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Comparative Assessment of Oxalate Content in Selected Locally Most Consumed Vegetables and Foods in Otukpo Local Government Area of Benue State, Nigeria

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Abstract: In recent times, researchers are hard-pressed to determine which chemical substances present in an individual's diet and internal body system are working together to promote good health or are conspiring to create illnesses. The persistently increasing cases of hyperoxaluria-linked illnesses like kidney stones, bone deformations, and arthritis have led to strident and persistent research reports on oxalates in vegetables and foods. Thus, assessment of total oxalate contents of highly consumed vegetables and foods in Otukpo Local Government Area of Benue State has been carried out. Hot extraction and High-Performance Liquid Chromatography methodology were used for the investigation. The total oxalate contents of the samples in milligrams were; 5.40±0.01634; 6.80±0.08185; 50.20±0.16432; 8.60±0.24495; 80.40±0.16432 for Bitter Leaf, Pumpkin Leaf, Okra, Yam and Cassava respectively and for White Maize, Brown Maize, White Rice, and Brown Pepper were 5.40±0.16432; 4.73±0.12490; 38.00±0.08185; 26.57±0.10114, 38.80±0.08185, respectively. From the results obtained, all the samples investigated showed low oxalate content and the dried samples have higher electrical conductivity but lower amount of oxalate compared to the fresh samples. Also, fresh cassava has the high oxalate content, followed by fresh okra and the order is: Cassava > Okra > Brown Pepper > White rice > Sweet Potato > Yam > Pumpkin > Bitter Leaf/White Maize > Brown Maize. The determination of oxalate contents of the samplesto create awareness of health risks associated with the consumption of vegetables and foods with high oxalates is the aim of this investigation.

Keywords: Hyperoxaluria, kidney stone, arthritis, content, illnesses

Introduction

The strident and persistent scientific researches' reports on oxalic acid and other oxalate chemical substances in edible fruits, vegetables and food substances are meant to create the necessary awareness on the possible health risk associated with the consumption of fruits, vegetables and food substances with high content of oxalic acid and other oxalate mineral salt substances. It has been well established that organic acids such as a poly-hydric oxalic acid is a common naturally occurring chemical substance in living organisms including microbes, plants, animals and man [1, 2]. Although oxalic acid and its related oxalate mineral salts are not essential human body's nutrients, the human body usually produce oxalate compounds on its own or through other metabolic reactions involving other organic compounds such as vitamin-C (ascorbic acid) and carbohydrates (e.g. glyoxylate) undergoing partial oxidation [1, 2]. More recently, Minesh [3] has reported that oxalates also known as oxalic acid, are naturally occurring compounds in plants and that human beings eat them in food and also that, human bodies equally make them as well. Of all the oxalate mineral salts formed by oxalic acid or its ionized ions ($C_2O_4^{2-}$), calcium oxalate in particular is of great concern because of its potential health risk it poses to man. The human body contains essential metal ions including calcium ions (Ca^{2+}) for proper functioning of bones and if a large amount of oxalate is accumulated in the body through dietary intake, it may result in the formation of calcium oxalate in the body. Unfortunately, being an insoluble form of oxalate salt, calcium oxalate usually finds its way to the kidney through the body fluid and becomes accumulated in the kidney thereby resulting in kidney stones [4, 5, 6]. Uniquely, calcium oxalates are also the most common and abundant bio-minerals found in the plant kingdom and widely recognized in the life sciences due to their common association with kidney stone disease (urolithiasis) [7].

Meanwhile, it has been extensively reported that oxalates are widely distributed in plant foods in readily water-soluble form as potassium, sodium and ammonium oxalates and insoluble calcium oxalates [8]. In an extensively independently investigations [9, 10] have reported that vegetables and legumes which are commonly consumed among all kinds and conditions of people in all parts of the world and which constitute an important component of every day diet of millions of people in the world are natural sources of metallic oxalates. Foods consumed as daily diets are of course, the main sources of nutrients required by the human body. Interestingly, it has been unequivocally asserted that, the vegetables are inevitable good sources of certain minerals, vitamins, dietary fiber and antioxidant phyto-chemicals, whereas the legumes in addition to all these benefits, also largely contribute towards meeting the daily human body's protein and other essential and non-essential minerals requirements [9, 10]. Plainly, as reported elsewhere, Holloway *et al.* [11] on their part have equally reported that plant foods are considered as the major dietary source of both soluble and insoluble oxalates. That; oxalates are widely found in many plant species and they also occur as end products of metabolism, both in soluble and insoluble forms. Supporting many assertions in literature, Neuza *et al.* [6] have equally underscored that oxalates as chemical substances are present in a wide range of foods, with plant products being the main sources of dietary oxalates [12]. In addition, as well established and guaranteed fact, [13] has equally pointed out that, plant species and some cultivars may accumulate significant quantity of oxalates in their tissues up to 400-600 mg/100g fresh weight (FW), while other ranges from 700-900 mg/100g FW. Interestingly also in that regard, many studies have been conducted and reports have asserted that, the oxalates content of food can vary depending on the variety, growth, season, soil conditions, time of harvest and any other factors [14, 15, 16], oxalate-rich plants include fat hen ("lamb's quarters"), sorrel, soybean**s,** potatoes, raspberries, dates, navy beans, beets, almonds and several other oxalic species including spinach which has been reported to a very high oxalate content, with reported values as high as 1145 mg/100g of wet weight sample. High oxalate foods, vegetables and fruits have continued to receive the highest rating of great potential health risk factor to humans from researchers [3].

