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Nutrient and Antinutrient Contents of Cassava Steeped in Different Types of Water for Pupuru Production*

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Abstract: Cassava (*Manihot esculenta*, Cranz) tubers were processed into pupuru, a locally fermented cassava product using different types of water viz: well water, stream water, sterile water and warm (40°C) water. The protein content (11.32%) of the cassava sample steeped in stream water was highest and significantly different ($p = 0.05$) when compared to the other samples. The antinutrients viz: cyanide, phytate and tannin were also lowest in the cassava sample steeped in stream water. However, this sample contains *E. coli*, which makes it microbiologically unsafe. The cassava samples steeped in warm and well waters show high protein (10.41 and 9.37%, respectively) and reduced antinutrient contents when compared with the control (raw cassava samples). Local processors of pupuru are advised not to use stream water that is microbiologically unsafe. Well and warm water may be used since it also increase the protein and reduce the antinutrient contents of the pupuru considerably.

Key words: Nutrient, antinutrient, cassava, steeped, pupuru

Introduction

Pupuru, a fermented cassava product, is consumed by at least 4-6 million people in Nigeria and more in some African countries (Odetokun *et al.*, 1998). It is prepared from cassava (*Manihot esculenta*, Cranz), hence a high carbohydrate food material with low protein content (Okpokiri *et al.*, 1995). The traditional processing of cassava into pupuru involves peeling of tubers, steeping of peeled tubers in stream water and fermentation of the tubers for 4-6 days. The fermented cassava mash is removed from the water into a jute bag to drain off the water. It is then ground between the two hand palms, molded into balls and fire dried. The dried ball has brownish to black outer coating and can be made into pupuru flour by peeling the outer coating and sieving. The flour can be reconstituted in boiled water to pupuru meal.

Generally, traditional carbohydrate foods such as cassava play an important role in African diet. However, cassava as a major source of diet is limited by its low protein and antinutrient contents (Hahn, 1992; Oboh *et al.*, 2002). Fortification of cassava with proteinous plants has been developed to upgrade the protein content of cassava (Odetokun *et al.*, 1998; Reade and Gregory, 1975). Moreover, fermentation process has been reported to result to pH decrease and organic acid production, which had lead to the hydrolysis of linamarin and evolution of gaseous hydrocyanic acid with subsequent reduction in cyanide level of cassava meal (Aboua, 1995).

Locally, rural women process cassava into pupuru by steeping peeled cassava tubers in stream water and fermenting it for 4-6 days. This method of processing does have some side effects such as fouling the water and also increasing the level of microbial contaminants in the fermented cassava product. The present study was therefore aimed to assess the nutrient and antinutrient contents of cassava steeped in four different types of water viz: well, stream, sterile and warm (40°C) waters for pupuru production. The microbial qualities of the pupuru samples obtained were also assessed.

Materials and Methods

Source and Processing of Cassava Tubers

Cassava tubers were obtained from a farm in Akure, Ondo State. The tubers were peeled; washed and 1000 g each was steeped in four different waters viz: Well Water (WW), Sterile Water (StW), Warm Water (WaW) and Stream Water (SrW) for six days. After fermentation, the water was drained off and the cassava crushed between the palms, molded into balls and fire dried.

Microbial Analysis

Nutrient agar (Oxoid) and Potato Dextrose agar (Oxoid) were used to isolate bacteria and fungi associated with the fermentation of cassava for six days. Bacteria plates on nutrient agar were incubated at 37°C for 24 h while fungi plates were incubated at 26±1°C for 3-5 days. Standard microbiological methods were used for the characterization of bacteria and fungi isolates (Buchanan and Gibson, 1974; Barnet and Hunter, 1972).

Chemical Analysis

The proximate composition (ash, fat, crude fibre) of the fermented cassava for pupuru production were determined using standard Association of Official Analytical Chemists (AOAC, 1984) method. Protein content was determined using Microkjeldah method ($N \times 6.25$). Percentage soluble carbohydrate was determined by subtracting the sum of percentage ash, crude fibre, crude protein, crude fat and moisture content from one hundred. Tannin, phytate and cyanide level were evaluated by the methods of Makkar *et al.* (1993), Wheeler and Ferrel (1971) and De Bruijn (1971), respectively.

