

# Calcium, Phenol, minerals and proximate content in relation to infection and weight loss in *Solanum tuberosum* L. incubated with *Botryodiplodia theobromae* Pat

## Abstract

Irish potato, *Solanum tuberosum* L. is grown as a staple in temperate, subtropical and tropical areas of the world today. The tubers are available in Nigeria markets but high costs prevail making them affordable by the middle class and the rich. Tubers are cooked and consumed after purchase as fast deterioration and sprouting disallow keeping on the shelf. Potato tubers were sampled from three major markets namely Kuto, Lafenwa and Osiele in Abeokuta town (capital of Ogun state, in the south west area of Nigeria), to determine tuber calcium content in relation to infection by *Botryodiplodia theobromae*. Proximate content, phenol and other essential mineral contents of the tubers were compared after analysis. The infection experiment was of 3 treatments (the three markets), and 7 replicates arranged in complete randomized design. The experiment lasted 14 days because of deterioration. Proximate content levels were appreciable, even though there were no significant differences in any of them, in tubers from the three markets. Carbohydrate the energy component was relatively higher than other components with an average of 80% dm. Fat was relatively low compared to other components with an average of less than 0.5% dm. Crude fibre and ash were over 9%, 6% and 3% respectively. K varied significantly thus: 69.58 (Lafenwa)>66.75/62.64mg/100gdm (Kuto/Osiele). N varied significantly thus: 1241.7 (Lafenwa)>1214.3 (Kuto)>1169mg/100gdm (Osiele). S varied significantly thus: 914.67 (Osiele)>890.33 (Lafenwa)>879.67mg/100gdm (Kuto). P varied significantly thus: 72.39 (Osiele)>71.63 (Kuto)>68.92mg/100gdm (Lafenwa). Fe varied significantly thus: 1.11/1.05mg/100gdm (Osiele/Kuto)> 0.96mg/100gdm (Lafenwa). Calcium was not significantly different in tubers from the three markets, and the range was 15.60 to 16.96mg/100gdm. Magnesium was also not significantly different in the three markets, and had a range of 62.64 to 69.58mg/100gdm. Infection and weight loss, each of which were over 45% are quite high for a 14-day incubation with *Botryodiplodia theobromae* a major pathogen of *Solanum tuberosum* tubers. Weight loss was highest in Kuto tubers (65.42%) and was lower in Osiele tubers (34.01%). Weight loss in Lafenwa tubers (48.62%) was not significantly different from those of Osiele or Kuto. Infection however decreased with increase in phenol content. Infection increased thus: 47.82% (Lafenwa)<57.70% (Osiele)<65.48% (Kuto), whereas phenol content decreased thus: 306.98mg/100gdm (Lafenwa)>270.93mg/100gdm (Osiele)>179.79mg/100gdm (Kuto). There were correlations between four minerals including calcium and infection as well as two proximate components and infection, but which were not significant as follows: Ca and infection ( $r = -0.0006$ ), N and infection ( $r = -0.0059$ ), P and infection ( $r = -0.0370$ ) as well as K and infection ( $-0.0465$ ); Dry matter and infection ( $-0.3945$ ), fat and infection ( $r = -0.1331$ ). It is necessary to vary in subsequent research, the four minerals Ca, N, P and K in different proportions to ascertain optimal levels commensurate to lowest infection and weight loss by *B. theobromae* and other rot pathogens, as well as to correlate various levels of these minerals with phenol or phenolic compounds in the tubers. This mineral nutrition strategy will establish the necessary standard for storage or export tuber quality for *Solanum tuberosum* varieties in the favourable region (Plateau State versus middle belt) of growth in Nigeria.

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## Introduction

*Solanum tuberosum* L. (Irish potato) is an annual herbaceous dicotyledonous plant in the family Solanaceae. It is reported to have originated from the highland of Bolivia in South America and had been spread to areas like Spain, England and Ireland as far back as 1963 by Spanish Explorers. It was introduced to Nigeria by European

miners in Jos Plateau early in the 20<sup>th</sup> century.<sup>1</sup> *Solanum tuberosum* is both a food and cash crop, produced mainly in Plateau State, a high altitude region of Nigeria. Considering its growth cycle of about 95 days respectively shorter than other root and tuber crops that are staples such as cassava and yam, management of low soil fertility and good storage practices could raise the level of production optimally. The post-harvest susceptibility of potatoes to microbial rot pathogens

