prosperity and general well – being (Lucas and Giles, 2003).

It is therefore imperative to find out the dietary pattern, and its role in nutritional status, of primary school children in rural and urban local government areas (LGAs) in Lagos State, a cosmopolitan setting with generally higher standards of living as well as influences from urbanization, westernization and globalization.

The objectives of the study were to describe the dietary pattern of primary school pupils in an urban and rural local government area of Lagos State, Nigeria and to examine the relationship between dietary pattern and prevalence of underweight, overweight, wasting and stunting among primary school pupils in the urban and rural local government area of Lagos State, Nigeria.

METHODOLOGY

This cross-sectional comparative study adopted a multistage random sampling which included children aged between six and twelve years in primary schools in randomly selected urban and rural LGAs in Lagos State. Sample size was determined using sample size formula for comparing proportions with confidence level

set at 95%, power of 80% and to detect a difference of 11% between both arms of the study yielded a sample size of 240 per group. The 20 LGAs in Lagos State were stratified into urban and rural, giving 16 urban and four rural LGAs (Ukoli et al, 1993), from which one urban (Ikeja) and one rural (Ikorodu) LGA was randomly selected from each of the strata. Thereafter, one ward was selected by simple random sampling from each of the selected LGAs from which one public and one private primary school were randomly selected in each of the two selected wards from the list of approved public and private primary schools in Lagos State, previously obtained from the Ministry of Education, Lagos State Secretariat, Alausa, Ikeja. An arm of each of the six grades in each school was selected at random via ballot and all pupils in the selected arms were interviewed and examined. Thus, a total of 529 pupils were included in the study comprising of 265 pupils for the urban arm and 264 for the rural arm.

Ethical clearance was obtained from the Research and Ethics Committee of the Lagos University Teaching Hospital. In addition, approval was obtained from the Lagos State Local Government Education Authority (LGEA) as well as from the individual school authorities. Written consent to interview and examine each child was obtained from their parents/caregivers via letters sent through the children. Child assent was then obtained from children whose parent/caregiver gave consent for their participation.

Data was collected from each of the selected pupil with the use of an interviewer-administered questionnaire which obtained information of socio-demographics, dietary history/pattern, food frequency and anthropometric measurements of the pupils. Prior to the survey, a certified anthropometrist of the International Society for the Advancement of Kinanthropometry (ISAK) (Anwer and Awan, 2003) trained the research assistants in obtaining anthropometric measurements from children after which the questionnaire was pre – tested in one public and one private primary school each in Surulere LGA (urban) and Badagry LGA (rural) in Lagos state.

The weight or body mass of each pupil was measured using a Seca® electronic weighing scale while their heights were measured with the use of a customized stadiometer. Calibration of the Seca® electronic weighing scale was undertaken every morning, before data collection, using reference weights of known mass. The GPM® Anthropometer was used for the calibration of the customized stadiometer. Anthropometric measurements (weight and height) were obtained from each pupil according to the ISAK standard protocol. The measurements were converted to nutritional indices: weight for age (W/A), weight for height (W/H) and height for age (H/A), based on percentage of reference median using The United States National Centre for Health Statistics (NCHS) standard recommended by the World Health Organization (WHO) as an international reference standard; age as at last birthday, as reported by each child, was verified from school's register of date of birth. In obtaining their weight/body mass (Ivanovic et al, 1986) each pupil was measured privately, standing still and bare footed, lightly dressed in their underwear. Their weights were taken in the morning, as it is known that there are diurnal variations in weight (Julia et al, 2004), and was recorded to the nearest tenth of a kilogram. The height or stretch stature (Ivanovic et al, 1986) was taken as the maximum vertical distance from the feet to the vertex of the head, which technically is the highest point on the skull when the head is held in the Frankfort plane. In this position, the subject is looking straight, the line joining the inferior margin of the eye socket with the tragion (notch above tragus) of the ear is horizontal or at right angles to the long axis of the body, arms hanging naturally by the sides, heels together and both heels touching the base of the stadiometer. The heels, buttocks and upper part of the back and back of the head were in contact with the stadiometer. Each subject looked straight ahead and took a deep breathe. The headpiece was brought down firmly, 'crushing' the hair and making contact with the vertex. The measurement was read off before the

Table 1. Socio – demographic characteristics of pupils in rural compared with urban area.

Variable	Location rural N (%) N = 264	Urban N (%) N=265	Statistic
Age (years)			
6	19 (7.2)	37 (14.0)	$\chi^2=11.83$
7	40 (15.1)	45 (17.0)	P=0.07
8	37 (14.0)	30 (11.3)	-
9	37 (14.0)	38 (14.3)	-
10	51 (19.3)	36 (13.6)	-
11	37 (14.0)	46 (17.4)	-
12	43 (16.3)	33 (12.5)	-
Sex			
Female	147 (55.6)	139 (52.4)	$\chi^2=0.55$
Male	117 (44.4)	126 (47.6)	P=0.46
Religion			
Christianity	177 (67.5)	232 (87.5)	$\chi^2=31.64$
Islam	87 (32.5)	33 (12.5)	P<0.001
Ethnicity			
Igbo	30 (11.4)	76 (28.7)	$\chi^2=162.97$
Yoruba	222 (84.1)	87 (32.8)	P<0.001
Others	12 (4.5)	102 (38.5)	-
School type			
Private	127 (48.1)	131 (49.4)	$\chi^2=0.09$
Public	137 (51.9)	134 (50.6)	P=0.76

subject exhaled, and then stepped away from the stadiometer. Measurements were taken to the nearest tenth of a centimeter.

In order to ensure that precise measurement had been taken, it was essential to determine the quantity of measurement error intrinsic to this study, and to ensure that this was within internationally accepted limits. The technical error of measurement (TEM) was used as a measure of validity (Anwer and Awan, 2003; Julia et al, 2004).

Data analysis

Data entry, validation and analysis were done using the EPI INFO epidemiological software package (version 6.04) (WHO, 1997). Categorical variables were presented in frequency distribution tables and / or charts; Appropriate summary statistics were also generated for the discrete variables: the Student's t – test was used for comparison of means while the Chi Square test was used to compare rates, ratios and proportions. Yates correction of Chi Square was done for tables with cells having expected values less than five (Adamu et al, 2012). Underweight, overweight, stunting and wasting were classified using z scores.

RESULTS

Socio – demographic characteristics

Five hundred and twenty nine pupils participated in the

study comprising of 264 (49.9%) pupils from Ikorodu local government area (rural); and 265 (50.1%) pupils from Ikeja local government area (urban). Over half, 289 (54.1%), of the respondents were females giving an overall male: female ratio of 1:1.2. Table 1 shows the socio – demographic characteristics of pupils in rural area compared with the urban. The pupils in the rural area were not significantly different from those in the urban area in sex, age and school type but were significantly different by religion and ethnicity. Respondents in rural area were of predominantly Yoruba extraction while the urban were more evenly distributed. There were more Christians in the urban schools (87.5%) than rural schools (67.5%).

Prevalence of underweight, stunting and wasting

Table 2 shows the weight for age of pupils in the rural and urban areas based on weight for age z scores (WAZ). Almost half (49.6%) of the pupils in the rural area were underweight compared with 15% pupils in the urban area. Thus, the prevalence of underweight was significantly higher in the rural area compared with the urban area. Conversely, the prevalence of overweight

Table 2. Weight for age of pupils in rural and urban areas.

WAZ	Rural frequency (%)	Urban frequency (%)	Statistic
<-2	131 (49.6)	40 (15.1)	$\chi^2=156.0$
-2 to +2	133 (50.4)	150 (56.6)	P<0.001
> +2 to +3	0 (0.0)	40 (15.1)	-
> +3	0 (0.0)	35 (13.2)	-
Total	264 (100.0)	265 (100.0)	-

Table 3. Height for age of pupils in rural and urban areas.

HAZ	Rural frequency (%)	Urban frequency (%)	P-value
< -3	43 (16.3)	13 (4.9)	$\chi^2=71.6$
-3 to < -2	91 (34.5)	31 (11.7)	P<0.001
-2 to +2	130 (49.2)	221 (83.4)	-
Total	264 (100.0)	265 (100.0)	-

Table 4. Weight for height of pupils rural and urban areas.

WHZ	Rural frequency (%)	Urban frequency (%)	P-value
< -3	12 (4.5)	6 (2.3)	$\chi^2=9.97$
-3 to < -2	52 (19.7)	30 (11.3)	P=0.007
-2 to +2	200 (75.8)	229 (86.4)	-
Total	264 (100.0)	265 (100.0)	-

and obesity was significantly higher in the urban than the rural area with 15.1% overweight and 13.2% obese pupils in the urban area, but none in the rural area. Table 3 shows the height for age of pupils in the rural and urban areas based on height for age z scores (HAZ). The overall prevalence of stunting in the rural area (50.8%) was significantly higher than that of the urban area (16.6%). The degree of stunting ranged from moderate (34.5% rural vs 11.7% urban) to severe (16.3% rural vs 4.9% urban). Table 4 shows the weight for height of pupils in the rural and urban areas based on weight for height z scores (WHZ). The prevalence of wasting was significantly higher in the rural area compared to the urban as almost a quarter (24.2%) of the rural pupils were found to be wasted as opposed 13.6% urban pupils. About a fifth (19.7%) of the pupils in the rural area were moderately wasted compared with about a tenth (11.3%) pupils in the urban area. In like manner, severe wasting in the rural LGA was found to be 4.5% compared to 2.3% in the urban area. In Table 5, all the mean nutritional indices (weight for age z scores, height for age z scores, and weight for height z scores) were significantly higher among pupils in the urban area compared with the rural area.

Dietary consumption

Table 6 shows the frequency of fruits, vegetables, bread,

noodles, milk and egg consumption by pupils in rural and urban areas. Rural-urban comparisons were significantly different for the various food groups: Fruits 176 (66.7%) versus (vs) 90 (33.3%); vegetables 219 (83%) vs 167 (63.1%); noodles 215 (81.4%) vs 132 (49.8%); regularly consumed milk 150 (56.8%) vs 179 (68.5%); wheat bread 6 (2.7%) vs 54 (20.4%) and regular consumption of eggs 26 (9.9%) vs 191 (72%).

Table 7 shows the consumption of snacks, sweets and soft drinks among pupils in rural and urban areas. Regular consumption of snacks (4 to 7 days a week) and sweets were not significantly different (206 (76.9%) and 5 (1.9%) vs 7 (2.6%) respectively vs 205 (77.4%). Regular consumption of soft drinks was significantly different (66 (25.0%) vs 162 (61.1%).

Relationship between dietary patterns and nutritional status

Tables 8, 9 and 10 represent the regression of weight for age, height for age and weight for height respectively on frequency of dietary consumption (in days per week). The equations representing the association in the form $Y = a + b_1X_1 + b_2X_2 + \dots + b_nX_n$ are:

$W/A = 82.515 - 1.641 \times \text{freq. of wheat bread} + 0.729 \times \text{freq. of egg} - 1.96 \times \text{freq. of fruit} + 1.256 \times \text{freq. of noodles} + 1.180 \times \text{freq. of milk} + 0.312 \times \text{freq. of veg} + 0.701 \times \text{freq. of white}$

Table 5. Comparison of mean nutritional indices of pupils according to location.

Indices	Location		Statistic
	Ruraln=264	Urbann=265	
WAZ mean (SD)	-1.1 (0±1.0)	0.2 (0±1.5)	t=12.038, p<0.001
HAZ mean (SD)	-1.1 (0±1.2)	0.2 (0±1.4)	t=11.525, p<0.001
WHZ mean (SD)	-1.2 (0±1.2)	0.3 (0±1.6)	t=6.58, p<0.001

Table 6. Frequency of consumption of fruits, vegetables, bread, noodles, milk and egg among pupils in rural and urban areas.

Frequency of fruit consumption	Rural	Urban	P-value
Regularly (4-7 / 7 days)	176 (66.7)	90 (33.9)	$\chi^2=56.5$
Occasionally (1-3 / 7 days)	88 (33.3)	175 (66.1)	P<0.001
frequency of vegetable consumption			
Regularly (4-7 / 7 days)	219 (83.0)	167 (63.1)	$\chi^2=26.7$
Occasionally (1-3 / 7 days)	45 (17.0)	98 (36.9)	P<0.001
Frequency of white bread consumption			
Regularly (4-7 / 7 days)	181 (68.6)	184 (69.5)	$\chi^2=0.27$
Occasionally (1-3 / 7 days)	74 (28.0)	74 (27.9)	P=0.87
Never	9 (3.4)	7 (2.6)	-
Frequency of wheat bread consumption			
Occasionally (1-3 / 7 days)	6 (2.3)	54 (20.4)	$\chi^2=43.1$
Never	258 (97.7)	211 (79.6)	P<0.001
Frequency of noodles consumption			
Regularly (4-7 / 7 days)	36 (13.7)	123 (46.4)	$\chi^2=67.9$
Occasionally (1-3/ 7 days)	215 (81.4)	132 (49.8)	P<0.001
Never	13 (4.9)	10 (3.8)	-
Frequency of milk consumption			
Regularly (4-7 / 7 days)	150 (56.8)	179 (68.5)	$\chi^2=6.47$
Occasionally (1-3 / 7 days)	114 (43.2)	86 (32.5)	P=0.01
Frequency of egg consumption			
Regularly (4-7 / 7 days)	26 (9.9)	191 (72.1)	$\chi^2=211.7$
Occasionally (1-3 / 7 days)	156 (59.1)	49 (18.5)	P<0.001
Never	82 (31.0)	25 (9.4)	-

bread consumption W/A=118.880-2.806 x freq. of wheat bread+2.499 x freq. of egg+0.722 x freq. of fruit-2.052 x freq. of noodles-6.575 x freq. of milk+2.926 x freq. of veg+0.671 x freq. of white bread consumption (for rural and urban areas respectively).

H/A=94.969-0.929 x freq. of wheat bread+0.148 x freq. of egg-0.322 x freq. of fruit+0.786 x freq. of noodles +0.026 x freq. of milk+0.354 x freq. of veg+0.441x freq. of

white bread consumption H/A=103.361-0.503 x freq. of wheat bread+0.701 x freq. of egg+0.299 x freq. of fruit-1.130 x freq. of noodles-1.193 x freq. of milk+0.90 x freq. of veg-0.032 x freq. of white bread consumption (for rural and urban areas respectively).

W/H=88.556-1.567 x freq. of wheat bread+0.344 x freq. of egg-1.587 x freq. of fruit +1.367 x freq. of noodles+1.456 x freq. of milk+0.560 x freq. of veg+0.943

Table 7. Frequency of consumption of snacks, sweets and soft drinks among pupils in rural and urban areas.

Frequency of snacks consumption	Rural	Urban	P-value
Regularly (4-7 / 7 days)	206 (76.9)	205 (77.4)	$\chi^2=4.5$
Occasionally (1-3 / 7 days)	46 (17.4)	54 (20.3)	P=0.11
Never	15 (5.7)	6 (2.3)	-
Frequency of sweet consumption			
Regularly (4-7 / 7 days)	190 (71.9)	165 (62.3)	$\chi^2=5.7$
Occasionally (1-3 / 7 days)	69 (26.2)	93 (35.1)	P=0.06
Never	5 (1.9)	7 (2.6)	-
Frequency of soft drink consumption			
Regularly (4-7 / 7 days)	66 (25.0)	162 (61.1)	$\chi^2=76.3$
Occasionally (1-3 / 7 days)	166 (62.9)	97 (36.6)	P<0.001
Never	32 (12.1)	6 (2.3)	-

Table 8. Relationship between frequency of dietary consumption (in days per week) and weight for age (w/a) of pupils in rural and urban areas.

Frequency of consumption (in days per week)	Rural				Urban			
	Coefficient	Std Error	F-test	P-Value	Coefficient	Std Error	F-test	P-Value
Wheat bread	-1.641	2.275	0.5202	0.471404	-2.806	2.735	1.0522	0.305980
Egg	0.729	1.915	0.1447	0.703992	2.499	2.308	1.1716	0.280101
Fruit	-1.965	1.341	2.1479	0.143996	0.722	2.481	0.0846	0.771395
Noodles	1.256	2.085	0.3630	0.547364	-2.052	2.519	0.6635	0.416097
Milk	1.180	1.276	0.8554	0.355903	-6.575	1.970	11.1390	0.000971*
Vegetables	0.312	1.329	0.0552	0.814418	2.926	2.303	1.6141	0.205077
White bread	0.701	1.241	0.3194	0.572475	0.671	2.040	0.1082	0.742475
Constant	82.515	13.262	38.7131	0.000000	118.880	13.572	76.7261	0.000000

*Significant variations

x freq. of white bread consumption
 $W/H=109.868-2.786 \times \text{freq. of wheat bread}+2.500 \times \text{freq. of egg}+0.822 \times \text{freq. of fruit}-2.001 \times \text{freq. of noodles}-6.765 \times \text{freq. of milk}+2.789 \times \text{freq. of veg}+0.766 \times \text{freq. of white bread consumption}$ (for the rural and urban areas respectively).

DISCUSSION

This study was carried out to determine the effects of dietary patterns on the nutritional status of primary school children.

Adequate nutrition promotes good nutritional status and thus satisfies the requirement for good physical health hence the risk of malnutrition is increased with unhealthy dietary habits and practices (Adamu et al., 2012). Nutritional status has a great impact on the learning

capacity of children, on their productivity as adults as well as and on their quality of life in general (Flynn et al., 2006).

The food frequency questionnaire revealed that majority of the pupils consumes an adequate number of meals/day (average of four to five). However, more pupils in the urban area consume more of carbohydrate foods than fruits and vegetables relative to their rural counterparts. In like manner, the consumption of fizzy sugary drinks among the urban pupils (61.1%) was more than two-times that of the rural pupils (24%). This was contrary to the findings of Wang, 2001 obtained among African-Americans where he found low consumption of soft drinks. Egg and milk consumption by pupils in the rural area was poor as only a tenth of the pupils reportedly consume egg regularly (at least 4-7 days/week) and just a little over half consume milk regularly. This is not encouraging as these are protein of animal sources

Table 9. Relationship between frequency of dietary consumption (in days per week) and height for age (h/a) of pupils in rural and urban areas.

Frequency of consumption (in days per week)	Rural				Urban			
	Coefficient	Std error	F-test	P-Value	Coefficient	Std error	F-test	P-Value
Wheat bread	-0.929	0.916	1.0286	0.311444	-0.503	0.621	0.6563	0.418613
Egg	0.148	0.771	0.0370	0.847599	0.701	0.524	1.7890	0.182235
Fruit	-0.322	0.540	0.3559	0.551348	0.299	0.563	0.2820	0.595882
Noodles	0.786	0.840	0.8757	0.350265	-1.130	0.572	3.9063	0.051178
Milk	0.026	0.514	0.0026	0.959433	-1.193	0.447	7.1147	0.008133*
Vegetables	0.354	0.535	0.4376	0.508900	0.900	0.523	2.9633	0.086384
White bread	0.441	0.500	0.7781	0.378539	-0.032	0.463	0.0048	0.944903
Constant	94.969	5.340	316.274 2	0.000000	103.361	3.080	1126.1431	0.000000

*Significant variations.

Table 10. Relationship between frequency of dietary consumption (in days per week) and weight for height (w/h) of pupils in rural and urban areas.

Frequency of consumption (in days per week)	Rural				Urban			
	Coefficient	Std error	F-test	P-Value	Coefficient	Std error	F-test	P-value
Wheat bread	-1.567	2.648	0.7775	0.367034	-2.786	2.305	1.0877	0.305670
Egg	0.344	1.467	0.3887	0.766783	2.500	2.178	1.1897	0.296655
Fruit	-1.587	1.356	2.7544	0.186543	0.822	2.456	0.1566	0.865543
Noodles	1.367	2.357	0.8658	0.678664	-2.001	2.751	0.7656	0.457899
Milk	1.456	1.678	0.7865	0.456803	-6.765	1.785	11.1366	0.000712*
Vegetables	0.560	1.778	0.0567	0.778538	2.789	2.454	1.6167	0.200087
White bread	0.943	1.468	0.4798	0.567865	0.766	2.056	0.1562	0.755678
Constant	88.556	12.267	36.6781	0.000000	109.868	11.597	75.7001	0.000000

*Significant variations.

which are readily available and essential for the growth and development of children. This result is in tandem with that obtained from the study in Nagpur, India by Vinod et al. (2006) where they found low consumption of protein among children of parents in the low socioeconomic class. The

frequency of milk consumption may have contributed significantly to the variations in the nutritional status across the three indices (weight for age, height for age and weight for height) of the pupils in the urban area only. In this study, the mean weight and height of pupils in the urban

area were significantly higher than those in the rural areas.

Undernutrition (underweight, stunting and wasting) was prominent among primary school children in Lagos State, Nigeria, especially in the rural area. This was in agreement to the report of

similar studies in Nigeria and other developing countries (Oninla et al., 2007; Abdul Azzez & Devi, 2012; Senbanjo et al., 2013; Duru et al., 2015; Tadesse and Alemu, 2015). This could be as a result of degenerating living conditions and poor socioeconomic standards in the rural area. Malnutrition, presenting as underweight, stunting and wasting was found to be significantly higher among primary school pupils in the rural area (49.6, 50.8 and 24.2%, respectively) compared with pupils in the urban area (15.1, 16.6 and 13.6%, respectively). Consequently, the mean z-score for weight for age, height for age and weight for height were each found to be significantly lower among the rural pupils than urban pupils ($p < 0.001$). This finding was in agreement with a similar study carried out in Ile – Ife, Osun State which also found the prevalence of underweight, stunted and wasted to be consistently higher among pupils in the rural area compared to pupils in the urban area (Olumakaiye et al., 2010).

