International Journal of Pharmacology, Phytochemistry and Ethnomedicine *Submitted: 2020-05-16*
ISSN: 2297-6922, Vol. 17, pp 1-16 *Revised: 2021-06-10*
doi:10.18052/www.scipress.com/IJPPE.17.1 *Accepted: 2021-07-08*
CC BY 4.0. Published by SciPress Ltd, Switzerland, 2021 *Online: 2021-10-26*

Vitamins Composition in *Clarias gariepinus* Fish Body Parts (Liver, Muscle, Head): Reporting on Samples on Fresh, Smoked- Dried and Dry Extract Bases

Adeyeye Emmanuel Ilesanmi[1,a*] and Olatoye Rauf Abioye[2,b]

[1,2] Department of Chemistry, Ekiti State University, Ado-Ekiti, Nigeria

[a]e-mail: adeyeyeilesanmi2012@gmail.com, [b]abioyerauf@gmail.com

Keywords: *Clarias gariepinus,* true value, vitamin concentration.

Abstract. An investigation into the vitamins composition levels in *Clarias gariepinus* fish was carried out and reported in dry extract/fresh; dry extract / smoked-dried on individual vitamins and the sum of the whole vitamins. Parts investigated were liver, muscle and head. Whereas fresh and smoked-dried data were laboratory results, the dry extract portions were calculated and reported as dry extract /fresh sample, dry extract / smoked-dried sample for liver, muscle and head. Results obtained ran thus and all values were in mg/100g vitamin where d = difference, CV% = coefficient of variation and % difference = % value that shows what made dry extract value greater than its reported comparison:

i. dry extract/fresh, % d = 74.5 (all), CV% = 84.0 (all), in liver;

ii. dry extract/smoked, % d =24.5 (all), CV% = 19.7 (all), in liver;

iii. dry extract/fresh, % d = 74.3 (all), CV% = 83.6 (all), in muscle;

iv. dry extract/smoked, % d = 10.2 (all), CV% =7.60 (all), in muscle;

v. dry extract/fresh, % d = 68.5 (all), CV% = 73.7 (all), in head;

vi. dry extract/smoked, % d = 9.10 (all), CV% = 6.74 (all), in head;

vii. dry extract/fresh, % d = 71.9- 74.5, CV% = 79.4 - 82.4 in total vitamin body load;

viii. dry extract/smoked, % d = 9. 69- 24.5, CV% = 7.20 - 19.7 in total vitamin body load;

ix. dry extract (fresh) – dry extract (smoked), %d = 69.6 - 82.0 in liver;

x. dry extract (fresh) – dry extract (smoked), %d = 72.3 - 76.3 in muscle;

xi. dry extract (fresh) – dry extract (smoked), %d = 62.9 - 75.2 in head;

xii. dry extract (fresh) – dry extract (smoked), %d = 69.7- 79.0 in total vitamins body load.

Among the dry extract values calculated from fresh samples and subjected to chi-square (χ^2) values, significant values were observed in vitamins B_6, C, A, B_1, D, E and total at α=0.05. In the dry extract values from smoked samples, only three significant χ^2 values in vitamins A, E and total were observed. In reflection to vitamin concentration levels, percentage higher levels in dry extracts (from fresh) had these trends: liver (74.5%) > muscle (74.3%) > head (68.5%) whereas from smoked, we had liver (24.5%) > muscle (10.2%) > head (9.10%). Also total vitamin body load from dry extract (fresh) was 71.9-74.5% difference and dry extract (smoked) was 9.69 -24.5% difference. It should be noted that liver occupied the higher part of the range in the two comparisons, like 74.5% (fresh) and 24.5% (smoked).

Introduction

From the time past, fish has always served as a source of human food. Fish has come to be available to human beings through catching. Fish is a source of concentrated protein with high level of lysine thereby making fish suitable for supplementing high carbohydrate diet. Fish farming being practiced since about 3,500 BCE in China [1] has become increasingly important in many countries. On the whole, about one – sixth of the world's protein is estimated to be provided by fish [2]. Fish is known to be good sources of vitamins such as riboflavin, thiamine, vitamins A and D, as well as phosphorus. The proportions of thiamine, riboflavin, vitamins A and D as well as phosphorus are considerably elevated in some developing countries and regions that depend heavily on the sea [2]. The fishery sector is assumed to contribute about 3.5% of Nigeria's Gross Domestic Product (GDP) and also provides direct and indirect employment to more than six million people [3].

The African catfish is a specie of catfish of the family Clarridae (air breathing fishes) and its scientific name is *Clarias gariepinus* [4]. The catfish is named after its environment, the Gariep river, the Hottentot name of the Orange river, South Africa and the ability of the fish to live for a long time out of water [4]. Generally, catfish are a diverse group of ray finned fish, named for their prominent barbells that resemble a cat's whiskers.

Hot smoking normally exposes foods to smoke and heat within a controlled environment. Such item is hung first to develop a pellicle, and then smoked. Sometimes foods that had been hot smoked are often reheated or cooked, they are typically safe to eat without further cooking. Hot smoking usually occurs with the temperature range of 52^0C – 80^0C (126^0F - 176^0F) [5]. Within this temperature range, foods are fully cooked, moist and flavourful. In Nigeria, traditional direct smoking system using traditional kiln, wire gauze on steel drum are almost exclusively utilized.

Vitamins are known organic chemical compounds which are found in small amounts in natural foodstuffs, they are needed and / or required by an organism as a vital nutrient to sustain life. They play an important role in normal metabolism process, growth and vitality. Vitamins can be sourced either from our diet which is the major source for them or from vitamins supplements [6]. Many recent studies proved that incidence of cancer, diabetes, obesity and heart diseases in older people is directly related to insufficient supplement of vitamin in nutrition during childhood and adulthood [6]. The importance of receiving a balanced diet each day comes from the fact that our bodies do not have the ability to synthesize vitamins.

Vitamins are generally classified into two main broad categories which are: water soluble vitamins (B_{12}, C, B_9, niacin, B_3, B_1, B_2, B_6, B_5) and fat soluble vitamins (K, E, D, A) [6].

Intrinsic parameters of food include pH, moisture, oxidation – reduction potential (presence or absence of oxygen), nutrient content, occurrence of antimicrobial constituents and biologic structure. Water is an important part of all body functions and processes, including digestion and elimination. When you are on a diet, water also acts as a weight-loss aid because it can help you eat less. Entrapped (bound, adsorbed, immobilized) water is water held in muscle by being entrapped by the structural features of the muscle cell. For example water held in the myofibrils and water held in the cell itself by the cell membrane (sarcolemma) [7]. Such water is not free to act as solvent for salts and sugars. It can be frozen only at very low temperatures. It exhibits no vapour pressure. Its density is greater than water. The water molecules are bound to polar groups or ions on molecules such as starch, pectins and proteins; this water is held firmly [8].