Importantly, it has been recognized that a great many diseases particularly chronic ones originate from interplay of many factors and sentinel event often points to the fact that certain environmental factors and dietary components are greatly contributing to the occurrence of these diseases. Hence, in recent times, researchers are hard-pressed to decide which consumable diet and how many of the elements present in an individual's dietary intake and internal body system are working together to help the human body to maintain good health or create illness. Foods eaten by humans serve as "fuel" and thus are the primary sources of humans' energy and in addition to the essential mineral nutrients contained in them, they also provide though in minute amount, other mineral substances such as phenolics, antioxidants, vitamins [9]. Essentially, increased intake of plants derived phenolics through daily diets is thought to lower the risk of age-related chronic diseases such as cardiovascular diseases and cancer [9]. However, these foods are equally well known according to Savage *et al*. [9] and [17], to contain anti-nutritional constituents such as oxalates, which can reduce the bioavailability of those food nutrients as they are being recognized as inhibitors of minerals bioavailability in the human body. Stereotypically, in line with global researchers' views, the concomitant variety of health risks due to dietary consumption of high amount of oxalate in humans can lead to inimical health challenging conditions including interference with essential macro mineral ions such as calcium and other nutritional minerals' absorption and kidney stone formation- a health condition otherwise described as nephrolithiasis or urolithiasis [6]. In fairly recent times, particularly in line with clinical researches' reports and other scientific investigative reports, excess oxalate in the human body has been linked with hyperoxaluric health condition which manifests as urinary excretion of excess oxalate in human urine [18]. The hyperoxaluria condition may further bring considerable strain in human health thereby causing severe bone deformations, pains, pathological fractures, and arthritis which could further deteriorate resulting to joint effusions and arthralgias [6, 18].

To this end, it is generally preferable to identify that consumption of foods, vegetables and fruits with high profile of oxalate contents increase the risk of kidney stones and as reported that, about one in ten people (ratio of 1:10) are strongly believed to be affected by kidney stones, even though some people are at more risk than others [18]. When oxalate levels are high, there's a greater chance that, the oxalate ions will react and bind to calcium ions, thereby forming insoluble calcium oxalate that could results in kidneystones. Furthermore, another common expressible risk of high oxalate in the human body is the lowering of mineral nutrients' absorption [9, 17]. Because oxalates bind to minerals like calcium, iron, and even magnesium, excess amounts can prevent the human body system from absorbing other beneficial nutrients in the digestive tract from daily diets intake. As rightly pointed in Upton & Graber [19], people often do not know that they are administering into their body potential health risk substances through their daily dietary intake or people do not even remember that they have been exposed to a potentially harmful chemical agent or they may remember but fail to see it relevance and thus do not bother for any possible remedies or precautions. That, a considerable period of time may elapse between exposure to a hazard and the onset of disease (latency period). Years of accumulated exposure or consumption of a chemical substance and continued consumption may be required before symptoms of induced diseases and the damage done are expressed. Thus, depending upon the human nature, individual variation in the way people react to substances is another key factor that also strongly determine how people response to potential health risk factors particularly dietary health risk factors, as people often vary widely in their response to even known harmful substances. Since many reports on health risk associated with oxalate are characterized by high accumulations of oxalate in the human tissues, several seemingly harmless quantities of oxalate can accumulate and interact to produce markedly more serious effects [19]. Typically, all these factors may conspire in one way or the other with human failure to avoid or reduce the intake of such potential health risk substance to provoke illnesses and that in addition often makes it difficult for physicians to link cause and effect even for disease known to have environmental or dietary origin.

In addition, the disconcerting reality is that at extreme conditions, severe systemic oxalosis may occur and this is a case where excess oxalate is dialyzed and accumulate in the bloodstream resulting in multiple health implications including serious damage to the eyes, joints, bone marrows, skin and other important organs and tissues [20]. Acute kidney injury, due to blockage of the urinary tube linking the urethra and the kidney has equally been reported due to calcium oxalate formed from excess oxalate in the human body [21]. Although a lot more in terms of wider implications of excess oxalate in the human body have been reported, however what seems to be the cardinal issue of great concern is the precipitation of insoluble calcium oxalate as kidney stone which may lead to the blockage of renal and urinary tubes, vascular necrosis and hemorrhage and that can exacerbate the condition thereby resulting further in devastating health conditions described as anuria, uremia, and a complete kidney failure [6, 20, 22]. Worst still, being anti-nutrient chemical substances, excess oxalates in the body can impede the bioavailability of some nutrients in the body since they can be chemically bound to metallic ions, such as calcium, magnesium, or iron, thereby reducing their metabolic availability, absorption and systemic use [23, 24]. From the forgoing, it be can added that, according to Yimer *et al.* [25] salt formed from oxalic acid is known as an oxalate; soluble salts are absorbed by the body and form strong chelates with dietary calcium, inhibiting its absorption; and at higher doses consumed, it causes calcium deficiency diseases such as osteomalacia, rickets and kidney stones.

