PROXIMATE AND MINERAL CHARACTERISTICS OF NIGERIAN LOCAL CHEESE (*WARA*)

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Abstract: The report here contained the analytical characteristics of the proximate and mineral evaluation of Nigerian local cheese popularly called *wara* prepared from the local extract coagulant from *Calotropis procera*. Analyses were on dry weight basis. The proximate values exhibited high levels of the following (g/100 g): crude fat, crude protein, crude fibre (probably from *C. procera*), dry matter, organic matter and total fatty acid (TFA) (converted from the crude fat) but low levels for carbohydrate, total ash and moisture content. Whereas total gross energy from crude fat + crude protein + carbohydrate was 404 kcal/100 g and 1718 kJ/100 g, corresponding TFA energy of 156 kcal/100 g and 643 kJ/100 g respectively were recorded. The UEDP% was moderately high at 19.6 – 19.8. Nutritional minerals of significance in the samples (mg/100 g) were: Fe, Cu, Mn, Zn, Ca, Mg, K, P all totalling 2039 mg/100 g. High percentage mineral levels were observed for Ca (10.7%), K (59.8%) and P (26.6%) with lowest coming from Cd (3.43×10^{-5}%). Among the mineral ratios, Ca/Mg was the only ratio that was close to the ideal (6.07/6.67); the high ratios observed for the toxic minerals were advantageous. The calculated mineral safety index (MSI) was compared to the standard MSI, values showed that the $MSI_C < MSI_T$ for all the minerals (Na, Mg, P, Ca, Fe, Se, Zn and Cu); percentage differences ranged from 10.1 (Fe) to 99.7 (Na). All the differences were positive towards MSI_T meaning that none of the minerals in *wara* would be harmful to its consumer(s).

Keywords: Natural coagulant, *wara*, chemical characteristics

Introduction

Cheese is a product from the processing of milk (Scott, 1986). Cheese is used as a form of preserving essential nutrients in milk and it is used to be an excellent source of nutrients such as proteins, fats, minerals and vitamins (Omotosho *et al.*, 2011). In some milk-producing countries, a large portion of the milk produced is used for cheese making (Davies and Law, 1984); in African countries, about one-third of the total volume of milk is used for cheese making (FAO, 2002). In Nigeria, there is widespread traditional manufacture of soft cheese locally called *wara*. *Wara* is made by boiling cow milk with some added coagulant to curdle the milk protein resulting in coagulated milk protein and whey (the liquid remaining after milk has been curdled and strained). This milk protein is then skimmed off from the wey and sold as *wara* (https://www.pulse.ng/lifestyle/food-travel/wara-this_loc). As sometimes portrayed *wara* is not cheese. It is milk curds achieved by adding a coagulant to fresh milk whereas cheese is achieved through a process of ageing pressed milk curd. It may be called a local *Tofu*. *Wara* is not a product of fermentation. *Wara* is eaten in various forms such as raw cheese, flavoured snack, sandwich filling or fried cake (Omotosho *et al.*, 2011).

The use of vegetable extracts as milk coagulants in soft cheese processing has been known traditionally in many parts of West Africa such as Nigeria and the Republic of Benin (Aworth and Muller, 1987). Milk coagulants of plant origin have over-ridden the use of animal rennet. It has been concluded that the yield and quality of cheese is said to be determined by the quality of the milk used and the type of coagulants. Plant sources of coagulants include sunflower (Aworth and Muller, 1987), pineapple (O'Connor, 1993), several plant preparations of *Cynara cardunculus* (Vieira de Sá and Barbosa, 1972), $CaCl_2$ (Omotosho *et al.*, 2011). In Nigeria, *tofu*, a coagulated soymilk is produced at household level using coagulants such as $CaCl_2.2H_2O$, alum and steep water (from pap produced from maize) (Oboh and Omotosho, 2005). The coagulant used in cheese making is said to have a dual role. The primary function is to coagulate milk to produce cheese curd that is, converting liquid milk to a gel form. This conversion is catalysed by different proteases (Green, 1984). In addition, a small proportion of the coagulant is carried over into the cheese. The residual coagulant remains proteolytically active in most aged cheeses and plays an important role in the development of texture and flavour (Law, 1987).

The Nigerian cheese, *wara,* is usually manufactured from coagulant derived from the juice of *Calotropis procera* (Sodom Apple leaves: *bomubomu* in the Yoruba language). *Calotropis procera* (Aiton) W. T. Aiton (Asclepiadaceae) has a common name of Auricula Tree. Auricula tree is large, erect shrub growing 4 metres or more tall. It is cultivated and wild. It is a plant of the semi-arid tropics and subtropics, found growing best in areas where the mean annual rainfall is in the range of 300 – 400 mm. Established plants are drought – resistant. Often dominant in areas of abandoned cultivation especially sandy soils in areas of low rainfall. Plant has milky latex throughout, latex contains a proteolytic enzyme called caloptropaine. The latex is used as an absortifacient (The Wealth of India, 1950), spasmogenic and carminative properties (Sharma, 1934). This study would reveal the nutritional attributes (proximate and mineral characteristics) of *wara* produced from the local coagulant of *Calotropis procera.*

Materials and Methods

Collection of samples

Samples of *wara* were purchased from the main market of Ado-Ekiti, Ekiti State in the southern part of Nigeria. Purchase was made from the Bororo women that hawk *wara* in Ado –Ekiti. The *wara* is always in about measured sizes; five of such sizes were purchased for analysis.

Sample treatment

The samples were cut into smaller bits and spread on aluminium tray. The tray was put in an oven and sample oven-dried till constant weight was achieved. The sample was then homogenised, sieved using 200 mm mesh size and kept in plastic bottles in the refrigerator (2.8^{o}C).

