NISEB Journal Vol. 17, No. 3. September, 2017 1595-6938/2017
Printed in Nigeria (2017) Society for Experimental Biology of Nigeria
http://www.nisebjournal.org

In vitro Buffering Capacity of Some Nigerian Cereals, Legumes and Starchy Staples

[1]Ogedengbe OO, *[2]Aihie EO, [1]Okhaeifo M, [1]Mmaduka CT, and [1]Oboh HA

[1]Department of Medical Biochemistry, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria
[2]Department of Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria.

Abstract
Food components in a diet can influence buffering capacity (BC) in vivo. A knowledge of the in vitro BC of some indigenous staples may guide the choice of food. The in vitro BC of some Nigerian starchy staples; Cassava, 'Garri', Rice, Ripe plantain, 'Semovita' (Golden penny), Starch, Unripe plantain, wheat (Honeywell) and white yam; Legume seeds; brown and white cowpea (Vigna ungiuculata), common beans (Phaseolus vulgaris), groundnut (Arachis hypogea), pigeon pea (Cajanus cajan) and soya beans (Glycine max) and Cereal grains; millet (Pennisetum glaucum), sorghum (Sorghum bicolor), white and yellow maize (Zea mays) were assessed. The processed and dried samples were homogenized in deionized water and filtered to obtain an extract used to determine the pH. The in vitro BC were determined by titrating the different extracts respectively with 1M HCl or 1M NaOH. The results revealed that pigeon pea had the highest BC (3.05±0.03). Cereals had low BC of 0.56±0.09 to 0.60±0.01. The BC of the starchy staples ranged from 0.40±0.11 for starch to 3.18±0.21 for 'Tapioca'. Legumes had the highest BC when compared with other staples. This study revealed that legumes have alkalinizing effects in vitro and its use could be effective in nutritional interventions for therapeutic purposes.

Keywords: Buffering capacity, pH, Nigerian staples

Introduction
Starchy root tubers, legumes and cereals are the major sources of food staples in Nigeria. They form the bulk of food purchased and eaten by the teeming population [1]. The root tubers are yams, cassava, potatoes, cocoyam which can be peeled and boiled, fried or processed into flour. It is subsequently dissolved in hot water to form a thick paste. The bolus of the paste is usually eaten with a sauce or soup [2, 3]. Legumes (cowpeas, pigeon peas, groundnuts) and cereals (millet, sorghum, guinea corn and rice) are dry seeds. Legumes can be boiled, processed into flour and made into pudding called moin-moin' or fried to make bean cakes called 'Akara' [4]. Nigerian cereals are usually processed to flour and eaten as gruel.
The typical Nigerian diet is rich in starchy root vegetables and cereals, with limited consumption of legumes, proteins, fruits and vegetables. The consequences of eating a larger proportion of these staples and refined foods could lead to nutritional disorders like obesity, cardiovascular diseases and cancer [5]. Several researches [6, 7, 8] have reported that a diet rich in carbohydrates would lead to acidity of the gastro intestinal tract which produces as much as 100 mEq of acid per day; that is almost twice what the body can handle. [9]. This means that on a daily basis our bodies have to use the mineral (calcium. phosphate, potassium and sodium) that make up our bones to neutralize the overabundance of acids in our bodies [10]. Prolonged acid imbalances can lead to either metabolic acidosis or alkalosis. It is pertinent to keep the H^+ concentration, and the products of our metabolic system within a particular narrow limit for optimal function via buffering systems in the body [11]. The buffering systems prevent extreme changes in pH by removing or releasing H^+ in cases of acidosis or alkalosis respectively. The maintenance of acid-base homeostasis is a vital process in human health [12]. The pH levels (acid-alkaline measurement) of human body fluid affect the metabolic process of the internal and the external cells of the body [13]. Foods possess a chemical property termed buffering capacity, which allow them to resist changes in pH due to the food constituents such as phosphate salts, carboxylic acids and minerals [14]. Some foods are alkalizing while others are acidifying; the potential of a particular food to produce an acidifying or alkalizing effect is determined primarily by the availability of its constituent nutrients [15].
Over-indulgence in westernized diets, high intake of processed and carbohydrate laden foods coupled with low intake of fruits and vegetables could lead to increased acid in the gastrointestinal tract resulting in metabolic acidosis, decrease bicarbonate concentration and consequently a fall in pH; that leads to the onset of some cancers and chronic diseases [16,17]. The buffering capacity of Nigerian fruits and vegetables and the effect of an alkalizing diet have been reported [18]. This study was carried out to assess the *in vitro* buffering capacities of some Nigerian staples.