The emergence of overweight and obesity in the urban area may be attributable to the fact that Lagos State is more cosmopolitan compared with other states in the same region, with generally higher standards of living, and a lot of influences from urbanization, westernization and globalization resulting in change of diet and pattern of physical activity of children and adults alike (Ekekezie et al., 2012; Ajayi et al., 2015). In this study, pupils in the urban area also had high consumption of energy – dense soft drinks, snacks and sweets, which could be responsible for the overweight and obesity. The better nutritional status of the urban pupil relative to the rural pupil could be a reflection of the expected higher social class of the urban pupil which probably enabled them to have better nutrition because though the frequency of consumption of fruits, vegetables, white bread and noodles was significantly higher among pupils in the rural area, the quantity and quality consumed by pupils in the urban area was probably higher as a result of their higher socioeconomic status. Furthermore the consumption of protein – rich foods, milk and eggs, (which are relatively more expensive) was significantly more frequent among pupils in the urban area. Other studies have also shown that there is inadequate consumption of daily food in the rural area, coupled with children's diets being inadequate in quality as well as in quantity throughout the year (Rohlen, 2002; Berenson, 2005). There are more food-insecure households in rural areas, and reducing the portion size of food is a common coping-strategy (Adebayo and Abegunrin, 2013; Edeh and Gyimah-Brempong, 2015; Ezeama et al., 2015; Toriola, 1998). Some studies have also shown higher prevalence rate of intestinal parasites among the rural pupils. This could contribute to their lower nutritional status (Usfar et al., 2007; Ogbonna et al., 2004; Quihui et al., 2004).

Another probable reason for the disparity in nutritional status between the rural and urban areas may be due to inadequate food intake in the rural area, not only because

of lower socioeconomic status, but also because most of the food produced is rendered even more inadequate as they are taken to the cities to sell and to earn relatively more money (Hadju et al., 1995; Chukwuma, 2014). Also, it is possible that due to urban – rural migration there are less people left in the rural areas to farm and produce food, as many have drifted to the urban areas in search of 'greener pastures'.

The pupils in the urban area accounted for all the over-nutrition observed probably because they could afford more nutritious food in addition to energy – dense drinks, snacks and sweets (Senbanjo et al., 2014). The pupils in the rural area accounted for most of the undernutrition observed, probably because the quality and quantity of food they consume was less (Oyemade et al., 1981). Also probably because they consumed energy – dense drinks and sweets as a substitute to balanced meal and not in addition to their inadequate meals (Rohlen, 2002). Though the pupils in the rural area ate carbohydrate foods such as bread and noodles more frequently than those in the urban area, they ate much less of protein - rich foods such as eggs and milk, which are more important for a child's growth and development (Osinusi and Oyejide, 1987). This is probably because protein – rich foods are more expensive and beyond the reach of those in the rural area. The form of bread consumed most in both rural and urban areas was the white bread which is less nutritious than the wheat bread (Berkey et al., 2005). Infact, 79.6% of pupils in the urban area and 97.7% in the rural area had never eaten wheat bread. Consumption of milk was found to be associated with nutritional status in the urban area in this study: 43.0% of pupils in the urban area consumed milk daily compared with only 8.3% in the rural area.

A study conducted on school-aged children in Nigeria (Afolabi et al., 2015) also revealed that children in the urban areas had poor eating habits coupled with sedentary life style as reflected in their practice of excessive media (TV and internet) exposure accounting for a 10 and 4% prevalence of overweight and obesity respectively. Majority of the pupils snack regularly, with over 66% snacking at least 4 to 5 days in a week. It is possible that snacking has a positive influence on the pupils' nutritional status as it has been observed in other studies with primary school children. Also, Adamu et al. (2012) observed that most of the upper primary school pupil who are underweight, snack just once in a day whereas those who are normal, overweight or are at risk of becoming overweight snack twice or more in a day.

CONCLUSION

This study found the prevalent rates of underweight, stunting and wasting in the rural area to be 49.6, 50.8 and 24.2% respectively; while in the urban area they were 15.1, 16.6 and 13.6% respectively. In addition

however, there was over-nutrition in the urban area: 15.1% were overweight and 13.2% were obese. Dietary consumption of milk was found to be associated with nutritional status in the urban area. In the rural area, there were no significant variations across the food groups as all variables of interest were generally base, thus none was a significant determinant of nutritional status.

The study recommended comprehensive public health intervention measures, including a comprehensive nutritional education programme for pupils, their parents and teachers to promote nutritional health with the necessary political will of governments at all levels to ensure sustainability; establishment or improvement where it already exists, of adequately financed and well managed school health programmes including well supervised school meals that include milk drink daily especially in the rural areas.

Conflict of interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Comparative and quality analyses of different tomato brands sold in major markets in Ibadan, Nigeria

Onyeaghala A. A.^{1*}, Ijagbone I. F.² and Kalu O. Chima²

¹Unit of Chemical Pathology, Department of Medical Laboratory Science, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria.

²Nigerian Institute of Science Laboratory Technology, Samonda, Ibadan, Oyo State, Nigeria.

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Tomato paste is consumed in many homes. It is a good source of lycopene and vitamin C. These are antioxidants which fight against free radicals and oxidative stress. It had been reported that tomato pastes imported into Nigeria were often substandard and adulterated with starch, but these claims have not been supported using evidence based study. The aim of the study was to assess the quality of different tomato brands made in Nigeria and those imported into the country using biochemical parameters. Twenty eight different tomato products were identified in the market. Of these numbers, sixteen were randomly selected. Five tins and sachets each of these products were purchased respectively and used for analyses. All the products sampled were registered with the National Agency for Food and Drug Administration and Control. There were significant differences in moisture, Lycopene, total sugar, starch, vitamin C and acidity among all products ($P < 0.05$). Of all the tin tomato products analyzed, 14 (87.5%) contained sugar as against 2 (12.5%) which were sugar-free and 13 (81.25%) contained starch as against 3 (18.75%) which were starch-free. The starch contained in the tomato brands was not declared in over 75% of the products. The biochemical contents of tomato brand made in Nigeria were of comparable quality with those made in US and Italy ($P > 0.05$). However, tomato brands imported from China were significantly higher in total sugar and starch, but significantly lower in lycopene ($P < 0.05$). Variations in content were observed between tin and sachet of the same brand and between different batches of the same product. This study provides scientific evidence that tomato products made in Nigeria are better in biochemical quality than products imported from China. The need to patronize and support local manufacturers while tightening regulatory and registration processes of products imported into the country becomes imperative.

Key words: Tomato, quality assurance, biochemical analysis, Free radicals, oxidative stress, antioxidants.

INTRODUCTION

Tomato is an annual vegetable commonly consumed in Nigeria. It is a widely distributed crop which can be

consumed fresh, cooked or processed into a paste or syrup, otherwise called a tomato puree. Tomato crop can

*Corresponding author. E-mail: aaonyeaghala@gmail.com.

grow in several climates and this confers on the crop its ability to survive at various temperatures. More than 30% of global tomato is grown from the Mediterranean (Ahmet and Vedat, 2009). However, a greater percentage also comes from Turkey which is located in the East of the Mediterranean (Ahmet and Vedat, 2009). According to Powell et al. (2003), tomato is grown with a view to consuming it fresh or processing it into other forms such as paste or puree. A good number of tomato paste products currently sold in Nigeria are mostly supplied from China and other Asian Countries.

Studies had shown that lycopene from processed tomatoes were better absorbed than lycopene from fresh tomato (Anonymous, 1997). This suggests that processed tomato products such as tomato paste, tomato sauce and ketchup are better sources of this antioxidant. It has also been reported that lycopene from tomato paste is 2.5 times more bioavailable in humans than lycopene from fresh tomatoes especially when boiled with oil, a common medium in which tomato is prepared in this region of the world (Anonymous, 1997; Ahmet and Vedat, 2009). Recent reports have shown that lycopene helps prevent the development of some cancers, such as prostate cancer (Anonymous, 1997). This implies that commercially sold processed tomato paste should contain a good amount of this phytochemical which should be able to benefit humans when consumed. Several parameters have been used to assess the quality of tomato paste. These include: The consistency, total solids content, titratable acidity, pH and levels of sugar (Ahmet and Vedat, 2009, Lu et al., 2014).

Well-structured studies which examine the quality of different tomato brands sold in Nigeria are limited. However, available but unvalidated reports have repeatedly shown that many tomato brands imported into the country were adulterated with starch and colorants.

Umeofia (2016) a leading chief executive in one of the tomato manufacturing companies in Nigeria, has lamented that the country loses huge sums of money to importation of fake tomato products. He reported that a good number of tomato products imported into the country from Asia were often adulterated with starch which could have adverse effects on the health of Nigerians.

Shehu (2013) reported that Nigeria had turned into a dumping ground for fake and low quality tomato products imported from Asia. He further reported that the spate of importation of adulterated tomato sauce in the name of tomato paste was of grave concern to stakeholders in the tomato industry. The infiltration of poor quality tomato products into the country has been attributed to the activities of unpatriotic marketers conniving with unethical foreign companies to bring into the country tomato paste loaded with starch and treated with food colour additives in order to achieve the deep red colour. The premium placed by the National Agency for Food and Drugs Administration and Control (NAFDAC) on the scrutiny of

drugs imported into the country to the detriment of food and cosmetics has also enhanced the activities of unpatriotic marketers of tomato products (Shehu, 2013).

The importation of fake tomato products is not only a problem in Nigeria. The Ghana Standards Board (Daniel, 2015) has reported that most tomato brands sold in Ghana contained starch and sugar which have raised eyebrows and safety concerns among the consumers of such products. The reports showed that the products had labels which indicated that they contained modified starch, tomato paste, sugar and some acidic component, but there were no indications of the relative quantities of those ingredients, a situation which has raised concerns over the quality of the products and the possible health implications related to their consumption.

The consequences of consuming fake and substandard products are better imagined than experienced. The effects and several deaths resulting from *My Pikin* teething powder episode in Nigeria can never be forgotten. The high prevalence of several diseases such as cancer, hypertension, diabetes, cardiovascular disorders, kidney and liver diseases are evident within the country. Several manufactures have been forced to close their businesses and companies due to preference of Nigerians to imported good even when such products are unhealthy and substandard when compared with local made products.

Nigeria is the biggest economy in Africa. Premised on her economic size and huge population, manufacturers all over the world have hinged on these to bring in several products into the country with a view to increasing sales and making more profit. The desire for imported goods has driven many Nigerians to relegate to the background several products manufactured locally; even when it has been hypothesized that local made goods were better than imported products. On this account, several manufactures from across the globe have brought in fake and substandard products into the country believing that since most Nigerians prefer cheap products to costlier ones, reducing the quality of such products could attract more purchase and patronage. This has predisposed the nation to become a dumping ground for several substandard, fake and unhealthy products. With poor regulatory systems, poor adherence to quality management systems and porous borders, there is no doubt that it is the responsibility of researchers to assess the quality of several products sold in the nation's markets with a view to generating evidence based data and information which could be helpful in strengthening regulatory agencies, formulate better polices that will benefit the nation, improve public health and quality of life of citizens.

Considering the lack of documented information on the quality assessment of imported and locally produced different tomato paste sold in the Nigeria market, this study was conducted to bridge this gap. The objectives of the study were to determine the biochemical parameters

contained in selected local and imported tomato brands, to ascertain if imported tomato brand was better than those manufactured and packaged locally, to determine if there were differences in content between tin and sachet of the same tomato brand and to ascertain if there were variations in content between different batches of the same tomato brand. The information generated would help consumers of tomato products to make an informed decision when choosing available products.

MATERIALS AND METHODS

Study area and site

Different brands of tomato paste sold in two major markets in Ibadan, Agbeni and Bodija were identified. In all, 28 different tomato brands were identified. Of these brands, 16 of them were selected at random. Five tomato tins each of selected brands were purchased from randomly selected retailers of the tomato products. Tomato brands, having corresponding sachet were also selected. This gave a total of 80 tins and 50 sachets of different tomato brands including Hunt, a tomato paste manufactured and approved by the United States Food and Drug Administration (USFDA). Both tins and sachets of selected tomato brands were all registered with the National Agency for Food and Drug Administration and Control (NAFDAC). Products endorsed by the Nigeria Industrial Standards (NIS) were noted and documented. The manufactured and expiry dates, country of origin and batch numbers of all products were noted and recorded.

Justification for market selection

Agbeni and Bodija markets are the melting points of major commercial activities not only for people living in Ibadan, but for many other states in the South West geo political zone. These markets are major commercial nerves for major distributors and consumers in Oyo state and other nearby South Western states. Majority of house hold products are deposited at these markets by different producers from where many retailers and users come to repurchase both for resale and household consumption.

Sample size calculation

Studies examining the quality of different brands of tomato paste sold in Nigeria are limited. One of the outcomes for the determination of the quality of tomato is the quantity of lycopene contained in the tomato paste. The study conducted by Maria et al. (2014) in Mexico on quality parameters and bioactive compounds of red tomatoes at different post-harvest conditions reported a standard deviation of 1.5 lycopene in tomatoes. Using this information, the number of tomato paste which we used for this study was calculated using the following formular (Fleiss, 1986):

$$N = \frac{2(Z_{\alpha} + Z_{\beta})^2 \delta^2}{(\mu_1 - \mu_2)^2}$$

Where: Z_{α} = standard normal deviate corresponding to 2 sided level of significance of 5% =1.96; Z_{β} = standard normal deviate corresponding to 80% power = 0.84; N = number of samples; δ = standard deviation; $(\mu_1 - \mu_2)$ = assumed difference in means between the two groups considered significant. It is assumed to be

50% of the standard deviation.

Therefore, the minimum number of tomato samples used was calculated thus:

$$N = \frac{2 \times (1.96+0.84)^2 \times (1.5)^2}{(0.75)^2}$$

$$N = \frac{2 \times 7.84 \times 2.25}{0.56}$$

$$N = 62.72$$

Therefore the minimum number of tomato samples was 63. In order to make room for easy generalization of information, the sample size was increased to 130 tomato samples.

Selection criteria

The inclusion and exclusion criteria for all tomato brands used for the study were as follows.

Inclusion criteria

1. Presence of NAFDAC number
2. Availability of Batch Number
3. Availability of Manufactured and Expiry Dates
4. Indication of country of production or packaging
5. Must either be in tin and/ or sachet

Exclusion criteria

1. Absence of NAFDAC Number
2. Non-availability of Manufactured and Expiry Dates
3. No inscription of country of origin
4. Absence of batch number

Laboratory analysis

All biochemical parameters associated with this study were analyzed at the Nigerian Institute of Science Laboratory Technology (NISLT) Samonda, Ibadan. The following analyses were performed in all the samples: Moisture content, total solids, lycopene, total sugar, starch content, vitamin C and total acidity. All parameters were measured using the approved and validated method of Association of Official Analytical Chemists (AOAC, 2000).

Laboratory methods

Analysis of starch and sugar

Protocol summary: Starch was first hydrolysed to sugar. The sugar was determined using the Lane and Eynon method. Result was expressed in g/100 g (w/w) of tomato paste. The level of starch was determined by multiplying the sugar value by a factor of 0.9. The value obtained was expressed in g/100 g of tomato paste. The presence of starch was qualitatively confirmed using the iodine method. (AOAC, 2000: 923.09). Ethical approval for this study was obtained from the Oyo State

Vitamin C

Protocol summary: Ascorbic acid was oxidized to dehydroascorbic

acid by bromine water in the presence of acetic acid. After coupling with 2, 4, Dinitrophenyl- hydrazine, DNPH, the solution was treated with 85% H₂SO₄ to produce a red complex. The colour formed was measured spectrophotometrically at 521 nm. The intensity of colour formed was directly proportional to the concentration of vitamin C present in the sample. The result obtained was expressed in mg/100 g of tomato paste (Rahman et al., 2006).

Total solids (AOAC, 2000)

Protocol summary: The total solids content is a measure of the amount of paste remaining after all the water has been evaporated in an oven. The mass of the residue is expressed as a percentage (%) of the original mass of paste. The Gravimetry, weight difference recorded after removal of moisture from the tomato paste by oven-drying is a function of the total solids. Data obtained was expressed in g/100 g (w/w) of paste (AOAC 2000).

Moisture (AOAC, 1995)

Summary of protocol: The moisture content was determined by measuring the mass of tomato paste before and after the water was removed by evaporation in an oven. The mass of the residue is expressed as a percentage (%) of the original mass of paste. Gravimetry, weight difference after removal of moisture by oven-drying was determined. Data was expressed in percentage (w/w) (AOAC, 1995).

Determination of total acidity (AOAC, 2000)

Protocol summary: The volume of 0.1 M solution of NaOH required to neutralize a standardized and measured amount of sample is the function of its acidic content. Since citric acid is the commonest acid present in fruits, volume of NaOH consumed is directly proportional to the citric acid present in the sample. The data obtained was expressed in g/L of citric acid (AOAC, 2000).

Spectrophotometric determination of lycopene

Protocol summary: Lycopene was extracted using hexane, ethanol and acetone mixture. The extract was measured spectrophotometrically at 503nm. The data obtained was expressed in mg/100 g (Anonymous, 2016).

Data analysis and management

Data collected from biochemical analysis were analyzed using SPSS version 20 statistical software. Data obtained were expressed as mean and \pm standard deviation (\pm SD) for quantitative variables. Paired t-test was used to test the significance of the difference between mean values of laboratory results. One way Analysis of Variance (ANOVA) was used for comparison of means between groups. Where required, appropriate graphical application was used to express data and non-parametric analysis was used to determine the degree of significance between means.

Ethical issues

Ethical approval for this study was obtained from the Oyo State Ministry of Health Ethical Committee (Approval Reference AD13/479/1016 of February, 2016) All samples were blinded during the sampling and analytical phases of the study, but were unblinded

during the reporting stage. This was performed to eliminate bias. Confidentiality of data was maintained in all stages of the study.

RESULTS

The mean concentration and comparative analysis of various biochemical contents among different tin tomato products is shown in Table 1. It was evident that there were significant differences in moisture, lycopene, total sugar, starch, vitamin C and acidity among all products ($P < 0.05$). Figure 1 shows percentage tomato products that contained and those that did not contain sugar. Of all the tin tomato products analyzed, 14 (87.5%) contained sugar in varying degrees against 2 (12.5%) which were sugar free. Of all the tin tomato products analyzed, 13 (81.25%) contained starch in varying degrees as against 3 (18.75%) which were starch free (Figure 2). Among the tomato brands which contained starch, such was not declared in a good number of the tomato products (Figure 3).

Figures 4 to 8 show the mean concentrations of lycopene, starch, sugar, total acidity and total solids contained in sachets of different tomato brands.

A comparative analysis in biochemical parameters between tomato products made in Nigeria and those imported from other countries such as China, Italy and USA using ANOVA showed that there were significant differences in moisture ($P = 0.008$), lycopene ($P = 0.001$), total sugar ($P = 0.000$) and starch ($P = 0.000$) (Table 2). Post Hoc analysis of tomato products made in Nigeria and those from other countries showed that tomato brands made in the country were of comparable biochemical quality in lycopene, total sugar and starch (if any) when compared with products from USA and Italy ($P > 0.05$). Tomato products imported from China were consistently and significantly higher in total sugar, starch, but significantly lower in lycopene content ($P < 0.05$) respectively (Table 3). Comparative analysis of tins and sachets of the same tomato brand revealed that significant differences ($P < 0.05$) were observed among some tomato brands. In Gino tomato paste, significant differences were observed in moisture, total sugar and starch content of the tomato brand ($P < 0.05$; Table 4). In Hanno tomato paste, differences were observed in the starch and total acidity contents of the tomato product ($P < 0.05$; Table 5). In Tasty Tom, significant differences were observed in total solid and total acidity contents of the tomato products ($P < 0.05$; Table 6). In Derica, there was significant difference in the lycopene content between tin and sachet of the same product ($P < 0.05$; Table 7). There was no significant difference in both the tin and sachet of Ric Giko and Promo tomato brands ($P > 0.05$; Tables 8 and 9) respectively. Comparative biochemical analysis between different batches of the same tomato brand showed that in Gino and Derica tomato brands, there were significant differences in total solid content of the tomato brands ($P = 0.002$, Tables 10 and 11) respectively.

Table 1. Comparative analysis of various biochemical contents among different tin tomato products.

Parameter	Tomatoes (N=80) For all products ** Products Packaged in Nigeria								F-Value	P-value
	*RG	BR	DOCK	HAN	GIN	ROS	*TAI	ST..Rit		
Moisture (g/100 g)	70±0.9	72±2.07	71±2.19	70±3.21	73±3.16	70±3.36	71±2.82	69±2.68	2.03	0.027*
T Solid g/100 g)	30.49±6.6	28.85±6.02	22.62±3.35	25.25± 14.01	23.55± 7.70	28.74± 5.01	24.23±9.51	26.00±7.76	1.49	0.13
Lycopene (mg/100 g)	9.88±1.38	6.50±0.73	7.83±2.09	6.71± 2.70	8.79±1.26	8.57±1.53	9.25±1.08	7.74±1.00	2.23	0.007*
T. Sugar (g/100 g)	0	21.9± 2.55	27.32± 2.55	29.58±1.48	11.88± 1.02	30.9± 1.29	21.32± 1.82	20.92± 0.96	287.1	0.000*
Starch (g/100 g)	0	19.76±0.73	24.62±2.32	26.04±0.23	10.74±0.91	27.88±1.16	19.24±1.62	18.88±0.84	354.27	0.000*
Vit C (mg/100 g)	30.00±2.60	23.44± 4.05	16.64±3.4	20.74±1.14	21.84±1.85	25.26±2.18	20.86±3.47	17.88±4.22	8.44	0.000*
T.Acidity (g/L)	0.24±0.16	0.18±0.01	0.16±0.03	0.17±0.02	0.28±0.02	0.18±0.00	0.17±0.03	0.19±0.030	3.39	0.000*

Parameter	Tomatoes (N=80) For all products ** Products Packaged in Nigeria								F-Value	P-value
	DER	DE-G	L79	TT	POM	CIA	*LUN	HUN		
Moisture (g/100 g)	73±3.11	69±2.51	70±3.03	71±3.51	73±3.40	71±2.68	73±3.78	76±3.03	2.03	0.027*
T Solid g/100 g)	30.67±10.44	28.49±5.77	26.58±5.21	39.03±10.66	28.05±3.84	21.69±6.75	24.56±2.15	20.53±2.31	1.49	0.13
Lycopene (mg/100 g)	8.49±1.87	7.54±0.80	7.07±2.11	9.11±1.25	8.63±1.25	8.56±0.72	9.85±0.98	9.49±1.47	2.23	0.007*
T. Sugar (g/100 g)	0	31.1±1.80	21.16±1.27	31.23±1.80	0.46±0.35	21.88±2.12	12.62±1.31	12.00±0.00	287.1	0.000*
Starch (g/100 g)	0	28.02±1.61	19.08±1.13	28.13±2.73	0.14±0.31	19.72±1.92	11.58±1.18	0	354.27	0.000*
Vit C (mg/100 g)	17.84±2.85	27.30±3.24	13.3±2.77	27.23±3.35	22.82±7.29	16.28±5.51	17.84±2.22	26.26±2.26	8.44	0.000*
T.Acidity (g/L)	0.26±0.06	0.29±0.09	0.31±0.11	0.22±0.06	0.30±0.03	0.25±0.05	0.33±0.01	0.22±0.08	3.39	0.000*

*P<0.05 (Significant); RG = Ric; Giko; BR = Brisk; DOCK= Docker; HAN = Hanno; GIN = GINO; ROS = Rosa; TAI =Taima; St.Rit = St Rita; DER = Derica; DEG = De Gold; L79 = L79; TT =Tasty Tom; POM = Pomo; CIA; Ciao; LUN = Luna; HUN = Hunt.