Proximate and mineral analyses

Proximate analysis

Moisture content was estimated by drying at 100^{o}C in ventilated oven to express moisture in g/100 g. Crude protein (N x 6.25) of the sample was evaluated by micro-Kjeldahl method (AOAC, 2006). Total lipid was determined by the Soxhlet extraction method using petroleum ether (AOAC, 2006). Crude fibre and total ash contents were determined following the methods outlined in AOAC (2006). The carbohydrate was calculated based on Muller and Tobin (1980);

Carbohydrate (g/100 g) = 100 – [g (protein) + g (lipids) + g (fibre) + g (total ash)] ………………………………. (1)

The calorific value was calculated based on Atwater factors:

Gross energy (kJ/100 g and kcal/100 g) = (Protein x 17/4) + (Lipid x 37/9) + (Carbohydrate x 17/4) ……………….. (2)

Calculation of total fatty acid in the crude fat:

FACID (g/100 g EP) = TL (g/100 g EP) x XFA ………… (3)

Total lipid (TL = crude fat) level was multiplied by a conversion factor of 0.945 to convert to total fatty acids (Anderson, 1976). This gave total fatty acids (TFAs) and non-fatty acid lipids.

Other calculations

i. The percentage contribution to the total energy due to protein (PEP), due to total fat (PEF) and due to carbohydrate (PEC) as PEP%, PEF% and PEC% respectively were calculated.
ii. The percentage utilizable energy percent due to protein (UEDP%) assuming 60% protein energy utilization was also calculated.
iii. Approximate sample weight equivalents to the energy requirements of adults and infants from the proximate values.
iv. Water balance requirements for complete protein metabolism.

Mineral analysis

The minerals were analysed from the solution derived by first ashing samples at 550^{o}C. The filtered solutions were used to determine Na, K, Ca, Mg, Zn, Fe, Mn, Co, Cu, Pb, Se, Cd and Ni by means of atomic absorption spectrophotometer (Buck Scientific Model- 200A/210, Norwalk, Connecticut 06855) and phosphorus was determined colorimetrically by spectronic 20 (Gallenkamp, UK) using the phosphovanado molybdate method (AOAC, 2006). All chemicals used were of British Drug House (BDH, London, UK) analytical grade. The detection limits for the metals in aqueous solution had earlier been determined using the methods of Varian Techtron (1975) giving the following values in µg/ml: Fe (0.01), Cu (0.02), Na (0.002), K (0.005), Ca (0.04), Mg (0.002), Zn (0.005), Mn (0.01), Pb (0.08), Ni (0.02), Se (0.15), Cd (0.01) and Co (0.05). The optimal analytical range was 0.1 – 0.5 absorbance units with coefficient of variation from 0.9 – 2.21%.

The percentage mineral values were calculated. Mineral ratios were evaluated as appropriate and the milliequivalent ratio [K/(Ca + Mg)] was evaluated (Hathcock, 1985). The mineral safety index (MSI) of appropriate minerals were evaluated (Hathcock, 1985).

Result and Discussion

Proximate composition

The proximate constituents (g/100 g) of the samples were shown in Fig. 1. The moisture content of the sample was relatively high at 7.70 g/100 g. On comparison, the moisture content was higher than these literature values: *Numidia meleagris* (2.99 $\pm$ 1.75 g/100 g) (Adeyeye and Adesina, 2014); in duck organs (2.88 $\pm$ 1.40 g/100 g) (Adeyeye, 2020) and in the pouch rat, moisture mean was 3.23 $\pm$ 2.02 g/100 g (Adeyeye and Adesina, 2018). The relative high moisture content might not ensure a long shelf life for the sample against microbial attack/spoilage; this is because most Nigerians do not enjoy stable electricity supply that could have assisted in the preservation of samples. The dry matter (DM) content of the *wara* sample was 92.3 g/100 g, was lower than the DM in the organs of the duck (95.22 – 95.5 g/100 g) (Adeyeye, 2020), the DM was a reflection of the moisture content of the sample. The organic matter (OM) of the *wara* sample was 87.9 g/100 g. This value compared favourably to the values observed in four fresh water fishes of *Mormyrops delicious* (86.4 g/100 g), *Bagrus bayad* (75.0 g/100 g), *Synodontis budgetti* (84.0 g/100 g) and *Hemichronis fasciatus* (76.0 g/100 g) (Abdullahi and Abolude, 2002). However, the *wara* was lower than these literature values (g/100 g): duck-hen samples (91.45 – 99.27) (Adeyeye and Adesina, 2018); ostrich muscle (98.97) (Sales and Hayes, 1996) and trunk fish (91.07) (Adeyeye and Adamu, 2005). The ash content of the sample was 4.40 g/100 g. The literature ash content was lower to the *wara* ash content value (g/100 g): total mean ash in duck was 1.66 $\pm$ 1.40; it was 2.34 $\pm$ 2.80 in *N. meleagris;* mean ash value in organs of pouch rat was 1.65 $\pm$ 1.67 (Adeyeye and Adesina, 2018). The ash content of any sample is a reflection of the mineral content of such a sample. Since the ash content of *wara* was greater than the literature compared values, then the *wara* would be richer in mineral content than cited literature samples. The fibre content of *wara* was high at 8.50 g/100 g. Both duck samples, guinea fowl samples and pouch rat samples had no detectable level of fibre. The high fibre in *wara* could very much likely had come from the plant coagulant used to curdle the protein in the milk. The crude protein was high at 33.4 g/100 g. Depending on which part of the other animal organs (duck and pouch rat) was compared to the *wara* protein value, that of the *wara* protein would either be lower or higher, seen as follows (g/100 g), *wara*/literature compared: *wara*/duck eyes (33.4/18.2); *wara*/duck skin (33.4/3.24); *wara*/pouch rat eyes (33.4/7.11); *wara*/pouch rat skin (33.4/2.70); *wara*/*N. meleagris* skin (33.4/1.08); *wara/N. meleagris* eyes (33.4/17.7); *wara*/other duck organs (33.4/71.6 – 81.5) and *wara*/other pouch rat organs (33.4/54.0 – 85.8). The total crude fat content in *wara* was 18.4 g/100 g. This level was higher than the reports in the crude fat levels of 0.23 – 5.60 g/100 g in the organs of duck-hen; in the pouch rat, fat range was 0.210 – 4.62 g/100 g and the range was 0.120 – 3.55 g/100 g in guinea fowl (Adeyeye and Adesina, 2018); muscle of turkey had a value of 2.12 g/100 g and the turkey skin had a value of 12.1 g/100 g (Adeyeye and Ayejuyo, 2007); ostrich muscle (with skin) was 12.6 g/100 g and beef (22.3 g/100 g) (USDA, 1979); USDA, 1986) and kilishi (a beef product) had a value of 14.2 g/100 g (Adeyeye *et al*., 2020). When the crude fat was converted to the total fatty acid (TFA), the TFA value of *wara* was 17.4 g/100 g whereas other lipids was 1.00 g/100 g. The implication of this would be that the sample was high in TFA but very low in other lipids like sterols, phospholipids, spingolipids, etc. The carbohydrate level in *wara* was relatively low with a value of 27.6 g/100 g. In literature, the following carbohydrate levels had been reported in animal organs (g/100 g): eyes of Muscovy Duck-hen (80.2 g/100 g) and skin (95.8 g/100 g); muscle of duck-hen (6.19 g/100 g) whereas other organs in duck-hen ranged from 13.0-24.5 g/100 g (Adeyeye, 2020), this range being lower to the present *wara* value. In guinea fowl, skin had carbohydrates value of 98.3 g/100 g and followed by 80.6 g/100 g in guinea fowl eyes but in the fowl muscle as 5.46 g/100 g (Adeyeye and Adesina, 2014), again this was lower to *wara* values. In pouch rat, skin had 94.6 g/100 g, eyes (88.7 g/100 g) and muscle (6.83 g/100 g) (Adeyeye *et al.*, 2020). Minus the eyes and skin which carbohydrate values were much higher than in *wara*, *wara* had much higher carbohydrate levels than all the muscle values compared.