Wikipedia [9] has described the dry matter as a measurement of the mass of something when completely dried. The dry matter of plant and animal material would be its solids, i. e. all its constituents excluding water. The dry matter of food would include carbohydrates, fats, proteins, vitamins, minerals and antioxidants (such as thiocyanate, anthocyanin and quercetin). Carbohydrate, fats and proteins, which provide the energy in foods (measured in kilocalories or kilojoules), make up 90% of the dry weight of a diet [10]. Water content in foods varies widely. Many foods are more than half water by weight: boiled oatmeal (84.5%), cooked macaroni (78.4%), boiled eggs(73.2%), boiled rice (72.5%), white meat chicken (70.3%) and sirloin steak (61.9%) [11]; fruits and vegetables are 70-95% water. Most meats are on average about 70% water; fresh meat has water content of 70% and a water activity of 0.98 [12]. Breads are approximately 36% water [13]; reference [12] reported water in bread as 40% as well as flour (14%) and pasta (10%) [12]. Some foods have a water content of less than 5%, e.g., peanut butter [13], crackers and chocolate cake[14] and potato chips is 2% [12]. Water content of dairy products is quite variable; butter (15% water), cow̓s milk (88-86% water), Swiss cheese (37% water) [13]. Water content of milk and dairy products varies with the percentage of butterfat so that whole milk has the lowest percentage of water and skimmed milk has the highest [9]. In the sugar industry the dry matter content is an important parameter to control the crystallization process and is often measured on-line by means of microwave density meters [15].

The nutrient of foods, animal feeds or plant tissues are often expressed on a dry matter basis i.e. as a proportion of the total dry matter in the material. For example, a 138g apple contains 84% water (116g water and 22g dry matter per apple) [16]. The potassium content is 0.72% on a dry matter basis, i.e. 0.72% of the dry matter is potassium. The apple, therefore, contains 158mg potassium (0.72/100 x 22g). Dried apple contains the same concentration of potassium on a dry matter basis (0.72%), but is only 32% water (68% dry matter). Hence 138g of dried apple contains 93.8g dry matter and 675mg potassium (0.72/100 x 93.8g). When formulating a diet or mixed animal feed, nutrient or mineral concentrations are generally given on a dry matter basis; it is therefore important to consider the moisture content of each constituent when calculating total quantities of the different nutrients supplied [17]. A substance in the feed, such as a nutrient can be referred to on a dry matter basis (DMB) to show its level in the feed (e.g., ppm). When nutrient levels in different feeds are considered on dry matter basis (rather than as-is basis) makes a comparison easier because feeds contain different percentages of water. This also allows a comparison between the level of a given nutrient in dry matter and the level needed in an animal's diet [18]. Dry matter intake (DMI) refers to feed intake excluding water content.

The purpose of this study was set at determining the vitamin contents of African catfish. Laboratory reports on analysis of food substances usually give results in " wet" (fresh), "dry" or as-is value. For a nutrient in food, the initial concentration measured in the laboratory is considered an as-is or "wet" or "fresh" basis result, because no calculations have been made to compensate for the moisture content of the sample. When a "dry" value is reported, the laboratory might have measured the moisture content of the sample to a constant weight. However both "wet" and "dry" values can be calculated further by calculating the concentration based on the percent solids present in the sample. This is either called "dry matter basis" or "dry extract basis"; this value puts all moisture content to similar level since only the "entrapped" or "bound" water remained. Because of our national peculiarity (with poor and unstable electricity power supply), laboratory results were obtained on both fresh (wet) and smoked (dry) bases and both later calculated on dry extract basis based on the percent solids present in the samples. This new dry extract value was now compared to fresh and dried results respectively to observe the levels of vitamins in liver, muscle and head as affected by the third procedure. Based on the data values, an hypothesis was generated that "significant difference does not occur between the values reported in fresh, dry and dry extract basis in the body parts of *Clarias gariepinus.* It is anticipated that this type of determination of the vitamin contents of this fish will provide necessary information on the nutrient value of this food for both consumers and researchers who work on nutrient Tables and will also guide the farmers who cultivate this specie in terms of the feeding requirements.

Materials and Methods

Fish samples

Five pieces of matured *Clarias gariepinus* were harvested from a local fish vendor that managed fish ponds located in Basiri quarters of Ado-Ekiti, Ekiti State, Nigeria in the month of December, 2017. On cropping, the live fish samples were found to weigh an average of 1.40kg. Fresh fish samples were sacrificed and dissected to get the head, muscle and liver. The harvested fish samples were later cleaned and transferred into a frozen container for transportation to the laboratory. The parts: head, muscle and liver that were used for the analyses were patiently separated and then stored in a freezer prior to sample preparation. These parts were later withdrawn from the freezer, rinsed with deionized water, ground by agate mortar and then blended with an Excella Mixer blender.

The fish parts were also smoked using the African traditional method of smoking called charcoal smoking. The fishes were smoked for about 12h at moderate to high temperature (80^0C-110^0C) (to avoid dry matter loss that can result from heat generation [19]) during which turning over was done within an interval of 30min to achieve uniform smoking. The smoked samples were further dried at low temperature overnight over the ember to ensure that the samples were properly dried. The smoking process involved placing a piece of cardboard over the fishes so as to trap the smoke directly on the fish. The fish parts were then homogenized using a mortar and pestle before being blended.

The homogenized samples (smoked and fresh) were stored in glass bottles and kept in a refrigerator at less than 4^0C for subsequent analyses. Determinations were in duplicate. Samples designations were as FL, FM, FH, FT, DL, DM, DH and DT where F=fresh, L= liver, M= muscle, H= head, D= dry and T= total.

Simultaneous analysis of fat and water soluble vitamins

The samples were withdrawn from the < 4^0C compartment in the laboratory and placed on the bench to allow acclamitization to the laboratory conditions.

Extraction of water-soluble vitamins

Each sample was grinded with the aid of the laboratory mortar and pestle. Accurately weighed value of 0.100g of ground sample was quantitatively transferred into 100ml volumetric flask and 80ml deionized water added. In about 15min of ultrasonic extraction, water was added to the volumetric flask mark.

