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Variation in proximate and resistant composition of unprocessed and processed form of two Nigerian foods: A case study of *Usi* and *Okpa*

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ABSTRACT: Foods are processed to increase the taste, safety and shelf life. Though the benefits of processing cannot be overemphasized, it is necessary that nutrient loss is minimal. This study assesses the variability in nutrient quality of two foods (*Okpa and Usi)* in Southern Nigerian from their unprocessed forms to their commonly eaten forms. The proximate, resistant starch and mineral content of unprocessed (Bambara groundnut flour and grated cassava) and processed forms (*Okpa* and *Usi*) were analysed. Results of the proximate analysis revealed a significant reduction ($p < 0.05$) in ash, fibre and carbohydrate content in both samples after processing. A significant ($p < 0.05$) increase in lipid and moisture content were also observed in both samples. Processed *Usi* samples showed a significant ($p<0.05$) difference in resistant starch content ($p<0.05$) from its unprocessed forms. Phosphorous, iron and calcium content were ($p<0.05$) reduced in *Usi* samples. A significant ($p<0.05$) reduction in zinc and phosphorous content were observed in *Okpa* sample after processing. The study shows that processing methods employed had minimal adverse effects on nutrient and resistant component of food samples.

Keywords: *Okpa*, *Usi*, cassava, Bambara groundnut

Introduction

Nowadays, the aim of consumption of foods is not limited to preventing hunger; it is advocated that food consumed should provide adequate nutrients and have some health benefits (Marc, 2020). In order to achieve this aim, there is increased focus in regulating food processing and preparation methods. Food processing/preparation has advantages of increasing digestibility and improving food flavour or quality by destruction of oxidative enzymatic reactions and anti-nutrient components (Oluoch *et al*., 2012), studies have also shown some detrimental effects on the nutritional and functional properties of foods (Oghabaei and Prakesh 2015, Arinola and Adesina 2014). Boiling has been reported to cause significant reduction in soluble solids, vitamins and minerals through leaching (Heninway, 2012). Food processing/preparation methods have been shown to be one of the principal factors affecting the resistant content of foods (Niba, 2003). Resistant starch (RS) content of foods, though not regarded to be of nutritive value, is considered a useful property of food (Jyothsna and Hymavathi, 2017) According to Asp (1992), resistant starch has been defined as 'the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals'. There are four types of resistant starch. RS1 are starches that physically trapped in food matrix and cannot be hydrolysed. RS2 are starches known to resist hydrolysis by enzymes due to some properties e.g. its high amylose, content. RS3 also known as retrograded starch is formed after cooling of gelatinized starch. RS4 are chemically modified starch (Jyothsna and Hymavathi, 2017 Sáyago-Ayerdi *et al.,* (2011) which has been reported to lower caloric content of foods, cholesterol reducing actions and protection against colorectal cancer. Other reports had associated a reduction in glucose and insulin concentrations with an increase in resistant starch in food (Behall and Hallfrish, 2002; Robertson *et al*., 2003; Park *et al*., 2004). Several scientific studies have revealed beneficial roles of resistant starch in health

With the current trends in the nutrition, there is need for more studies of food processing / preparation methods of our staple foods. This will provide data that can be used in minimising nutrient loss in foods or serve as basis for enrichment of our foods. The scientific evidence obtained will also help ensure that the nutritional and other useful properties in our foods are still of significant value after preparation (Arinola and Adesina 2014).

Usi and *Okpa* are staple foods eaten in South-South and South-East zones of Nigeria. *Okpa* is prepared and enjoyed by most Igbo people especially children who eat it as a snack (Ndidi *et al.,* 2014; Okeke *et al*., 2008). It is prepared by cooking Bambara groundnut into steamed pudding which could be eaten at any meal time (Okeke *et al*.,2008) *Usi*, a by-product in the production of *garri,* is a local delicacy among the Urhobo and Isoko people of Delta State (Ekeh, 2015; Osagie and Eka 1998).

The aim of this study is to assess the variation in proximate values, resistant starch and mineral composition in raw (Bambara groundnut flour and grated cassava) and processed forms (*Okpa* and *Usi*).

Materials and Methods

Preparation of Food Samples

Cassava and Bambara groundnut were purchased from Edjemovyanwe Market in Oghara, Delta State and Nsukka Market in Enugu State respectively.