More recently, of all the humanhealth challenges linked to high level of oxalic acid and its salt compounds formed in the human body, it is becoming more worrisome as more global attention has been on kidney stone and also especially the osteomalacia, and a complete kidney failure which have been extensively reported to be caused by high oxalate contents in edible fruits, vegetables and different varieties of foods [1]. Unarguably, much of what dietary experts and researchers have reported about the role of diet and diet-oxalate contribution to the human body, as well as high-risk practices such as eating foods, vegetables and fruits with high oxalate content profile in the development of kidney stone depends upon long-term consumption of such foods, vegetables, and fruits and the subsequent accumulation of oxalate in the body tissues. Equally worthy to note is the fact that, investigators are now frequently able to infer the way in which certain chemical substances behave or react in the human body system and whether such reactions are either detrimental with a disturbing pattern of increased health risk or beneficial to the body for proper functioning of the internal organs and tissues of the body. Notably, several seemingly harmless minute quantities of oxalic acid in dietary substances such as foods, vegetables and fruits can interact with other chemical substances in body's fluid to produce markedly more serious reactions and chemical compounds that may become injurious to human health.

Following up the reported linkage between high oxalate level in the human body and the associated health risks, there are significant research activities in this area of high level of oxalate as potential health risk factor and one area of such very active research activities in recent years is in the determination of oxalate contents in foods, vegetables and fruits from different parts of various countries of the world. Therefore, determination of oxalate content in foods is very important especially to people without any health challenge and also to patients with kidney stone problems.

It seems reasonable to suggest that the attempts of lowering the concentration of oxalate in the human body may include cooking of high-oxalate foods and vegetables to lower their oxalate content before consumption as especially, the soluble oxalates easily lose their concentration during cooking and boiling also works especially well for this purpose [9]. We ardently believe that the standard of maintaining a healthy living can also be enhanced by cultivating a habit of drinking enough water daily to help the body system to flush out excess oxalates. Although it may be practically impossible to completely avoid eating certain foods rich in oxalates especially as it is a well-known fact that, leafy greens, legumes, and other foods high in oxalates are equally very rich in other beneficial essential nutrients, however, balancing high-oxalate foods with other fruits and vegetables will be of immense help to ensure good health. Additionally, Minesh [3] has further suggested that, taking a recommended amount of calcium which may help to bind oxalates during digestion may equally help to reduce oxalate level in the human body. More so, reduction of excess sodium and sugar intake, which may contribute to kidney stones at high levels and taking the recommended amount of vitamin-C (excess vitamin-C can increase oxalic acid production in your body) are equally advisable for possible reduction of oxalate level in body [3]. Despite these facts, oxalate is not typically consumed daily in high concentrations and there are other constituents in foods which have a protective role against kidney stone formation, such as phytate, potassium and antioxidant phytochemicals like polyphenols [9]. From another report, it is equally worthwhile to become aware that, frequent taking of antibiotics, can lessen the good bacteria in the human gut that helps in converting oxalates to other chemical compounds and can consequently lead to oxalates to build up human body [18].

There is also another good piece of evidence that at least helps to reduce the general fear of fast attainment of excess oxalate-load in the human body and that is; oxalate-load can equally be reduced by certain bacteria such as *Oxalobacter formigenes* which dependon oxalates as a source of energy, and the utilization of oxalates by these bacteria helps to lessen oxalate excess-build-up in the body [3]. The bacteria and other microorganisms such as Escherichia coli, *Bifidobacterium spp*., and Lactobacillus *spp*., enhance the biodegradation of oxalates to other substances like carbon (IV) oxide and formate and energy and therefore helping to reduce the level oxalates in the body fluids [26]. Meanwhile, even as some people have less of the oxalates degrading microbes while other people may have more of this bacteria than others, it is important to add that, some processes employed during the cooking of food such boiling, steaming, soaking, and processing with calcium sources are means through which oxalates content especially the soluble oxalate of vegetables, fruits and foods can be predominantly reduced, hence, minimizing the heightening fear of accumulation of high level of oxalate in the body through daily dietary consumption. Emphasizing our insistence on people prioritizing dietary intake due to the health challenges related-impacts on human health as a result of excess accumulation of oxalate in the human body, it is extremely important for people to either control the amount of oxalate present in foods usually prepared as meals, or make a wise choice of consuming daily diets with very low oxalate contents' profile especially for people with kidney disorders, gout problems, rheumatoid, arthritis or specific kinds of chronic vulvaric pains [2]. Again, much as it may not be completely possible to control the amount of oxalate in edible leafy green vegetables, fruits and foods used as daily diets, however, it is extremely necessary for people to control diets in terms of selecting the kind of vegetables, fruits and foods based on their oxalate contents profile. There are available reports that have identified fruits, vegetables and foods with low oxalate contents and those with high oxalate contents. For instance, Minesh [3] has reported that banana, cashews, peanuts, and walnuts, pumpkin and sunflower seeds, sweet potatoes, broccoli, kidney beans, blueberries and blackberries are foods and vegetable with low oxalate content [18]. Others include yam, butter, vegetable oil, margarine, Salad dressing, milk, buttermilk [3, 18].