Extraction of fat-soluble vitamins

An accurately weighed 0.125g of ground sample was transferred into 10ml volumetric flask and 8ml of CH_3OH-CH_2CL_2 (1:1, v/v) was added to the volumetric flask mark. The prepared sample solution was stored in the dark; and diluted if necessary. Prior to injection, the solutions were filtered through a 0.2µm filter (Millex- GN).

Optimized chromatographic conditions

Water- and fat-soluble vitamins were separated simultaneously under the following optimized chromatographic conditions combined with value switching, double injection, envelope-injection and wavelength switching.

Column

Acclaim PA, 3µm, 120A, 3.0 x 150mm for fat soluble. Acclaim C18, 3µm, 120A, 3.0 x 150mm for water soluble.

Column temperature

25^0C

Mobile phase

For water-soluble vitamin determination

(A) 25 mm phosphate buffer (dissolved ~ 3.4g KH_2PO_4 in 100ml water, and adjust pH to 3.6 with H_3PO_4).

(B) CH_3CN. Mobile phase A (7:3 v/v).

For fat-soluble vitamin determination

(A) CH_3OH- CH_3CN (8:2, v/v).

(B) Methyl tert-betyl ether (MTBE)

Injection volume

10µl (Dionex, Technical Note 89) (www. dionoex.com).

Data results

The chromatographically determined values were reported for fresh and dry samples respectively. However, for this work, both fresh and oven-dried sample results were further processed to convert the machine values to calculated "dry extract" values. This would bring the values of the vitamins to the same level of comparison. Therefore, to calculate the concentration on a dry extract basis, the following formula was used:

$C_D = C_W / P_S \times 100$

C_D = concentration corrected for dry extract.

C_W = fresh or dry weight concentration as the case may be.

P_S = solid concentration (i.e. 100 - moisture content).

Statistical evaluation

Both descriptive statistics and inferential statistics were used in processing the available data. For the descriptive statistics, mean, standard deviation (SD) and coefficient of variation (CV%) were employed to study the differences between the comparisons of the samples. It also involved the calculation of the differences / percentage differences in the compared groups. For the inferential statistics, the individual vitamin values were compared between the liver, muscle, head, total body load and subjected to chi- square (χ^2) statistics to find out if significant differences existed among the vitamins within the body parts. Critical level of statistics was set at α= 0.05(n-1) df [20].

Chemical compounds

Chemical compounds studied in this article were: Retinol (Pubchem CID: 445354); Cholecalciferol (Pubchem CID: 5280795); Cyanocobalamin (Pubchem CID: 5311498); alpha-Tocopherol (Pubchem CID: 14985); 3-Hydroxy-vitamin K (Pubchem CID: 5280540); Niacin (Pubchem CID: 983); Riboflavin (Pubchem CID: 493570); Pyridoxine (Pubchem CID:1054); Thiamine (Pubchem CID:1130); Folic acid (Pubchem CID:6037); Pantothenic (Pubchem CID: 6613); Ascorbic acid (Pubchem CID: 54670067) [21].

Results

Dry extract values compared with fresh values in liver, muscle, head and total body load of vitamins

In Table 1, we depicted the dry extract from fresh values as compared to the original fresh value results. Table 1 column 2 was the dry extract whereas column 3 was the corresponding fresh value. In all the vitamin and total body load, the dry extract was consistently higher than the corresponding fresh value. In column 5, the percentage differences between the values in columns 2 and 3 were shown. These percentage difference values were consistently high at a uniform value of 74.5%. Also of note were the CV% values in column 8 which were all high and similar at 84.0%. Columns 2 and 3 were of likely equivalent values under different physical states of liver, values only being different due to level of free moisture difference.

Table 2 resembled the observations in Table 1, but here muscle was involved. Compared to Table 1 in columns 5 and 8, slight differences were observed: column 5 gave a consistent value of 74.3% as the percentage difference as against 74.5% in Table 1 (liver) and values in column 8 were consistently at 83.6% (CV%) as against 84.0% in Table 1 (liver). Both percentage differences and CV% values were close in Tables 1 and 2 with respective values of 74.5 / 74.3 and 84.0 / 83.6 and just difference of 0.20% and 0.40% respectively.

Table 3 concerned values for the head. Here partial constant percentage difference of 68.5% was observed with only one vitamin (B5) being 64.3%; for CV% we also observed partial constant value of 73.7% whereas only vitamin B5 had CV% of 66.9. The percentage difference and CV% in the head were slightly lower than both in the liver and the muscle. The percentage difference values in the head were a reflection of the vitamins composition in the head.

Table 4 showed the total body load of the vitamins between the dry extract and the fresh levels. The percentage differences had various values ranging from 71.9% (lowest) – 74.5% (highest), these values were slightly within the region of the values of percentage differences as observed in the liver (Table 1) and the muscle (Table 2). Similar observations were made in the CV% values with values of 79.4% (lowest) - 83.9% (highest) being within the values in column 8 of Tables 1 and 2.

Dry extract values compared with smoke-dried values in the body parts of *C. gariepinus* and total body load of vitamins

Table 5 began the series of observations made between dry extract and smoke-dried sample values. The columns 5 and 8 were much lower than the corresponding columns of Table 1- 4 because much water had been lost in the smoke-drying process. The dry extract/smoke- dried observations for the liver were depicted in Table 5. Percentage difference values were consistent at 24.5% and CV% values were also consistent at 19.7%.

Table 6 showed consistent percentage differences of 10.2% and consistent CV% of 7.60%. The Table 6 contained the data values of vitamins in the muscle. Both values of 10.2% and 7.60% were correspondingly much lower than the values of 24.5% and 19.7% in the liver (Table 5).

In Table 7, the comparison was for the head region. Percentage differences here were consistent at 9.10% and consistent CV% of 6.74%. Unlike in the observation between Tables 5 and 6 where we had high disparity of values, values between Tables 6 and 7 were close; in comparison for Tables 6/7, we had 10.2% / 9.10% (percentage difference) and 7.60% / 6.74% for the CV% values.

The Table 8 was for the total vitamin body load under this dry extract / smoke-dried mode. Like we had in Table 4, the percentage differences were not consistent and values ranged from 9.69 - 24.5 (all within the range of liver and muscle) and CV% values of 7.20 – 19.7 which were all outside the CV% of the head.

In Table 9, values of dry extracts from fresh and dry samples were substracted from each other and the percentage differences recorded. The results ran thus: liver (69.6% - 82.0%), muscle (72.3% - 76.3%), head (62.9% - 75.2%) and body load (69.7% - 79.0%). These values were difficult to be arranged in both ascending and descending order, values being highly comparable.