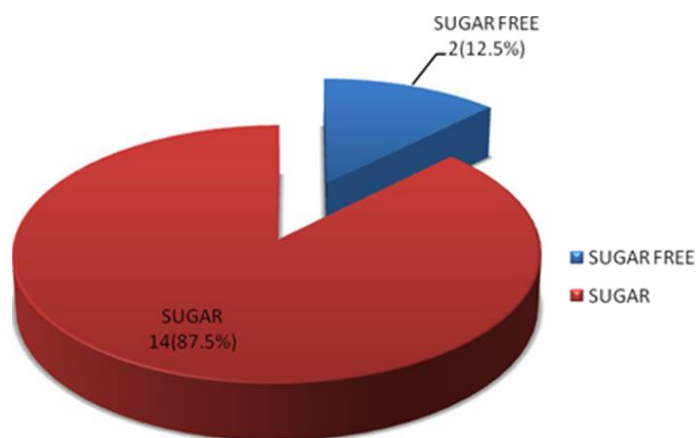


Figure 1. Chart showing percentage tomato products that contained and those that did not contain sugar.

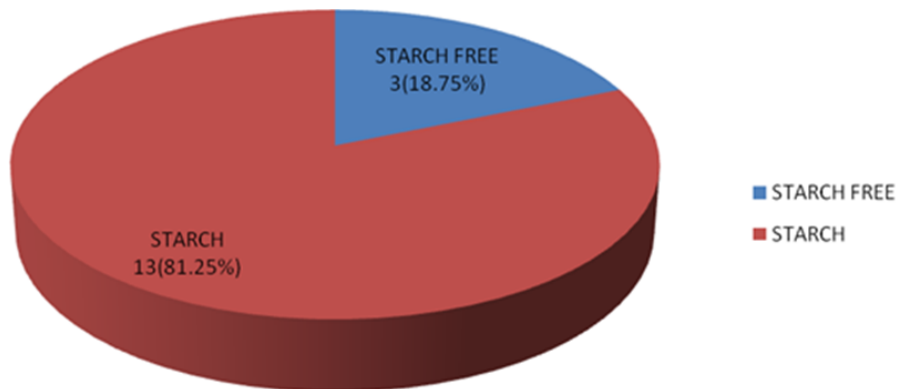


Figure 2. Chart showing tomato products that contain and those that did not contain starch.

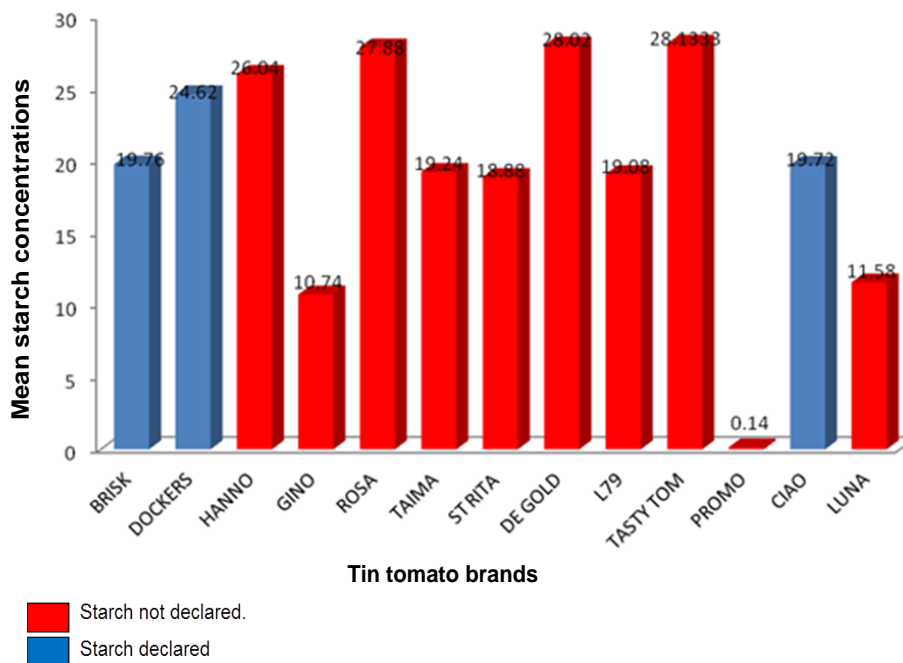


Figure 3. Declaration of starch contained in different tomato brands.

In Ric-Giko tomato brand, there was no observed significant batch variations in biochemical parameters ($P > 0.05$) (Table 12). In Dangote tomato brand, significant differences were observed in total solid ($P = 0.025$), vitamin C ($P = 0.011$) and total acidity ($P = 0.001$) (Table 13).

DISCUSSION

Tomato pastes are often consumed everyday by many homes including those in South West Nigeria. They are good sources of lycopene and vitamin C, phytochemicals

which have been implicated in the fight against cancer and other disorders initiated by oxidative stress. Premised on their high demand, it becomes imperative that tomato products sold in markets should be of the best international and acceptable standards.

Several biochemical parameters have been used to assess the quality of tomato. This includes the total acidity, sugar, moisture and total solids. However, it is expedient that all tomato pastes solely produced from pure tomato seed should contain a high percentage of lycopene and vitamin C. Our study showed that the various tomato brands investigated contained various levels of these biochemical substances. This could imply

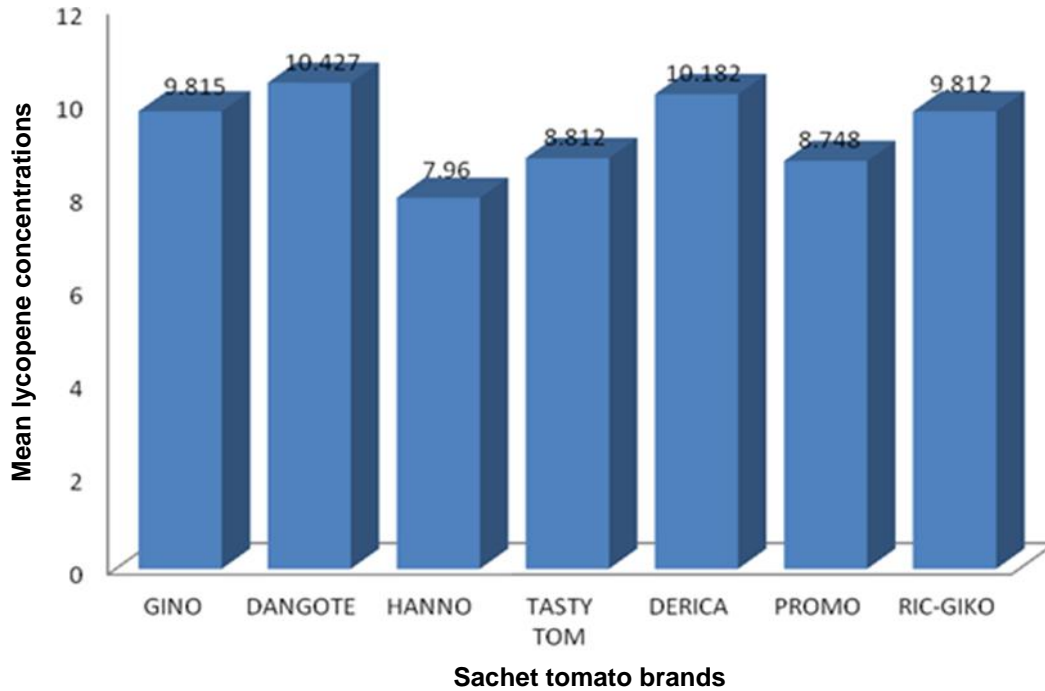


Figure 4. Mean concentration of Lycopene (mg/100 g) in sachets of different tomato brands.

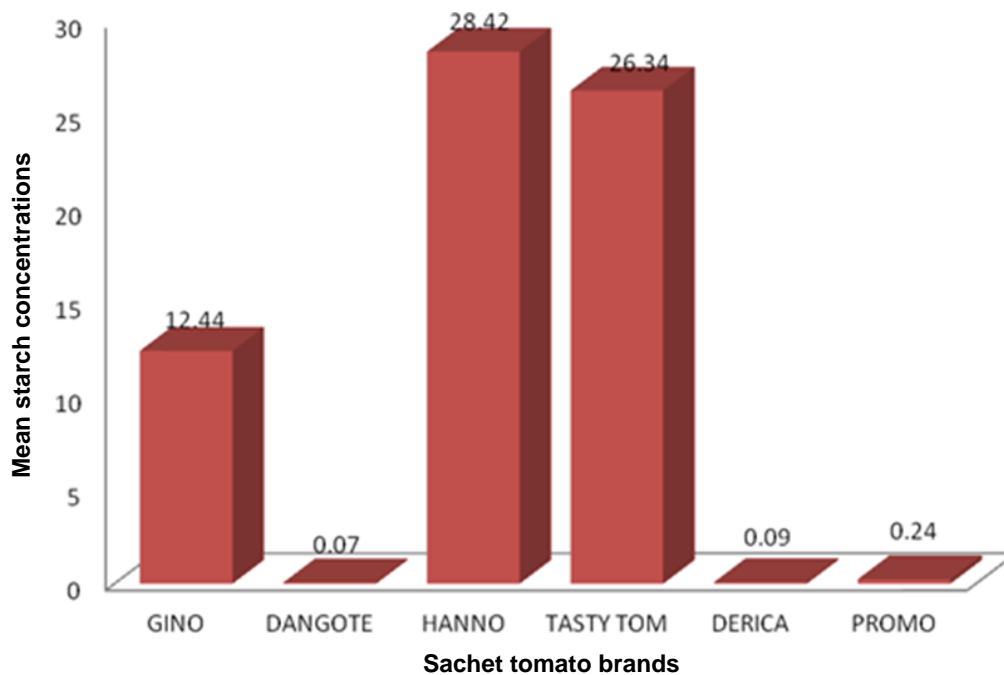


Figure 5. Mean concentration of Starch in (g/100 g) sachets of different tomato brands.

that each of the tomato brands was made from different tomato seed which, depending on region and level of ripening, could contain varying levels of these biochemicals. Of all the tomato tin brands investigated, all

contained sugar except Rick Giko and Derica tomato products. It is important to note that the Sugar and Starch contained in Promo tomato brand was negligible and could be as a result of contamination during the

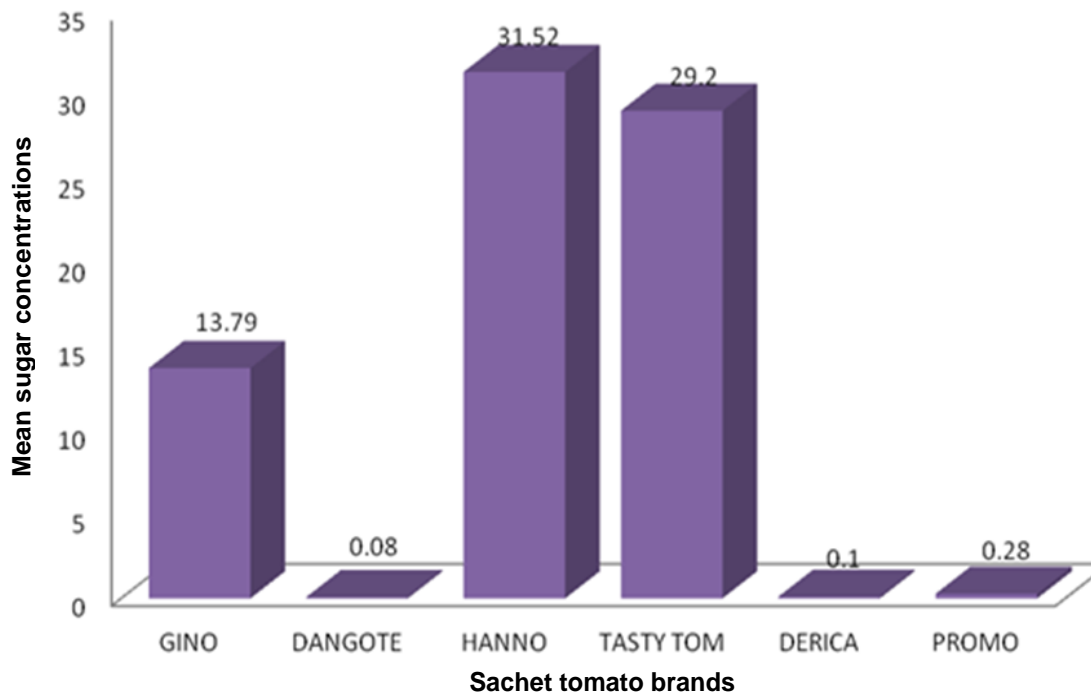


Figure 6. Mean concentration of Sugar (g/100 g) in sachets of different tomato brands.

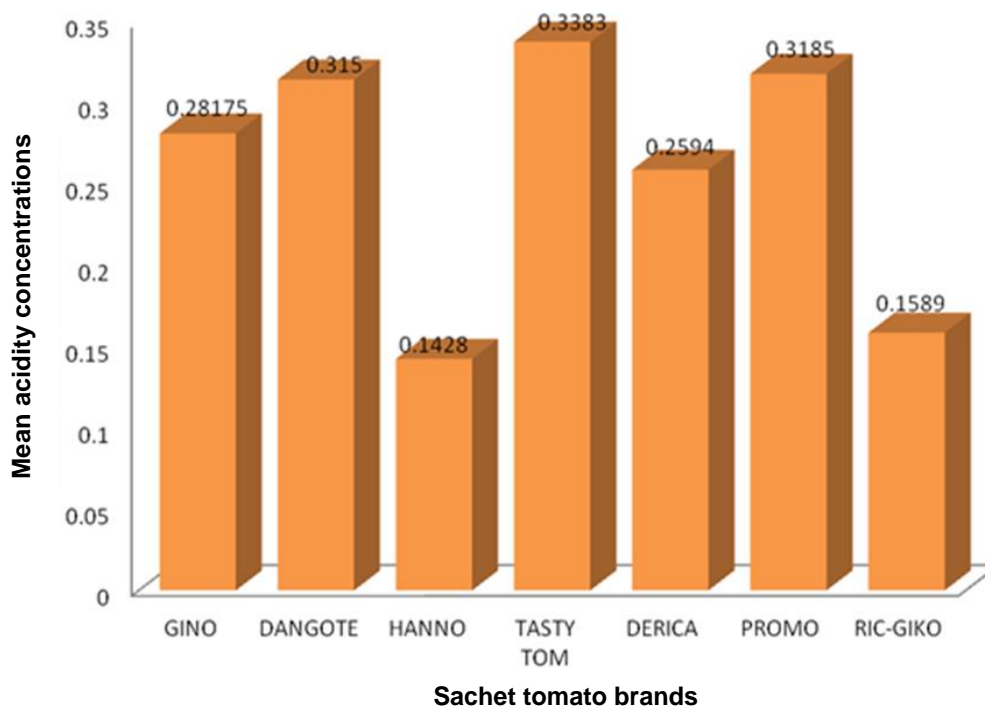


Figure 7. Mean concentration of total acidity (g/L) in sachets of different tomato brands.

production process. Since most of the tomato products contained starch, it is possible that the presence of sugar could either be as a result of hydrolysis of starch or

deliberately added during the manufacturing process with a view to improving quantity and taste. This is however subject to further investigation for proper elucidation. The

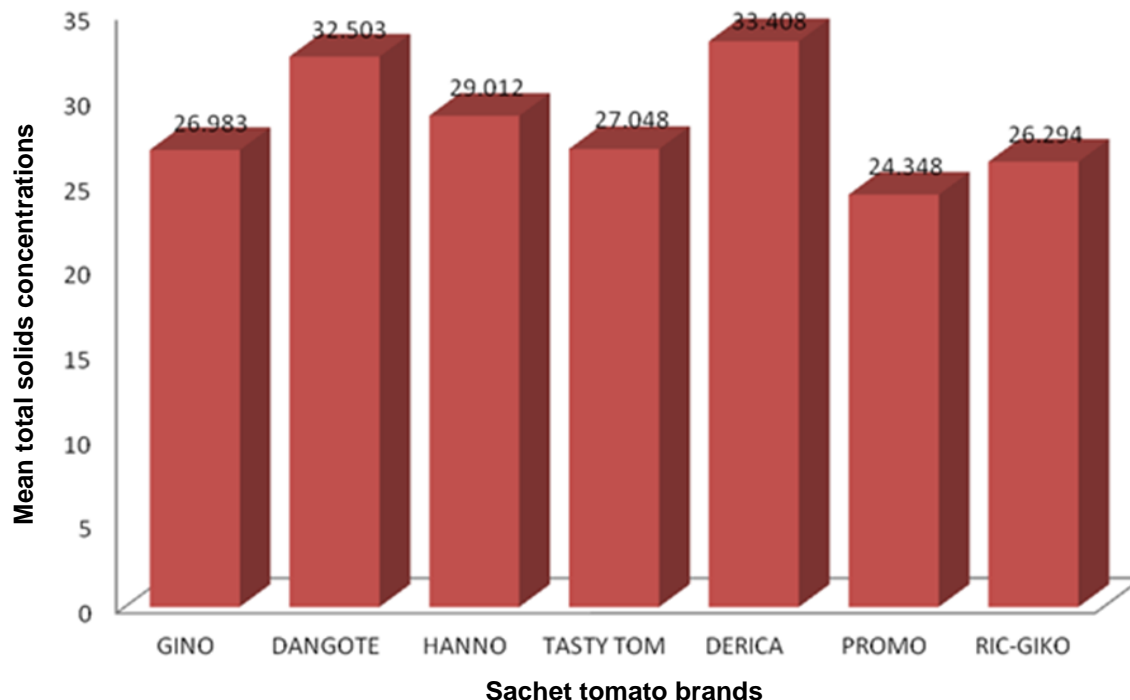


Figure 8. Mean concentration of total solid (g/g) in sachets of different tomato brands.

Table 2. Biochemical parameters between tomato products made in Nigeria and products from other countries (China, Italy and USA).

Parameter	Tomatoes (N=80) For all products				F-value	P-value
	NIG =15	CHINA =53	ITALY=5	USA=5		
Moisture	71.13± 3.45	70.66 ± 3.14	73.20±3.11	75.23± 3.05	4.260	0.008*
T Solid	26.43± 6.97	26.72± 7.67	30.76± 10.44	20.53± 7.61	1.571	0.204
Lycopene	9.66± 1.12	7.87± 1.62	8.49±1.87	9.49± 1.47	6.189	0.001*
T. Sugar	11.31± 9.13	22.22± 9.31	0.000	12.00± 0.00	14.838	0.000*
Starch	10.27± 8.25	19.97± 8.33	0.000	0.000	20.926	0.000*
Vit C	22.90± 5.95	20.92± 5.64	17.84± 2.85	26.26± 2.26	2.534	0.063
T.Acidity	0.25± 0.11	0.23± 0.07	0.26± 0.06	0.22± 0.10	0.418	0.741

*P<0.05 (Significant).

sugar contained in Hunt and brisk tomato products were declared, while the starch contained in other products were not declared. Hunt is a tomato paste made and approved by the USFDA. It is a regulatory requirement in US that all products made and sold in the United State should declare all chemicals known or unknown before being approved by FDA. Furthermore, brisk tomato paste be made for export to US market which necessitated the declaration of the sugar contained in the paste.

The findings from this study showed that most of the products investigated contained starch except Ric Giko, Derica, Dangote and Hunt tomato products. It is also worthy of note that majority of such tomato brands were

those imported from China for Nigeria Market. Previous reports have shown that different tomato brands especially those manufactured and imported from China contained starch (Shehu, 2013; Daniel, 2015; Umeofia and Umeofia, 2016]. This study supports their claims.

Derica and Hunt tomato brands were products made from Italy and US respectively. Products from these countries are highly regulated and should conform to regulatory requirement prior to marketing. This finding also implies that Rick Giko tomato brand is consistent with Hunt, Derica and Dangote tomato products in content and quality.

Starch should not be a component of quality tomato

Table 3. Post Hoc analysis of tomato products made in Nigeria and those from other countries.

Variable	Country of origin	Country	Mean difference	P-value
Moisture	NIGERIA (Mean SD) 71.13± 3.45	China (70.66±3.14)	0.473	1.00
		Italy (73.20±3.11)	-2.067	1.00
		USA (75.23±3.05)	-4.467	0.053*
Lycopene	NIGERIA 9.66± 1.12	China (7.87±1.62)	1.791	0.001*
		Italy (8.49±1.87)	1.170	0.887
		USA (9.49±1.47)	0.172	1.000
Total sugar	NIGERIA 11.31± 9.13	China (22.22± 9.31)	-10.907	0.000*
		Italy (0.000)	11.31	0.088
		USA (12.00± 0.00)	-0.6866	1.000
Starch	NIGERIA 10.27± 8.25	China (19.97± 8.33)	-9.704	0.000*
		Italy (0.000)	10.273	0.081
		USA (0.000)	10.273	0.081

*P< 0.05 (Significant).