Scientists have long known that the human health is basically affected by complex, and interrelated factors of heredity and environmental components especially what is eaten, drank and applied to the human body like cosmetics. In any case, it is essential to reecho that no matter how harmful a substance may be humans are at the risk if they are exposed to it at basically high concentration or for a lengthy period of time. Furthermore, an individual's reaction to chemicals depends on a variety of factors which are mostly environmental and hereditary including health condition, previous exposure, and the nature of a chemical [19]. More often however, the work of many contemporary researchers such as Witting *et al.* [20] have equally increased people's knowledge of the risk involved in excessive consumption of diets, fruits and vegetables that have established profile of high oxalate content and certainly, the recognition of the health risk association with high oxalate contents in the human body as a potential health risk factor makes caution essential. Particularly, caution is essential in applying the conclusions of a study based on information about the induced-illnesses caused to individuals. Importantly, it is generally preferable as specifically reported by PanelQuan-Yuan *et al.* [1], that patients with kidney stone problems should control dietary oxalate intake to less than 40–50 mg per day and it is further recommended by the American Dietetic Association [18]. Equally very important to note is the fact that, most people bridle when they are presented with risk comparisons that juxtapose or put side by side, a voluntary risk and an involuntary risk [19]. A risk could be termed involuntary especially when the risk taker, primitively does not know anything about the contents of a consumable good or synthetic product used. Therefore, determination of oxalate content in foods is very important especially to patients with kidney stone problems [1]. This again, substantiates and justifies the purpose of this investigation to make available at public domains the highly experimental scrutinized information of these investigative findings.

Fundamentally, it should also be noted however that, so many risk factors are involved in the development of an illness that observational and descriptive studies can only suggest, but not prove, in totality the possible connections. Therefore, it is critical to recognize the fact that, the task of establishing links between a dietary content as a potential health risk in to human health and the induced health effects is obviously not a simple one and more often than not, it is an arduous process that can take many forms; can be complicated by myriad of factors; and can require a great deal of time, experimental processes, resources, and deductive insight [19]. Very importantly, the human nature plays a role in the assessment of risk [19] and the cold facts of fatality and (injury) surgical operations performed on people to remove kidney stones, due to consumption of foods, vegetables and fruits with high oxalate contents, are simply not enough to convince other people that eating certain foods, vegetables and fruits having high oxalate contents may pose serious health risk. It is not surprising that the human sense of judging of risks of things is also affected by psychological and cultural factors, deep-seated fears, moral and spiritual values, habitual pattern of thoughts, as well as by the manner in which information about risks is being communicated to people [19]. To this end, when judging their own health especially in regard to what is consumed as daily diets, for example, most people believe that their own fate will be better than that of their neighbors-a fallacy in reasoning that underlies the difficulty of convincing people to change even the most dangerous habits, such as eating meals that are highly rich in oxalate contents [19]. Although many people are convinced that, it is scientifically proven true that consumption of foods, vegetables and fruits with high oxalate contents are potential health risks based on the available and witnessed health challenges of people, however, there are still a lot of people who are yet to consciously agree with the numerous research reports on this regard.

The 21st century has presented modern technology which makes possible research reports more substantially accurate and devoid of a complex web of disagreement within the scientific domains of researchers. As reported by many contemporary researchers in both their investigative reports and reviews, over the years, various sensitive methods have been employed for the determination of oxalate in foods, vegetables and fruits and despite having different features, the different methods have common extraction procedures [6]. Particularly, extraction and analytical conditions of HPLC and UV–Vis spectrophotometry have been reviewed in Neuza *et al.* [6] and their observations are buttressed by information from other observations through experimental usage of the technique for analysis of different sample by other contemporary researchers. Accurate measurement to adequately determine the concentration of oxalate in foods is extremely dependent on its extraction methodology and which is the first step in oxalate analysis [10]. Accordingly, the methods for determination of oxalate include titration [1], capillary electrophoresis (CE), gas chromatography [27], high performance liquid chromatography (HPLC) [9] and enzymatic analysis. In addition, PanelQuan-Yuan *et al*. [1] have reported oxidase-based colorimetric determination of oxalate as a simple and fast method with very high sensitivity and selectivity. Honow and Hesse [12] reported HPLC-enzyme reactor method (HPLC-ER) and Roshini [2] reported the determination of oxalate content of vegetables in rural market by redox titration using potassium permanganate solution.

Again, in addition, acknowledging the limited knowledge of majority of consumers of foods, vegetables and fruits about oxalates and equally not realizing the fact that excessive oxalates content in such edible foods, vegetables and fruits may be detrimental to human health especially in the area where this investigation is carried out, it is these expatiated facts that justify the interest of this study to assess the concentration or content level of oxalates in locally most consumed foods and vegetables in Otukpo Local Government Area of Benue State in Nigeria. Hence, the data obtained from this study can serve as evidence to the consumers and to the policy makers in policy development to create awareness for compliance by people in order to know the health risk associated with high oxalates content in foods, vegetables and fruits and equally identify to consumers in both rural and urban areas, those foods, vegetables and fruits with high oxalates content profile. This work therefore, describes the quality of different locally most consumed foods and vegetables in Otukpo Local Government Area of Benue State in Nigeria in terms of oxalates content. More importantly, the content of oxalates in foods, vegetable and fruits should be routinely evaluated and this may include collecting both fresh and dried harvested samples of foods, vegetables and fruits available in an area for analysis in order to ascertain the safety of those edible substances in terms of their oxalates content. It is therefore, envisaged that the result of these findings will in addition serve as a model extricate for profiling of oxalates content of foods, and vegetable locally consumed in Otukpo Local Government Area of Benue State in Nigeria.