In the Table 10, dry extract values calculated from fresh samples of the *C. gariepinus* body parts were subjected to chi- square (χ^2) analysis. The critical value was 5.99 at α=0.05 (n-1)df. Values from these vitamins were each greater than 5.99: vitamins B6, C, A, B, D, E and totals. Without considering the totals, both water- and fat-soluble vitamins shared the significance level at 3/3 or 50%/50%.

In Table 11, the exercise of Table 10 was repeated between dry extract calculated from smoke-dried samples. However, only two vitamins had $\alpha_{calculated} > \alpha_{Table}$ of 5.99 at $\alpha = 0.05$ (n-1)df. These two vitamins were fat-soluble and they were vitamins A and E; also totals was also significant.

Discussion

Dry extract values compared with fresh values

The values under the above title were shown in the Table 1 (liver), Table 2 (muscle), Table 3 (head) and Table 4 (total body load). Twelve vitamins were analysed for, whilst four of them were fat-soluble vitamins, eight others were water-soluble and number thirteen being the total body load of the vitamins.

In Table 1 where the dry extract fresh/fresh values were depicted, liver was the body part considered. The two most concentrated vitamins were fat-soluble vitamins E (97.3/24.8mg/100g) and A (20.5 / 5.22mg/100g). The other two fat- soluble vitamins were low at 1.71 / 4.36 e-1 mg/100g (K) and 4.42e-1 / 1.13e-1mg/100g (D). The only vitamin B complex member that was significant was B_3 (11.5 / 2.92 mg/100g). B-complex vitamins above trace levels were B_6, B_1, B_2, and both B_9 and B_{12} (when values were converted to µg levels); however, vitamin C was even below trace level. These results showed that the liver could be described as the home for fat-soluble vitamins. Humans, other primates, guinea pigs and some bats, birds and fish lack a liver enzyme, L-gulono-υ-lactone oxidase, hence such animals cannot synthesize vitamin C in their liver [22]. The total vitamins were 135/34.5mg/100g.

Table 2 was replication of Table 1 but muscle had taken the position of the liver. As in Table 1, fat-soluble vitamins still occupied a pride of place as they occupied the first two highest positions in the Table. They were vitamins E (33.6/8.65mg/100g) and D (14.0/3.59mg/100g). In Table 1, vitamin E was in the most concentrated position as we have in Table 2 (although with lower concentration). The value of vitamin D had significantly improved in Table 2 moving from 4.42 e-1/1.13 e-1 mg/100g (Table 1) to 14.0/3.59 mg/100g in Table 2. Generally, vitamin B-complex members had good show in the muscle. Such members were vitamins B_3 (8.35/2.15mg/100g), B_6 (11.2/2.87mg/100g), B_1 (11.6/2.98mg/100g) and B_{12} (1.77/4.55 e-1 mg/100g). These vitamins had low to very low levels of concentration: A, B_2, B_9, K and B_5. Totals had value of 89.9/23.1mg/100g. Vitamin C had great improvement as a component of the muscle with value of 8.07/2.07mg/100g.

The dry extract fresh/fresh in the head had the values depicted in Table 3. Vitamins E and D led the concentration level in the Table 3. It had now become very clear that *Clarias gariepinus* would serve as good sources of vitamins E in both liver, muscle and head whereas the muscle and the head would serve well the supply of vitamins D and E in the diet. Other vitamins found at reasonable level in the *C.gariepinus* body parts were vitamins B_3, B_6, B_1 and B_5. The muscle and the head would supply average level of vitamin C. Total vitamins concentration in the head was 58.5/18.4mg/100g. Among the Tables 1, 2 and 3 vitamins concentration we have: (89.9/23.1mg/100g) muscle < liver (135/34.5mg/100g) » head (58.5/18.4mg/100g). We have these ratio relationships: liver : muscle

(1.00:1.50); liver : head (1.00:2.31); and muscle: head (1.00:1.54). These ratios further demonstrated the closeness in the values of vitamins in the liver and the muscle and their being far away from the head vitamins concentration. People who had taken the head of *C. gariepinus* as a delicacy might not be gaining much of the vitamins as compared to the liver and the muscle.

Table 4 showed the summary of the values from Tables 1-3 as it comprised the total value of each type of vitamin and their overall totals. The concentration train went thus: E > B_3 > D > A> B_1> B_6> C > B_{12} > K > B_5 > B_2 > B_9.

Dry extract values compared with smoke-dried values

In Table 5, the liver vitamin levels under this mode were depicted. The fact that the dry extract values were generally higher than the smoke-dried samples showed that not all the water had been removed through this drying process. It might be possible that such fish samples could still be subjected to mould growth/spoilage. The concentration trend followed what we have observed in Table 1. The percentage difference of 24.5 was relatively low, also the CV% of 19.7 was low and showed how close the values were to each other. Total vitamins was 38.6/29.1mg/100g.

Table 6 for the muscle was a reflection of Table 2. However, the percentage difference in Tables 5 and 6 were not as close as we observed in Tables 1 and 2. For example in Tables 1 and 2, both percentage difference and CV% had these respective values: 74.5/74.3 and 84.0/83.6 but in Tables 5 and 6 we had 24.5/10.2 and 19.7/7.60.

In the Table 7, head values were shown. This should also be a reflection of Table 3. However, the percentage difference and the CV% values were much closer to Table 6 values: 10.2/9.10 and 7.60/6.74. It would appear the effect of heating was much on the fish samples. Could this be due to what has been called dry matter loss which can result from heat generation as caused by microbial respiration? This decreases the content of nonstructural carbohydrate, protein, and food energy [19]. Could this had happened in these samples?

Table 8 followed the trend in Table 4. However, in the concentration train, vitamin B_6 > B_1, hence that interchange distrupted the pattern of concentration as seen in Table 4. Also the differences were close at values of 9.69 – 24.5% and CV% of 7.20 – 19.7.

Dry extracts from wet/dry extracts and smoke-dried compared

In Table 9, differences between the two different dry extract values were compared. On vitamin basis from liver, muscle, head and totals, we have these percentage difference ranges : B_3(66.5 – 72.3%); B_6 (67.4 – 72.6%); A (66.6- 73.0%); B_1 (70.0- 74.5%); B_2 (66.7-75.3%); D (66.8- 76.3%); E (66.2- 73.2%); B_9 (66.8- 74.7%); K (73.4- 82.0%); B_5 (62.9- 74.9%); B_{12} (66.3- 74.3%) and totals (67.1 – 73.6). In all the ranges shown above, the head had the lowest value of the range except in vitamin K whereas the higher end of the range was shared between liver and muscle values. This further confirmed the closeness between the values in between the liver and the muscle but also the lower values in the head.