As changes occur in dietary, nutritional status and age of an animal, appreciable shifts occur in the tissue compartments, water and protein levels (Cowgwill, 1958). For the efficient utilization and conservation of food within the human body, water is indispensable (Snively and Wessener, 1954), it is because the water content of the body changes with the type of diet (White House Conferences, 1932). This important connection of water with other food substances is the fact that the biochemical basis for the relationship arises from the fact that the water deficit created by protein metabolism is about seven times that for equivalent calories of carbohydrates or fat. Therefore, in young children an increase in calories from carbohydrates causes hydration; whereas an increase in calories from proteins causes dehydration (Pratt and Snyderman, 1953). The increased output of ketones and acids that accompanies a shift to high fat diets is associated with increased water loss that can be offset by increase in carbohydrate intake. Protein quality as well influences the degree of tissue hydration. Albanese (1959) had estimated grammes of water needed for complete metabolism of 100 calories of some food substances. Food materials (protein, starch and fat) all have preformed water of 0.00 in each case; water gained by oxidation: 10.3 (protein), 13.9 (starch) and 11.9 (fat); lost in dissipating heat: 60.0 for each of the food materials; water lost in excreting end products (1 calorie of protein requires 3.0 ml of water for the excretion of the urea and sulphate formed from it, 1 g of ash requires 65 ml of water for its excretion): 300 (protein), both 0.00 in starch and fat; deficit: 350 (protein), 46 (starch) and 48 (fat). The energy equivalent of this part of discussion would be elaborated in Fig. 2 discussion.

Total energy contributions (kJ/100 g and kcal/100 g) by the proximate composition components had been depicted in Fig. 2. The percentage energy contribution levels could also be seen in Fig. 2. Contribution parts in kJ/kcal/100 g and percentage value: crude fat, 166 kcal/100 g (44.1%) /681 kJ/100 g (39.6%); crude protein 134 kcal/100 g (33.2%)/568 kJ/100 g (33.1%); carbohydrate, 104 kcal/100 g (25.7%)/469 kJ/100 g (27.3%) and total, 404 kcal/100 g (100%)/1718 kJ/100 g (100%). The total energy value in *wara* sample corresponded about similar value in the heart value of Muscovy Duck-hen with value of 1713 kJ/100 g (1.713 MJ) or 404 kcal/100 g but much higher than in the gizzard with value of 1644 kJ/100 g (1.644 MJ) or 388 kcal/100 g (Adeyeye, 2020). This energy level of 1.72 MJ/100 g was better than 1.61 – 1.71 MJ in eight organs of guinea fowl, turkey muscle and skin (1.33 - 1.37 MJ) but lower to the levels in sheep lean meat (2.06 MJ), lean pork (2.29 MJ) but better than kilishi meat (1.66 MJ) (Adeyeye and Adesina, 2014; Adeyeye and Ayejuyo, 2007; Fornias, 1996; Adeyeye *et al.*, 2020). Other literature total energy values showed that *wara* was still better than them as shown: cereals (1.3 – 1.6 MJ/100 g) (Paul and Southgate, 1978); energy levels in 10 organs of African giant pouch rat (1.63 – 1.70 MJ/100 g) (Adeyeye and Adesina, 2018). The *wara* metabolisable energy showed it to be a source of dense energy. The PEF%, PEP% and PEC% in Fig. 2 showed that PEF% > PEP% > PEC%. In duck-hen organs, the trend was PEF% (0.5027 – 12.1) < PEC% (6.32 – 96.2) < PEP% (3.25 – 81.7) (with 5/9 or 55.6% being in the range of 72.1 – 76.8%) (Adeyeye, 2020). The trend in the duck-hen was in the reverse case compared to the *wara* trend with its value falling within the range of the values in the duck-hen. In kilishi the trend was PEC%(2.36) < PEF% (31.7) < PEP% (66.0) (Adeyeye *et al.*, 2020). The PEF% of 39.6 (kJ/100 g) and 41.1 (kcal/100 g) compared favourably with recommended level of 30% (NACNE, 1983) and 35% (COMA, 1984) for total fat intake, this is still reasonable for people wishing to adopt the guidelines for a healthy diet. This is more so when it is noted that the EP (g/100 g) (edible portion) of the crude fat was 17.4 with energy equivalent of 156 kcal/100 g and 643 kJ/100 g which gave a 37.4% on kJ/100 g basis which was close to 35% and 38.6% on kcal/100 g basis which was comparable also to 35%. PEC% is important because carbohydrate is the primary source of metabolizable energy. Its adequacy would enhance the metabolizable activity of both protein and fat (NACNE, 1983; COMA, 1984).