Meanwhile it appears that, there is a dearth of information with regard to oxalates content in locally most consumed foods and vegetables in Otukpo, as research on this area is limited and several studies reported elsewhere which have also revisited the analysis of foods, vegetables and fruits using different methodologies for determination of oxalates' content in foods, vegetables and fruits in recent years even including those using newly available techniques, none of such studies have been reported in literature in the area of the present investigation. Again, it is boldly envisaged that, this report will continue to be a very visceral reminder that humans need to work toward a future without unnecessary avoiding the consumption of foods, vegetables and fruits just because of fear of possible accumulation of high oxalate level in the body but wisely and selectively avoid dietary intake of foods, vegetables and fruits well known to have profiles of high oxalate contents. Being one of the most recently referenced methods used for the determination of oxalates in varieties of substances because of its high sensitivity, accuracy, versatility, and reliability, despite being expensive [6], HPLC was optimally used in this study and the results reported here unfold the allure of HPLC methodology. Also, this investigation report might serve as an invaluable information resource to the indigenous people of Benue State, Nigeria and the world at large concerning oxalate contents of locally grown vegetables and food plants. In particular, it is unarguably agreed that this technique is particularly useful when oxalate extraction is concerned and requires with high degree of accuracy.

We hope that this investigation will provide a valuable source of information about the health implications linked to the dietary intake of foods, vegetables and fruits highly rich in oxalates content, but we also hope that it will help people feel confident, rather than being terrified, about the prospects of living in today's world that is evidently believed that a man's daily diets are strong contributors to his or her overall state of health. The report of this investigation is presented in order to guide people on the steps to take to safeguard their health by distinguishing foods, vegetables and fruits that pose serious potential health risks and therefore, take control their dietary intake. In that regard, we continue to believe that there are goals any designed scientific research must accomplish regardless of the approach, emphasis, or the techniques used. Central to this belief is an approach to this investigation, with the view that, when the human senses and knowledge concerning an issue or a potential health substance are purified through availability of first-hand information, then people can engage in the service of making conscious effort to prioritize what they eat in order to live a healthier life. This study in addition, therefore highlights an important issue confronting those seeking to battle or avoid dietary health risks and the report of the present investigation is presented in order to guide people on how to safeguard their health by distinguishing foods, vegetables and fruits with low oxalate content as their preferred daily dietary intake. Fundamentally, in this investigation, a discussion of the current knowledge on oxalate analysis, its extraction conditions, specific analytical methods commonly employed in oxalate extraction, reported occurrence in foods, and its health implications are presented. In addition, a brief conclusion and further perspectives on whether high-oxalate foods are truly problematic and can be seen as health threats to humans are equally highlighted.

Materials and Methods

Samples collection and preparation

Samples collection and preparation

Fresh succulent bitter leaves (*Vernonia Spp*) and pumpkin leaves (*Cucurbita moschata*) were bought at a local market, Tiv market at Otukpo, Otukpo Local Government Area of Benue State Nigeria, from two different sellers. Also, the other samples, okra (*Abdelmoschus esculentus*), yam (*Dioscorea esculenta*), Cassava (*Manihot esculenta*), white maize (*Zea mays*), brown maize (*Zea mays*), rice (Oryza sativa), sweet potato (*Ipomea batatus*) and brown pepper (*Capsicum spp*) were purchased from local sellers at the same Tiv market.

Preparation of dry sample of pumpkin and bitter leaves

The 40 carefully selected bitter leaves and pumpkin leaves respectively were first washed with tapped water thrice and then with distilled water twice. Thereafter, separately, the leaves were chopped into very tiny pieces and were dried in an electric oven at 40^oC for 20 minutes to avoid a complete loss of moisture content of the leaves. The dried chopped bitter leaves and pumpkin leaves were further grinded and carefully sieved into finely powered form and then stored in a sterilized poly-ethene bag.

Preparation of fresh (wet) sample of pumpkin and bitter leaves

The 25 selected freshly succulent bitter leaves and pumpkin leaves respectively were washed thrice with tapped water and twice with distilled water, then chopped into very tiny sizes. The chopped fresh leaves were separately stored in sterilized poly-ethene bags and preserved in a refrigerator at 20^oC for 45 minutes before being used for extraction of oxalate.

Preparation of dry sample of okra and brown pepper

Fifteen (15) sizeable fresh okra and brown pepper respectively were carefully selected at random and then washed with tapped water thrice and with distilled water twice. Thereafter, separately, they were sliced into very tiny pieces and were dried in an electric oven at 45^oC for 40 minutes to avoid a complete loss of moisture content of the samples. The dried okra and brown pepper were further grinded separately and carefully sieved into finely powered form and then stored in a sterilized poly-ethene bag.

Preparation of wet (fresh) samples of okra and brown pepper

Fifteen (15) sizeable fresh okra and brown pepper respectively were carefully selected at random and then washed with tapped water thrice and with distilled water twice. Thereafter, separately, the were sliced into very tiny pieces and were separately stored in sterilized poly-ethene bags and preserved in a refrigerator at 20^oC for 45 minutes before being used for the analysis.

Preparation of fresh sample of different foods

The wet (fresh) sample of different foods was prepared by peeling off the bark (for yam, cassava and potato) and then washed thoroughly with distilled water thrice and with deionized water. Thereafter, the food samples were sliced into smaller sizes. The fresh food samples were milled and then 100 ml of deionized water was added to 100 g of each food sample and the extract was properly filtered into a clean corked bottle and then stored for in a refrigerator at 30^oC for 60 minutes before being used for the oxalate analysis.

Preparation of dry sample of different foods

The dry sample of each food was prepared by first of all, peeling off the bark (for yam, cassava and potato) and then washed thoroughly with distilled water thrice and with deionized water. Thereafter, the food samples were sliced into smaller sizes and then dried in an electric Thermostat oven (DHG9030 with temperature RXNGE) at 60^oC for 45 minutes. The dried samples were further milled, sieved to obtain their powder forms and then 100 g of each sample weighed was dissolved in 100 ml of deionized water. For maize and rice, one cup (milk can) was and grinded, sieved and 100 g of the powdered samples each were measured and dissolved in 10 ml of deionized water and preserved in a refrigerator at 25^oC.