Usi (Locally known as Cassava Starch)

Cassava tubers were grated and filtered through a clean cloth. The filtrate was left undisturbed for 24 hours to sediment. A portion of the filtrate was prepared for consumption based on local practice by mixing with water. Then, one ml of palm oil per gram of filtrate was added and heated in a saucepan while stirring. The filtrate was stirred continuously for ten minutes until a thick yellow gel was formed.

Okpa

Bambara groundnuts were oven dried at 80^{o}C to reduce the anti-nutrients according to the method of Onimawo and Akpojovwo (2006). The dried seeds (Bambara groundnut) were milled into flour and sieved to obtain fine particles of flour. Then 10g of palm oil per 100g was rubbed into the flour. Forty three litres of water was added to the flour per 100grams to make a paste. A blend of tomatoes, pepper and onions was added to the paste as well as salt and two seasoning cubes. The paste was poured into plastic containers and steamed for two hours.

Proximate Analysis

The moisture, crude protein, fibre, ash, lipid and carbohydrate were determined according A.O.A.C (1995).

Determination of Mineral Content

Analysis of potassium and sodium content of the samples was carried out using flame photometry, while phosphorus was determined by the phosphovanado-molybdate method (AOAC, Association of Official Analytical Chemists 2005). The other elemental contents (Ca, Mg, Zn and Fe) were determined, after wet digestion of sample ash with an Atomic Absorption Spectrophotometer.

Determination of Resistant Starch

Resistant starch was determined using the method described by Mcleary and Monaghan (2002). *In vitro* resistant starch is that starch which is not hydrolyzed by incubation with α-amylase. Amyloglucosidase is added to avoid inhibition by by-products of amylase digestion. Hydrolysis products were extracted with 80% ethanol and discarded. The resistant starch was then solubilized with 4M potassium hydroxide and hydrolyzed with amyloglucosidase. The resistant starch is hydrolyzed to glucose. Glucose concentration is determined using glucose oxidase assay.

Hydrolysis of Non-Resistant Starch

The assay was performed using a commercially available kit for resistant starch determination (Megazyme International Ireland Ltd., Bray, Co. Wicklow, Ireland).

Measurement of Resistant Starch via Glucose Oxidase Test

A magnetic stirrer bar (5 x 15 mm) was added to each tube containing the pellet and two militers of 2M KOH. The resistant starch (RS) fraction was re-suspended and dissolved by stirring for 20 min in an ice water bath over a magnetic stirrer. Eight milliliters of 1.2M sodium acetate buffer (pH=3.8) and 0.1 ml of amyloglucosidase were added, mixed and incubated in a water bath for 30 minutes at 50 °C. For samples containing greater than 10% RS content, tube contents were transferred to a 100 ml volumetric flask, adjusted to volume with water and mixed. 10 mililiters aliquot was centrifuged at 1000 g for ten minutes. Samples containing less than 10% resistant starch were not diluted and the tube was centrifuged directly at 1000 g for ten minutes.

Aliquots (0.1 ml) were transferred to two glass test tubes (either the diluted or the undiluted aliquots) and were treated with three mililiters glucose oxidase-peroxidase- aminoantipyrine (GOPOD) reagent. Samples were incubated for 20 minutes at 50 °C and absorbance read at 510 nm against a reagent blank. Glucose solution (1.0 mg/ml) was used as a standard.

Statistical Analysis

Data are represented as Mean ± S.E.M (n = 6). Significance of difference between raw and processed form of a sample was tested by Student t-Test, Turkey-Kramer test, using the GraphPad Instat Version 5 (GraphPad Software Inc. San Diego, California U.S.A.). Statistical Significance wasset at $P < 0.05$.