**Corresponding Author's Email: eghe.aihie@uniben.edu*

Materials and Methods

Sample preparations

Cereal grains; millet (*Pennisetum glaucum*), sorghum (*Sorghum bicolor*), white and yellow maize (*Zea mays*) and legume seeds; brown and white cowpea (*Vigna ungiuculata*), common beans (*Phaseolus vulgaris*), groundnut (*Arachis hypogea*), pigeon pea (*Cajanus cajan*) and soya beans (*Glycine max*) were commercially purchased from New Benin market, Benin City, Edo State. Raw weight of 0.7kg of each cereal grain was picked and soaked with 1.75L distilled water for two days, the soak water was discarded after 24 hours and the soaking process repeated. The samples obtained thereafter were milled with 1.75L distilled water in a Moulinex blender (UK), sieved with a net mesh to remove the shaft. The filtrate was allowed to settle, supernatant discarded, and the sediment was poured in a sieve bag and allowed to drain. The sediment (0.4kg) of each sample was processed as 'pap': by making paste with de-ionized water and 1.5L of boiled distilled water was added to the paste to form a thick gruel.

Raw weight of 0.5kg of each legume seed was picked (to remove sand and debris), washed and rinsed with normal water and de-ionized water respectively. The seeds were cooked to softness. Doneness was established when the cooked grains was pressed easily between two fingers. The cooked legumes were drained to remove the water. The processed 'pap' and cooked legumes were consequently oven dried (searchtech Laboratory dry oven, USA) at 72ºC to constant weight. The dried 'paps' and legumes were blended with NAKAI Electric blender (Japan) to flour and stored in airtight containers.

Starchy staples; Cassava, 'Garri', Rice, Ripe plantain, 'Semovita' (Golden penny), Starch, Unripe plantain, wheat (Honeywell) and white yam were purchased from Edaiken market, Uselu, Benin City, Edo State. 1kg dry flour of wheat, unripe plantain flour ('Amala'), 'garri', 'fufu' and semovita was added to 1.5L boiling de-ionized water and stirred continuously to make paste. Wet starch (0.75kg) was dissolved in de-ionized water with few drops of red oil and stirred to paste. The paste was cooked over a hot flame to form a firm jelly-like consistent paste. Peeled cassava, yam and ripe plantain were washed and rinsed with normal and de-ionized water respectively and allowed to cook for 30 minutes in boiling water. The paste formed from the starchy staples and the cooked yam and ripe plantain were oven dried (Searchtech Laboratory dry oven, USA) at 70ºC to constant weight, blended to flour with NAKAI Electric blender (Japan) and stored in airtight containers. The soft boiled cassava was cut into 2cm rectangular size and soaked in de-ionized water for 2 days and the resultant product 'tapioca' was also oven dried and processed to flour.

Five (5) grams each of the blended samples were dissolved in 100ml of deionized water for 15 minutes with continuous stirring with a glass rod. The solutions were filtered through 10mm filter paper and filtrates were collected and used for assay immediately.

Two grams of commercial antacid tablet Danacid (Dana Drugs Limited, Nigeria) containing 250mg magnesium trisilicate, 120mg dried aluminum hydroxide and peppermint flavor was dissolved in 100ml deionized water and used as control.