Table 4. Comparative analysis between gino Tin vs Gino sachet.

Variable	Tin (N=5)	Sachet (N=10)	T test	P value
Moisture	73.0±3.16	76.5±2.17	-2.536	0.025*
Total Solid	23.5±7.7	26.98±5.28	-1.0213	0.326
Lycopene	8.79±1.26	9.47±2.23	-0.8196	0.427
Total Sugar	11.88±1.02	13.79±1.20	-3.0337	0.010*
Starch	10.74±0.98	12.44±1.09	-2.9681	0.011*
Vitamin C	21.84±1.85	26.18±5.12	-1.8076	0.094
Total Acidity	0.28±0.02	0.28±0.03	0.1790	0.861

*P< 0.05 (Significant).

Table 5. Comparative analysis between Hano Tin vs Hano Sachet.

Variable	Tin (N=5)	Sachet (N=5)	T test	P value
Moisture	70.4 ± 3.20	70.2± 3.11	1.000	0.3739
Total solid	25.25± 14.0	29.01±5.28	-0.9030	0.417
Lycopene	6.71± 2.70	7.96± 2.26	-0.7950	0.471
Total sugar	29.58±1.48	31.52± 1.89	-2.4334	0.0717
Starch	26.04± 0.23	28.42± 1.69	-2.8623	0.0458*
Vitamin C	20.74± 1.14	20.67± 1.56	0.128	0.9039
Total Acidity	0.17±0.21	0.14±0.11	7.3232	0.001*

*P< 0.05 (Significant).

paste. Nevertheless, when they form part of the biochemical components, regulatory processes should mandate that such be declared so that consumers are adequately informed about their content before making an informed decision. From this study, the starch content

of many of the tomato products was not declared.

This presents the products as though they only contained tomato paste, thus creating a wrong impression to the buyers of such products. In situation where manufacturers have declared the quantity of starch

Table 6. Comparative analysis between Tasty Tom Tin vs Tasty Tom sachet.

Variable	Tin (N=5)	Sachet (N=5)	T test	P value
Moisture	70.67± 3.51	75.7±2.51	-2.343	0.058
Total Solid	39.03± 10.66	27.04±2.23	2.555	0.043*
Lycopene	9.11± 1.25	8.81±2.63	0.1819	0.862
Total Sugar	31.23± 3.02	29.2± 1.16	1.398	0.211
Starch	28.13± 2.73	26.34± 1.03	1.3735	0.219
Vitamin C	27.23±3.35	16.44± 1.68	-0.5053	0.6314
Total Acidity	0.22±0.06	0.33±0.05	-2.4732	0.048*

*P< 0.05 (Significant).

Table 7. Comparative analysis between Derica Tin vs Derica sachet.

Variable	Tin (N=5)	Sachet (N=10)	T test	P value
Moisture	73.2± 3.11	76.0± 2.40	1.934	0.075
Total Solid	30.67± 10.44	33.4± 10.68	0.471	0.646
Lycopene	8.49± 1.87	10.18±0.60	2.663	0.020 *
Total Sugar	0.00±0.00	0.1±0.2	1.016	0.328
Starch	0.00±0.00	0.09±0.19	1.033	0.320
Vitamin C	17.84±2.85	18.25± 2.23	0.306	0.764
Total Acidity	0.26±0.06	0.26±0.07	-0.253	0.804

*P< 0.05 (Significant).

Table 8. Comparative Analysis between Promo Tin vs Promo Sachet.

Variable	Tin (N=5)	Sachet (N=5)	T test	P value
Moisture	73.2± 3.96	75.2±2.95	-1.291	0.266
Total Solid	28.05± 3.84	24.34± 4.78	1.204	0.295
Lycopene	8.63±1.25	8.74±1.63	-0.113	0.916
Total Sugar	0.16±0.35	0.28±0.38	-0.429	0.690
Starch	0.14±0.31	0.24±0.33	-0.412	0.702
Vitamin C	22.82±7.29	14.78±2.27	2.244	0.08
Total Acidity	0.30±0.03	0.31±0.27	-1.0330	0.360

*P< 0.05 (Significant).

Table 9. Comparative analysis between rick giko tin vs Ric Giko sachet.

Variable	Tin (N=5)	Sachet (N=5)	T test	P value
Moisture	69.8± 4.08	71.0±0.70	0.753	0.493
Total Solid	30.49±6.62	26.2± 4.92	-2.0687	0.107
Lycopene	9.88± 1.38	9.81± 0.22	-0.129	0.903
Total Sugar	0.00±0.00	0.00±0.00	-	-
Starch	0.00±0.00	0.00±0.00	-	-
Vitamin C	30.00±2.6	29.2±3.57	-0.748	0.496
Total Acidity	0.24±0.16	0.20±0.11	-1.086	0.338

P< 0.05 (Significant).

Table 10. Comparative analysis between Gino tomato products of different batches.

Variable	Batch 1 (N=5)	Batch 2 (N=5)	T test	P value
Moisture	76.4±3.21	76.6±0.55	-0.126	0.906
Total Solid	22.40±1.39	31.56±2.92	-7.214	0.002*
Lycopene	8.27±2.99	11.36±0.33	-2.289	0.083
Total Sugar	13.36±0.99	14.22±1.34	-0.985	0.380
Starch	12.06±0.93	12.82±1.22	-0.944	0.398
Vitamin C	29.34±5.56	23.02±1.78	2.409	0.074
Total Acidity	0.27±0.03	0.29±0.04	-2.217	0.091

*P< 0.05 (Significant).

Table 11. Comparative analysis between Derica tomato products of different batches.

Variable	Batch 1 (N=5)	Batch 2 (N=5)	T test	P value
Moisture	75.2±3.11	76.8±1.30	-1.000	0.374
Total Solid	23.93±4.73	42.88±3.13	-7.681	0.002*
Lycopene	10.28±0.86	10.08±0.25	0.551	0.611
Total Sugar	0.20 ±0.28	0.00±0.00	1.581	0.189
Starch	0.18±0.25	0.00 ±0.00	1.616	0.181
Vitamin C	16.74 ±2.04	19.76±1.18	-2.689	0.055
Total Acidity	0.22±0.09	0.30±0.03	-1.780	0.149

*P< 0.05 (Significant).

Table 12. Comparative analysis between Ric –Giko tomato products of different batches.

Variable	Batch 1 (N=5)	Batch 2 (N=5)	T test	P value
Moisture	71.0±0.71	69.8±4.09	0.735	0.493
Total Solid	26.29±4.93	30.49±6.63	-2.069	0.107
Lycopene	9.81±0.23	9.89±1.39	-0.129	0.903
Total Sugar	0.00 ±0.00	0.000±	-	-
Starch	0.00±0.00	0.00 ±0.00	-	-
Vitamin C	29.2±3.57	30.0±2.60	-0.747	0.496
Total Acidity	0.16±0.02	0.24±0.16	-1.086	0.339

*P< 0.05 (Significant).

Table 13. Comparative analysis between Dangote tomato products of different batches.

Variable	Batch 1 (N=5)	Batch 2 (N=5)	T test	P value
Moisture	74.6±5.86	77.0±0.71	-0.862	0.438
Total solid	26.24±5.59	38.77±2.79	-3.485	0.025*
Lycopene	10.81±2.65	10.04±0.12	0.664	0.543
Total sugar	0.00 ±0.00	0.16±0.36	-1.000	0.374
Starch	0.00±0.00	0.14 ±0.31	-1.000	0.374
Vitamin C	16.54±1.48	11.64±1.48	4.406	0.011*
Total Acidity	0.34±0.01	0.29±0.02	7.913	0.001*

*P< 0.05 (Significant).

contained in their tomato paste, it is the responsibility of the buyer to make an informed decision before purchasing such products. Approving products which contained high concentration of starch and reduced concentration of Vitamin C and lycopene is highly inimical to consumers' health. Several diseases have been linked to obesity and excessive generation of free radicals. It is possible that consumption of tomato paste loaded with starch and lower in lycopene and vitamin C could be contributing to this high incidence of obesity and associated medical problem prevalent in the country. This is subject to further investigation using a well-structured clinical study. All the products which contained starch had NAFDAC registration and endorsement, but without NIS endorsement. This could imply that products with NIS endorsement could be more consistent in quality and safety. This requires further investigation.

It is worrisome that the nation's regulatory agency should approve and register products which could be inimical to people's health without adequately evaluating such products for quality and safety using established and acceptable guidelines. From this study, the need for reviewing, strengthening and tightening regulatory processes for registration of tomato products imported into the country becomes very evident.

In every manufacturing process especially for food industry, it is expected that manufacturers adhere strictly to the principles of good manufacturing practice while putting in place good quality assurance measures. From our finding, it was evident that there were variations in tin and sachet of the same tomato brand except those of Ric-Giko.

Consistency in tin and sachet of the same tomato product usually do imply that such products might have been processed following good manufacturing practice guideline which are in consistent with good quality assurance measures. In products that showed variation in tin and sachet of the same tomato brand, this could be attributed to either differences in manufacturing process, limited to lack of or poor implementation of established quality assurance measures. It could also be attributed to different locations where the tin and sachet of the same tomato brand were produced. It is possible that many of the sachet tomato brands sold in Nigeria are packaged locally while the tin of the same brand is packaged overseas before they are imported into the country for sales. This could account for the difference observed in tomato brands which exhibited such variations. Lack of variations observed among different batches of the same tomato product could also be attributed to good consistency in quality in various batches of the same tomato brand.

Conclusion

Based on the objectives of this study and the biochemical information obtained, it was evident that the quality of

tomato brand made in Nigeria compared relatively well with tomato brands made in United States and Italy. Imported tomato brands especially those from China were of poorer quality than those manufactured and packaged within the country. However, in most tomato products investigated, there were variations in content between tin and sachet of the same brand and between different batches of the same product.

This study provides scientific evidence that local made tomato products are better in quality and consistency in biochemical parameters than products imported from China. It was also very evident that Ric-Giko tomato products showed good consistency between batches as well as between tin and sachet of the same product. Unlike Ric Giko tomato products which had both NAFDAC and Nigerian Industrial Standards (NIS) endorsement, many of the products which were of poor quality had only NAFDAC registration, but lacked NIS endorsement. The need to patronize, encourage and support local manufactures while tightening regulatory processes in the registration of products imported into the country becomes very imperative and this should not be compromised if the health, consumer right and well-being of the people are to be preserved.

Conflict of interest

This study is free from all forms of conflict of interest. It was performed with a view to assessing the quality of different tomato products sold and consumed by many Nigerians. Mentioning of products in this article was solely for providing scientific information. It is not intended to disapprove any product, but to provide information which could strengthen regulatory processes and enable consumers have adequate information to make informed decision.

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Full Length Research Paper

Microbial population and physico-chemical composition of an African Fish based flavouring agent and taste enhancer

Janvier Mélégnonfan Kindossi*, Victor Bienvenu Anihouvi, Opportune O. D. Akpo-Djenontin, Générose Vieira-Dalodé, Mathias Hounsou, Noël Houédougbe Akissoé and Djidjoho Joseph Hounhouigan

Department of Nutrition and Food Science, Faculty of Agronomic Sciences, University of Abomey-Calavi, 01 BP 526, Cotonou, Benin.

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Sixty samples of a traditional flavouring agent and taste enhancer (FATE) locally referred to as *Lanhouin* obtained by spontaneous fermentation of cassava fish (*Pseudotolithus* sp.) and king fish (*Scomberomorus tritor*), used as traditional condiment to enhance the flavour of many dishes were purchased from processing sites and markets, for physico-chemical and microbiological characterization using standard methods. FATE samples exhibited similar water activity level (0.75-0.77), variable pH values (6.88-7.68), variable amounts of dry matter (43.4-47.2 g/100 g), salt (18.7-26.6 g/100 g DM), protein (49.2-53.8 g/100 g DM), lipid (10.8-47.4 g/100 g DM), thiobarbituric acid reactive substances (24.8 to 27.1 mg malonaldehyde/kg DM), total volatile nitrogen (453.6 to 618.6 mg N/100 g DM) and acidity index (1.7 to 4.9 g oleic acid /100 g DM), various organic acids and histamine contents within acceptable limit of 20 mg/100 g for 87% of samples analysed. For all these chemical components, significant differences ($p < 0.05$) were observed between fish species and between sampling places. Total viable counts were ranged between 3.6 to 4.2 Log cfu/g. No *Salmonella* and *Listeria monocytogenes* were found in any FATE sample. The technological flora such as lactic acid bacteria were enumerated (1.2 Log cfu/g) in 42% of samples while coagulase negative *Staphylococci* were found in all the FATE samples (2.9-3.9 Log cfu/g).

Key words: King fish, cassava fish, flavouring agent, *Lanhouin*, fermentation, quality characteristics.

INTRODUCTION

Lanhouin, a traditional fermented fish-based condiment is processed in the coastal areas of West African countries

*Corresponding author. E-mail: jkindossi@gmail.com. Tel: 00229 96 81 44 20.

including Benin, Togo, Ghana, Nigeria and Côte-d'Ivoire. It is mostly used as taste enhancer and flavouring agent in many types of dishes (Anihouvi et al., 2005; Kindossi et al., 2012). The production of Lanhouin is essentially based on endogenous knowledge, laborious and time consuming. The raw materials used for Lanhouin production include the fish and the salt, and the fermentation is spontaneous and uncontrolled (Anihouvi et al., 2012). Different processes and different types of fish are used to produce Lanhouin, but the end-product seems apparently the same. For the production, the fresh fish is scaled, gutted, washed and left for ripening during 8 to 11 h before the matured fish is treated with salt and allowed to ferment for 3 to 9 days. So, the various technologies applied are still artisanal, and consequently the quality of the final product is unpredictable. In addition, the conditions of production are not likely to guarantee its harmlessness. Moreover, the most significant operations such as ripening and fermentation are not well defined, nor controlled whereas they determine the final quality of Lanhouin (Anihouvi et al., 2005; Kindossi et al., 2012). In order to improve the process and quality of Lanhouin, it would be necessary to characterise this product on both microbiological and physico-chemical aspects. The current investigation aims to assess the quality of Lanhouin obtained from two types of fish mainly used for its commercial production.

MATERIALS AND METHODS

Sample source and sampling

A total of 60 samples of Lanhouin made with cassava fish (*Pseudotolithus sp.*) and king fish/spanish mackerel (*Scomberomorus tritor*) were randomly collected in sterile stomacher bags from 12 retailers in market at Comé and Djodah cities and from nine (09) processing sites in Grand-Popo municipality in the southern region of Benin. The Lanhouin samples were transported to the laboratory in an ice box filled with dry ice and maintained at 4°C. The microbiological analyses were performed within 24 h. The remaining samples were kept at 20°C for physico-chemical and biochemical analyses.

Microbiological analyses

Ten (10) g of each Lanhouin sample were introduced aseptically in a sterile stomacher bag and 90 ml of sterile diluent containing 0.1% peptone (Oxoid L37, Basingstoke, Hampshire, England), 0.8% sodium chloride (NaCl) (Merck KGaA, Germany) with pH adjusted to 7.2 was added. The mixture was then homogenised for two min, using a Stomacher (Lab-Blender, Model 80, Seward Medical, London, UK) (1999). One ml of the suspension was serially used for microbial counts according to ISO norms.

Total viable counts (TVC), Lactic Acid Bacteria (LAB) and *Enterobacteriaceae* were enumerated using Plate Count Agar (PCA, Oxoid CM0325, Basingstoke, Hampshire, England), de Man, Rogosa, Sharpe agar (MRS, Oxoid CM0361, Basingstoke, Hampshire, England) and Violet Red Bile Glucose Agar (VRBG, Oxoid, CM0485, Basingstoke, Hampshire, England) respectively.

Yeasts and moulds were enumerated using Yeast Extract Agar (Oxoid CM0019, Basingstoke, Hampshire, England) supplemented with chloramphenicol (Oxoid SR0078E, Basingstoke, Hampshire, England) and the inoculated plates were incubated at 25°C for 3-5 days (ISO-7954 1988). PCA (ISO-4833 2003) and MRS (ISO-15214 1998) plates were incubated at 30°C for 72 h. *Enterobacteriaceae* plates were incubated at 37°C for 24 h (ISO-21528 2004). *Escherichia coli*, *Bacillus cereus*, *Clostridium perfringens* and *Staphylococcus aureus* were enumerated according to ISO methods using Tryptone bile glucuronide (TBX, CM0945, Basingstoke, Hampshire, England), *Bacillus cereus* agar base (Oxoid, CM0617, Basingstoke, Hampshire, England), TSC & SFP (Oxoid CM0587, Basingstoke, Hampshire, England) supplemented with egg yolk emulsion (SR0047, Basingstoke, Hampshire, England) and TSC supplement (SR0088, Basingstoke, Hampshire, England), and Baird Parker agar base (Oxoid CM0275, Basingstoke, Hampshire, England) supplemented with egg yolk tellurite emulsion (SR54, Basingstoke, Hampshire, England). The inoculated plates were incubated at 44°C for 24 h (ISO-16649 2001), 30°C for 48 h (ISO-7932 2004) and 37°C for 24 h (ISO-6888 1999; ISO-7937 2004) respectively.

Salmonella were investigated on Xylose-Lysine-Desoxycholate Agar (Oxoid CM0469, Basingstoke, Hampshire, England) after pre-enrichment of 25 g of sample in buffered peptone (Oxoid CM 509 Basingstoke, Hampshire, England) and selective enrichment in Rappaport-Vassiliadis Broth (Oxoid CM 669 Basingstoke, Hampshire, England) and Muller-Kauffmann Tetrathionate Novobiocin broth (MkTTn, Oxoid CM 1048, Basingstoke, Hampshire, England) (ISO-6579 2002).

Listeria monocytogenes were examined on Palcam Agar Base (Oxoid CM0617, Basingstoke, Hampshire, England) and Chromogenic *Listeria* Agar Base (Oxoid CM1084 Basingstoke, Hampshire, England) after pre-enrichment of 25 g of sample in Fraser Broth Base (Oxoid CM 0895 Basingstoke, Hampshire, England) and *Listeria* Enrichment Broth Base (Oxoid CM 0863 Basingstoke, Hampshire, England) (ISO-11290 2004).

Determination of physico-chemical characteristics

pH of samples was measured with a pH meter (Hanna Instrument HI 9318) according to reference method (ISO-2917 1999). Water activity (*a_w*) was measured with a thermo-hygrometer recorder C056696 (Rotronic Hygrolab 2, 8303 Bassersdorf) according to the method described by Anihouvi et al. (2006). Protein content was determined according to reference method (ISO-937 1978). Total volatile nitrogen (TVN) was estimated using perchloric acid extraction and steam distillation method (Ababouch, 1995). Lipid was determined according to Folch method (Folch et al., 1957). Thiobarbituric acid reactive substances (TBARS) and Acid index were determined according to Pearson (1976) and AFNOR (1993), method NF T. 60-204. Sodium chloride content (NaCl) was determined by measuring the chloride ion concentration with a chloride analyser (Corning MKII model 926, Sherwood Scientific Ltd, UK) after extraction in 0.3 N nitric acid.

Determination of organic acids

Samples preparation

Approximately 150 mg of each Lanhouin sample were suspended in 1 ml of 5 mM H₂SO₄ and mixed thoroughly for 30 min using vortex mixture. After centrifugation of the food suspension at 4140 rpm for 5 min, the supernatant was collected and filtrated through a 0.45 µm microporous membrane before the determination of

organic acid contents (Mestres et al., 2004).

HPLC equipment

Organic acids were determined using an HPLC (Knauer system, Germany) equipped with a Rheodyne 7125 injector, an on-line solvent degasser with LPG Smartline manager 5050 (ADA110606103, Knauer, Germany), Smartline RI Detector 2300 (n°110542, Knauer, Germany) and Spectra system UV2000, a Knauer system controller Smartline, a pump 1000 (n°111235, Knauer, Germany), a Supelcogel H 59304-U Column (30 cm x 7.8 mm ID, Bellefonte, PA, USA) with Supelguard C610H pre-column (5 cm x 4.6 mm ID) and a 20 µl injector loop (Rheodyne, Cotati, CA, USA).

Chromatographic conditions

The analysis was carried out isocratically at a flow rate of 0.6 ml/min., employing as mobile phase water adjusted to pH 2.1 with metaphosphoric acid. The column was thermostated at 30°C. Injection volume was 20 µl and organic acids were detected at 210 nm. Citric, malic, lactic, formic, acetic, propionic acids were identified by retention and spectral data (Mestres et al., 2004).

Determination of biogenic amines

Samples preparation

Approximately 50 mg of each Lanhoun sample were suspended in 3 ml of 0.4 M perchloric acid solution, shaken for 15 min and centrifuged at 2500 × g for 20 min at room temperature. The supernatant was collected and filtrated through Whatman paper n°1.

Derivatisation of sample extracts

Two hundred and fifty (250) µl aliquot of each sample extract was mixed with 50 µl of 2 M sodium hydroxide and 75 µl saturated sodium bicarbonate (Na₂CO₃). Five hundred (500) µl of a dansyl chloride (Dns-Cl) solution was prepared by ultrasonic dissolution of 5 mg Dns-Cl (1-dimethylamino naphthalene-5-sulfonyl chloride, Sigma Aldrich) per 1 ml acetone (Fisher Scientific) and the mixture was then thoroughly vortexed for 1 min. For derivatisation, the mixture was incubated in water bath at 60°C for 45 min in the dark. After this incubation period, 25 µl of 25% ammonium hydroxide was added to remove the excess of dansyl chloride and the mixture was again incubated for 30 min at room temperature in the dark. After this second incubation period, 375 µl of acetonitrile was added to the mixture. Finally, the mixture was filtered through 0.45 µm pore-size filter (Millipore Co., Bedford, MA) and injected into the chromatographic column (Mah et al., 2002; Zaman et al., 2010).