Analysis of Data

The data gathered were analysed using one-way analysis of variance and Duncan multiple range test compared means (Zar, 1984).

Results

The microorganisms found to be associated with the cassava steeped in the different water sample is shown in Table 1. Most of the fungi involved in the fermentation were isolated from the second day of fermentation.

Table 1: Microorganisms Isolated from pupuru produced by steeping cassava in different types of water

Microbial isolates	Well water	Warm water	Sterile water	Streamwater
<i>Micrococcus luteus</i>	-	+	-	-
<i>Pseudomonas aeruginosa</i>	+	-	+	-
<i>Corynebacterium pyogenes</i>	-	-	-	-
<i>Acinetobacter antracis</i>	+	-	-	+
<i>Staphylococcus aureus</i>	+	+	+	+
<i>Bacillus subtilis</i>	+	-	-	+
<i>Escherichia coli</i>	-	-	-	+
<i>Bacillus cereus</i>	-	-	-	+
<i>Aspergillus niger</i>	+	-	-	-
<i>Aspergillus flavus</i>	-	-	-	+
<i>Lactobacillus fermentum</i>	-	-	-	+
<i>Penicillium species</i>	+	-	-	-
<i>Saccharomyces cerevisiae</i>	-	+	+	+
<i>Geotrichum candidum</i>	+	+	+	+
<i>Rhizopus species</i>	-	-	-	+

-: Absent; + Present

Table 2: Percentage proximate composition of pupuru produced by steeping cassava in different types of water

Sample	Ash	Moisture	Carbohydrate	Protein	Crude fat	Crude fibre
Raw	0.95±0.01 ^a	66.06±0.02 ^d	26.06±0.02 ^d	4.26±0.02 ^a	0.45±0.01 ^a	2.27±0.06 ^a
WaW	2.50±0.03 ^d	63.30±0.02 ^c	18.66±0.82 ^b	10.41±0.71 ^{bc}	1.59±0.13 ^d	2.83±0.06 ^{ab}
WW	1.60±0.02 ^c	61.31±0.10 ^a	22.49±0.94 ^c	9.37±0.67 ^{bc}	0.95±0.15 ^b	3.65±0.30 ^{bc}
StW	2.68±0.06 ^e	67.10±0.01 ^e	15.87±0.84 ^a	8.65±0.78 ^b	1.23±0.01 ^c	4.18±0.20 ^c
SrW	1.28±0.02 ^b	62.84±0.78 ^b	19.98±0.60 ^b	11.32±0.62 ^c	1.09±0.15 ^{bc}	3.50±0.17 ^{bc}

Raw: Raw cassava tuber; WaW: Warm (40°C) water; WW: Well Water; StW: Sterile Water; SrW: Stream Water. Values in column without the same superscript(s) are significantly different ($p \leq 0.05$)

Table 3: Antinutrients composition of pupuru produced by steeping cassava in different types of water

Sample	Cyanide (mg kg ⁻¹)	Phytate (mg/100 g)	Tannin (%)
Raw	5.38±0.37 ^d	225.64±0.00 ^c	0.52±0.00 ^f
WaW	2.39±0.20 ^b	206.44±9.60 ^c	0.15±0.01 ^b
WW	3.06±0.20 ^c	150.03±24.49 ^b	0.17±0.02 ^b
StW	2.03±0.00 ^b	169.23±0.00 ^b	0.10±0.06 ^{ab}
SrW	1.35±0.00 ^a	112.82±0.00 ^a	0.05±0.00 ^a

Raw: Raw cassava tuber; WaW: Warm (40°C) water; WW: Well Water; StW: Sterile Water; SrW: Stream Water. Values in column without the same superscript(s) are significantly different ($p \leq 0.05$)

The chemical compositions of the fermented cassava products are presented in Table 2 and 3. There was a significant ($p \leq 0.05$) increase in protein content of the cassava fermented into pupuru (Table 2). The protein content was however highest in the pupuru steeped in stream water (11.32%). Similarly, the fat content increase and the highest value was recorded in pupuru sample steeped in warm water (1.59%).