Chemical Analysis

The buffering capacity of Nigerian staples was assessed as described previously [19]. Acidity of each filtrate from the cereals, legumes and starchy staples was evaluated using an HANA electronic pocket-sized pH meter (USA) at room temperature. Filtrates with pH greater than 4 was titrated against 1M HCl until the pH decreased to 2 (pH of human stomach) while those with lower than 4 were titrated with 1M NaOH until the pH increased to 4 (normal distal duodenum pH). The final pH reading was measured and recorded within 30second – 1minute after titrating with either acid or base.

Buffering Capacity (BC) was calculated thus: $\frac{Total\ volume\ of\ Acid/Base \times Molarity\ of\ Acid/Base}{pH\ unit\ change}$

Results and Discussion

Nigerian cuisines are known to include dishes comprising mostly of staples such as yams, rice, cassava and plantain; which are combined with hot peppery stews, legumes and a variety of vegetables and possibly some meat, fish and poultry [20]. The scarcity and expense of proteins in Nigeria among the poor has resulted in the consideration of vegetable proteins like legumes (Nuts, beans, peas and lentils) as an alternative source of proteins [20].

Cereals and legumes are rich sources of nutrients especially when used as whole grains. They are known to be essential source of energy, carbohydrate, protein, fibre, micronutrients like Vitamin B and E and some minerals like Na, Mg, and Zn [21, 22]. The grains are often processed to useful end products that vary in the nutritional composition *in vivo* [22].

The results obtained from this present study show that 'Eba' and starch have low buffering capacity. Cereals have a buffering capacity ranging from 0.56 ± 0.09 to 0.60 ± 0.02 for white maize to Millet with no significant difference ($P < 0.05$). This could be attributed to the loss of most nutrients (dietary fibre, minerals, vitamins, polyphenols, phytic acids etc.) stored particularly in the aleurone layers and germ of the cereals during processing of the cereals into paste 'Ogi'. It has been reported that differential milling and refining of cereals can lead to bran and germ separation and loss which consequently accounts for the reduction of available essential nutrients largely concentrated in this region of the grains [23].

Table 1: pH, volume of acid and buffering capacity of cereals

Cereals	**pH**	**Volume of acid (ml)**	**Buffering Capacity**
Millet	5.40 ± 0.06^{b}	2.03 ± 0.09^{b}	0.60 ± 0.02^{b}
Sorghum	5.27 ± 0.09^{b}	1.97 ± 0.03^{b}	0.60 ± 0.01^{b}
White Maize	4.93 ± 0.24^{bc}	1.60 ± 0.17^{b}	0.56 ± 0.09^{b}
Yellow maize	4.43 ± 0.12^{b}	1.47 ± 0.09^{b}	0.60 ± 0.01^{b}
Control (Antacid)	9.27 ± 0.03^{a}	11.13 ± 0.18^{a}	1.44 ± 0.02^{a}

Results are expressed in triplicate determination of mean ± SEM.
Values in the same column with different superscript are statistically different @ P<0.05.

Table 2: pH, volume of acid and buffering capacity of legumes

Legumes	**PH**	**Volume of acid (ml)**	**Buffering Capacity**
Brown Cowpea	7.03 ± 0.03^{c}	9.73 ± 0.54^{ad}	1.87 ± 0.10^{bc}
Common Beans	7.50 ± 0.06^{b}	14.00 ± 1.12^{bc}	2.55 ± 0.21^{de}
Groundnut	7.03 ± 0.12^{c}	11.40 ± 2.25^{ab}	2.23 ± 0.40^{cd}
Pigeon Pea	7.30 ± 0.06^{b}	16.17 ± 0.34^{c}	3.05 ± 0.03^{e}
Soya beans	7.50 ± 0.06^{b}	6.77 ± 0.03^{d}	1.22 ± 0.01^{a}
White cowpea	7.03 ± 0.03^{c}	8.40 ± 0.55^{ad}	1.59 ± 0.10^{ab}
Control (Antacid)	9.27 ± 0.03^{a}	11.13 ± 0.18^{ab}	1.44 ± 0.02^{ab}

Results are expressed in triplicate determination of mean ± SEM.
Values in the same column with different superscript are statistically different @P<0.05.