Oxalate analysis

Extraction of natural oxalate from fresh pumpkin and bitter leaves

According to Gupta *et al*. [28] there are different methods often used to obtain oxalate extract from food and vegetable samples, however, hot extraction method was preferred over the cold method and was used for the extraction of the oxalate compound from pumpkin and bitter leaves samples. Hot method was preferably used because it usually gives a high content of oxalate extract than the cold method [10, 29]. Twenty-five (25) freshly cut bitter leaves stored in the refrigerator at 20^oC for 30 minutes were removed and pounded using local mortar and pestle and thereafter, the mixture was hand squeezed to obtain the green liquid extract. The green liquid extract obtained was first filtered using Whatman#1 Filter paper and further filtered using vacuum pump filter machine and then 250 cm^3 portions of the liquid extract were measured and transferred into 500 cm^3 flasks and 50 cm^3 of 2.0 $mol.dm^{-3}$ HCl was added (for total oxalate extraction) and 50 cm^3 of de-ionized water was added (for soluble oxalate extraction). The flasks were placed in a shaking water bath at 80^oC for 30 minutes. The extracts were further diluted with 50 cm^3 distilled de-ionized water and then transferred into 15 ml centrifuge tube and centrifuged at 4200 rpm for 10 minutes. The supernatants were filtered using Whatman#1 filter paper and kept frozen until the time of oxalate analysis. Each sample was extracted in triplicate.

Determination of total oxalate in prepared samples extracts

High Performance Liquid Chromatography (HPLC) equipment was used to determine the total oxalate contained in the pumpkin and bitter leaves extracts. The

HPLC of extracted oxalic acid was carried out on a 250 x 4.6 mm reverse phase column (Supelco). The mobile phase was 0.25% dehydrogenate phosphate and 0.0025M tetrabutylammonium hydrogen sulphate buffered at pH of 2.0 with ortho-phosphoric acid. The equipment consisted of dual piston HPLC pump (model LC-10A, Shimadzu, Japan), with a UV detector set at 210nm. The extracts of each of the samples (1.0 ml) were injected into the 1.0 ml HPLC auto-sample vials in duplicate and were led into the column and eluted at a flow rate of 1.0 ml/min. The oxalic acid peaks were eluted at 10 minutes and were used to extrapolate the content of oxalate in the samples' extracts. The extracts were analysed for oxalate in triplicates.

Statistical analysis

All data are presented as means ± SD. The means and standard deviations were determined for each food sample and the relative uncertainty for each sample result was extrapolated while the mean of each result was compared and inferred accordingly for the oxalate content of each sample to create a common profile for easy identification of values that are above the recommended daily value or tolerable upper intake limit.

Standard Deviation (SD) = $\frac{\sqrt{\sum(xi-\bar{x})2}}{N-1}$; where x_i = Individual value; x = Mean value; N = Number of measurement /observations.

Results and Discussion

The physicochemical properties of the samples are presented in (Table 1). From the results, it is observed that, the fresh (wet) samples have high pH values compared to the dried sample and this could be as a result of water content. As the water content is reduced in dried samples, they become acidic and their pH values become decreased. It is important to note that, the pH of foods, vegetables and fruits is a function of ionizable acids present in them which can be released as hydrogen ions that may give acid foods, vegetables and fruits their peculiar sour flavour [30]. Additionally, the results show that, all the samples investigated are low-acid foods and vegetables, since their pH values are high ranging from (5.10 -6.70) for fresh samples and (4.80-6.66) for dried samples. Again, it seems reasonable to also establish that, the pH of foods, vegetables and fruits depends on a number of factors including the soil, season of maturity, duration of and the variety or species of foods, vegetables and fruits. At this point it should also be noted that as important part of the physical properties of the samples, the electrical conductivity of the samples increases significantly with increase in temperature as shown in the results of conductivity and standard temperature in degree Celsius/Kelvin (^{o}C/K) in Tables 1 and 2. Furthermore, the results are in agreement with the reports of Blahovee [31] and Banti [32] and expectedly, the electrical conductivity of the samples decreases at about 1.35% in brown maize to 11.22% in okra depending upon the pH. Unarguably, this observation may be attributed to the nature of food and vegetable which is a strong factor that influences the electrical conductivity of biological materials [32]. Another possible factor that may influence the electrical conductivity of the samples is storage duration and electrical conductivity increases with extended storage period and temperature [31]. Generally, the pH of the samples decreases with increase in conductivity and vice versa.