The result of the antinutrients viz: cyanide, phytate and tannin are presented in Table 3. There was a significant reduction in the cyanide content when compared to the raw cassava. The lowest cyanide level was however recorded for pupuru sample steeped in stream water (1.35 mg/100 g). The same trend was observed in the distributions of phytate and tannin in the pupuru samples produced by steeping cassava in different types of water. The phytate and tannin levels were however lowest and significantly different ($p \leq 0.05$) in pupuru obtained by steeping cassava in stream water when compared to other samples.

Discussion

Cassavas is usually processed into various forms in order to increase the shelf life of the products, facilitate transportation and marketing, reduce cyanide content and improve palatability (Hahn, 1992). One of such processed form of cassava is pupuru. It is the traditional diet of the Ikales and Ilajes in the south west region of Ondo state, Nigeria. A similar cassava product that resembles pupuru is kum kum that is popular in Cameroon (Numfor and Ay, 1987). A total of 15 microbial isolates were obtained from the cassava samples fermented into pupuru using different types of water (Table 1). Ten of this isolates including *E. coli* were isolated from cassava sample steeped in stream water. Some of the microbial isolates are *Corynebacterium pyrogenes*, *Saccharomyces cerevisiae*, *Geotrichum candidum*, *Penicillium* species and *Aspergillus* species. Nwachukwu and Edward (1987) had earlier reported that the following microorganisms are involved in lafun, a similar fermented cassava product. The organisms include *Geotrichum candidum*, *Aspergillus niger*, *penicillium* species, *Leuconostoc* species and *Corynebacterium* species. The other organisms isolated (Table 1) are likely to be contaminants from the environments from where the waters were obtained.

The result of the proximate composition (Table 2) shows increase in protein and fat contents of the samples when compared to the raw cassava. The protein content of the cassava steeped in stream water was the highest (11.32%) and significantly different ($p \leq 0.05$) from the other samples. The increase in protein content of cassava fermented products could be attributed to the possible secretion

of some extracellular enzymes into cassava mash in an attempt to utilize the cassava starch as a source of carbon (Akindahunsi *et al.*, 1999). Sauti *et al.* (1987) reported that the growth of mould on the peeled/sliced cassava root increases the protein content of the final products three to eight times. Increase in the microbial mass may also account for the increase in the protein content of the pupuru produced by fermenting cassava.

An increase was also observed in the fat content of the pupuru produced by steeping cassava in different types of waters. Akindimula and Glantz (1998) suggested that fungi could produce microbial oil during the course of fermentation. *Aspergillus flavus*, *A. niger* and *Penicillium species* isolated from cassava sample may have increased the fat content of the fermented cassava (pupuru).

The antinutrients (cyanide, tannin and phytate) composition of the cassava fermented into pupuru was lower and significantly different ($p \leq 0.05$) from the raw sample. The antinutrients level of the pupuru sample obtained from cassava steeped in stream water was however the lowest (Table 3). Fermentation of cassava had been reported to significantly reduce the antinutrients level (Aboua, 1995; Oboh *et al.*, 2002). The cassava fermented into pupuru could be considered safe in terms of tannin and cyanide poisoning. This is because the level of these antinutrients is far below the reported deleterious level of 0.76-0.09% and 30 mg kg⁻¹ for tannin and cyanide as reported by Aletor (1993) and Akinrele *et al.* (1962), respectively. The reduction of the phytic acid will also make nutritionally essential minerals available. Phytic acid had been reported to interfere with Ca, Fe, Mg and Zn absorption as a result of its ability to chelate divalent cationic minerals (Nelson *et al.*, 1968).

Locally, pupuru is produced by steeping cassava in stream water. The present study reveal an increase in the protein and reduction in the antinutrient contents of cassava steeped in different types of water, with the sample steeped in stream water having the best result. However, the presence of *E. coli* in the cassava sample steeped in stream water is of major health concern. Local processors of pupuru are therefore advised to use well and warm water for steeping the cassava to be fermented into pupuru since the pupuru samples obtained from these waters have considerably high protein and low antinutrients contents.

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