Table 3: pH, volume of acid and buffering capacity of carbohydrate staples

Samples	**pH**	**Volume of acid (ml)**	**Buffering Capacity**
Amala	6.53 ± 0.03[c]	14.23 ± 1.21[a]	3.14 ±0.25[c]
Eba	5.07 ± 0.07[e]	1.67 ± 0.09[d]	0.54 ± 0.02[d]
Fufu	4.43 ± 0.03[f]	2.00 ± 0.06[d]	0.82 ± 0.03[bd]
Plantain	4.67 ± 0.03[f]	3.37 ± 0.03[cd]	1.27 ± 0.02[b]
Rice	6.87 ± 0.03[c]	6.60 ± 0.15[c]	1.36 ± 0.03[b]
Semovita	6.00 ± 0.03[d]	1.53 ± 0.07[d]	0.38 ± 0.02[d]
Starch	4.67 ± 0.03[f]	1.07 ± 0.29[d]	0.40 ± 0.11[d]
Tapioca	5.30 ± 0.15[e]	10.57 ± 1.22[b]	3.18 ± 0.21[c]
Wheat	6.03 ± 0.03[d]	1.53 ± 0.09[d]	0.38 ± 0.02[d]
Yam	7.37 ± 0.03[b]	11.30 ± 0.12[ab]	2.11 ± 0.03[a]
Antacid (Control)	9.27 ± 0.03[a]	11.13 ± 0.18[ab]	1.44 ± 0.02[ab]

Results are expressed in triplicate determination of mean ± SEM.
Values in the same column with different superscript are statistically different @ P<0.05.

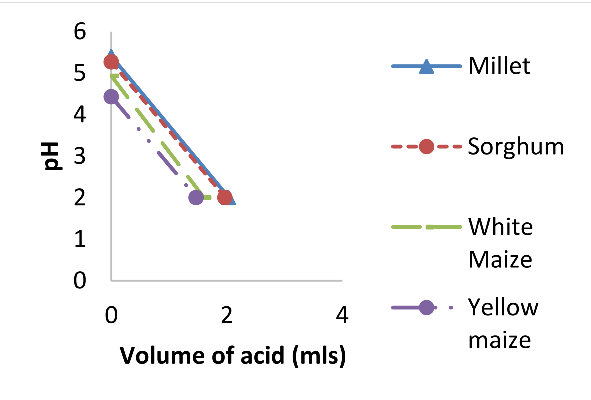


Figure 1: Titration curve for cereals

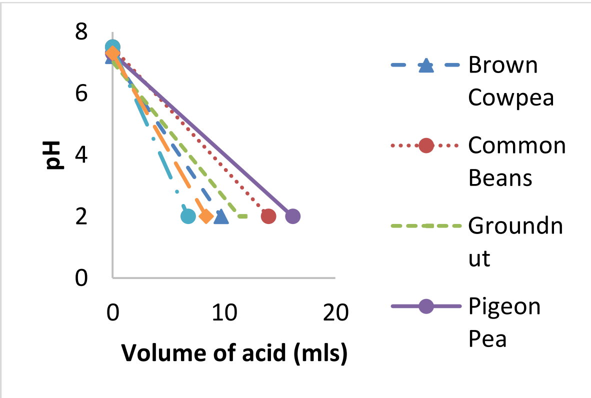
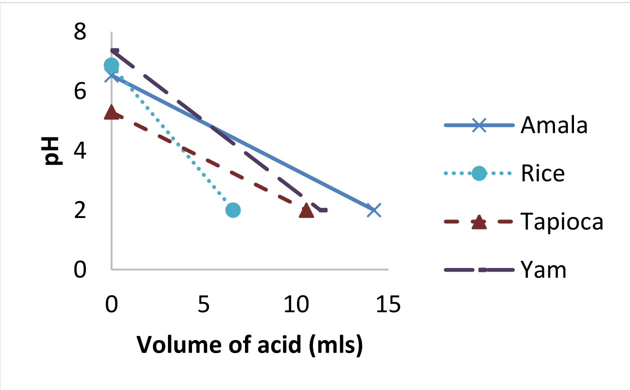