Note: Statistical test was done between taw/unprocessed forms of one sample i.e. raw/unprocessed usi and processed usi; raw / unprocessed forms of *okpa* and processed form of okpa,

Results

Table 1 shows the percentage proximate composition of *Usi* and *Okpa* before and after preparation.. The processing of these foods by cooking resulted in processed *Okpa* (OKP) having significantly ($p<0.05$) reduced levels of fibre (0.88 ± 0.45%), ash (3.87 ± 0.10%) and carbohydrates (54.75 ± 2.35%); while the lipids (14.80 ± 0.84%) and moisture (54.25 ± 0.11%) contents were significantly ($p<0.05$) increased in contrast to the unprocessed form (OK). The processed *Usi* (USP) counterpart had no significant ($P>0.05$) difference in protein (4.65 ± 0.43%), lipid (3.36 ± 0.21%), ash (1.55 ± 0.34%), fibre (6.15 ± 2.23%) contents and carbohydrate content (84.32 ± 3.00%) when compared with the unprocessed form (US).

Table 1: Proximate Composition of Usi and Okpa per 100grams dry weight

	US	**USP**	**OK**	**OKP**
Protein	2.15±0.06	4.65± 0.43*	22.2± 3.01	20.55±0.15
Lipid	1.82± 0.16	3.36±0.21*	5.18±0.24[a]	14.80± 0.84[v]
Fibre	8.13± 0.19	6.15± 2.23*	9.40± 0.30[a]	0.88± 0.45 *
Ash	0.91± 0.02	1.55± 0.34*	7.12 ±0.04	3.87± 0.10*
Carbohydrates	86.99± 2.45	84.32± 2.00	61.02±1.72	54.75± 2.35[a]

Analyses were carried out in triplicates and values expressed as mean ±SEM. US = Unprocessed / Raw *Usi;* USP = Processed *Usi;* OK=Unprocessed / Raw *Okpa;* OKP= *Okpa* (processed); * in processed forms within a row indicates a significant ($p<0.05$) difference from raw/ unprocessed form.

The resistant starch content of *Usi* and Okpa are presented in Table 2. The resistant starch content of unprocessed *Usi* (US) was found to be highest amongst the food samples ($p<0.05$). The processing of *Usi* and *Okpa* revealed significant differences ($p<0.05$) between raw (US) and processed *Usi* (USP) as well as raw (OK) and processed (OKP) *Okpa*.

Table 2: Resistant Starch Content of Raw and Processed Forms of *Usi* and *Okpa*

Food Sample	**Resistant starch content g/100g**
US	10.26± 0.68
USP	7.56± 0.23*
OK	6.54± 0.45
OKP	5.30± 0.78

Analyses were carried out in triplicates and values are expressed as mean ±SEM. US= Raw / unprocessed *Usi;* USP= Processed *Usi;* OK=Raw / unprocessed *Okpa;* OKP= processed *Okpa*;); * in processed forms within a row indicates a significant ($p<0.05$) difference from raw/ unprocessed form.

Table 3 shows the elemental composition of raw and processed *Usi* and *Okpa* samples.

It was also observed that concentration of elements reduced after processing except for potassium and sodium where increase in concentrations occurred after processing. Magnesium content were not significantly ($p>0.05$) altered after processing of both samples (16.66±0.32; 23.613±0.05) and (14.34±0.29; 22.85±0.11). Calcium content reduced significantly ($p<0.05$) in *Usi* (6.63±0.34)) samples after processing (3.50±0.11). The potassium content of processed forms of *Usi* and *Okpa*; (77.92±1.63; 166.73±0.09) were not significantly ($p>0.05$) higher unprocessed forms (74.04±0.73; 154.1±0.60). Sodium of unprocessed *Usi* and *Okpa (*15.21±0.66 ;86.38±0.17) were significantly ($p<0.05$) lower than their processed forms (51.04±0.31; 138.8±0.56). Processing also reduced significantly ($p<0.05$) the phosphorus content (1.38±0.05; 3.55±0.01), (1.00±0.001; 1.33±0.01) of both samples. Iron and zinc content of *Usi* were not significantly ($p>0.05$) reduced (19.35±0.31; 0.370±0.002); (17.20±0..04; 0.36±0.001), whereas there was a significant ($p<0.05$) reduction in *Okpa* samples (23.94±0.16; 0.53±0.001) (19.15±0.13; 0.39±0.005).