In terms of energy need, the daily energy requirement for an adult is between 2500 – 3000 kcal which depends on one's physiological state whilst it is 740 kcal in infants (Bingham, 1978). This translated to mean that an adult would have to consume between 619 – 743 g to make up the energy range of 2500 – 3000 kcal whilst infants would require 183 g of *wara* to satisfy their energy needs. These adult and infant quantity requirements were highly comparable to the following literature values, *wara*/literature values (adult): *wara*/kilishi (619 – 743/634 – 760 g) and *wara*/guinea fowl organs (619 – 743/649 – 733 g); for the infants, we have *wara*//kilishi (183/188 g) and *wara*/guinea fowl organs (183/193 g) (Adeyeye and Adesina, 2014). However, *wara* value was lower than these literature values: *wara*/*Acanthurus monroviae* (619 -743/733 – 880 g) (adults) and 183/220 g (infants); *wara/ Lutjanus goreensis (*619 -743/735 – 882 g) (adults) and 183 / 221 g) (infants) (Adeyeye *et al.*, 2016); in *Callinectes latimanus*, it was 915 g (adults minimum) and 271 (infants) (Adeyeye *et al.*, 2014); 786 – 944 g (muscle) and 761 – 913 g (skin) of turkey to meet adult requirements but 233 g (muscle) and 325 g (skin) in infants (Adeyeye and Ayejuyo, 2007). From Fig. 2, 134 kcal/100 g energy from the *wara* protein would require 402 ml of water for complete metabolism. Hence, water deficit = 350/100 x 134 = 469 ml; hence 469 – 402 = 67.0 ml. (This is because 100 calories have water deficit of 350 ml). This showed that little water (just above the volume of sachet water of 50.0 ml: 67 ml > 50 ml by 17 ml) would always be needed for consumption in taking the diet of only *wara*. In the organs of duck-hen, water requirements for proximate compositions of three head organs, three visceral organs, muscle and skin, water balance requirement ranged between 6.48 – 160 ml (Adeyeye, 2020).

The utilizable energy due to protein (UEDP%) for *wara* (assuming 60% utilization) was 19.6/19.8 (kcal/100 g/kJ/100 g). the recommended value for an adult man who requires about 55 g protein per day with 60% utilization is 8% (lower than in *wara*). This value of 19.6/19.8 fell within some of these literature values: 1.95 – 49.0 (duck- hen organs); 56.4 and 40% (turkey muscle/skin, respectively); 12.1 - 28.8% (female and male exoskeleton), 12.5 – 23.8% (female and male flesh) and 13.8 – 17.9% (female and male whole body) of West African fresh water crab (*Sudananautes africanus africanus)* (Adeyeye and Ayejuyo, 2007; Adeyeye *et al.*, 2010). In guinea fowl, UEDP% ranged from 0.650 – 51.6 (Adeyeye and Adesina, 2014); in pouch rat, range was 1.62 – 51.9 (Adeyeye and Adesina, 2018) whereas in kilishi, range was 39.2 – 39.6 (Adeyeye *et al.*, 2020). The high UEDP% from the sample showed that the sample had protein concentration in terms of energy that would be more than enough to prevent protein energy malnutrition in children and adult fed solely on the sample as the main source of protein.

Furthermore in Fig. 2 we have values for energy of crude fat, total fatty acids (TFAs) and other lipids; it showed that the TFA in the crude fat far outstripped the other lipids (like phospholipids, sterols, spingolipids, etc.). Whereas % TFA/total lipid was 94.6, the other lipids/total lipid was 5.4. Incidentally, a particular end of the above observation tallied almost exactly to one end of the range in duck-hen organs; it follows (*wara*/duck-hen organs): *wara*/duck organs (94.6/56.1 – 94.6, TFA%) and 5.4/5.50-43.9, other lipids%) (Adeyeye, 2020).

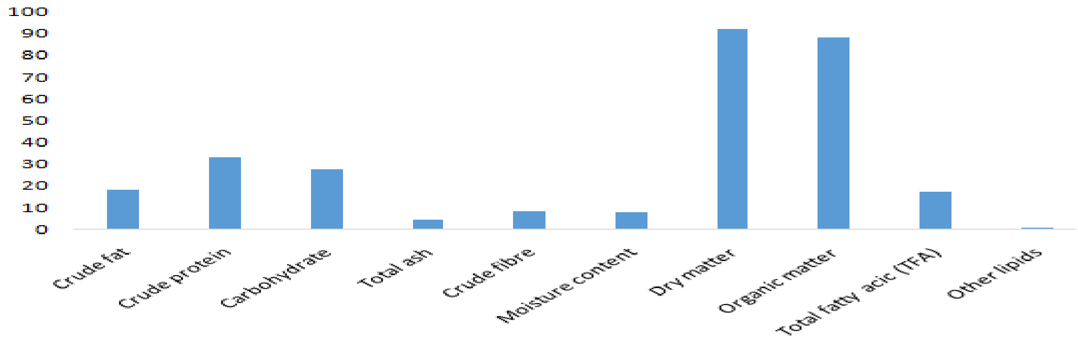

Fig. 1: Proximate composition of *wara* (g/100g dry weight)