Table 1: Physiochemical properties of raw (fresh) samples

Sample	Physicochemical Properties of Wet/Fresh Samples		
	pH	Conduct.mS/cm	Temp. (^{o}C)/K
Bitter Leaf	5.77	2.80	25/ 298
Pumpkin Leaf	5.20	2.90	25.5/298.5
Okra	6.05	0.98	25/298
Yam	6.70	1.004	27.7/300.7
Cassava	5.80	3.60	27.5/300.5
White Maize	6.50	0.76	28/301
Brown Maize	6.70	0.74	28/301
White Rice	6.36	0.70	28/301
Sweet Potato	5.60	1.05	27.8/300.8
Brown Pepper	5.10	1.12	27/300

Table 2: Physiochemical properties of dry samples

Sample	Physicochemical Properties of Dry Samples		
	pH	Conduct. mS/cm	Temp. (^{o}C)/K
Bitter Leaf	5.20	2.99	26/299
Pumpkin Leaf	5.00	3.03	26/299
Okra	6.01	1.09	25.8/298.8
Yam	6.60	1.05	27.9/300.9
Cassava	5.50	4.0	28/301
White Maize	6.50	0.82	28/301
Brown Maize	6.50	0.75	28/301
White Rice	6.30	0.74	28/301
Sweet Potato	5.30	1.09	27.9/300.9
Brown Pepper	4.80	1.15	27.4/300.4

Table 3: Oxalate content per 100g of raw (fresh) sample

Sample	Mean of total of Oxalate in (mg)	Result of Total Oxalate/100g); n=3 (mg)	Relative Uncertainty
Bitter-Leaf	5.40	5.40±0.01634,	0.303%
Pumpkin	6.80	6.80±0.08185,	1.204%
Okra	50.20	50.20±0.16432	0.327%
Yam	8.60	8.60±0.24495	2.848%
Cassava	80.40	80.40±0.16432	0.204%
White Maize	5.40	5.40±0.16432	3.043%
Brown Maize	4.73	4.73±0.12490	2.641%
White Rice	38.00	38.00±0.08185	0.215%
Sweet Potato	26.57	26.57±0.10114	0.381%
Brown Pepper	38.80	38.80±0.08185	0.211%

Table 4: Oxalate content per 100g of dry sample and relative uncertainty

Sample	Mean of Total Oxalate in (mg)	Result of Total Oxalate/100g) n=3 (mg)	Relative Uncertainty
Bitter-Leaf	5.00	5.0±0.10	2.0%
Pumpkin	6.20	6.20±0.10	1.6%
Okra	48.60	48.60±0.10	0.206%
Yam	7.70	7.70±0.10	1.30%
Cassava	79.77	79.77±0.153	0.192%
White Maize	4.90	4.90±0.10	2.041%
Brown Maize	4.50	4.50±0.265	5.89%
White Rice	37.70	37.70±0.10	0.27%
Sweet Potato	25.80	25.80±0.10	0.388%
Brown Pepper	38.30	38.30±0.10	0.261%

The results obtained as concentration of oxalate from raw (freshly) and dried (powder) food and vegetable samples are shown in Tables 3 and 4. The results indicate that oxalate content in both the fresh and dried samples (Tables 3 and 4) were lower than the lethal dose of 450 mg/100g oxalate recommended in and even the concentration in cultivated vegetables and food plants reported in [13]. The low oxalate in the investigated food and vegetable samples even with the optimization of the condition of extraction using cold method of extraction may be due to the soil conditions where the samples were cultivated, or due to climatic conditions, region, time of harvest and maturity, the period and condition of storage which are some common factors that influence oxalate content in foods, vegetables and fruits. Also, soaking in water and washing the samples during preparation may also attribute to the low concentrations as oxalic acid is readily soluble in water and can easily be washed out from those samples in addition to boiling, steaming, and processing with calcium sources which are some procedures mostly used to reduce the content of soluble oxalates from substances. Additionally, method of analysis may have influence on the value of oxalate concentration obtained. Comparatively, of all the samples analyzed, cassava and okra seem to have high oxalate concentrate of 79.77±0.153 and 48.60±0.10 mg/L respectively (Figs 1 and 2), and these results are equally in agreement with most reports in the literature.