Table 3: Mineral Composition of raw and processed forms of *Usi* and *Okpa*

Mineral (mg/100g)	US	USP	OK	OKP	RDA
Magnesium,	16.660±0.32	14.343±0.29	23.613±0.05	22.85±0.11	30–130[a], 240–360[b],320–340[c]
Calcium,	6.63±0.34	3.50±0.11*	13.916±0.14	12.54±0.21	210-800[a],800[b],1200[c]
Potassium	74.04±0.73	77.92±1.64	154.1±0.60	166.72±0.88	400–3000[a], 4700[bc]
Sodium	15.21±0.66	51.04±0.31*	86.38±0.17	138.80±0.56*	120[a], 1500[bc]
Phosphorus	1.38±0.05	1.00±0.001*	3.55±0.01	1.33±0.01*	100–500[a],1250[b],700[c]
Iron	19.35±0.31	17.20±0..04	23.94±0.16	19.15±0.13*	0.27–11[a],8–15[b],10[c]
Zinc	0.370±0.002	0.36±0.001	0.53±0.001	0.39±0.005*	2–5[a],11[b],15[ac]

Analysis were carried out in triplicates and values expressed as mean ± SEM. US= unprocessed/raw Usi; USP = Processed *Usi*; OK= Unprocessed/ Raw Okpa; OKP= cooked *Okpa*. RDA= recommended daily allowances.); * in processed forms within a row indicates a significant (p<0.05) difference from raw/ unprocessed form.
Culled from the United States Department of Agriculture (USDA) and can be accessed via http://www.nap.edu/. [a] Infants; [b] children; [c] adults.

Discussion

Usi, predominantly consists of carbohydrates as reported by Omoregie and Osagie (2011). Our previous studies (Isiosio *et al*. 2015) and current study have been consistent with this finding. The processing of grated cassava in into *Usi* by gelatinization had no substantive effect effects on carbohydrate content. Earlier reports (FAO, 1998) indicated no loss of carbohydrate by gelatinization; instead digestibility of carbohydrate is increased. Our previous report had shown that 93.58% of carbohydrates are hydrolysable by the human digestive system (Isiosio *et al.*, 2015). Heating and gelatinization did not affect the nutrient composition of *Usi*. The carbohydrate content of Bambara groundnut flours depending on processing method range from 55% -68% (Oyeleke *et al.*, 2012; Ndidi *et al.*, 2014), the carbohydrate content observed is in agreement with these studies. The carbohydrate content reduced after processing, this may be due to the sieving during processing and loss of some low molecular weight carbohydrates (FAO. 1998). A study has reported the presence of reducing carbohydrates in Bambara groundnut (Oyeleke *et al.*, 2012), reducing carbohydrates are usually are low molecular weights.

Raw and processed *Okpa* had high protein content as observed in this study. These values were lower than values reported by Okonkwo and Opara (2010), but similar results were obtained by Onimawo *et al*., (2007) and Oyeleke *et al*.,(2012) . Variation in protein content may be attributed to differences in species of Bambara used for the preparation of the *Okpa*. The high protein content of *Okpa* makes it suitable for enhancing growth and maintenance of integrity of the cell (Okonkwo and Opara, 2010). In this study, the processing of *Okpa* which included dehulling of testa, sieving of the flour and cooking had no significant effect (p>0.05) on the protein content, but there was significant reduction (p<0.05) in crude fibre and ash content of *Okpa*.

It was observed in this study that the raw foods (raw *Okpa* and raw *Usi*) have higher resistant starch content than the processed forms (*Okpa* and *Usi*). This is consistent with studies by Sajilata *et al*., (2006) in which they showed that raw starches generally have higher resistant starch content than processed forms. The most plausible mechanism of action was that gelatinization of starch improved accessibility to digestive enzymes (Sajilata *et al.*, 2006). Gelatinization may account for the decrease in the RS 1 content of *Usi* after processing. With increased gelatinization, the processed, Usi still had a high resistant content when compared with cooked legumes, root and tuber crops (Isiosio *et al.*, 2015; Fabbri *et al*., 2016). The processing method employed involved heating the raw starch in a saucepan till it is completely gelatinized, thereafter, the food is cooled. During cooling process, there could be formation of another type of resistant starch (RS 3) (Aigester, 2009). This study did not observe any significant change (p>0.05) in the resistant starch content of raw and processed *Okpa*. This observation is in contrast to reports by Niba (2003) showing that heat treatment reduced resistant starch content of foods. Fabbri *et al*.,(2016) reported the formation RS3 in legumes after cooling.