Chromatographic conditions

The same HPLC unit described above was used for biogenic amines determination. Chromatographic separation of biogenic amines was carried out according to the procedure developed by Mah et al. (2002) with minor modifications. The analysis was performed using Column Kromasil 100-5C18 (250 x 4.6 mm E72991) (Rheodyne, Cotati, CA, USA) with water (solvent A) and acetonitrile (solvent B) as the mobile phases at the flow rate of 0.8

ml/min. The program was set for a linear gradient starting from 50% of solvent B to reach 90% of the solvent at 19 min. The sample volume injected was 20 µl and the sample was monitored at 254 nm. Histamine, cadaverine, putrescine and spermidine were identified by retention and spectral data.

Statistical analysis

Data were analysed using Statistica (version 6, StatSoft France, 2004) and significance was accepted at probability $p < 0.05$ with one-way analysis of variance (ANOVA) by using least significant difference method of Fisher. Correlations between variables were evaluated using principal component analysis with XLSTAT software (version 2011, Addinsoft, Paris, France).

RESULTS AND DISCUSSION

Physico-chemical characteristics of Lanhoun samples

The results of physico-chemical analyses of Lanhoun samples are summarized in Table 1. The dry matter content of all samples varied from 43.9 to 47.2 g/100 g for cassava fish and from 43.4 to 45.4 g/100 g for king fish Lanhoun samples. These values agree with those previously reported for Lanhoun samples (49.9 and 43.4 g/100 g from cassava fish and king fish respectively) (Anihouvi et al., 2006), Momoni samples (44.60-48.58 g/100 g) (Sanni et al., 2002; Essuman, 1992) and salted anchovy samples (45.84 g/100 g) (Hernández-Herrero et al., 1999). The dry matter content of market Lanhoun samples processed with cassava fish was significantly higher ($p < 0.05$) than that collected from processing sites. These variations in dry matter contents could be the result of variable drying conditions, duration between processing time and sampling time, level of salt and type of salt used during the processing (Anihouvi et al., 2006). However, the moisture content seems to be an inexact indicator of the susceptibility of a product to undergo microbial spoilage. A key factor, which determines the microbial stability of foods, is water activity (*aw*) (Anihouvi et al., 2006).

The water activity (*aw*) of all Lanhoun samples varied from 0.74 to 0.75 and 0.75 to 0.77 for cassava fish and king fish Lanhoun samples respectively, which were slightly above 0.70 as reported by Anihouvi et al. (2006). These values are too high to prevent enzymatic activity and microbial proliferation including food poisoning bacteria during storage. These values of *aw* classed Lanhoun samples as intermediate moisture content product (Maltini et al., 2003).

pH values of samples ranged between 7.11 and 7.40 and between 6.88 and 7.68 for cassava fish and king fish Lanhoun samples respectively. Whatever the species of fish, the pH of samples collected from the processing sites was significantly ($p < 0.05$) lower than that of the

Table 1. Physico-chemical characteristics of *Lanhouin* samples collected from markets and processing sites (Means \pm Standard Deviation).

Parameter	Cassava fish		King fish	
	Processing sites (n=18)	Market (n=12)	Processing sites (n=18)	Market (n=12)
Water activity (Aw)	0.74 \pm 0.02 ^a	0.75 \pm 0.02 ^a	0.75 \pm 0.03 ^a	0.77 \pm 0.02 ^a
Dry matter (g/100 g)	43.9 \pm 2.1 ^b	47.2 \pm 2.4 ^a	43.4 \pm 1.9 ^a	45.4 \pm 2.8 ^a
pH	7.11 \pm 0.34 ^a	7.40 \pm 0.24 ^b	6.88 \pm 0.72 ^a	7.68 \pm 0.19 ^b
NaCl (g/100 g DM)	26.6 \pm 6.6 ^b	19.3 \pm 5.0 ^a	23.5 \pm 12.1 ^a	18.7 \pm 7.6 ^a
Protein (g/100 g DM)	53.8 \pm 7.6 ^a	49.3 \pm 8.1 ^a	52.6 \pm 4.9 ^a	49.2 \pm 6.8 ^a
Lipid (g/100 g DM)	12.2 \pm 7.0 ^a	10.8 \pm 7.4 ^a	42.8 \pm 17.0 ^a	47.4 \pm 21.1 ^a
TBARS (mg malonaldehyde / kg DM)	27.1 \pm 3.2 ^a	25.5 \pm 1.9 ^a	26.7 \pm 3.7 ^a	24.8 \pm 2.7 ^a
TVN (mg N / 100 g DM)	453.6 \pm 125.3 ^a	457.9 \pm 132.4 ^a	553.6 \pm 94.5 ^a	618.6 \pm 174.8 ^a
Acidity index(% oleic acid DM)	1.8 \pm 0.4 ^a	1.7 \pm 1.1 ^a	4.3 \pm 1.0 ^a	4.9 \pm 1.4 ^a

^{a,b}: Means with different letters according to each row and each species of fish are significantly different ($p < 0.05$); n: number of samples analysed; DM : dry matter.

samples collected at market level. This could be due to the fact that the samples collected from processing sites were freshly made while the ones collected at market level were probably old. Previous studies on *Lanhouin* and *Momone*, a *Lanhouin* like-product have shown that a potential problem of these products is the continuous bacterial and enzymatic activity after processing, leading to the rise of pH values during storage (Anihouvi et al., 2006). These pH values recorded agree with those reported on *Lanhouin* samples from cassava fish (7.3) and king fish (7.6) (Anihouvi et al., 2006). The pH values of both fish *Lanhouin* were slightly higher than those of *Momoni* (6.47-6.56) (Sanni et al., 2002) and *Adjuevan* (5.20-6.10) (Koffi-Nevry et al., 2011).

The salt contents of *Lanhouin* samples varied from 19.3 to 26.6 g/100g DM for cassava fish *Lanhouin* and 18.7 to 23.5 g/100 g DM for king fish *Lanhouin*. The salt content of cassava fish *Lanhouin* collected at market level was significantly lower ($p < 0.05$) than that of cassava fish *Lanhouin* collected from processing sites. But for king fish, no significant difference was observed between the salt content of *Lanhouin* samples collected from processing sites and that of *Lanhouin* samples collected at market level. The variations in salt contents could be attributed to the fact that the amount of salt used during processing is not standardized, so varied from one processor to another (Kindossi et al., 2012). The values of salt content were slightly higher than those reported during a previous study (14.63 g/100 g DM for cassava fish and 10.42 g/100 g DM for king fish *Lanhouin*) collected in the Atlantic municipalities of Benin (Anihouvi et al., 2006); but these values agree with the salt contents of 25 g/100 g DM and 20.1 g/100 g DM obtained on the laboratory samples of *Lanhouin* made with cassava fish and king fish respectively (Dossou-Yovo et al., 2011).

The protein contents varied from 49.3 to 53.8 g/100 g DM and 49.2-52.6 g/100 g DM in all *Lanhouin* samples prepared with cassava fish and king fish respectively. For both fish, there was no significant difference ($p > 0.05$) between the protein content of market *Lanhouin* and that of *Lanhouin* collected from processing sites. The protein values obtained for all samples agree with those reported in *Lanhouin* samples obtained with cassava fish (46.9–59.3 g/100 g DM) and king fish (48.2 to 68.2 g/100 g DM) (Anihouvi et al., 2006) and in *Momoni* in general (36.0-49.1 g/100 g DM) (Sanni et al., 2002) and *Momoni* samples made with king fish (49.0 to 57.7 g/100 g DM) (Nketsia-Tabiri and Sefa-Dedeh, 2000).

The lipid contents varied from 10.8 to 12.2 g/100 g DM and 42.8 to 47.4 g/100 g DM for cassava fish *Lanhouin* and king fish *Lanhouin* samples respectively. For each species of fish there was no significant difference ($p > 0.05$) between the lipid contents of all *Lanhouin* samples collected from market and from processing sites. But the lipid contents of king fish *Lanhouin* were significantly ($p < 0.05$) higher than those of cassava fish *Lanhouin*. The difference in lipid contents of *Lanhouin* samples prepared with the two species of fish is due to the fact that king fish is a fatty fish while cassava fish is a lean fish (Huss, 1988; Love, 1997).

Thiobarbituric acid (TBARS) contents ranged between 25.5 and 27.1 mg malonaldehyde/kg DM for cassava fish *Lanhouin*, and between 24.8 and 26.7 mg malonaldehyde/kg DM for king fish *Lanhouin* samples. No significant difference ($p > 0.05$) was observed in the thiobarbituric acid contents for all *Lanhouin* samples whatever the species of fish used. Thiobarbituric acid numbers provides an indication of onset of lipid oxidation (Hernández-Herrero et al., 1999). The current values of thiobarbituric acid recorded on *Lanhouin* samples were higher than values of 10.6 to 12.2 mg malonaldehyde/kg

Table 2. Organic acids composition of *Lanhouin* samples collected from markets and processing sites (Means±Standard deviation).

Organic acids (mg/g DM)	Cassava fish		King fish	
	Processing sites (n=18)	Market (n=12)	Processing sites (n=18)	Market (n=12)
Lactic acid	2.9±2.8 ^a	1.5±1.9 ^a	4.3±1.5 ^a	3.5±0.9 ^a
Citric acid	1.9±2.3 ^a	1.9±2.0 ^a	1.3±1.7 ^a	0.6±0.9 ^a
Malic acid	1.1±2.1 ^a	1.2±1.9 ^a	0.5±1.2 ^a	0.4±0.7 ^a
Formic acid	1.2±1.6 ^a	0.9±1.9 ^a	0.5±0.5 ^a	1.0±1.8 ^a
Acetic acid	5.6±1.4 ^a	4.7±2.2 ^a	4.6±1.2 ^a	4.6±1.7 ^a
Propionic acid	1.3±1.8 ^a	1.1±1.4 ^a	3.3±4.8 ^a	2.4±1.4 ^a

^{a,b,c}: Means with different letters according to each row and each species of fish are significantly different ($p < 0.05$); n: number of samples analysed; DM: dry matter.

for cassava fish *Lanhouin*, and 16.6 to 21.7 mg malonaldehyde/kg DM for king fish *Lanhouin* reported by Anihouvi et al. (2006). However, lower values in TBARS of 6.9-7.4 mg malonaldehyde/kg DM have been reported by Oduor-Odote and Obiero (2009) on smoked fish. The total volatile nitrogen (TVN) contents of samples varied from 453.6 to 457.9 mg N/100 g DM for cassava fish *Lanhouin*, and 553.6 to 618.6 mg N/100 g DM for king fish *Lanhouin*. No significant differences ($p > 0.05$) were observed for the TVN contents of all *Lanhouin* samples within and between species. Level of TVN in fish is generally used as spoilage indicator due to bacterial and enzymatic action, leading to proteins degradation and a low nutritional value of the end product (Anihouvi et al., 2006; Hernández-Herrero et al., 1999). These values of TVN agree with those reported by Anihouvi et al. (2006) in *Lanhouin* samples made with cassava fish (530.5–650.0 mg N/100 g DM) and king fish (827.6-898.2 mg N/100 g DM).

The acidity index contents in cassava fish *Lanhouin* (1.7 to 1.8 g oleic acid/100 g DM) were lower than those of king fish *Lanhouin* (4.3 to 4.9 g oleic acid/100 g DM). For each species no significant difference ($p > 0.05$) was recorded for the acidity index of all *Lanhouin* samples. These values of acidity index were lower than those reported for a previous study on market samples of *Lanhouin* (Anihouvi et al., 2006). High acidity index content is an indication of microbial and enzymatic spoilage such as lipases activities (Anihouvi et al., 2006; Hernández-Herrero et al., 1999). The acceptable limit of acidity index is about 0.5-1.5 oleic acid/100 g (Saritha and Patterson, 2012). Moreover, according to Daramola et al. (2007), in most fish oils, the rancidity is noticeable when the acidity index is between 0.5-1.5 oleic acid/100 g. The organic acids composition in *Lanhouin* is summarized in Table 2. Levels of lactic acid varied from 1.5 to 2.9 mg/g DM and 3.5 to 4.3 mg/g DM for cassava fish *Lanhouin* and king fish *Lanhouin* respectively. No significant difference ($p > 0.05$) was recorded for lactic acid contents in all *Lanhouin* samples collected from market

and processing sites within species but significant difference ($p < 0.05$) was noted between species. Lactic acid contents in *Lanhouin* samples analysed were lower than those reported in other salted and fermented fish products (16 mg/g DM) (Kuda et al., 2002), salted fish (48.10 mg/g DM), fish sauce (aji-no-susu) (57.14 mg/g DM) (Kuda et al., 2009). Levels of acetic acid varied from 4.7 to 5.6 mg/g DM and 4.6 mg/g DM were determined for cassava fish and king fish *Lanhouin* samples respectively. The levels of acetic acid contents were higher than other organic acids determined in the *Lanhouin* samples. These values of acetic acid are in agreement with those reported by Essuman (1992) in fermented fish from Mali. No significant difference ($p > 0.05$) was recorded for acetic acid contents in all *Lanhouin* within and between species. Other organic acids such as citric, malic, formic and propionic acids were also detected in all *Lanhouin* samples, although their amounts were very low.

Various biogenic amines including histamine, putrescine, cadaverine and spermidine were detected in variable amounts in the *Lanhouin* samples analysed (Table 3). Histamine content in cassava fish *Lanhouin* varied from 10.1 to 33.0 mg/100 g. No significant difference ($p > 0.05$) was observed for histamine contents in all cassava fish *Lanhouin*. Independently to sampling place, histamine contents less than 20 mg/100 g was obtained in 87% of *Lanhouin* samples made from cassava fish (*Pseudotolithus* sp.) while 3 and 10% of samples showed histamine levels ranging between 20-40 mg/100 g and exceeding 40 mg/100 g respectively. Regarding *Lanhouin* samples obtained from king fish (*Scomberomorus tritor*), their histamine contents varied from 23.7 to 31.2 mg/100 g. The histamine contents in king fish *Lanhouin* collected from market was not significantly different ($p > 0.05$) with those of king fish *Lanhouin* collected from processing sites. According to the European Union regulation (CE n°853/2004), for fishery products which have undergone enzyme maturation treatment, on 9 samples analysed, the average histamine

Table 3. Biogenic amines determined in *Lanhouin* samples collected from markets and processing sites (mean \pm standard deviation).

Biogenic amines (mg/100 g ww)	Cassava fish		King fish	
	Processing sites (n=18)	Market (n=12)	Processing sites (n=18)	Market (n=12)
Histamine	33.0 \pm 23.8 ^a	10.1 \pm 3.4 ^a	31.2 \pm 14.6 ^a	23.7 \pm 13.0 ^a
Cadaverine	165.1 \pm 25.0 ^a	170.5 \pm 31.3 ^a	323.8 \pm 141.2 ^a	104.5 \pm 24.0 ^a
Putrescine	33.9 \pm 6.0 ^a	29.1 \pm 6.2 ^a	37.2 \pm 6.3 ^b	16.1 \pm 4.5 ^a
Spermidine	56.5 \pm 23.1 ^a	77.2 \pm 25.9 ^a	47.4 \pm 8.7 ^a	69.6 \pm 36.9 ^a

^{a,b}: Means with different letters according to each row and each species of fish are significantly different ($p < 0.05$); n: number of samples analysed; ww: wet weight basis.

Table 4. Microbial quality of *Lanhouin* samples collected from markets and processing sites (Means \pm Standard deviation).

Parameter (Log CFU/g)	Cassava fish		King fish	
	Processing sites (n=18)	Market (n=12)	Processing sites (n=18)	Market (n=12)
Total viable count	3.7 \pm 0.6 ^a	4.0 \pm 0.7 ^a	3.6 \pm 0.5 ^a	4.2 \pm 0.2 ^a
<i>Enterobacteriaceae</i>	<1 ^a	1.6 \pm 1.4 ^b	<1 ^a	<1 ^a
<i>Escherichia coli</i>	<1 ^a	<1 ^a	<1 ^a	<1 ^a
<i>Bacillus cereus</i>	1.7 \pm 0.9 ^b	<1 ^a	1.6 \pm 0.9 ^b	<1 ^a
<i>Staphylococcus aureus</i> and CPS	1.6 \pm 0.5 ^a	1.9 \pm 0.2 ^a	1.8 \pm 0.4 ^a	1.5 \pm 0.3 ^a
<i>Clostridium perfringens</i>	1.0 \pm 0.8 ^a	<1 ^a	<1 ^a	1.4 \pm 1.3 ^a
Yeasts and moulds	1.4 \pm 1.0 ^a	1.7 \pm 0.9 ^a	<1 ^a	1.8 \pm 1.0 ^b
Lactic acid bacteria (LAB)	1.0 \pm 0.9 ^a	1.2 \pm 0.9 ^a	1.2 \pm 0.9 ^a	<1 ^a
Coagulase negative <i>Staphylococci</i> (CNS)	3.5 \pm 1.3 ^a	2.9 \pm 0.6 ^a	3.9 \pm 1.0 ^b	3.2 \pm 0.6 ^a
<i>Listeria monocytogenes</i> *	Absent	Absent	Absent	Absent
<i>Salmonella</i> *	Absent	Absent	Absent	Absent

^{a,b}: Means with different letters according to each row and each species of fish are significantly different ($p < 0.05$); n: number of samples analysed; * search in 25 g sample.

content must be 20 mg/100 g or less; no more than 2 samples may have levels between 20 mg and 40 mg/100 g; and no sample may have a level above 40 mg/100 g. In this respect, 67% of king fish *Lanhouin* contained histamine levels less than 20 mg/100 g, 3% of them had histamine contents of 20 mg/100 g, and 13% had histamine contents ranging between 20-40 mg/100 g, while 17% showed histamine levels higher than 40 mg/100 g. Summarizing, both *Lanhouin* samples collected from processing sites and markets do not comply with the regulatory indicated above in terms of histamine content. These results showed that the type of fish can impair the production of histamine. Moreover, in a previous study (Anihouvi et al., 2006) where *Lanhouin* samples were purchased from the processors and from the markets, the histamine contents in the majority (75%) of samples, mainly the ones prepared with king fish, exceeded the recommended level of 20 mg/100 g stipulated by the Australian Food Standards Code (AFSC, 2001). Furthermore, the levels of putrescine,

cadaverine and spermidine found in the *Lanhouin* samples during the current study were high. This may increase the toxic effect of histamine as both putrescine, and cadaverine are known to potentiate histamine toxicity (Lehane and Olley, 2000; Houicher et al., 2013; Tsai et al., 2006); also the toxicity of histamine can be increased by the presence of other biogenic amines (spermine, spermidine, dopamine and agmatine) which can have a synergistic effect (Duflos, 2009; FAO/WHO, 2012). Concerning putrescine and cadaverine no safe levels have been not yet set for human consumption (FAO/WHO, 2012).

Microbial population of *Lanhouin* samples

The microbial population of *Lanhouin* samples summarized in Table 4 revealed that the total viable counts (TVC) of these samples varied from 3.7 to 4.0 Log cfu/g and 3.6 to 4.2 Log cfu/g for all *Lanhouin* processed

with cassava fish and king fish respectively. Such levels of TVC were within the acceptable limit of 5 Log cfu/g (ICMSF, 2011; Fernandes, 2009). No significant difference was observed for TVC recorded in all Lanhouin samples within and between species. *Enterobacteriaceae*, considered as faecal contamination indicator were detected in few numbers (<1-1.6 Log cfu/g) in 20% of market samples obtained from both King fish and cassava fish, while *E. coli* count (Log cfu/g) was lower than 1 for all the samples. For fish and meat products, the authorised limit about *Enterobacteriaceae* is 3 Log cfu/g (ICMSF 2011). Thus, all the Lanhouin samples comply with this regulation. Similarly, low counts (Log cfu/g) of *B. cereus* ranging between less than 1 and 1.7 Log cfu/g were enumerated in 34% of samples. *S. aureus* and other Coagulase Positive *Staphylococcus* (CPS) were also detected in all the samples, and their loads varied from 1.5 to 1.9 Log cfu/g. The authorized level of *S. aureus* and CPS stipulated by European Commission for fish products is between 2 and 3 Log cfu/g (EC/n°2073 2005). In this regard, all the Lanhouin samples comply with this regulation, with 72% of Lanhouin samples having *S. aureus* and CPS loads less than 2 Log cfu/g and only 28% of Lanhouin samples with loads between 2 and 3 Log cfu/g. The relatively high level of *S. aureus* in some Lanhouin samples was probably due to the lack of good manufacturing practices and the quality of salt used, since previous studies by Anihouvi et al. (2006) showed the absence of *S. aureus* in Lanhouin and Momone, a Lanhouin-like Ghanaian fermented fish. Indeed, according to Kindossi et al. (2012), solar salt is the main type of salt used for salting in the sampling zones and solar salt is known for its poor microbiological quality (Plahar et al., 1999). According to Varnam and Evans (1991) *S. aureus* load can reach high levels of 5 Log cfu/g in products prepared under bad hygienic conditions and can cause food poisoning (ICMSF, 1986). Yeasts and moulds were enumerated in 67% of samples with loads ranging between less than 1 and 1.8 Log cfu/g, while Lactic acid bacteria (LAB) were found at a level of 2.2 Log cfu/g in 42% of samples. As expected, Coagulase Negative *Staphylococci* (CNS) was found in all Lanhouin samples (2.9-3.9 Log cfu/g). In contrast, pathogenic bacteria such as *Salmonella* and *L. monocytogenes* were not detected in any sample.

Correlation between physico-chemical and microbiological characteristics of Lanhouin samples

The NaCl contents of samples analysed were significantly ($p<0.05$) and negatively correlated with water activity ($r = -0.78$) and pH ($r = -0.67$). These correlations indicate that NaCl was used in order to reduce water activity (a_w) and pH. These actions of NaCl retard or eliminate the growth of proteolytic bacteria during fermentation. It was reported

that variable levels of salt can reduce considerably a_w and decrease pH (Nout, 2001; Kose and Hall, 2011).