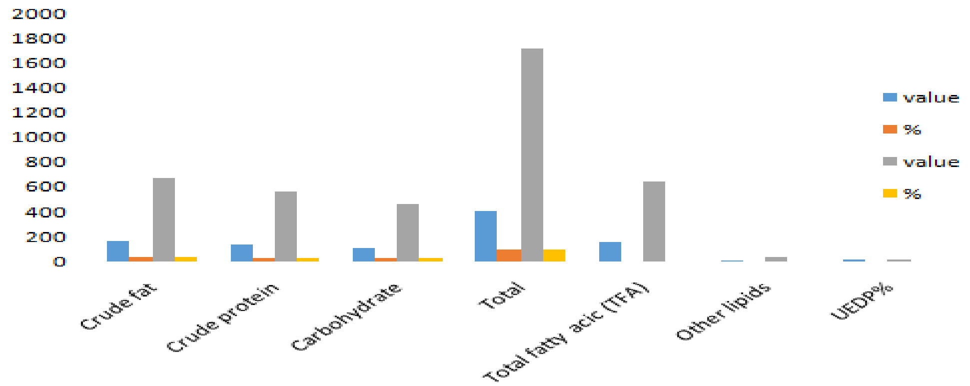

Fig. 2: Energy contribution by relevant proximate components of *wara*

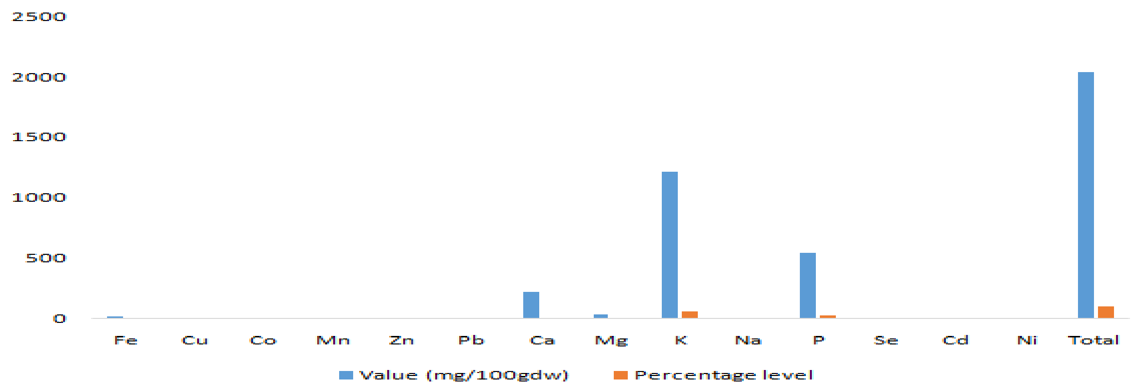

Fig. 3: Mineral composition of *wara*

Mineral composition

In Fig. 3, we have the mineral values and their percentage levels. Minerals of major and significant values were (mg/100 g): Fe, Mn, Cu, Zn, Ca, Mg, K and P. Total weight of the minerals observed was 2039 mg/100 g. For significant minerals observed in the sample, their percentage (%) levels were: Fe (0.6608), Cu (0.0726), Mn (0.1077), Zn (0.2048), Ca (10.7), Mg (1.77), K (59.8) and P (26.6). On the other hand in kilishi, the significant minerals were Fe, Zn, Ca, Mg, K, P and Na with total minerals being 2225 mg/100 g (Adeyeye *et al.*, 2020). The percentage mineral levels trend in *wara* had this: K (59.8) > P (26.6) > Ca (10.7) > Mg (1.77) > Fe (0.6608) > Zn (0.2048) > Mn (0.1077) > Cu (0.0726) whereas the trend was K (44.3) > P (35.1) > Na (14.4) > Mg (3.67) > Ca (1.33) > Zn (8.23e-1) in kilishi (Adeyeye *et al.*, 2020). Minerals of very low level percentages were (%): Co (7.85×10^{-5}), Pb (5.40×10^{-5}), Se (8.98×10^{-4}), Cd (3.43×10^{-5}) and Ni (5.64×10^{-4}). Nutrient minerals in this report were Ca, Mg, Na, K, Cu, Zn, P, Fe, Mn, Se and Co. Nutrient minerals had been extensively studied; they have been well defined and are considered essential for many biological functions in the human body. They play key roles in such metabolic processes as muscular activity, endocrine function, reproduction, skeletal integrity and overall development. On the other hand, toxic minerals in this report were Cd and Pb (Fig. 3). The toxic minerals or "heavy metals" are well-known for their interference upon normal biochemical function. They are commonly found in the environment and therefore are present to some degree, in all biological systems. However, these metals clearly pose a concern for toxicity when accumulation occurs to excess (Trace Elements, Inc.).

Minerals of high/moderate content values were, mg/100 g (percentage value): Fe, 13.5 (0.6608), Mn, 2.20 (0.1077), Zn, 4.18 (0.2048), Ca, 219 (10.7), Mg, 36.0 (1.77), K, 1219 (59.8) and P, 543 (26.6). Minerals at trace levels were Cu, Pb, Na, Se, Cd and Ni with concentration values of 0.0007 – 1.48 mg/100 g (3.43 x 10^{-5} to 0.0726%). On comparison, minerals of high content values in kilishi were: mg/100 g (percentage value): Mg, 81.6 (3.67), K, 985(44.3), P, 781(35.1) and Na, 320 (14.4) whereas moderate levels were Zn, 18.3 (8.23e-1), Fe, 8.62 (3.88e-1) and Ca, 29.6 (1.33). Others, such as Cu, Mn, Pb, Se, Cd, Ni, and Co were all in traces with percentage value range of 8.99e-6 to 1.44e-2 (Adeyeye *et al.*, 2020). These minerals: Fe, Cu, Co, Mn, Zn, Mg, Na, Se and Ni should be sourced from other animal protein sources when *wara* serves as the main source of animal protein. The presence of these minerals: Pb, 0.0011 mg/100g (5.40 x 10^{-5}%) and Cd, 0.0007 mg/100 g (3.43 x 10^{-5}%) showed that both minerals were at ultra-trace levels and their presence in *wara* could be an evidence of the onset of pollution of the environment.