Figure 2: Titration curve for Legumes.



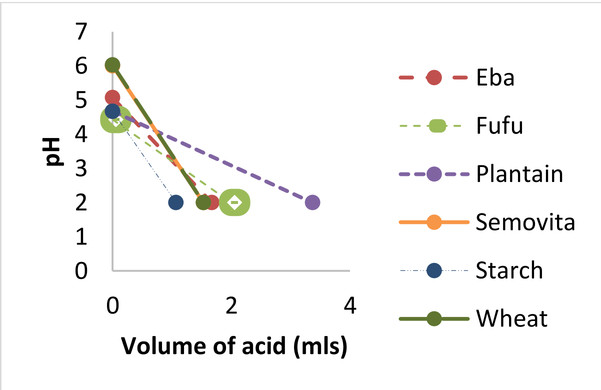


Figure 3: Titration curve for carbohydrate staples with with low buffering capacity

Figure 4: Titration curve for carbohydrate staples high buffering capacity

The legumes however were shown to have buffering capacity that ranged from 1.22 ± 0.01 to 3.05 ± 0.03 for Soya bean to Pigeon pea. These buffering capacities were however higher than those observed in the cereals and antacids (control) except soya beans. The observed increase could be attributed to the mode of processing the legumes by heat treatment. The bran and germ portion were intact. The presence of proteins could also account for its neutral to slightly alkaline initial pH reading.
The starchy staples like 'Semovita', wheat, 'Eba' and 'fufu' showed low buffering capacity ranged from 0.38 ± 0.02 to 0.82 ± 0.03. These could be attributed to processing of these food samples to flour. Refined wheat flour is known to have decrease concentration of protein, fat, ash calcium, zinc, iron, vitamin B (thiamine, riboflavin), tannin, soluble and insoluble dietary fibre. The Processing methods leads to product devoid of fibre and minerals. The particle size of pulverized fibre is also reduced by the different processing methods which improves starch content and digestibility [22, 23]. This could be responsible for the low BC of 'semovita', 'eba', starch and wheat which are not significantly different [24]. Starchy staples like plantain (ripe), rice, yam, 'amala' (unripe plantain flour) and Tapioca had varying buffering capacity that ranged from 1.36 ± 0.03 to 3.18 ± 0.21. The high buffering capacity of amala could be due to the presence of polyphenols in the flour which is alkaline in aqueous solution [25]. The Nigerian food composition table reported unripe plantain flour (amala), boiled yam and ripe plantain contain 2.11g, 1.05g and 1g of ash content respectively [26]; thus, the high buffering capacity of 3.14 ± 0.25, 2.11 ± 0.03 and 1.27 ± 0.02 in amala, boiled yam and ripe plantain respectively could be ascribed to their respective ash content. Minerals are known to play vital role in acid-base balance and could modulate pH shift [27]. Eating these starchy staples with african sauces containing vegetables known to have high BC [18] will provide additional benefits by neutralizing acids produced from starchy staples.

Conclusion

Legumes, 'tapioca' and 'amala' have high buffering capacity that could be used as adjuvant in modulating the effect of acid imbalance. They could also be choice of food for nutrition intervention with the aim of alkalising diet for therapeutic purposes. There is a need to investigate the *in vivo* buffering capacity of these dietary staples and their efficacy.

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