The protein contents of Lanhouin samples were significantly and negatively correlated with dry matter ($r = -0.72$, $p<0.05$) and *Enterobacteriaceae* ($r = -0.64$, $p<0.05$), and yeast and moulds ($r = -0.69$, $p<0.05$); and significantly and positively correlated with Coagulase Negative *Staphylococcus* (CNS) ($r = 0.68$ $p<0.05$). These correlations indicate that the reduction of protein value in the product was due to the protein degradation by the proteolytic activity of these microorganisms.

The lipid contents were significantly and positively correlated with TVN ($r = 0.80$, $p<0.05$), acidity index ($r = 0.97$; $p<0.05$) and propionic acid ($r = 0.76$; $p<0.05$). These high correlations indicate that fish lipids are highly unsaturated fatty acids (Halamíčková and Malota, 2010; Rael et al., 2004) and therefore, can be easily oxidized (Vidotti et al., 2011). Oxidation can affect the nutritional quality of the product by making the proteins and amino acids unavailable and also making the product unpalatable. These results showed that high lipid content carries away an increase in TVN and propionic acid contents of Lanhouin samples.

The lactic acid content was significantly and negatively correlated with yeast and moulds ($r = -0.72$, $p<0.05$), and *Enterobacteria* ($r = -0.79$, $p<0.05$), and positively correlated with CNS ($r = 0.71$, $p<0.05$). These higher correlations indicated that the lactic acid produced during the fermentation has an inhibitory effect on *Enterobacteria*, and yeast and moulds, but its presence promotes the development of CNS. These correlations confirmed the inhibitory effect of organic acids on pathogenic microorganisms (Rhee et al., 2011; Hall, 2002).

TBARS content was significantly and positively correlated with *B. cereus* counts ($r = 0.97$, $p<0.05$) and significantly and negatively correlated with yeasts and moulds counts ($r = -0.70$, $p<0.05$) and negatively correlated with lipid but not significantly ($r = -0.26$, $p>0.05$). These correlations indicate that lipid oxidation is not dependent up only on lipid substrate but depends also on microbial activities. The same result was observed as relation between TBARS and lipid during the processing of herring (Undeland et al., 1998; Undeland and Lingnert, 1999). Yeast and moulds reduce lipid oxidation and consequently TBARS contents in Lanhouin. Other studies showed that fungi and yeast were used to reduce lipid oxidation during the processing of fermented fish meal (Khodanazary et al., 2013; Yano et al., 2008). Histamine was significantly and positively correlated ($p<0.05$) with cadaverine ($r = 0.67$). This correlation indicates that the toxicity of histamine was influenced by the presence of cadaverine. This is in agreement with the findings of Naila et al. (2010) and Kose and Hall (2011) who reported that the toxic effect of histamine is increased by the presence of other biogenic amines such

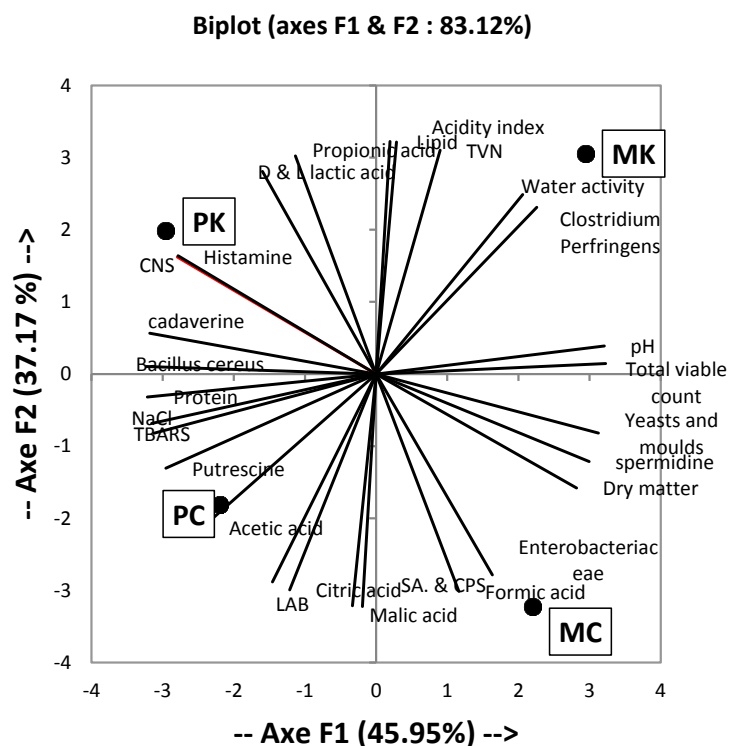


Figure 1. Principal component analysis on physico-chemical characteristics and microflora of *Lanhouin* samples collected from markets and processing sites. TBARS = Thiobarbituric acid reactive substances; TVN = total volatile nitrogen, TVC = total viable count; SA&CPS = *Staphylococcus aureus* and Coagulase Positive *Staphylococci*; CNS = Coagulase-Negative *Staphylococci*, LAB = lactic acid bacteria, MK = King fish *Lanhouin* from market, MC = Cassava fish *Lanhouin* from market; PC = Cassava fish *Lanhouin* from processing site, PK = king fish *Lanhouin* from processing site.

as cadaverine and putrescine. *B. cereus* was significantly and positively correlated with spermidine ($r = 0.71$, $p < 0.05$) and positively correlated with histamine but not significantly ($r = 0.44$, $p > 0.05$). But yeast and moulds was significantly and negatively correlated with putrescine ($r = -0.76$, $p < 0.05$). These correlations indicate that *B. cereus* contributes to the formation of histamine and spermidine, but the presence of yeasts and moulds prevent the production of putrescine in *Lanhouin*. These observations are in agreement with Moreno-Arribas et al. (2000) and Kim et al. (2009) who reported a correlation between these biogenic amines indicated above and the presence of lactic acid bacteria during wine making, and the presence of *enterobacteria* during fish storage at a temperature greater than 4°C respectively. Similar observations were reported by various authors who indicated that some lactic acid bacteria and other microorganisms such as *Acinetobacter*, *Aeromonas*, *Bacillus*, *Clostridium*, *Escherichia* and *Pseudomonas* possessed amino acid decarboxylase activity and took

part to the production of biogenic amines in fermented foods (Bover-Cid et al., 2001; Moreno-Arribas et al., 2000; Moreno-Arribas and Carmen Polo, 2008; Ntchimani et al., 2008; Kim et al., 2009).

Principal correspondence analysis on physico-chemical and microbiological characteristics of *Lanhouin*

The principal correspondence analysis (PCA) performed on the physico-chemical and microbiological characteristics of *Lanhouin* samples resulted in two axes accounting for 83.12% of the total variation, of which 45.95% was explained by the first axis (axis F1) and 37.17% by the second (axis F2) (Figure 1). Regarding the first axis, the cassava fish *Lanhouin* (MC) and the king fish *Lanhouin* collected from market (MK) were located in the right-hand part of the Figure 1, were the most dried samples, have the highest count of *Clostridium*,

Enterobacteria, *S. aureus* and CPS, and yeasts and moulds, and the highest content of citric acid, malic acid, formic acid and spermidine. This suggested that the market Lanhouin samples contained more spoilage microorganisms due to the fact that markets Lanhouin were more handled by the sellers and the customers at the selling places during purchasing.

Regarding the second axis, the cassava fish Lanhouin and the king fish Lanhouin collected from processing sites mainly located in the left hand part of the Figure 1, have the highest contents of histamine, cadaverine, putrescine; protein, TBARS, NaCl, acetic acid and the highest loads of CNS, lactic acid bacteria, *Bacillus cereus*. Among this group of micro-organisms, numbers are considered as biogenic amines producers (Kim et al., 2009; Suzzi and Gardini, 2003; Halasz et al., 1994). In addition, the highest contents of histamine in newly processed Lanhouin collected from processing sites could also be attributed to the fact that the amount of biogenic amines formed is influenced by factors such as the availability of free amino acids, which level may be high in newly processed Lanhouin than old and more dried Lanhouin collected from markets, although the water activity level of both Lanhouin samples appeared similar (Kerr et al. 2002).

Conclusion

The total viable counts of all Lanhouin samples are within acceptable limit. However, the presence of some bacteria such as *Clostridium perfringens* even though in few numbers in some samples showed that Lanhouin processing, handling and selling conditions need to be improved. The study also showed that protein, lipid and acidity index contents in Lanhouin samples were within acceptable limits but thiobarbituric acid (TBARS) contents were high as a reflection of some forms of spoilage. In addition, the pH values of the majority of samples were above 7. Acetic, citric and lactic acids are the predominant organic acids present in Lanhouin samples. For all Lanhouin samples the water activity (*a_w*) values were slightly above 0.70. These levels of *a_w* are relatively low to prevent enzymatic activity and microbial proliferation including food poisoning bacteria during storage. So, for the reengineering activities, one of the suitable manners to upgrade the quality of Lanhouin is to find a way to lower the pH of Lanhouin during processing. Low pH combined with low *a_w* and appropriate packaging could allow the production of safe Lanhouin and improve as well the preservation of Lanhouin during storage.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Sensory evaluation of wild-captured and pond-raised Tilapias in Malawi

Allain S. Joram¹ and Fanuel Kapute^{2*}

¹Bunda College Campus, Lilongwe University of Agriculture and Natural Resources, P. O. Box 219, Lilongwe, Malawi.

²Department of Fisheries Science, Mzuzu University, P/Bag 201, Luwingu, Mzuzu 2, Malawi.

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A sensory evaluation study was carried out to answer the question: "Are pond raised fish inferior to wild fish?" Lake Malawi Chambo - Tilapia (*Oreochromis karongae*) and Shire River Tilapia (*Oreochromis shiranus*) were processed into various products. Panelists evaluated preferences of the fish based on four sensory attributes namely: flavour, smell, taste and texture using a blind sample scoring. Results demonstrated that consumers were indifferent in their preference for fish from the wild and pond raised ($P>0.05$) suggesting that processed fish from both sources are equally liked. Acceptability of wild and pond raised *O. karongae* increased for processed products ($P<0.05$) while processed *O. shiranus* was liked indifferently irrespective of source ($P>0.05$). The study concludes that the disparity that consumers have regarding preference between wild and pond raised tilapia could merely be subjective, and hence, rejecting the hypothesis that pond raised fish are inferior to wild fish. A further observation is that presenting pond raised fish in a processed form could help in removing consumer bias consistent with sensory acceptability of food thereby improving its marketability and consequently, profitability. Processing also aids in value adding to realize more economic and nutritional benefits from fish. Findings from this study could be useful in planning and designing efficient marketing strategies for promotion of farmed fish.

Key words: *Oreochromis karongae*, *Oreochromis shiranus*, consumer behavior, fish presentation, unprocessed fish.

INTRODUCTION

Tilapias are the most important and commonest farmed fish species in Malawi due to the ease of management. They require cheap and locally available feed hence mostly raised by small scale and low income farmers.

The experimental fish in this study, *Oreochromis shiranus* and *O. karongae* are two of the four tilapia species for aquaculture in Malawi, others being *O. mossambicus* and *Tilapia rendalli*. Though fish production from aquaculture

*Corresponding author. E-mail: fkapute@gmail.com Tel: +265 999 916110.

is growing at an increasing rate due to many factors such as the declining catches from the wild versus increasing population, pond raised fish still receive a comparatively low rating from consumers than wild fish. There is a widely accepted traditional belief among consumers in Malawi (pers. obs) and elsewhere (Gaviglio and Demartini, 2009; Vanhonacker et al., 2013; Claret et al., 2012, 2014) that farmed fish do not taste as good as fish from the wild and also that fish from ponds have a muddy smell.

The general consumer attitude and myths is that pond raised fish are inferior to lake (wild) fish also reported by Schlag and Ystgaard (2013). Such assertions are counterproductive because consumer acceptance is crucial for market success of aquaculture produce (Vanhonacker et al., 2013). Pond raised fish thus, attract lower market prices than fish from the wild and hence removing the incentive of investing into commercial aquaculture in Malawi. It is known nevertheless, that knowledge about a product is the major motivational factor for purchasing functional foods (Frewer et al., 2003), while lack of knowledge is the major reason of not consuming functional foods (IFIC, 1999). Claret et al. (2014) noted that people perceive clear differences between farmed and wild fish and, that beliefs related to quality favor wild fish, while those related to availability and price favor farmed fish. This is the pattern of challenges also reported by Schlag and Ystgaard (2013) that when consumers only consider non-scientific concerns such as trust and nature, they prefer wild to farmed fish. That explains why consumer preference of farmed fish products may hinder production and marketing of fish from aquaculture. The reason given is that consumers have less trust in the production and consumption of farmed fish than in wild fish, perceiving the former as unnatural and unfamiliar. Schlag and Ystgaard (2013) suggests that it is imperative to incorporate moral and ethical risk dimensions in communication because these are the important aspects that influence consumer preference of wild over farmed fish.

This study was therefore carried out to compare consumer preferences between processed products from two indigenous tilapia fish species collected from the wild (lake) and pond raised.

MATERIALS AND METHODS

Preparation of recipes

The study used fish of average weight 150 g. *O. karongae* (Lake Malawi Chambo) and *O. shiranus* (Shire River tilapia) were obtained from Lake Malawi and Bunda College Aquaculture Fish ponds, respectively. The fish were washed in clean water, scaled and eviscerated and prepared as meatballs, fillets and whole fish cut into pieces which were later deep fried in edible cooking vegetable oil. Some of the cut pieces were boiled.

Sensory evaluation

A total of pre-trained 30 panelists (college students) of ages between 19 and 24 were invited to carry out a sensory evaluation of the prepared fish recipes and indicate their preferences on four sensory attributes namely: taste, color, flavor and texture. After training and pre-testing, panelists were screened based on their consistency to score the samples. Recipes were arranged on tables in a Nutrition Laboratory assigned blind random codes such as A, X, C, W etc. covering all samples to avoid bias. Each panelist was asked to taste each recipe by rinsing their mouths with water before and after each taste (Oduor-Odote et al., 2010; Daramola et al., 2007), as well as using a different fork between tastes to avoid interactions of the sensory characteristics. Panelists explained their sensory judgments by way of filling a designed form after the tasting and before moving on to the next sample (recipe) from the hedonic scale. The scores on the hedonic scale ranged from 1-5 as 1 = Dislike extremely, 2 = Dislike moderately, 3 = Neither like nor dislike, 4 = Like moderately and 5 = Like extremely.

Ethical considerations

In order to adhere to consumer ethical conduct regarding eating of foods, panelists were well informed about the type of fish species which they would taste and the source of the fish including the mode of processing e.g. the type of cooking oil used. Panelists therefore freely accepted to be part of the study with full knowledge of the process.

Data analysis

Data were analyzed using SPSS for Windows version 15.0. Descriptive statistics: frequencies, percentages, and means were used to report the data. Sample means were compared using one way analysis of variance (ANOVA) at 5% level of significance.

RESULTS

Mean sensory scores for lake (wild) and pond raised *O. karongae* and *O. shiranus* are 3.726 ± 0.023 , 3.408 ± 0.0129 ; 3.505 ± 0.006 and 3.611 ± 0.020 respectively (Figure 1).

Results show significant differences ($P < 0.05$) in consumer preference between wild and farmed *O. karongae* (Tables 1 and 2; Figures 2 and 3). *O. shiranus* was liked indifferently irrespective of source ($P > 0.05$) (Figure 2). It was also observed that when *O. shiranus* was processed, consumer preference in flavor, smell, taste and texture did not differ ($P > 0.05$) between fish collected from the wild and pond raised. Meat balls and fillets from pond raised *O. shiranus* were favoured more compared to wild fish ($P < 0.05$) (Table 1).

Significant differences ($P < 0.05$) were observed in flavor and taste between wild and pond raised *O. karongae* but no differences in smell and texture ($P > 0.05$) (Figure 3).

Smell, flavour and taste did not contribute much to consumer preference of wild *O. karongae* ($P > 0.05$) while texture was the main driver for such ($P < 0.05$). For pond raised *O. karongae*, consumer preference was mainly

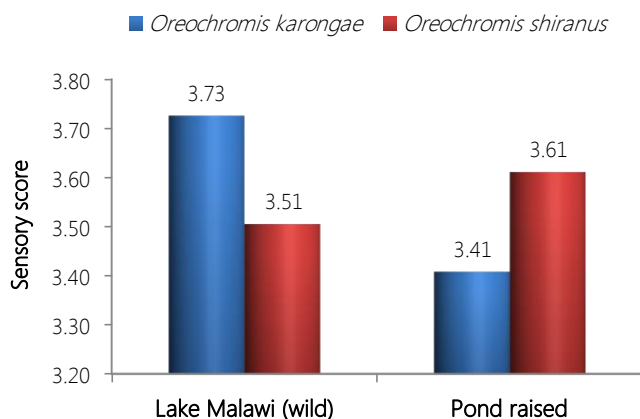


Figure 1. Mean sensory scores for wild and pond raised *Oreochromis karongae* and *Oreochromis shiranus*.

Table 1. Mean sensory scores for processed wild and pond raised *Oreochromis shiranus*.

Type of product	Source	Sensory score			
		Taste	Flavour	Smell	Texture
Boiled	Wild	3.96±0.16 ^a	4.00±0.15 ^a	3.82±0.17 ^a	3.96±0.20 ^a
Boiled	Pond	3.55±0.21 ^b	3.51±0.18 ^b	3.58±0.18 ^b	3.86±0.20 ^a
Fried	Wild	3.88±0.21 ^a	3.88±0.19 ^a	3.44±0.18 ^a	3.88±0.21 ^a
Fried	Pond	3.41±0.22 ^b	3.55±0.24 ^b	3.44±0.20 ^a	3.72±0.24 ^b
Meat balls	Wild	2.73±0.23 ^b	2.80±0.22 ^a	2.63±0.20 ^a	2.96±0.22 ^a
Meat balls	Pond	2.75±0.24 ^a	2.55±0.17 ^b	2.55±0.18 ^b	2.58±0.23 ^b
Filleted	Wild	4.35±0.10 ^a	4.10±0.16 ^a	3.96±0.21 ^a	3.96±0.20 ^a
Filleted	Pond	4.06±0.17 ^b	3.86±0.19 ^b	3.93±0.16 ^b	3.86±0.19 ^b

Means with same superscript in a column are significantly not different ($P>0.05$).

Table 2. Mean sensory scores for processed wild and pond raised *Oreochromis karongae*.

Type of product	Source	Sensory score			
		Taste	Flavour	Smell	Texture
Boiled	Wild	3.75±0.16 ^b	3.62±0.21 ^b	3.27±0.15 ^b	3.68±0.18 ^b
Boiled	Pond	4.32±0.18 ^a	4.25±0.20 ^a	4.32±0.17 ^a	4.39±0.18 ^a
Fried	Wild	3.55±0.21 ^b	3.42±0.20 ^b	3.29±0.20 ^b	3.32±0.21 ^b
Fried	Pond	3.75±0.22 ^a	3.60±0.21 ^a	3.60±0.19 ^a	3.92±0.19 ^a
Meat balls	Wild	2.24±0.19 ^a	2.44±0.19 ^a	2.93±0.20 ^a	2.93±0.23 ^a
Meat balls	Pond	2.06±0.17 ^a	2.31±0.19 ^a	2.24±0.14 ^b	2.82±0.22 ^a
Filleted	Wild	4.13±0.16 ^b	4.06±0.19 ^b	3.96±0.18 ^b	4.37±0.10 ^a
Filleted	Pond	4.62±0.15 ^a	4.44±0.13 ^a	4.10±0.18 ^a	4.10±0.20 ^b

Means for the same product with same superscript in a column are significantly not different ($P>0.05$).

influenced by taste, texture and flavour but not smell. Consumers neither liked nor disliked the smell/odor of wild and pond raised *O. karongae* (kite is pulled inside –

Figure 3). Without blind scoring, pond raised *O. karongae* would be rated low compared to wild. Interestingly, consumers highly liked the taste of pond raised *O.*

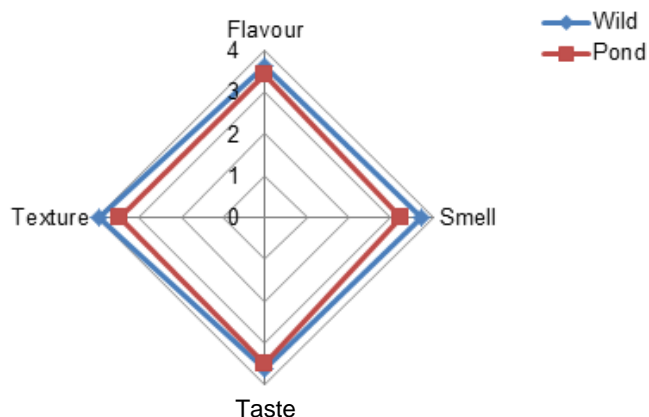


Figure 2. Consumer mean sensory scores for wild and pond raised *Oreochromis shiranus* flavor, smell, taste and texture.

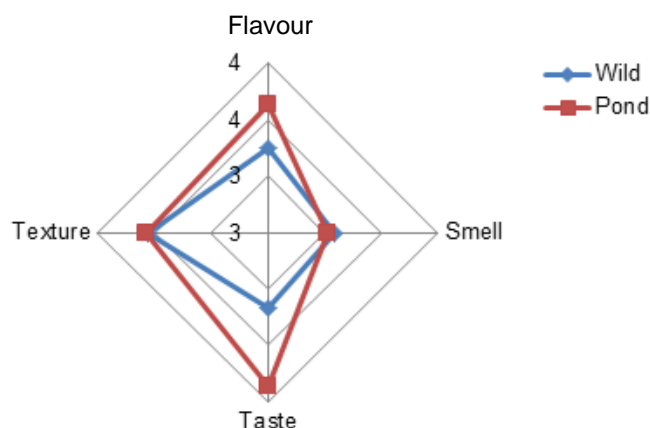


Figure 3. Consumer mean sensory scores for wild and pond raised *Oreochromis karongae*.

karongae (kite pulled outside - Figure 3) but were indifferent for wild *O. karongae*. With an exception of meat balls, boiled, fried and filleted pond raised *O. karongae* was significantly liked in all organoleptic attributes ($P < 0.05$) (Table 2).