Phosphorus plays an important role in the bones as well as in the cellular membranes as a component of the phospholipids building the membrane lipid bilayer. It is also a component of many intracellular compounds as nucleic acids, nucleoproteins and organic phosphate such as creatine phosphate and adenosine triphosphate. The total content of phosphorus in the human body is about 700 g of which 80.0% are found in bones, 10.9% in viscera and 9.00% in the skeleton muscle tissue (Mertinez-Valverde *et al.*, 2000; Ghosh and Joshi, 2008). Deficiency of phosphorus in the body leads to muscle disorder, metabolic acidosis, encephalopathy and alteration in bone mineralization as well as in cardiac, respiratory, neurological and metabolic disorders (Ghosh and Joshi, 2008). In many publications, P is suggested to be better sources from the following: between 204 and 230 mg/100 g in fish, mollusks, crustaceans, when compared to 176 mg/100 g in terrestrial meat (Tecon and Metian, 2013). Phosphorus in *wara* was much higher than the literature values but less than the value in kilishi, 781 mg/100 g (35.1%) (Adeyeye *et al.*, 2020).

Potassium is primarily an intracellular cation, most part being bound to protein and with sodium influences osmotic pressure and contributes to the normal pH equilibrium (Sanstead, 1967). Dietary lack of potassium is seldom found as both plants and animal tissues are rich sources of the mineral. In *wara*, K value was 1219 mg/100 g making a percentage of 59.8 of the total mineral analysed for showing that K was dominant quantitatively over ther minerals like P, Na, Ca and Mg. The value of 1219 mg/100 g of K in *wara* was higher than in kilishi (985 mg/100 g and 44.3%) (Adeyeye *et al.*, 2020). Sodium regulates water content of the body, aids in transport of CO_2 and maintains osmotic pressure to be effectively carried out, other sources of sodium must be present in the diet if *wara* would be the main source of animal protein; in *wara* value of sodium was just 1.47 mg/100 g(0.0722%) but high in kilishi, 320 mg/100 g (14.4%) (Adeyeye *et al.*, 2020). Magnesium level in *wara* was just average as its value was just 36.0 mg/100 g (1.77%); this being lower than kilishi at 81.6 mg/100 g (3.67%). Magnesium repairs and improves the growth of human body, maintains blood pressure, prevents tooth decay and helps to keep bones healthy. Value of zinc in *wara* was 4.18 mg/100 g less than in kilishi (18.3 mg/100 g and 8.23e-1%). Minimum Zn allowance per day is 15 – 20 mg zinc is a part of many enzymes, required for the body and wound healing. Selenium prevents cancer, poisonous effect of heavy metals and helps the body after vaccination (Ahmed *et al.*, 2018). Iron is one of the key minerals present in meat, it plays a vital role in human health and its deficiency causes several hindrances in the normal functioning of human body, particularly it disturbs child growth and development (Lozoff and Georgieff, 2006). Iron is available in a number of foodstuffs and occurs in two forms like heme and non-heme iron. The former one comes from the haemoglobin and myoglobin, so it is present in animal foods only and has a high degree of bioavailability that could easily be absorbed in the intestinal lumen (Simpson and Mckie, 2009). Iron was high in *wara*, 13.5 mg/100 g (0.6608%). About 1-10% of Fe from plant sources is normally absorbed by the body (Bender, 1992) although this value can be improved upon when plants are consumed with meat. For example, the addition of meat to legume or cereal diet can double the amount of Fe absorbed and so contribute significantly to the prevention of anaemia, which is widespread in developing countries like Nigeria (Bender, 1992). Copper and Fe are present in the enzyme cytochrome oxidase involved in energy metabolism.

Calcium is an important mineral in human nutrition being important for bone density. Calcium salts provide rigidity to the skeleton and calcium ion play many roles in most metabolic processes (FAO/WHO, 2001). Nearly 99.0% of the calcium in the human body is found in the bones (Ghosh and Joshi, 2008). The recommended daily intake of calcium is about 400 – 500mg/day for adults. Compared to other minerals, Ca absorbance to the body is relatively inefficient, in general, only about 25.0% - 30.0% of dietary Ca is effectively absorbed (FAO/WHO, 2001). When the amount of Ca is adequate in the diet, Fe is utilized to better advantage; this is said to be an instance of sparing action (Fleck, 1976). Manganese (2.20 mg/100 g, 0.1077%) has always been found low in the foods consumed in Nigeria. For example; it was 1.9 ± 0.04 mg/kg (meat pie), 1.0 ± 0.00 mg/kg (doughnut), 29.0 ± 0.01 mg/kg (moin-moin) and cake (2.8 ± 0.01 mg/kg) (Adeyeye *et al.*, 2012).

The mineral ratios of *wara* were depicted in Table 1. Mineral ratios are often more important than the individual mineral levels themselves and this had been illustrated by the following statements by Vitale *et al.* as quoted by Watts (2010): "Determining nutritional interrelationships is much more important than knowing minerals levels alone. From a global standpoint, although dietary deficiency is at the more serious end of the spectrum, the opposite end, dietary excess and aberrations contribute to the burden of disease". "Mild and subclinical deficiencies of nutrients outnumber overt syndromes ten to one". There are two types of major ratios: (1) Significant ratios: If the synergistic relationship (or ratio) between certain minerals in the body is disturbed, studies show that normal biological functions and metabolic activity can be adversely affected. Even at extremely low concentrations, the synergistic and/or antagonistic relationships between minerals still exist which can indirectly affect metabolism (Trace Elements, Inc.); (2) Toxic ratios: It is important to note that individuals with elevated toxic levels may not always exhibit clinical symptoms associated with those particular toxic minerals. However, research has shown that toxic minerals can also produce an antagonistic effect on various essential minerals eventually leading to disturbances in their metabolic utilization. Four important properties of ratios had been enumerated:

- Ratios are often important than levels.
- Ratios represent homeostatic balances.
- Ratios are indicative of disease trends.
- Ratios are frequently predictive of future metabolic dysfunctions or hidden metabolic dysfunctions (ARL, 2012).

These significant ratios would now be further discussed: Ca/P, Na/K, Zn/Cu, Na/Mg, Ca/K, Ca/Mg and Fe/Cu. The Ca/Mg ratio is referred to as the blood-sugar ratio. Normal ratio is 6.67:1.00 but our value in Table 1 was 6.07 which were highly comparable to the ideal value. Calcium is required for the release of insulin from the pancreas whereas magnesium inhibits insulin secretion.