It was observed that Usi had low mineral content when compared to the recommended daily allowances for humans. It is essential that usi should be eaten with soups rich in minerals. Okpa is a rich source of minerals (Ndidi *et al*., 2014), this can be further enriched with the addition of vegetables in Okpa (Adumanya *et al*., 2012). Studies have revealed that food processing and culinary methods affect mineral content of food (Heinway, 2012; Ndidi *et al*., 2014, FAO, 1998), hence, it is not surprising that mineral content of *samples were* altered by processing. Changes (either higher or lower than the raw material contents) depend on the type of mineral, plant species and cooking method (Ndidi *et al*., 2014). Several studies have reported loss of magnesium during processing (Heinway, 2012; Wobeto, 2007), however, there was no significant change in magnesium content in our study. Magnesium loss in foods has been reported after roasting or boiling of samples (Heinway, 2012; Wobeto, 2007), this approach was not employed in this study.

Calcium is known to be useful in bone formation, maintenance and growth, tooth and blood clot formation (Onimawo and Egbekun 1998), hence, it is considered an essential micronutrient (Aremu and Ibrahim, 2014). In a study by Aguzue *et al*. (2013), concentration of calcium was reported to be 35.5mg/kg in cassava which is not consistent with calcium content of raw *Usi* (grated cassava). As mentioned earlier, raw *Usi* samples have been grated and sieved this may account for the disparity in calcium concentration. Calcium content was reduced after processing of *Usi* and *Okpa* which agrees with reports by FAO (1990) and Ndidi *et al*., (2014). These reports suggested that calcium in some root and tuber crops were significantly reduced by moist treatment. Potassium was the predominant mineral in raw and cooked Okpa which is in accordance with results by Abdulsalami and Sheriff (2017) and Ndidi *et al*., (2014). While other minerals are reduced, sodium and potassium increased after processing. Abiodun and Adepeju(2011) report on analysis of Bambara groundnut flour showed the possibility of presence of some minerals in seed coat of the legume, hence, sieving before preparation of Okpa further reduces these mineral thereby resulting in higher concentrations of minerals in the endoplasm of the seed. Furthermore, salt was added to Okpa during processing. Sodium play essential roles in the maintenance of osmotic pressure, acid-base balance in the body, relaxation of muscle cells, absorption of glucose, and transmission of nerve impulse (Onigbinde, 2005; Aremu and Ibrahim, 2014).

The reduction in phosphorous content of foods after processing have been reported previously (FAO, 1990; Ndidi *et al*., 2014), our results are consistent with these findings. According to Onigbinde (2005), phosphorus play a primary role in carbohydrate metabolism and in the formation, maintenance and growth of bones and tooth. The concentrations of iron and zinc in *for* raw and processed *Usi* (193.47mg/kg, 172.03±1.4mg/kg) are high when compared with 6.75mg/kg and 2.32mg/kg of iron and zinc documented by Aguzue *et al*. (2013) for cassava samples. However, there is paucity of data on the mineral content of *Usi*. The raw sample of *Usi* has been grated and filtered through a clean sieve cloth; this might have increased the concentration of these minerals. Processing did not significantly (P>0.05) affect iron and zinc levels in *Usi* whereas reports by FAO (1990) showed an increase in iron and a decrease in zinc when cassava is processed into lafun and garri. Processing Bambara groundnut flour into Okpa caused a reduction in iron. Ndidi *et al*., (2014) had reported an improvement in iron content on boiling bambara groundnut and a reduction when seed are roasted. This suggests that various processing methods do not exert same effect. Zinc concentrations were lowest of the seven minerals in raw and processed Okpa, this trend was also observed by Obieguna *et al*., (2019). The reduction in zinc content was observed after processing had also been by reported several studies (Ndidi *et al*., 2014, Feitosa *et al*., 2018)

Conclusion

The results of analysis indicates presence of the nutrients, mineral content and resistant starch in O*kpa* and *Usi*. *Usi* contains mainly carbohydrates, it is therefore necessary, the soup being eaten alongside should compensate for nutritional deficiencies. The resistant of starch in both samples may indicate some health benefits; however, further research is needed in the area. Our study suggests that processing caused minimal variation in proximate, resistant and mineral content of Usi and Okpa

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