Overall results nevertheless, show no significant differences in consumer preference between wild and pond raised fish ($P > 0.05$). The overall mean for all the sensory attributes was closer to 4 suggesting that the panelists liked moderately fish irrespective of the source from which they were collected.

DISCUSSION

If presented unprocessed, pond raised *O. shiranus* would

by far be out competed by the Lake Malawi Chambo (*O.s karongae*) because of its inferior appearance. A similar observation was reported by Gebrezgabher et al. (2015) that farm raised fish only attracted consumers in processed form. The fact that processed *O. shiranus* was liked indifferently irrespective of source (wild or pond), agrees with Costell et al. (2010) that consumer perception of food products and its acceptance or rejection is of a multi-factorial nature. Sensory characteristics present the strongest driver of fish consumption as well as one of the main barriers for acceptance of farmed fish (Claret et al., 2016). For example, Gaviglio and Demartini (2009) reported that a majority of the respondents thought that wild-caught product tastes better than farm-raised ones while yet others admitted not being able to distinguish the origin of fish by taste alone. This has been suggested to be one of the most common prejudices connected to farm-raised products. Similar findings have been reported by previous workers (Verbeke et al., 2007) that majority of the consumers do not perceive any differences between farmed versus wild fish concluding that consumers have difficulties in evaluating the quality of fish products. Possibly, this could point to the difficulty in explaining why consumer preference between wild and pond raised *O. karongae* was different while *O. shiranus* was liked indifferently irrespective of source. It is also not easy to provide an explanation for no differences in taste between wild and pond raised *O. karongae* but differences in taste and flavor. The multi-factorial nature of consumer food acceptance and rejection (Costell et al., 2010) and other factors may also have played a role for example, variations in the assessors' ability to judge the fish recipes. Disorders of taste and smell have proved difficult to diagnose and treat, often because of a lack of knowledge and understanding of these senses (<http://emedicine.medscape.com/article/861242/overview>). For example, one of the concerns reported by Lawless (1999) is whether odors and their mixtures are perceived as unitary or analyzable percepts. FAO (1998) stresses the need to assess the panelists before any sensory evaluation exercise. Notable areas of concern include but not least, whether the assessor is not anosmic (unable to perceive odors) - so that odors of decomposition and other defects will be perceived and described in a consistent manner. The assessor should also not be ageusic (unable to perceive basic tastes) - so that tastes associated with decomposition and other defects will be perceived and described in a consistent manner. These and many more attributes make sensory difficult and highly subjective. Kapute et al. (2012) observed that product declared unfit for consumption through sensory evaluation may still be nutritionally good. Overall results nevertheless, showed that there were no significant differences in consumer preference between wild and farmed (pond) fish suggesting that consumers

liked the fish moderately irrespective of the source from which they were collected. Results in this study agree with earlier reports where no significant differences were observed between wild and farmed fish (Jaffry et al., 2000; Vanhonacker et al., 2013; Claret et al., 2014). In fact, Vanhonacker et al. (2013) showed that fish origin does not seem to be a major issue or a criterion for differentiation and selection between wild and farmed fish. Apparently, this is contrary to what consumers would do without blind labeling of the samples. The assessment of blind-labeled product acceptability is one of the corner stone of sensory evaluation (Lawless and Heymann, 2010). Labeling which is usually by way of blind coding helps to remove subjectivity although it is known that blind coding of samples of products negatively biases taste perceptions and attitudes toward a food (Wansink et al., 2000). Consumer beliefs have significant influence regarding preference between farmed and wild fish. A generalized positive attitude towards wild fish appears to be the main driver of consumer beliefs about farmed versus wild fish. Claret et al. (2016) reported interesting findings that in the informed condition, participants preferred wild fish but scored the opposite when information was not provided to them. This suggests that consumers do not inherently dislike farmed fish.

CONCLUSION AND RECOMMENDATIONS

We conclude based on the study findings that discrimination of pond raised fish in Malawi is largely subjectivity of the consumers due to beliefs and thus, pond raised fish are as good as fish from the wild (lake). The hypothesis that pond raised fish are inferior to wild fish is therefore rejected. Study findings confirm challenges of using sensory evaluation as a sole method for testing superiority of foods and in this context - fish. Changing mindset of consumers is not a straight away issue. Results have also demonstrated that one way of avoiding consumer bias in pond raised fish is by processing into varied products. It is suggested that fish farmers should endeavor to sell their pond raised fish processed to favourably compete with fish from the lake. This could also be a way of value adding to realize more economic and nutritional benefits from fish. Findings could be useful in planning and designing efficient marketing strategies for promoting farmed fish.

Conflict of Interests

The authors have not declared any conflict of interest.

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Full Length Research Paper

Options for enhancing utilization of Jack beans (*Canavalia ensiformis*) in Tanzania

Bernadette M. Ndabikunze¹, Peter S. Mamiro^{1*}, George J. Ley² and Stewart J. Mwanyika¹

¹Department of Food Technology, Nutrition and Consumer Sciences, Sokoine University of Agriculture, Morogoro, Tanzania.

²Mlingano Agricultural Research Institute, Muheza, Tanga, Tanzania.

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Population increase is forcing mankind to look for alternative food sources from underutilized plants. Jack bean has been earmarked as one of these food sources. The only barrier for its utilization is the presence of inherent toxic compounds that should be removed, to make it edible to humans. A number of researchers have tried various ways in an effort to reach that goal. This study has also tried to perform a number of treatments on jack beans, which included soaking, treatment with trona (magadi soda) and germination. The samples of jack beans were brought from Mlingano Agricultural Research Institute and transported to the Sokoine University of Agriculture, Tanzania. Proximate analysis, mineral and phenolic compounds content were carried out on the treated samples. Acceptability tests were performed on products prepared from composite flour, made from 48 h. germinated jack beans. The products included porridges, breads and buns. Soaking results in lowering mineral concentrations. However, treatment with trona increased mineral profile. The levels of calcium, iron and zinc for the jack bean seeds analysed, gave 8.99, 3.83 and 1.76 mg/100 g, respectively. Proximate analysis revealed that moisture, protein, fibre, fat, ash and carbohydrate content were 4.6, 29.7, 5.2, 3.3, 3.4 and 53.9%, respectively. Phenolic compounds concentrations continued to decrease gradually with various treatments. Soaking had minimal effect in reducing phenolic compounds but, germination of jack beans for 48 h. had the highest (82%) reduction effect. There were significant differences ($P \leq 0.05$) in organoleptic properties with regards to breads and porridges but no significant differences ($P \geq 0.05$) in all organoleptic properties with respect to the buns. Panelists liked the buns much more compared to the breads and porridges. There were no complaints from all the panelists after consuming the jack bean based products. This outcome shows the potential of jackbean being used as a human food in the near future especially using germinated flour. The reports from researchers that jack beans are consumed by some ethnic groups and personal narrations of people who have consumed the beans, if followed up and comprehend the way they do their preparation, will be a great stride towards utilization of jack beans.

Key words: Jack beans, anti-nutritional factors, phenols, germination, soaking, trona.

INTRODUCTION

The population of the world is increasing at an alarming rate. Competition for food and feed for human and animal

consumption especially protein sources will continue to rise. Large population in developing countries suffers

varying degree of malnutrition because, protein rich food such as meat, milk, fish are expensive to acquire (Abitogun and Olasehinde, 2012; Adebowale et al., 2005). In addition, tropical developing countries are facing an increasing demand for protein rich foods due to increase in population, increased consumption of cereal based foods share and increased scarcity of fertile land. It is therefore imperative to look for other sources of proteins such as the underutilized legumes. One of these sources is *Canavalia ensiformis* commonly known as Jack bean. Raw *Canavalia* seeds contain about 300 g kg⁻¹ protein and 600 g kg⁻¹ carbohydrates (Rajaram and Janardhanam, 1992). Jack bean belongs to the kingdom Plantae, Division Magnoliophyta, Order Fabales, subsided Family Fabaceae/Leguminosae, Genus *Canavalia* and Species *C. ensiformis* (USDA, 2005). The genus *Canavalia* comprises 48 species of underutilized annual legumes widely distributed and indigenous to the tropics and they are rarely edible to man and under optimal agronomic conditions unless there are processed. However, India Doss et al. (2011a) reports that, mature beans are consumed by some tribal sects. In western countries this legume is used as a cover crop and the roasted seeds are ground to prepare coffee-like drink (Bressani et al., 1987). In Nigeria *C. ensiformis*, is currently grown as ornamental plant, planted near houses and allowed to trail on walls and trees, believed to repel snakes (Abitogun and Olasehinde, 2012). Many researchers have been investigating on how to make jack beans edible to human beings by using various treatments like heating, fermentation and extrusion but without obtaining favourable results (Justo et al., 1994). This is because there are a number of antinutritional factors present in jack beans, which restricts its utilization as human food. These factors include; thermo-stable factors (canavanine, concavalin, canavalin, canatoxin) and thermo-labile factors protease inhibitors, lectins and phytic acid (Carlini and Udedibie 1997; Udedibie and Carlini 1998).

Another study has been going on in Muheza district, Tanzania Mlingano Agricultural Research station on the use of *C. ensiformis* as green manure where the plant has shown potential in contributing about 98 kg N per ha to the soil and, increased maize yield from 1.4 to 2.5 t/ha and substantially reduced striga weed numbers (Figure 2). Okonkwo and Udedibie (1991) reported similar yields whereby a total yield of dry seeds reached up to 2.5 tons ha⁻¹. However, despite this outstanding performance by this technology, adoption by farmers around the research station has been very slow because farmers got discouraged for growing a crop that cannot be consumed. This study sought to look for options of utilization of jack beans for human consumption contrary to the way it is

being used now as green manure and cover crop.

METHODOLOGY

Raw materials and preliminary handling

About 40 kg of jack beans (Figure 3) were brought from Mlingano Agricultural Research Institute, Muheza, Tanzania and transported to Sokoine University of Agriculture, Department of Food Technology, Nutrition and Consumer Sciences. They were sorted by removing extraneous material. The beans were sealed into plastic bags and stored at room temperature ($\approx 25^{\circ}\text{C}$) until laboratory analysis. Acceptability studies were carried out on a number of recipes developed from jack beans. Various treatments were employed on jack bean seeds, in an effort to find ways that are normally practiced in households to make the beans utilizable. These treatments included soaking for varying time, boiling with/without outer coat removed, boiling with soda ash at various concentration and germination at varying times as presented in Table 1.

Oven dried jack bean seeds

About 2.0 kg of jack bean seeds were washed and oven dried at 105°C for 24 h.

Soaked jack bean seeds

A total of 8.0 kg of jack bean seeds were washed and soaked for 3 to 6 h. in distilled water to imbibe adequate water (ratio 1:4 bean: water) at room temperature ($\approx 25^{\circ}\text{C}$). About 2 kg each were given treatments as presented in Table 1. Decortication of the beans was done by hands after the soaking process.

Boiled jack bean seeds

About 12 kg of Jack beans were washed and boiled for 1 h (ratio 1:5 bean: water). The one hour was counted from when the water started to boil reaching a temperature of 100°C. About 2 kg each were given treatments as presented in Table 1.

Germinated Jack bean seeds

A total of 15.0 kg of jack bean seeds were washed and soaked for 8 h. (ratio 1:4 bean: water) in distilled water to imbibe adequate water at room temperature ($\approx 25^{\circ}\text{C}$). The seeds were then covered in a clean blotting paper germinated in the absence of light at a temperature of 37°C for 5 days. The germination process was stopped before the photosynthetic activity, by drying them in oven maintained at 105°C for 24 h. About 2 kg each were given treatments as presented in Table 1.

Proximate analysis of the samples

The bean samples for chemical analysis were ground to pass through a one millimetre screen in a Christy and Norris 20 cm laboratory Hammer Mill (London). Dry Matter percentage (%DM)

*Corresponding author. E-mail: petermamiro@yahoo.com, petermamiro@suanet.ac.tz.

Table 1. Sample Treatment

Sample	Sample treatment	Code
i	Control: Oven dried and milled	A
ii	3 h soaking, outer coat removed, oven dried and milled	B
iii	6 h soaking, outer coat removed, oven dried and milled	C
iv	3 h soaking, oven dried and milled	D
v	6 h soaking, oven dried and milled	E
vi	1 h boiled, outer coat removed, oven dried and milled	F
vii	1 h boiled, outer coat removed, oven dried and milled	G
viii	1 h boiled in 5 % Soda ash, oven dried and milled	H
ix	1 h boiled in 10 % Soda ash, oven dried and milled	I
x	1 h boiled in 5 % Soda ash, outer coat removed, oven dried and milled	J
xi	1 h. boiled in 10 % Soda ash, outer coat removed, oven dried and milled	K
xii	Bean seeds, soaked for 8 h., 24 h. germinated oven dried and milled	L
xiii	Bean seeds, soaked for 8 h., 36 h. germinated oven dried and milled	M
xiv	Bean seeds, soaked for 8 h., 48 h. germinated oven dried and milled	N
xv	Bean seeds, soaked for 8 h., 72 h. germinated oven dried and milled	O

was determined by drying the sample in an oven at 103 to 105°C for 24 h. Crude protein percentage (% CP) was determined by Kjeldahl method AOAC method No. 920.87 (AOAC, 1995) with the Kjeltex auto 1030 analyser, Tecator (Sweden) and percentage nitrogen obtained was used to calculate the % CP using the relationship: % CP = % N × 6.25 (Ahenkora et al., 1998). Ether extract percentage (% EE) was determined using the Soxhlet System HT- extraction technique AOAC method No. 922.06 (AOAC, 1995) and percentage ash (% minerals) was determined after the dry matter determination by incinerating the samples in a muffle furnace at 550°C for four hours. The ash was cooled in desiccators and then weighed. Crude fibre percentage (% CF) was determined by the fibre system and Weende method (AOAC, 1995). Nitrogen free extract percentage (%NFE) was calculated by difference: thus %NFE = 100 - (% moisture + %CP + % EE + % CF + % Ash).

Mineral content of the samples

Total mineral content of bean samples were carried out after dry ashing. The ashes of the various treated samples were dissolved in 10 ml of concentrated hydrochloric acid. Total calcium, iron and zinc were determined by atomic absorption spectrophotometer using an AOAC method No 970.12 (AOAC, 1995) and mineral concentrations were read from Shimadzu Atomic Absorption Spectrophotometer (AAS) (UNICAM 919, England).

Polyphenols in bean samples

About 0.5 g of milled bean sample was boiled with 20 ml distilled water at 100°C for 15 min. The resulting boiled sample was filtered using Whatman filter paper No. 1. Filtrate was then transferred to a 50 ml volumetric flask and diluted to 50 ml mark. 1ml of each sample extract and each standard concentration was taken into 100 ml volumetric flask in triplicate. Blank solution was prepared by taking 1 ml distilled water instead of the standard or sample. Exactly 70 ml of distilled water was added, followed by the addition of 5 ml of 2N Folin Ciocalteus reagent. The mixture was swirled and incubated at room temperature (25°C) for 10 min. Then 15 ml of Sodium Carbonate solution were added, diluted to 100 ml mark with distilled water and incubated for 2 h at room temperature.

Absorbance was read at 765 nm using Wagtech CECIL 2021 UV-visible spectrophotometer and the amount of polyphenols were calculated using the equation obtained from the standard plot (Gallic acid) obtained from Merk Schuchardt OHG, German.

Preparation of the taste panel products, acceptability studies and ethical clearance

Since it was risky to use Jack bean flour straight from the unprocessed seeds due to inherent toxicity, the products made from jack bean composite flour were porridges, buns and breads, which were obtained from jack bean that was germinated for 48 hours (Tables 1 to 3). This is because at 48 h the polyphenols were at their lowest level. The taste panel consisted of undergraduate students from the Department of Food Technology, Nutrition and Consumer Sciences. The undergraduate students have been trained to conduct sensory evaluations as part of their course and have been involved in several evaluations done frequently at the department. Ethical permission was provided by the Sokoine University of Agriculture.

Written consent was sought from the students, for their willingness to participate in the study. They were also informed of the possible effects that they might feel after ingesting the jack bean composite flour products such as feeling drowsy or tiredness. They were monitored for any physiological discomfort or any other ill feeling. The panellists were instructed to sip water before and after assessing each product. The panellists recorded sensory characteristics of each sample using a 7-point hedonic scale as described by (Lawless and Heymann, 2010) where 1 = like extremely, 2 = like very much 3 = like moderately, 4 = like slightly, 5 = neither like nor dislike, 6 = dislike slightly and 7 =dislike most. Variables tested were flavour, smell, taste, colour, and general acceptability.

The formulations, which were subjected to sensory evaluation included porridges, breads and buns (Tables 2, 3 and 4). The mixing ratio of either jack bean: maize or jack bean: wheat flour was 5, 10, 15, 20 and 25 to 95, 90, 85, 80 and 75, respectively. The total amount of the preparations was computed based on the aforementioned ratios. The composition of each preparation was based on how each formulation is often prepared in households.

Table 2. Preparation of composite flour (jack bean: maize) porridges.

Samples	Composition (%)			Code	
	Porridge	Bean flour	Maize flour		Total
Ratios (%)		5	95	100	JKPORRIDGE 0595
		10	90	100	JKPORRIDGE 1090
		15	85	100	JKPORRIDGE 1585
		20	80	100	JKPORRIDGE 2080
		25	75	100	JKPORRIDGE 2575

Table 3. Preparation of composite flour (jack bean: wheat) breads.

Samples	Composition (%)						Code	
	Breads	Bean flour	Wheat flour	Total	Yeast (g)	Salt (g)		Oil (ml)
Ratios (%)		5	95	100	10	7	20	JKBREAD0595
		10	90	100	10	7	20	JKBREAD 1090
		15	85	100	10	7	20	JKBREAD 1585
		20	80	100	10	7	20	JKBREAD 2080
		25	75	100	10	7	20	JKBREAD 2575

Table 4. Preparation of composite flour (jack bean: wheat) buns

Samples	Composition (%)						Code	
	Buns	Bean flour	Wheat flour	Total	Sugar (g)	Yeast (g)		Salt (g)
Ratios (%)		5	95	100	100	10	5	JKBUNS 0595
		10	90	100	100	10	5	JKBUNS 1090
		15	85	100	100	10	5	JKBUNS 1585
		20	80	100	100	10	5	JKBUNS 2080
		25	75	100	100	10	5	JKBUNS 2575

Data analysis

Data obtained were entered and subjected to statistical analysis using statistical package and service solution (SPSS) computer software version 16 to compute descriptive and inferential statistics. Descriptive statistics were run to obtain measures of central tendency. Analysis of variance of the results was done at 95% confidence interval ($P \leq 0.05$) using Turkey's Honestly Significant Difference. Homogeneity test was performed to determine homogenous sets.

RESULTS

Proximate composition of various jack bean treatments

The proximate composition of the various treatments employed on jack bean seeds are presented in Table 5: Moisture ranged from 3.83 ± 0.31 to $7.17 \pm 0.01\%$ while

protein ranged from 24.44 ± 0.03 to $29.69 \pm 0.07\%$. The fibre content varied a lot between treatments where the seed coat was removed and to those which the seed coat was retained. The fat, ash and carbohydrate contents did not differ much between treatments.

Mineral profile of various jack bean treatments

The mineral profile for the various jack bean treatments is presented in Table 6. Soaking resulted in lowering mineral concentrations. Removal of the outer coat further reduced mineral concentration probably due to leaching and some amount of minerals that were present in the seed coat. However, treatment with trona showed significant increase in minerals, which might have been contributed to the mineral composition of the trona. The increase was consistent with increase in trona concentration that is, 5% to 10%.

Table 5. Proximate composition of jack bean treated samples

Sample Code ¹	% Moisture	% Protein	% Fibre	% Fat	% Ash	% Carbohydrate
A	4.63 ± 0.02	29.69 ± 0.07	5.15 ± 0.28	3.28 ± 0.07	3.37 ± 0.01	53.88 ± 0.27
B	4.31 ± 0.02	28.2 ± 0.07	3.11 ± 0.13	3.20 ± 0.01	3.50 ± 0.01	57.51 ± 0.26
C	3.94 ± 0.05	28.07 ± 0.12	1.99 ± 0.02	3.18 ± 0.09	3.51 ± 0.02	59.30 ± 0.89
D	3.83 ± 0.31	26.07 ± 0.02	10.94 ± 0.49	3.39 ± 0.03	3.33 ± 0.11	52.43 ± 0.00
E	4.10 ± 0.25	29.57 ± 0.11	9.31 ± 0.00	3.21 ± 0.01	3.46 ± 0.03	50.31 ± 0.12
F	4.66 ± 0.45	25.07 ± 0.07	13.36 ± 0.66	3.38 ± 0.11	3.24 ± 0.01	50.27 ± 0.26
G	3.99 ± 0.05	28.21 ± 0.07	1.73 ± 0.34	3.21 ± 0.00	3.16 ± 0.05	59.68 ± 0.39
H	5.32 ± 0.00	29.4 ± 0.21	2.13 ± 0.14	3.27 ± 0.05	4.39 ± 0.01	55.45 ± 0.38
I	4.76 ± 0.02	25.02 ± 0.07	5.24 ± 0.63	3.28 ± 0.00	4.12 ± 0.04	57.56 ± 0.16
J	4.51 ± 0.17	29.33 ± 0.00	2.72 ± 0.07	3.95 ± 0.02	3.49 ± 0.01	55.98 ± 0.25
K	4.30 ± 0.17	28.01 ± 0.02	2.06 ± 0.21	3.14 ± 0.04	4.85 ± 0.44	57.61 ± 0.45
L	7.08 ± 0.01	25.87 ± 0.18	7.20 ± 0.14	3.15 ± 0.08	3.51 ± 0.09	53.17 ± 0.52
M	7.02 ± 0.01	24.82 ± 0.02	9.21 ± 0.02	3.21 ± 0.01	3.35 ± 0.00	52.36 ± 0.02
N	6.91 ± 0.03	25.47 ± 0.16	7.59 ± 0.49	3.33 ± 0.35	3.48 ± 0.09	53.20 ± 0.64
O	7.15 ± 0.01	24.44 ± 0.03	8.95 ± 0.09	3.14 ± 0.09	3.41 ± 0.07	52.88 ± 0.14

¹Check Table 1.**Table 6.** Mineral content of the various treated samples.