Table 1: Calculated mineral ratios of *wara*

Parameter	*Wara*	Ideal	Range
Ca/P	0.4030	2.6	1.6 - 4.6
Mg/P	0.0664	-*	-
Ca/Mg	6.07	6.67	3 - 11
Ca/K	0.1794	4.1	2.2 – 6.2
Zn/Cu	2.28	8	4.0 – 12
Fe/Cu	9.10	0.9	0.2 – 1.6
Ca/Pb	198766	84	42 – 163
Fe/Pb	12248	4.4	2.2 – 8.8
Fe/Co	8421	440	-
Na/Mg	0.0409	4.17	2.0 – 8.0
K/Co	761746	2000	-
Zn/Pb	3796	-	-
Na/K	0.0012	2.4	1.4 – 3.4
K/[(Ca + Mg)]	9.57	2.2	-

*No values available from literature

Magnesium is necessary to keep calcium in solution. Trends associated with Ca/Mg ratio; ratio (trend): 12 + [severe glucose (sugar) sensitivity]; 8.5 – 12 (imbalanced glucose metabolism); 6.68 – 10 (within optimal limits); 6.67 (IDEAL); 4.51 – 6.67 (within optimal limits); 4.50 – 3.3 (imbalanced glucose metabolism); below – 3.3 [severe glucose (sugar) sensitivity] (ARL, 2012). The Na/K ratio in Table 1 was 0.0012; the ratio is otherwise called life – death ratio because it is so critical. Na/K in addition to being life – death ratio, the ratio has the following properties:

- It is related to the sodium pump mechanism and the electrical potential of cells which is regulated by Na and K levels.
- Na is normally extracellular, while K is normally intracellular. If the ratio of these minerals is unbalanced, it indicates important physiological malfunctions within the cells.
- The Na/K ratio is intimately related to kidney, liver and adrenal gland function, and an imbalanced Na/K ratio is associated with heart, kidney, liver and immune deficiency diseases.
- The Na/K ratio is intimately linked to adrenal gland function, and the balance between aldosterone (mineralocorticoid) and cortisone (glucocorticoid) secretion (ARL, 2012).

Trends associated with Na/K ratio; ratio (trend): 6 + (severe elevation – inflammation and adrenal imbalance. High ratio can also be associated with asthma, kidney and liver problems. A high Na/K ratio is considered preferable to a low Na/K ratio); 4.5 – 6 (moderate elevation - tendency towards inflammation); 2.5 – 4.49 (mild elevation – good adrenal function); 2.5 (IDEAL); 2 – 2.5 (mild inversion – beginning of adrenal exhaustion); 1 – 2 (moderate inversion – kidney and liver dysfunction, allergies, arthritis, adrenal exhaustion, digestive problems, deficiency of hydrochloric acid); below 1.00 (severe inversion – tendency towards heart attack, cancer, arthritis, kidney and liver disorders) (ARL, 2012). In Ca/K ratio, the value in *wara* was 0.1794. Ideal Ca/K ratio is 4:1. Ca/K ratio is called thyroid ratio because Ca and K play a vital role in regulating thyroid activity. Ca/K characteristics:

- A Ca/K ratio of < 4.1 is indicative of increased thyroid activity.
- The thyroid gland is one of the major glands which regulate metabolic rate in the body. A hyperactive thyroid is associated with fast metabolism.
- When the thyroid (and adrenal) ratios are not normal, the efficiency of energy production in the body decreases. It is like an engine that is turning too slow or too fast – power output decline.

Trends associated with Ca/K ratio; ratio (trend): 32 + (severe low thyroid activity 75% + energy loss); 16 – 32 (sluggish thyroid 50 – 75% energy loss); 8 – 16 (moderate sluggish thyroid 25 – 50% energy loss); 4 – 8 (mild sluggish thyroid activity 10 – 25% energy loss); 4 (IDEAL – 100% energy); 2 – 4 (mild fast thyroid activity 10 – 25% energy loss); 1 – 2 (moderate fast thyroid activity 25 – 50% energy loss); below 1.0 (excessive thyroid activity 50% or more energy loss). Na/Mg ratio (0.0409 in *wara*) is referred to as the adrenal ratio because sodium levels are directly associated with adrenal gland function. Aldosterone, a mineral corticoid adrenal hormone, regulates retention of sodium in the body. In general, the higher the sodium level, the higher the aldosterone level. Na/Mg is also a measure of energy output, because the adrenal glands are a major regulator (along with the thyroid gland) of the rate of metabolism. Ideal Na/Mg ratio is 4.17:1. Trends associated with the Na/Mg ratio; ratio(trend): 16 + (extremely overactive adrenals 50% or more energy loss); 8 – 16 (moderate excessive adrenals 25 – 50% energy loss); 4.17 – 8 (mild excessive adrenal activity 10 – 25% energy loss); 4.17 (IDEAL 100% energy); 2 – 4.17 (mild sluggish adrenals 25 – 50% energy loss); below 1.0 (adrenal insufficiency 50% or more energy loss) (ARL, 2012). Using the Zn/Cu ratio is a much more effective method of evaluating Zn and Cu readings than considering either Cu or Zn levels alone. Zn/Cu in *wara* was 2.28 (less ideal of 8) (ARL, 2012). Symptoms of high Cu (excess) and/or low Zn (deficiency) include skin problems (acne, psoriasis, slow healing, eczema), emotional instability, "spaciness", detached behaviour, schizophrenia, PMS, reproductive problems, prostatitis, menstrual difficulties, depression and fatigue. Trends associated with the Zn/Cu ratio; ratio(trend): 16+ (severe copper deficiency or bio-unavailability of copper); 8+ to 6 (copper deficiency or unavailability); 8 (IDEAL); below 4.5 (copper toxicity); below 2(severe copper toxicity = excessive breakdown, emotional instability, Zn deficiency problems such as impotence, slow healing, loss of taste, smell, appetite and hair loss) (ARL, 2012). The ideal ratio of 0.9:1 and an acceptable ideal range from 0.2 to 1.6 had been reported for Fe/Cu ratio. The relationship between Fe and Cu are important for many reasons. They are involved in cellular respiration and electron transportation. Therefore, a distruption in their equilibrium can lead to serious consequences in normal cellular activity. An elevated Fe/Cu ratio leads to increased free radical production, particularly lipid peroxidation that can lead to mitochondrial damage. The corresponding reduction in copper increases the damage from superoxide radicals due to the suppression of Cu activated SOD. The ratio of Fe/Cu either high or low can also lead to neurological dysfunction affecting neurotransmitters and causing lipid peroxide damage within neurological tissues. An elevated or reduction in the Fe/Cu ratio is associated with a decrease in the utilization of Fe by affecting the ability to incorporated Fe into haemoglobin. In Table 1, Fe/Cu ratio was 9.10 as against 0.9 (the ideal). An elevated Fe/Cu ratio in the body may indicate a potential for chronic bacterial infection. A low Fe/Cu ratio can be associated with Fe deficiency as well as thyroid disturbance (Watts, 2010).