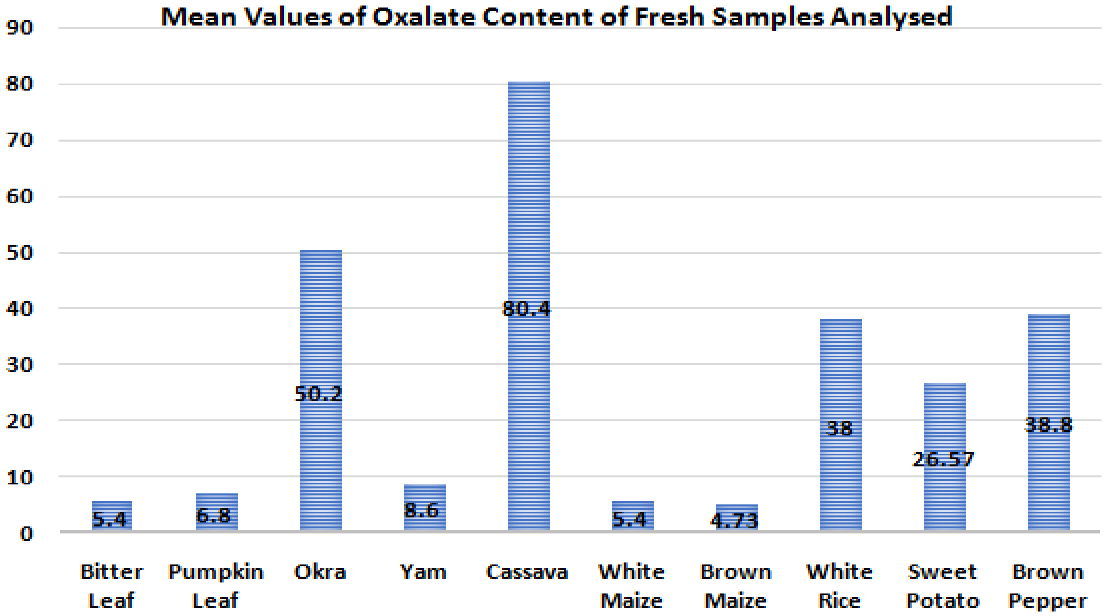

Figure 1: Chart of concentration of oxalate in analyzed fresh samples

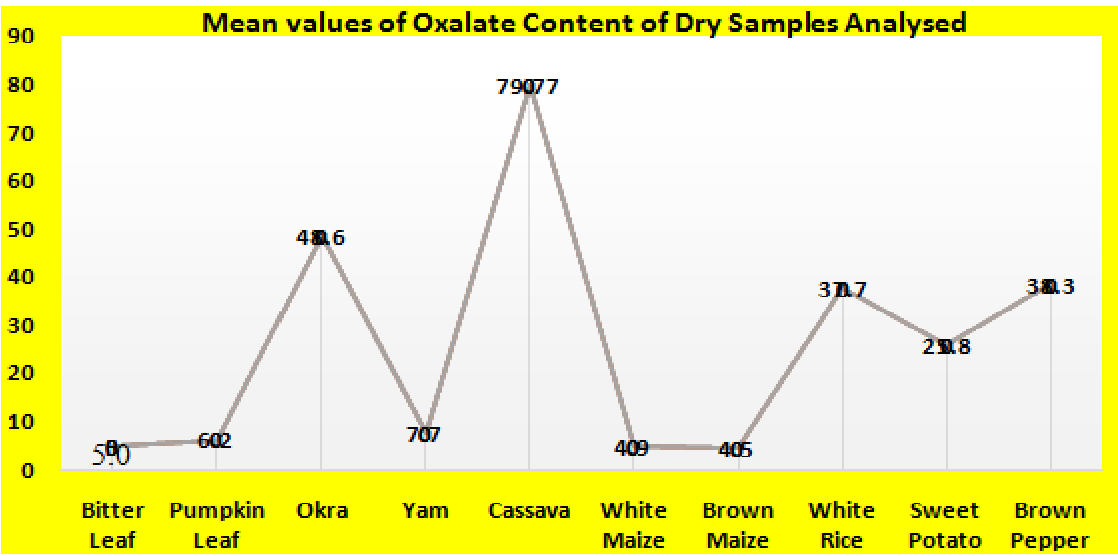

Figure 2: Chart of concentration of oxalate in analyzed dry samples

Thus, the results of the present study have revealed that, the locally most consumed vegetables and foods in Otukpo Local Government in Benue State of Nigeria are safe for consumption, considering their low oxalate contents and also, application of traditional cooking methods for preparation of these edible plants could further reduce the effects of their oxalic acid. Generally, it is a well-known fact that, the same food can be prepared differently either (raw, boiled, baked, fried, etc.) and this can lead to different oxalate results and the reported values for potato (*Solanum tuberosum*) are a great example to evidence the influence of cooking techniques on oxalate content. Specifically, boiling in particular has been associated with decreased oxalate content, especially soluble oxalates, due to its leaching and thermal degradation [5, 6, 13], as observed in spinach and vegetables. It is equally very expedient to consider the usual quantity of consumption of these foods even with their low oxalate contents, since excess and continuous daily intake can gradually lead to high oxalate content in the human body. For example, some aromatic plants, like parsley, contained high oxalate values but their daily intake is naturally much less than 100 g. Also, the method of food preparation for consumption has to be considered because some foods are not generally eaten in the form in which they were analyzed (for example raw beans or mushrooms).

Conclusion

Regular consumption of high-oxalate foods by healthy individuals as a part of a balanced and diversified diet and does not appear to cause health issues if daily consumption is from 50–200 mg/day, whereas for individuals susceptible to kidney stone formation dietary modification is crucial for its prevention [18]. For these individuals, it is recommended to limit the consumption of high-oxalate foods to less than 40–50 mg oxalate/day since they can present a health threat in these cases. Since absorption of oxalates is related to the quantity of soluble oxalates which are more bioavailable a simultaneous consumption of oxalate with calcium or magnesium can reduce its bioavailability and absorption due to the formation and fecal excretion of insoluble salts, lowering the health risk [18].

Additionally, the oxalate content of plants can vary according to their age, the season, the climate and the type of soil. In some plants, such as Rhubarb, oxalate content tends to increase as the plants mature, whereas, in other plants, e. g. Spinach, sugar beet leaves, and bananas, there is a large increase in oxalate content during the early stages of development, followed by a decrease as the plants mature [14].Also, foods which contain oxalates, such as fruits and vegetables, have a wide range of beneficial compounds that might outweigh possible negative implications on human health. Essentially, also boiling, steaming, soaking, and processing with calcium sources are some procedures to reduce the content of soluble oxalates, the most harmful oxalates. Research shows that restricting foods with high oxalates may no longer be practical. Most of these foods contain healthy nutrients that the body needs. Also, a large portion of the oxalate in urine comes from the body and not from food [3].

Are foods, fruits and vegetables with high oxalate contents truly problematic to human health? The answer is yes, if they are constantly eaten and at increased quantity which may lead to a build-up of excess oxalate in the body. Meanwhile, despite these facts, oxalate is not typically consumed daily in high concentrations and there are other constituents in foods which have a protective role against kidney stone formation, such as phytate, potassium, calcium, and antioxidant phytochemicals like polyphenols [24]. More so, in plants oxalates play role in ion balance, plant defense, tissue support, detoxification, light gathering and reflection.

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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