Sample Code ¹	Ca (ppm)	Fe (ppm)	Zn (ppm)
A	8.99±0.03	3.83±0.02	1.76±0.06
B	7.09±0.02	2.87±0.02	1.55±0.04
C	6.64±0.01	2.66±0.06	1.46±0.07
D	6.41±0.03	3.26±0.02	1.59±0.01
E	5.35±0.02	2.70±0.01	1.23±0.02
F	8.52±0.05	3.03±0.01	1.53±0.01
G	7.18±0.02	3.36±0.01	1.58±0.04
H	12.69±0.07	6.43±0.03	4.31±0.01
I	13.81±0.04	7.04±0.06	4.83±0.01
J	11.18±0.04	6.49±0.02	4.38±0.03
K	13.21±0.02	8.01±0.02	4.45±0.06
L	7.29±0.02	3.44±0.02	1.65±0.01
M	7.18±0.02	3.14±0.06	1.42±0.01
N	6.78±0.07	3.55±0.06	1.54±0.01
O	8.13±0.03	3.51±0.01	1.32±0.01

¹Check Table 1.

Phenolic compounds content of various jack bean treatments

The untreated sample had the highest phenol compounds (841 mg/100 g) as shown in Figure 1. With various treatments, the phenol concentrations continued to decrease gradually. Soaking had minimal effect compared to the control. However, with 3 h soaking the outer cover removed had a 50% phenolic reduction from the original concentration. Treatment with trona by boiling in 10% soda ash, outer coat removed, oven dried and milled showed even more reduction (72%) of phenolic

compounds. The 48 h germination showed a very significant reduction ($P \leq 0.05$) in phenolic compounds content compared to all other treatments reaching 150 mg/100 gm equivalent to 82% reduction. After the 48 h. germination the phenolic compounds seemed to gradually start to increase again.

Proximate composition of products made from composite flours

The proximate composition of the products made from

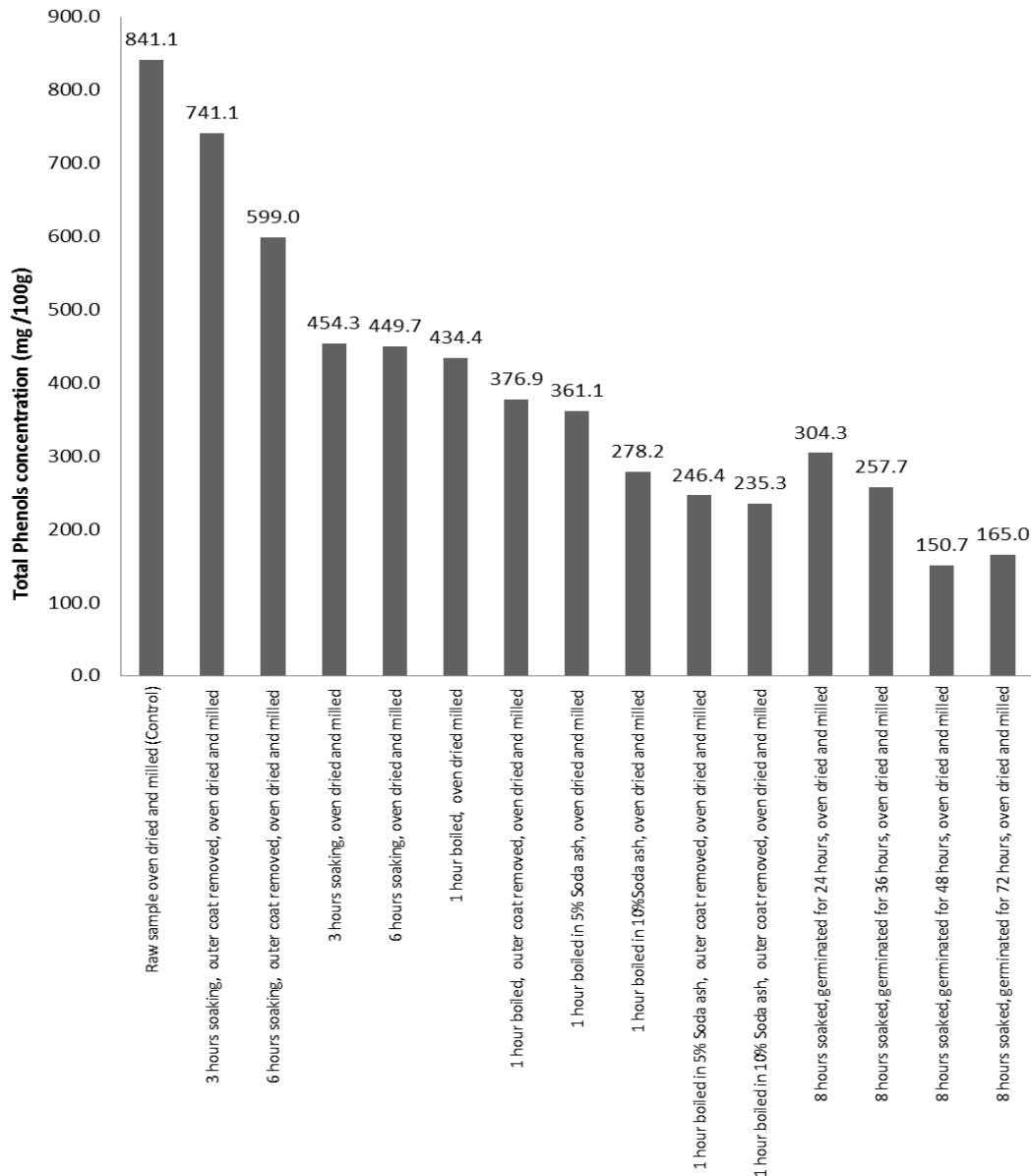


Figure 1. Phenolic compounds concentration for the various jack bean treatments.

composite flours from jack bean maize and wheat flour to prepare porridges, buns and breads are presented in Table 7. With regard to wheat flour and jack bean flour, they only had similar percent moisture, while the other proximate components differed significantly. The total phenols were significantly higher ($P \leq 0.05$) in jack beans compared to maize and wheat flour.

Sensory evaluation of products made from composite flours

Porridges JKPORRIDGE0595 and JKPORRIDGE 1585 were comparatively liked by the panellists, by giving an

average higher score 'liked very much' in all organoleptic attributes (Table 8). There were no significant differences ($P \geq 0.05$) in all porridges with respect to flavour and smell except for porridge JKPORRIDGE 2575. All the panellists scored Porridge JKPORRIDGE 2575 the least but most of them gave the porridge an average score of 4 'neither like nor dislike in flavour taste and general acceptability and disliked slightly the colour of the porridge.

There were varying results with regards to breads prepared with composite flours. Bread JKBREAD 0595, JKBREAD 1090, and C were not significantly different ($P \geq 0.05$) in all sensory attributes except Bread JKBREAD 1585 with respect to general acceptability (Table 10). The panellists gave Bread JKBREAD 2080



Figure 2. Jack bean plant in the field at Mlingano Agricultural Research Institute, Tanzania.



Figure 3. Jack bean seeds

and JKBREAD 2575 an average score ranging from 3 to 5 i.e. 'like moderately', 'like slightly', 'neither like nor dislike' and 'dislike slightly'. Bread JKBREAD 2575 was not appreciated by the panellists compared to the other breads since it was given an average score ranging 4 to 5 i.e. 'Like slightly', 'neither like nor dislike' and 'dislike slightly'. In all three products prepared, there was no panellist who came back with any physiological

discomfort or any other complaints of illness.

The panellists appreciated all the buns made from various combinations of the composite flours. The average score given ranged between 2 and 3 that is, 'Like very much and like slightly' (Table 9). There were no significant differences ($P \geq 0.05$) in all organoleptic properties. However, Buns JKBUNS 2575 were consistent in terms of decision by the panellists giving the

Table 7. Proximate composition, minerals and polyphenols for jack bean products (porridge, breads and buns).

S/N	Sample code ¹	Moisture %	Ash %	Fat %	Fibre %	Protein %	Calcium (mg)	Iron (mg)	Zinc (mg)	Total Phenols conc. (mg /100 g)
1	Wheat flour	10.9	0.5	0.8	12.2	9.0	653.2	23.2	16.7	65.2
2	Jack bean flour	4.5	4.3	1.8	22.8	26.4	9.1	3.9	1.82	148.9
3	Maize flour	12.6	0.4	2.7	8.7	0.2	175.1	13.2	3.5	61.4
4	JKPORRIDGE0595	87.9	0.1	1.7	10.8	0.0	217.7	13.8	7.1	23.1
5	JKPORRIDGE 1090	86.3	0.1	2.1	11.0	0.0	123.6	7.4	7.5	26.9
6	JKPORRIDGE 1585	87.3	0.0	5.0	11.5	0.0	140.2	9.2	6.7	40.9
7	JKPORRIDGE 2080	87.2	0.1	6.9	10.8	0.0	141.9	7.7	3.5	53.5
8	JKPORRIDGE 2575	88.0	0.1	8.6	10.8	0.0	152.2	12.8	8.6	106.9
9	JKBREAD 0595	34.1	0.7	1.6	10.2	0.8	143.9	2.8	0.9	38.0
10	JKBREAD 1090	32.8	1.7	2.5	10.1	1.0	136.2	2.7	0.7	44.6
11	JKBREAD 1585	33.7	1.2	2.5	10.3	0.8	138.4	4.6	0.9	75.3
12	JKBREAD 2080	34.5	1.3	4.2	10.3	1.9	161.5	5.3	0.7	111.1
13	JKBREAD 2575	33.6	1.8	4.5	11.2	1.8	174.3	4.7	0.7	120.8
14	JKBUNS 0595	35.5	0.5	1.2	8.8	1.9	211.7	6.9	5.1	18.5
15	JKBUNS 1090	36.4	0.9	2.1	8.6	4.1	155.5	8.5	5.7	56.2
16	JKBUNS 1585	34.4	1.1	2.9	8.3	3.0	165.5	8.0	6.4	77.8
17	JKBUNS 2080	37.6	0.9	3.2	9.6	3.2	196.0	9.5	6.6	91.1
18	JKBUNS 2575	40.4	1.4	3.7	9.7	2.9	207.0	13.1	6.7	120.6

¹Check Table 2,3 and 4**Table 8.** Composite flour porridge prepared using jack bean samples germinated at 48 h.

Sample Code *	Flavour	Taste	Smell	Colour	General acceptability
JKPORRIDGE0595	2.68±0.90 ^a	2.68±0.85 ^a	2.60±1.15 ^a	2.72±1.20 ^{ab}	2.56±0.96 ^a
JKPORRIDGE 1090	3.08±1.29 ^a	2.68±1.18 ^a	3.08±1.35 ^{ab}	2.52±1.04 ^{ab}	2.92±1.11 ^{ab}
JKPORRIDGE 1585	2.76±1.13 ^a	2.64±1.07 ^a	2.96±1.20 ^{ab}	2.28±1.13 ^a	2.68±0.90 ^{ab}
JKPORRIDGE 2080	3.36±1.32 ^a	3.44±1.29 ^a	3.76±1.42 ^b	3.28±1.27 ^b	3.52±1.50 ^{ab}
JKPORRIDGE 2575	4.40±1.41 ^b	4.72±1.59 ^b	5.04±1.33 ^c	3.48±1.47 ^b	4.04±1.36 ^c

1=Like extremely, 2=Like very much 3= Like moderately, 4=Like slightly 5=Neither like nor dislike, 6=Dislike slightly, 7=Dislike most. Mean scores with the same superscripts are not significantly different (P≤0.05). *Check Table 2

buns a score of 3 'Like slightly'.

DISCUSSION

Proximate composition and minerals profile

The result of the proximate composition of the jack beans analyzed in our study has not shown much difference compared with those reported by other scientists. Ajewole, (2002) reported that, the moisture content was 9.2% while crude oil, crude protein, crude fibre and carbohydrate contents were 2.8, 28.6, 5.3 and 51.3%, respectively. Seena and Sridhar (2004) analyzed jack

bean seeds, that consisted of 31.2%, 1.86%, 61.4% and 1580 kJ crude proteins, crude lipid, crude carbohydrates and energy, respectively. In other study done by (Sagarika et al., 1991) reported the proximate composition and nutritional quality of close variety of jack-bean *C. gladiata* (L.) to contain 29.2% crude proteins, 3.1% fat, 10.2% fibre and 53.2% carbohydrates on dry matter basis, respectively. Similarly, Doss et al. (2011b) reported, crude protein level ranging from 29.8 to 32.2 % as well as crude lipid (3.1 to 6%), crude fibre (7.34 to 9.98%), ash content (3.56 to 5.93%) and Nitrogen free extracts ranging from 50.77 to 54.28%. Equally, Abitogun and Olasehinde, (2012) found moisture content to be 2.19 ± 0.32%, protein content 20.97 ± 0.51%, crude fibre 2.55 ±

Table 9. Composite flour breads prepared using jack bean samples germinated at 48 h.

Sample Code ^ε	Flavour	Taste	Smell	Colour	General acceptability
JKBREAD 0595	2.21±1.34 ^a	1.82±1.19 ^a	2.00±1.53 ^a	1.95±1.10 ^a	1.95±1.55 ^{ab}
JKBREAD 1090	2.08±0.99 ^a	1.73±1.2 ^a	1.86±1.09 ^a	2.04±0.92 ^a	1.731±0.86 ^a
JKBREAD 1585	2.78±1.80 ^{ab}	2.73±1.5 ^a	3.00±1.44 ^a	2.52±1.53 ^{ab}	2.91±0.90 ^b
JKBREAD 2080	3.82±1.69 ^{bc}	4.13±1.57 ^b	5.08±1.34 ^b	3.47±1.70 ^{bc}	4.26±1.51 ^c
JKBREAD 2575	4.26±1.88 ^c	4.39±1.94 ^b	5.69±1.71 ^b	4.04±2.07 ^c	5.17±1.58 ^c

1=Like extremely, 2=Like very much 3= Like moderately, 4=Like slightly 5=Neither like nor dislike, 6=Dislike slightly, 7=Dislike most. Mean scores with the same superscripts are not significantly different ($P \leq 0.05$). ^εCheck Table 3.

Table 10. Composite flour buns prepared using jackbean samples germinated at 48 h.

Sample Code [†]	Flavour	Taste	Smell	Colour	General Acceptability
JKBUNS 0595	2.69±1.39 ^a	2.36±1.35 ^a	2.97±1.31 ^a	2.86±1.62 ^a	2.55±1.38 ^a
JKBUNS 1090	2.80±1.36 ^a	2.88±1.44 ^a	2.94±1.24 ^a	2.61±1.35 ^a	2.72±1.20 ^a
JKBUNS 1585	2.58±1.42 ^a	2.91±1.66 ^a	2.97±1.38 ^a	2.38±1.17 ^a	2.83±1.40 ^a
JKBUNS 2080	2.55±1.20 ^a	2.97±1.64 ^a	3.11±1.48 ^a	2.61±1.15 ^a	3.00±1.58 ^a
JKBUNS 2575	3.30±1.84 ^a	3.19±1.76 ^a	3.33±1.80 ^a	3.33±1.95 ^a	3.36±1.72 ^a

1=Like extremely, 2=Like very much 3= Like moderately, 4=Like slightly 5=Neither like nor dislike, 6=Dislike slightly, 7=Dislike most. Mean scores with the same superscripts are not significantly different ($P \leq 0.05$). [†]Check Table 4.

0.15%, ash content $3.45 \pm 0.96\%$, ether extract $10.23 \pm 1.61\%$ and carbohydrate levels $60.61 \pm 2.51\%$. Most researchers have reported values that do not differ significantly.

Mineral content of the jack beans analysis showed some differences from the information published by other researchers elsewhere. The levels of calcium, iron and zinc for the jack bean seeds analyzed in our study gave, 8.99, 3.83 and 1.76 mg/100g, respectively. Akingbade et al. (2009) found significantly high levels of iron 7.31 mg/100g than what was obtained in this study, but comparatively lower than zinc levels (0.73 mg/100g). Ajewole, (2002) report significantly higher levels of calcium (177 mg/100g), same levels of zinc (1.76 mg/100g), but comparative iron levels (4.27 mg/100 g) was slightly higher than what was obtained in our study. Abitogun and Olasehinde, (2012) in a study on nutritional evaluation of seed and characterization of crude jack bean found levels of calcium, iron and zinc to be 3.21, 0.83 and 2.9 mg/100g, respectively. The differences might arise from the soil type and mineral content where the plants were cultivated; a lot of variability are seen in various locations.

Phenolic compounds

In this study the raw jack beans contained about 860 mg/100 g of phenolic compounds. These high levels of phenolic compounds were similar to those found by Seena and Sridhar (2004) who reported higher levels in jackbean seeds to the tune of 1420 mg/100 g total

phenolics together with strong hemagglutination activity. In leguminosae family polyphenols are most commonly found in the cotyledons, and hence polyphenols have been found in dry bean (*Phaseolus vulgaris* L.), pea (*Pisum sativum*), chickpea (*Cicer arietinum* L.), faba bean (syn. broad bean, field bean; *Vicia faba* L.), cowpea (*Vigna unguiculata* L.) and lentils (*Lens culinuris* L.) at varying levels (Salunkhe et al., 1982). Germination process especially at 48 h. seems to decrease effectively in the concentration of phenolic compounds. In this study, germination decreased in phenolic compounds by 82 % from the original content. Similar results of 82 % phenolic content (mgGAE/100g) reduction in germinated jack beans were reported by Chaturvedi et al. (2015) who suggested that, germination process was viable and suitable for processing method, and could be recommended for the utilization of these underutilized legume. It has been studied that during germination, a multitude of enzymes are produced to hydrolyze their respective substrates.

These include even the other anti-nutritional factors, which are present in jack beans. According to Babar (1988), the application of dry heat to seeds and meal was not effective in inactivating the trypsin inhibitor and reducing the polyphenol content. Soaking for 24 h followed by cooking for 20 min, was equally effective in destroying the TI activity. Germination of jack bean seeds for 40 h. decreased the levels of TI and polyphenols by 31 and 35%, respectively. Reddy et al. (1985) observed that, since phenolic compounds and tannins are water-soluble, they may be eliminated by decortication soaking or cooking. The findings by Bhagya et al. (2006)

suggested that, considerable decrease of anti-nutritional factors in cooked *Canavalia cathartica* pods qualifies for human or livestock consumption. Cracked jack bean seeds cooked (100°C) in trona (NaCO₂.NaHCO₃.2H₂O) solution for one hour proved to be more effective as a method for inactivating the anti-nutritional factors in jack bean seeds (Udedibie and Carlini, 1998).

According to Mamiro et al. (2010) Magadi soda is often added to traditional foods such as dry cereals or grain legumes for the purpose of shortening the cooking times, improving taste and flavor. However, Udedibie and Carlini (1998) reported that, Concanavalin A (Con A), the most studied of plant lectins, appeared to be the most important toxic and antinutritional factor in the seed, being highly resistant to heat treatments and to proteolytic digestion in the gut. It required 3 h of cooking at 96°C or 45 min of pressure cooking; 48 h of soaking in water prior to cooking for 2 h.; 72 h of soaking in water prior to ordinary cooking for 1 h or pressure-cooking for 15 min, to completely eliminate it from the seed. However, Udedibie and Carlini (1998) insists that complete inactivation of Con A in the seed can be easily achieved, if the seed is broken into pieces and cooked for 1h or pressure-cooked for 15 min in a process known as crack and cook. Crack and cook treatment shows a significant effect in reducing the undetectable levels in the concentration of various anti-nutritional factors (Udedibie and Carlini, 1998).

A personal narration of a farmer who grows jack beans as a cover crop in Muheza district in Tanzania during a workshop session at Mlingano Agricultural Research Institute revealed that; he tried to boil the jack beans the way he does for the normal beans; but when they ate with the family, they felt drowsiness and headache. He however did not stop there, he continued to research further where he decided to boil and discard the water two times. This time when they ate the beans they did not feel drowsiness as felt before. He said this is how he prepares and utilizes the jack beans he grows in his farm with his family as of now (Wilbard Kavishe: Personal comm.). Traditionally, African mothers in most ethnic tribes used to boil beans and discard the boiling water once or twice with a probable reason of removing the beany flavor and other components. This process is supported by the long soaking process in which the longer the soaking the more phenolics reduction. Similarly, (Udedibie et al., 1996) reported that, a two-stage cooking is a practice commonly used locally for preparing certain poisonous foodstuffs, such as jack beans and sword bean (*Canavalia gladiata*) seeds, for human consumption.

Acceptability studies

The products that were prepared for acceptability studies are jack beans flour samples germinated for 48 h. This is

also assumed that, the other anti-nutritional factor will be hydrolyzed. The samples that were highly accepted were the buns, which were prepared by deep frying in oil. There was no significant difference ($P \geq 0.05$) with respect to all sensory attributes for the buns. This might be attributed to frying in oil as deep fried products which increases aroma and palatability. The other products i.e. porridge and breads, differed in some aspects. Since none of panelists came back with a report on any physiological discomfort or ill feeling, there is an indication that the 48 h. germination was possibly adequate in eliminating most of the anti-nutritional factors to a larger extent. This is probably one of the very few studies which have involved human subjects. However, Doss et al. (2011a) reports that mature beans are consumed by Indian tribal sects; Kurumba, Malayali, Irula and other Dravidian groups after cooking. According to Doss et al. (2011b), *C. ensiformis* ranks among the underutilized legumes that could ameliorate protein deficiency in human nutrition, particularly in developing countries.

Conclusion

The trials made on the acceptability of germinated jack-bean composite flours and the personal narration of the farmer shows, the possibility of the beans being used as a human food in the near future. Germination of the jack beans reduced to a greatest level the antinutritional factors embedded in the beans. The reports from researchers that jack beans are consumed by some ethnic groups like the tribal sects in India, if followed up and comprehend the way they do their preparation, will be a great stride towards utilization of jack beans.

Conflicts of Interests

The authors have not declared any conflicts of interests.

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