Chi-square values from dry extract from fresh samples

The values in Table 10 were the various χ^2 values for individual vitamins when compared across board between liver, muscle, head and totals. The critical value was set at α=0.05 at n-1(df) and had a value of 5.99. These vitamins had their $\alpha_{calculated}$ > α_{Table}: B_6, C, A, B_1, D, E and totals. Significant differences occurred among three fat-soluble and also among three water- soluble vitamins making a 50/50 percent sharing. The train of significance ran thus: E (64.5) >A (40.7) > total (31.6) > D (12.1) > B_1 (10.2) > B_6 (9.00) > B_1 (7.50). Other χ^2 values ranged between 1.06 – 1.92.

Chi-square values from dry extract from smoke-dried samples

Table 11 was a counterpart of Table 10 in terms of the statistical tool used to evaluate their various values. In Table 11, only three χ^2 values were significant; they were E (17.5), A (11.6) and totals (7.56). All other vitamins had their χ^2 values much lower than 5.99. All said and done *Clarias gariepinus* would serve as a major source of vitamins E and A particularly from their liver.

In Table 12, the weight range of the fish samples used was demonstrated, it also gave the mean weight, standard deviation and the percentage ratio of the head, muscle and liver in comparison with total mean weight of the cropped fish. The Table showed that the head was about 32.1%, muscle was 36.4%, liver was 13.6% and drained blood was about 17.9%. This showed that the liver occupied a reasonable portion of the total weight of *C. gariepinus* and so should not be neglected in nutrition.

After smoking, *C. gariepinus* smoke-dried weight was 2.89kg with total loss of 8.31kg moisture. This amounted to 74.2% of the moisture lost in the fresh catfish, which showed that catfish was about three quarter water and highly perishable. The value of 74.2% was comparatively close to the report of 73.68% by Olayemi *et al*. [23]. This is why it has always been urgent for the fish protection against destructive agents like microorganisms; hence the need for smoke-drying being the easiest and cheapest mode of fish preservation in Nigeria.

It is very much of interest to note that the inspection of the proximate analysis results gave an explanation to the various values reported for the percentage differences between dry extract versus fresh sample and also between dry extract versus smoke- dried samples. It has been explained in the above paragraph that 11.2kg of fish lost a value of 8.31kg of moisture which was equivalent to a percentage difference of 74.2%. A check on Tables 1, 2, 4 were all in this range of value for dry extract- fresh sample (percent) and value of 68.5% was very close to this in Table 3. In the case of dry extract- smoke - dried samples, as shown in Tables 5, 6, 7, the percentage differences were respectively: 24.5, 10.2 and 9.10. In the results for the proximate values for the liver, muscle and head, we had moisture content for liver as 24.5%, muscle as 10.2% and head as 9.10%. From these results, it could be concluded that the analytical value when subtracted from the calculated dry extract value would always yield in percentage value that would be equivalent to the original moisture content of that analytical value.

Conclusions

This study had established why *Clarias gariepinus* must be early preserved to avoid spoilage through the use of smoke - drying preservation in Nigeria. It also established the importance of the fish liver and muscle in being better contributors of vitamins particularly fat-soluble vitamins much more than the head which is a delicacy to many Nigerians. Significant differences existed in the values reported in dry extract, fresh and smoke-dried levels; this was contrary to the hypothesis propounded in the introduction. Finally it was revealed that calculation on dry extract basis would bring a percentage difference that would be equivalent to the moisture content of the original sample.

Table 1. Vitamin values (mg/100g) of wet liver sample substracted from dry extract values calculated from wet liver values (mg/100g) of *Clarias gariepinus*

Vitamin	Dry extract	Wet sample	Difference	% difference	Mean	SD	CV%
B_3	11.5	2.92	8.54	74.5	7.19	6.04	84.0
B_6	6.18e-1	1.58e-1	4.61e-1	74.5	3.88e-1	3.26e-1	84.0
C	1.33e-5	3.40e-6	9.92e-6	74.5	8.36e-6	7.02e-6	84.0
A	20.5	5.22	15.2	74.5	12.8	10.8	84.0
B_1	2.96e-1	7.54e-2	2.20e-1	74.5	1.86e-1	1.56e-1	84.0
B_2	1.32	3.37e-1	9.83e-1	74.5	8.28e-1	6.95e-1	84.0
D	4.42e-1	1.13e-1	3.29e-1	74.5	2.77e-1	2.33e-1	84.0
E	97.3	24.8	72.5	74.5	61.1	51.3	84.0
B_9	6.90e-4	1.76e-4	5.14e-4	74.5	4.33e-4	3.63e-4	84.0
K	1.71	4.36e-1	1.27	74.5	1.07	9.01e-1	84.0
B_5	1.72	4.38e-1	1.28	74.5	1.08	9.04e-1	84.0
B_{12}	4.33e-2	1.10e-2	3.22e-2	74.5	2.72e-2	2.28e-2	84.0
Totals	135	34.5	101	74.5	84.9	71.3	84.0

SD= Standard deviation; CV%= Coefficient of variation

Table 2. Vitamin values (mg/100g) of wet muscle sample substracted from dry extract values calculated from wet muscle values (mg/100g) of *Clarias gariepinus*

Vitamin	Dry extract	Wet sample	Difference	% difference	Mean	SD	CV%
B_3	8.35	2.15	6.20	74.3	5.25	4.39	83.6
B_6	11.2	2.87	8.30	74.3	7.02	5.87	83.6
C	8.07	2.07	6.00	74.3	5.07	4.24	83.6
A	2.64e-2	6.78e-3	1.96e-2	74.3	1.66e-2	1.39e-2	83.6
B_1	11.6	2.98	8.61	74.3	7.28	6.09	83.6
B_2	1.25e-1	3.21e-2	9.29e-2	74.3	7.86e-2	6.57e-2	83.6
D	14.0	3.59	10.4	74.3	8.77	7.33	83.6
E	33.6	8.65	25.0	74.3	21.1	17.7	83.6
B_9	1.98e-4	5.09e-5	1.47e-4	74.3	1.25e-4	1.04e-4	83.6
K	6.63e-1	1.70e-1	4.93e-1	74.3	4.17e-1	3.48e-1	83.6
B_5	5.33e-1	1.37e-1	3.96e-1	74.3	3.35e-1	2.80e-1	83.6
B_{12}	1.77	4.55e-1	1.31	74.3	1.11	9.29e-1	83.6
Totals	89.9	23.1	66.8	74.3	56.5	47.2	83.6