Oxidation types (ARL, 2012):

Fast oxidation: Ca/K ratio less than 4:1 and Na/Mg ratio greater than 4.17:1

Slow oxidation: Ca/K ratio greater than 4:1 and Na/Mg ratio less than 4.17:1

Mixed oxidation: Ca/K ratio greater than 4:1 and Na/Mg ratio less than 4.17:1

or

Ca/K ratio less than 4:1 and Na/Mg ratio greater than 4.17:1

The toxic ratios in Table 1 included Ca/Pb, Fe/Pb, Zn/Cd, etc. This section showed the relationships of the protective nutrient minerals relative to the heavy metals. There is no standard range associated with the toxic ratios, only a low or acceptable level. Since everyone is exposed and has heavy metals ever present in their body, the higher the toxic ratios the better (Table 1). However, there is no clinical significance of the ratios being double, triple or even ten times higher than the minimal acceptable level. As an example, the Ca/Pb ratio has an acceptable level of 84:1. Since Ca reduces Pb absorption and retention within the body, Ca is considered protective of excess Pb retention. The ratio of Ca to Pb should be 84 times higher than Pb in order to be protective or to prevent the adverse of lead within the body (Watts, 2010). A Ca/Pb ratio below 84:1 would indicate a potential for Pb interfering with metabolic processes. Heavy metal with nutrient mineral ratios that would lead to nutritional mineral protections would come from: Ca/Pb (198766/84), Fe/Pb (12248/4.4) and Zn/Pb (3796). Heavy metals interfere with normal metabolic processes due to their ability to displace nutritional minerals or poison enzymes function by their attachment to proteins.

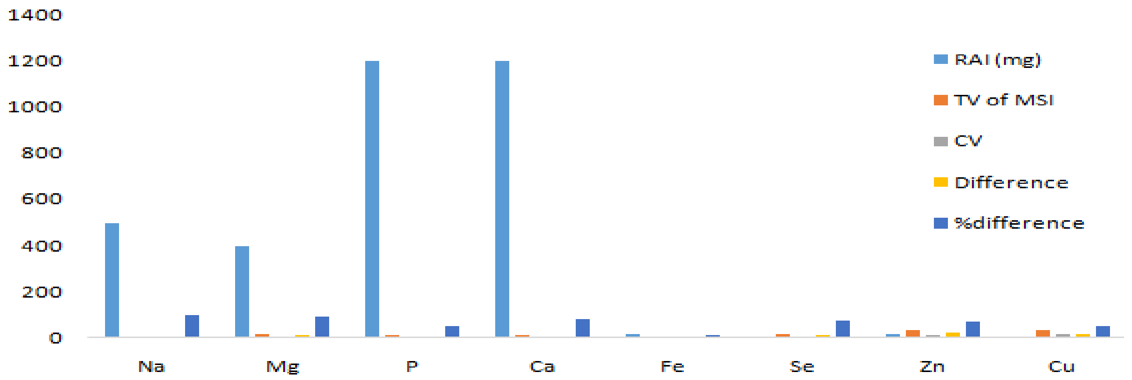

Fig. 4: Mineral safety index (MSI) of Na, Mg, P, Ca, Se, Zn and Cu of *wara* sample

The mineral safety index (MSI) of the appropriate minerals was shown in Fig. 4. The following information was provided: recommended adult intake (RAI), standard or Table value (TV) of MSI and the calculated *wara* MSI values shown as CV. The difference between TV – CV values were shown to be high to low but positive for the minerals (Na, Mg, P, Ca, Fe, Se, Zn and Cu) involved. The percentage differences ranged from 10.1 to 99.7 showing the standard values to be higher than the calculated values. Whereas the highest difference of 99.7% was observed in Na, the lowest was observed in Fe (10.1%) Since all the CV(MSI) < TV(MSI), then no deleterious effect could be felt by consuming *wara* (based on these TV standards). The CV (MSI) had the following explanation taking Ca as an example: the Ca RAI (column 2, Fig. 4) is 1200 mg, its minimum toxic dose (MTD) is 12000 mg or 10 times the recommended daily average (RDA) which is the MSI of Ca, this explanation works for other CV (MSI).

Conclusion

Wara can be eaten fresh, dried or fried and has high crude protein, moderate levels of crude fat, carbohydrate and crude fibre (due to the plant coagulant in its preparation). The crude fat had the distribution of high total fatty acids (TFAs) and low other lipids. The gross energy was moderately high at 404 kcal/100 g/1718 kJ/100 g. Energy distribution contribution trend was crude fat (41.1%) > crude protein (33.2%) > carbohydrate (25.7%). The UEDP% (19.6/19.8) would prevent malnutrition. The major minerals had these values (mg/100 g): K (1219) > P (543) > Ca (219) > Mg (36.0) > Na (1.47) while the nutritional minor minerals had (1.48). The toxic minerals were in ultra-trace levels. Among the mineral ratios, only Ca/Mg was close to the ideal (6.07/6.67) in nutritional ratios but all the toxic ratios were high and good (as body protector). The mineral safety index showed all the minerals involved were non-deleterious if consumed in *wara.*

Conflict of Interest

Authors have declared that there is no conflict of interest related to this work.

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