Table 3. Vitamin values (mg/100g) of wet head sample substracted from dry extract values calculated from wet head values (mg/100g) of *Clarias gariepinus*

Vitamin	Dry extract	Wet sample	Difference	% difference	Mean	SD	CV%
B_3	6.04	1.90	4.14	68.5	3.97	2.93	73.7
B_6	7.09	2.23	4.85	68.5	4.66	3.43	73.7
C	5.67	1.79	3.89	68.5	3.73	2.75	73.7
A	1.69e-2	5.34e-3	1.16e-2	68.5	1.11e-2	8.21e-3	73.7
B_1	7.11	2.24	4.87	68.5	4.67	3.44	73.7
B_2	8.91e-2	2.81e-2	6.10	68.5	5.86e-2	4.31e-2	73.7
D	8.49	2.67	5.82	68.5	5.58	4.11	73.7
E	22.0	6.92	15.1	68.5	14.5	10.6	73.7
B_9	1.42e-4	4.48e-5	9.74e-5	68.5	9.35e-5	6.89e-5	73.7
K	3.84e-1	1.21e-1	2.63	68.5	2.53e-1	1.86e-1	73.7
B_5	3.38e-1	1.21e-1	2.17e-1	64.3	2.30e-1	1.54e-1	66.9
B_{12}	1.29	4.05e-1	8.81e-1	68.5	8.46e-1	6.23e-1	73.7
Totals	58.5	18.4	40.1	68.5	38.5	28.3	73.7

Table 4. Total vitamin body load values (mg/100g) of wet substracted from dry extract values calculated from wet total body load values (mg/100g) of *Clarias gariepinus*

Vitamin	Dry extract	Wet sample	Difference	% difference	Mean	SD	CV%
B_3	25.9	6.97	18.9	73.0	16.4	13.4	81.3
B_6	18.9	5.26	13.6	72.1	12.1	9.62	79.8
C	13.7	3.86	9.89	71.9	8.80	6.99	79.4
A	20.5	5.23	15.3	74.5	12.9	10.8	83.9
B_1	19.0	5.29	13.7	72.1	12.1	9.68	79.8
B_2	1.53	3.97e-1	1.14	74.1	9.66e-1	8.04e-1	83.3
D	22.9	6.37	16.5	72.2	14.6	11.7	79.8
E	153	40.4	113	73.6	96.7	79.6	82.3
B_9	1.03e-3	2.72e-4	7.58e-4	73.6	6.51e-4	5.36e-4	82.4
K	2.76	7.28e-1	2.03	73.6	1.74	1.44	82.4
B_5	2.59	6.96e-1	1.89	73.1	1.64	1.34	81.5
B_{12}	3.10	8.71e-1	2.23	71.9	1.98	1.58	79.4
Totals	384	76.1	208	73.2	180	147	81.6

Table 5. Vitamin values (mg/100g) of liver dry sample substracted from dry extract values calculated from smoke dried liver values (mg/100g) of *Clarias gariepinus*

Vitamin	Dry extract	Dry sample	Difference	% difference	Mean	SD	CV%
B_3	3.48	2.63	8.54e-1	24.5	3.06	6.04e-1	19.7
B_6	1.87e-1	1.41e-1	4.57e-2	24.5	1.64e-1	3.23e-2	19.7
C	3.55e-6	2.68e-6	8.70e-7	24.5	3.12e-6	6.15e-7	19.7
A	5.82	4.40	1.43	24.5	5.11	1.01	19.7
B_1	7.53e-2	5.69e-2	1.85e-2	24.5	6.61e-2	1.31e-2	19.7
B_2	3.27e-1	2.47e-1	8.00e-2	24.5	2.87e-1	5.66e-2	19.7
D	1.10e-1	8.30e-2	2.69e-2	24.5	9.65e-2	1.91e-2	19.7
E	27.8	21.0	6.82	24.5	24.4	4.82	19.7
B_9	1.75e-4	1.32e-4	4.28e-5	24.5	1.53e-4	3.03e-5	19.7
K	3.07e-1	2.32e-1	7.53e-2	24.5	2.70e-1	5.32e-2	19.7
B_5	4.31e-1	3.26e-1	1.06e-1	24.5	3.79e-1	7.47e-2	19.7
B_{12}	1.11e-2	8.39e-3	2.72e-3	24.5	9.75e-3	1.92e-3	19.7
Totals	38.6	29.1	9.46	24.5	33.9	6.69	19.7

Table 6. Vitamin values (mg/100g) of muscle dry sample substracted from dry extract values calculated from smoke dried muscle values (mg/100g) of *Clarias gariepinus*

Vitamin	Dry extract	Dry sample	Difference	% difference	Mean	SD	CV%
B_3	2.31	2.08	2.36 e-1	10.2	2.20	1.67 e-1	7.60
B_6	3.06	2.75	3.12 e-1	10.2	2.90	2.22 e-1	7.60
C	2.07	1.86	2.11 e-1	10.2	1.96	1.49 e-1	7.60
A	7.12e-3	6.39e-3	7.26 e-4	10.2	6.76 e-3	5.14 e-4	7.60
B_1	3.16	2.84	3.22 e-1	10.2	3.00	2.28 e-1	7.60
B_2	3.43e-2	3.08e-2	3.50 e-3	10.2	3.25 e-2	2.47 e-3	7.60
D	3.30	2.97	3.37 e-1	10.2	3.13	2.38 e-1	7.60
E	9.02	8.10	9.20 e-1	10.2	8.56	6.50 e-1	7.60
B_9	5.34e-5	4.80e-5	5.45 e-6	10.2	5.07 e-5	3.85 e-6	7.60
K	1.76e-1	1.58e-1	1.80 e-2	10.2	1.67 e-1	1.27 e-2	7.60
B_5	1.45e-1	1.30e-1	1.48 e-2	10.2	1.37 e-1	1.05 e-2	7.60
B_{12}	4.78e-1	4.29e-1	4.88 e-2	10.2	4.54	3.45 e-2	7.60
Totals	23.8	21.3	2.42	10.2	22.5	1.71	7.60

Table 7. Vitamin values (mg/100g) of head dry sample substracted from dry extract values calculated from smoke dried head values (mg/100g) of *Clarias gariepinus*

Vitamin	Dry extract	Dry sample	Difference	% difference	Mean	SD	CV%
B_3	2.03	1.84	1.84e-1	9.10	1.93	1.30e-1	6.74
B_6	2.31	2.10	2.10e-1	9.10	2.21	1.49e-1	6.74
C	1.81	1.64	1.64e-1	9.10	1.72	1.16e-1	6.74
A	5.66e-3	5.14e-3	5.15e-4	9.10	5.40e-3	3.64e-4	6.74
B_1	2.13	1.94	1.94e-1	9.10	2.04	1.37e-1	6.74
B_2	2.97e-2	2.70e-2	2.70e-3	9.10	2.83e-2	1.91e-3	6.74
D	2.82	2.56	2.57e-1	9.10	2.69	1.81e-1	6.74
E	7.43	6.75	6.76e-1	9.10	7.09	4.78e-1	6.74
B_9	4.71e-5	4.28e-5	4.29e-6	9.10	4.50e-5	3.03e-6	6.74
K	9.51e-2	8.65e-2	8.66e-3	9.10	9.08e-2	6.12e-3	6.74
B_5	1.25e-1	1.14e-1	1.14e-2	9.10	1.20e-1	8.07e-3	6.74
B_{12}	4.33e-1	3.94e-1	3.94e-2	9.10	4.13e-1	2.79e-2	6.74
Totals	19.2	17.5	1.75	9.10	18.3	1.24	6.74

Table 8. Vitamin values (mg/100g) of total dry sample substracted from dry extract values calculated from smoke dried total values (mg/100g) of *Clarias gariepinus*

Vitamin	Dry extract	Dry sample	Difference	% difference	Mean	SD	CV%
B_3	7.83	6.55	1.27	16.3	7.19	9.01e-1	12.5
B_6	5.56	4.99	5.68e-1	10.2	5.27	4.02e-1	7.62
C	3.88	3.50	3.75e-1	9.69	3.69	2.65e-1	7.20
A	5.84	4.41	1.43	24.5	5.12	1.01	19.7
B_1	5.37	4.83	5.35e-1	9.96	5.10	3.78e-1	7.41
B_2	3.91e-1	3.04e-1	8.62e-2	22.1	3.47e-1	6.10e-2	17.5
D	6.23	5.61	6.21e-1	9.95	5.92	4.39e-1	7.41
E	44.3	35.9	8.42	19.0	40.1	5.95	14.9
B_9	2.75e-4	2.23e-4	5.25e-5	19.1	2.49e-4	3.72e-5	14.9
K	5.79e-1	4.77e-1	1.02e-1	17.6	5.28e-1	7.21e-2	13.7
B_5	7.02e-1	5.70e-1	1.32e-1	18.8	6.36e-1	9.33e-2	14.7
B_{12}	9.22e-1	8.31e-1	9.08e-2	9.85	8.77e-1	6.43e-2	7.33
Totals	81.6	68.0	13.6	16.7	74.8	9.63	12.9

Table 9. Vitamin values (mg/100g) of body parts of dry extract from dry smoked samples substracted from dry extract values from wet body parts of *Clarias gariepinus*

Vitamin	Source: Liver	Source: Muscle	Source: Head	Source: Total
	Dry extract (wet)-dry extract (smoked) and (% difference)	Dry extract (wet)-dry extract (smoked) and (% difference)	Dry extract (wet)-dry extract (smoked) and (% difference)	Dry extract (wet)-dry extract (smoked) and (% difference)
B_3	7.98 (69.6)	6.04 (72.3)	4.02 (66.5)	18.0 (69.7)
B_6	4.31e-1 (69.8)	8.11 (72.6)	4.77 (67.4)	13.3 (70.5)
C	9.77e-6 (73.3)	6.01 (74.4)	3.87 (68.1)	9.87 (71.8)
A	14.6 (71.5)	1.92e-2 (73.0)	1.11e-2 (66.6)	14.7 (71.5)
B_1	2.22e-1 (74.5)	8.43 (72.7)	4.97 (70.0)	13.6 (71.7)
B_2	9.93e-1 (75.3)	9.08e-2 (72.6)	5.94e-2 (66.7)	1.14 (74.5)
D	3.32e-1 (75.1)	10.7 (76.3)	5.67 (66.8)	16.7 (72.8)
E	69.5 (71.4)	24.6 (73.2)	14.6 (66.2)	109 (71.0)
B_9	5.14e-4 (74.7)	1.45 (73.0)	9.50e-5 (66.8)	7.54 (73.3)
K	1.40 (82.0)	4.87e-1 (73.4)	2.89e-1 (75.2)	2.18 (79.0)
B_5	1.28 (74.9)	3.88e-1 (72.8)	2.13e-1 (62.9)	1.89 (72.9)
B_{12}	3.22 (74.3)	1.29 (73.0)	8.54e-1 (66.3)	2.18 (70.2)
Totals	96.8 (71.8)	66.1 (73.6)	39.3 (67.1)	202 (71.2)

Table 10. Dry extract values (mg/100g) calculated from fresh samples of the body parts of *Clarias gariepinus* compared

Vitamin	Liver	Muscle	Head	Total	Mean	SD	CV%	Chi-square (χ^2)
B_3	11.5	8.35	6.04	25.9	8.62	2.72	31.5	1.71
B_6	6.18e-1	11.2	7.09	18.9	6.29	5.32	84.6	9.00*
C	1.33e-5	8.07	5.67	13.7	4.58	4.15	90.5	7.50*
A	20.5	2.64e-2	1.69e-2	20.5	6.83	11.8	173	40.7*
B_1	2.96e-1	11.6	7.11	19.0	6.33	5.68	89.8	10.2*
B_2	1.32	1.25e-1	8.91e-2	1.53	5.11e-1	7.01e-1	137	1.92
D	4.42	14.0	8.49	22.9	7.62	6.81	89.4	12.1*
E	97.3	33.6	22.0	153	51.0	40.5	79.5	64.5*
B_9	6.90e-4	1.98e-4	1.42e-4	1.03e-3	3.43e-4	3.01e-4	87.7	5.29e-4
K	1.71	6.63e-1	3.84e-1	2.76	9.19e-1	6.99e-1	76.1	1.06
B_5	1.72	5.33e-1	3.38e-1	2.59	8.63e-1	7.46e-1	86.4	1.29
B_{12}	4.33e-2	1.77	1.29	3.10	1.03	8.90e-1	86.2	1.53
Totals	135	89.9	58.5	384	94.6	38.7	40.9	31.6*

Calculations for Mean, SD, CV% and Chi- square involved only values from liver, muscle and head but excluded total; * = results significantly different at $\alpha = 0.05$(df, degree of freedom = n-1 =3-1 = 2; critical value = 5.99)

Table 11. Dry extract values (mg/100g) calculated from dry smoked samples of the body parts of *Clarias gariepinus* compared

Vitamin	Liver	Muscle	Head	Total	Mean	SD	CV%	Chi-square (χ^2)
B_3	3.48	2.31	2.03	7.83	2.61	7.72e-1	29.6	4.57e-1
B_6	1.87e-1	3.06	2.31	5.56	1.85	1.49	80.4	2.40
C	3.55e-6	2.07	1.81	3.88	1.29	1.13	87.2	1.96
A	5.82	7.12e-3	5.66e-3	5.84	1.95	3.36	173	11.6*
B_1	7.53e-2	3.16	2.13	5.37	1.79	1.57	87.8	2.76
B_2	3.27e-1	3.43e-2	2.97e-2	3.91e-1	1.30e-1	1.70e-1	131	4.45e-1
D	1.10e-1	3.30	2.82	6.23	2.08	1.72	82.8	2.85
E	27.8	9.02	7.43	44.3	14.8	11.4	76.9	17.5*
B_9	1.75e-4	5.34e-5	4.71e-5	2.75e-1	9.18e-5	7.19e-5	78.4	1.09e-4
K	3.07e-1	1.76e-1	9.51e-2	5.79e-1	1.93e-1	1.07e-1	55.5	1.19e-1
B_5	4.31e-1	1.45e-1	1.25e-1	7.02e-1	2.34e-1	1.71e-1	73.2	2.51e-1
B_{12}	1.11e-2	4.78e-1	4.33e-1	9.22e-1	3.07e-1	2.58e-1	83.8	4.32e-1
Totals	38.6	23.8	19.2	81.6	27.2	10.1	37.3	7.56*

Calculations for Mean, SD, CV% and Chi- square involved only values from liver, muscle and head but excluded total; * = results significantly different at $\alpha = 0.05$.

Table 12. Weight (kg) of cropped samples of *Clarias gariepinus*

Body part	Weight range	Total	Mean	SD[a]	Percentage[b]
(A) Whole fish	1.36 - 1.45	11.2	1.40	0.029	-
(B) Dressed parts*					
(i) Head	0.39 – 0.48	3.18	0.45	0.028	32.1
(ii) Muscle	0.46 – 0.56	4.08	0.51	0.029	36.4
(iii) Liver	0.17 – 0.22	1.55	0.19	0.018	13.6
(C) Blood drained	0.19 – 0.31	2.02	0.25	0.050	17.9

*Blood drained body parts; [a]SD= Standard deviation;
[b]Percentage = given mean body part weight / whole fish body weight x 100

References

[1] M. Spalding, Sustainable ancient aquaculture, National Geographic. 4 (2013) 111-114.

[2] G. S. Helfman, Fish conservation. A guide to understanding and restoring global aquatic biodiversity and fishery resources, Island Press, Washington DC, 2007.

[3] F. F. OIayemi, M. R. Adebayo, E. U. Bamishaye, E. F. Awagu, Proximate composition of catfish (*Clarias gariepinus*) smoked in Nigerian Stored Product Research Institute (NSPRI): developed kiln, Int.J. Food composition and Analysis. 3(5) (2011) 96-98.

[4] J.R.S. Vitule, V.M. Azevedo-Santos, V.S. Daga, D.P. Lima-Junior, A.L.B. Magalha, etc., Baril's drought: protect biodiversity, Science. 347 (6229) (2015) 1427-1428.

[5] N. Myrvold, Modernist cuisine, The cooking laboratory.1 (2011) 143-147.

[6] B.A.R. Hassan, Vitamins (importance and toxicity), Pharmaceutica Analytica Acta. 3(8) (2012) (Pharmaceut. Anal. Acta 3: e125.doi: 10.4172/2153-2435. 1000 e125).

[7] What is the difference between bound water, entrapped water, Aug 28 2019. https://swine.extension.org> what

[8] Food Chemistry and fish in nutrition: 2.1.3.1., Bound water ecoursesonline.lastri.res.in> view Last modified Tuesday, 28 February 2012.

[9] Wikipedia, Dry matter. https://wikipedia.org/wiki/Dry_matter

[10] Carbohydrates, proteins, and fats, The Merck Manual. http://www.merckmanuals.com/home/disorders_of_nutrition/overview_of_nutririon/carbohydrates_proteins_and_fats.html

[11] American Physiological Society, Physiology Reviews. 2(1922) 123-124.

[12] What is so special about water in food? The Cooking Science Guy. www.cookingscienceguy.com>...

[13] A.C. Brown, Understanding Food: Principles and Preparation, 3rd edition, Cengage Learning. (2007) p.25.

[14] R. Kava, Water Log, June 21, 2010, at the Wayback Machine, A.C.S.H., April 1 (2001).

[15] L. Bento, Microwave Sensors, Sucropedia. http: //www.sucropedia.com/? p=entries&a= read & id= E0093.

[16] S.E. Gebhardt, R.G. Thomas, Nutritive value of foods. www.ars.usda.gov. USDA. Retrieved 14 April, 2015.

[17] B.A. Reiling, Feed Dry Matter Conversions. www.ianr pubs.unl.edu. University of Nebraska Lincoln Extension. Retrieved 14 April. (2015).

[18] M.A. Wattiaux, Dairy Essentials, Chapt 2: Composition and Analysis of Feed, Babcock Institute. http://babcock.cals.wise.edu/?q=node/130)

[19] D.R. Buckmaster, Indoor Hay Storage: Dry Matter Loss and Quality Changes, June 11 (2010) at the Wayback Machine. Retrieved from " https://en.wikipedia.org/w/index.php?title = Dry_matter & oldid = 763039 095"

[20] R.A. Oloyo, Fundamentals of research methodology for social and applied sciences, R.O.A. Educational Press, Ilaro, Nigeria, 2001.

[21] Pubchem and the American Chemical Society, Reshaping Scholarly Communication, University of California, USA, 2005-05-31. Retrieved 2018-10-15.

[22] J.N. Counsel, D.H. Hornig, Vitamin C (Ascorbic acid), Applied Science Publishers, London, 1981.

[23] F.F. OIayemi, M.R. Adebayo, E.U. Bamishaye, E.F. Awagu, Proximate composition of catfish (*Clarias gariepinus*) smoked in Nigerian Stored Product Research Institute (NSPRI): developed kiln, Intl. J. Food Comp. Anal. 12 (2011) 9-16.