however, is regarded as the major challenge to Nigerian producers currently. This is reported to be related to the tuber calcium content.<sup>2</sup> Various forms of calcium fertilization in potatoes have improved potato post-harvest problems in the developed countries of the world. In-season calcium application has been reported to reduce bruising of *S. tuberosum* tubers during mechanical harvest.<sup>3</sup> Bruised tubers offer entry points for rot pathogens (both bacteria and fungi), that predispose to rapid deterioration, in transit or in storage. Internal brown spot and subapical necrosis of potato sprouts was reduced with calcium fertilization.<sup>4,5</sup> Erwinia soft rot was markedly reduced in potato tubers by calcium fertilization at pre-tillage or of the growing plant at tuberization as well as vacuum infiltration of the tubers before storage.<sup>6,7</sup> Potato shelf life have also been prolonged by immersion or spraying with calcium solution.<sup>8</sup> Calcium fertilization of *Solanum tuberosum* is not yet practiced in Nigeria. This study was carried out to determine the relationship between calcium content in fresh potato tubers from major markets in Abeokuta town (in Ogun State, South West Nigeria) in relation to infection after the incubation of the tubers with *Botryodiplodia theobromae*. The second objective of the study was to compare minerals, phenol content and proximate content in tubers from the three markets and their relationship to infection by *B. theobromae*.

## Materials and methods

### Sourcing of potato tubers

The tubers were sourced from three major markets in Abeokuta city. These are Lafenwa, Kuto and Osiele markets. Tubers were selected which were clean and without dark patches or disease spots.

### Processing of tubers and analysis

Tubers were washed in tap water and left to dry in labelled plastic trays under a low-speed fan on the laboratory bench top. Quadruplicate samples of the tubers were sliced into thin chips with a steel knife and transferred in the labelled trays to the COLPLANT (College of Plant Science and Crop Production) screen house. The trays were placed on top of raised, wooden, netted storage structures for two to three days for drying of the chips. The dried chips were then milled to powder with a waring 4-speed blender. The milled samples were poured into new large labelled envelopes and transferred to the Biological Sciences TETFund Laboratory and the Biotechnology Centre, FUNAAB (Federal University of Agriculture, Abeokuta) for analysis. Phenol content and calcium were analyzed as well as other minerals namely nitrogen, phosphorus, potassium, magnesium, iron and sulphur according to routine methods of the Association of Official Analytical Chemists.<sup>9</sup> Proximate composition, that is dry matter, moisture, ash, crude protein, crude fibre, fat and carbohydrate were also analysed. All analysis were according to routine methods of the Association of Official Analytical Chemists, A.O.A.C.<sup>9</sup>

### Infection and weight loss experiment

The experiment was arranged in complete randomized design, with three treatments which are the sources of tubers that is the three markets and seven replicates. Inoculation of tubers was done in an Inoculation structure (Hood) in the Crop Protection Laboratory in COLPLANT Building. Tubers were weighed and surface-sterilized with cotton wool soaked in 70% methylated spirit, at the point to be inoculated (inoculated site). Inoculation was with a 4mm cork borer, scalpel and pair of forceps. The latter had been sterilized to red-hot

over a lighted spirit flame bottle and left to cool, in a slanting position over another cork borer. The sterilized 4mm cork borer was used to bore a 4mm hole through the surface-sterilized inoculation site. The cut tissue was lifted out with the sterilized scalpel and forceps. Another sterilized and cooled 3mm cork borer was used to place a 3mm agar (potato dextrose agar) disc of a 10-day old pure culture (in a 9cm plastic petri dish) of *Botryodiplodia theobromae* in the hole made in the tuber. The cut out tissue was then replaced and the incision sealed with Vaseline (petroleum jelly). Incubation was for a period of 14 days. At the expiration of 14 days, the Vaseline was cleared from each tuber with spatula and cotton wool. Weights of the tubers were again measured with an electronic balance. A sharp knife was used to cut through the inoculation site in each tuber. Infected tissue was cut away from the two halves of the tuber on to a pre-weighed piece of aluminium foil. The weight of infected tissue was measured with an electronic balance.

### Percent weight loss was calculated with the formula

$$\% \text{ Weight loss (Y)} = \left( \frac{A-B}{A} \right) 100$$

Where A and B are weight of the tuber at the beginning and at the end of the experiment. Percent infection was calculated with the formula:

$$\% \text{ Infection} = \left( \frac{C}{A} \right) 100$$

Where C and A are corrected weight of infected tissue and weight of tuber at the beginning of the experiment respectively. C was calculated with the formula

$$C = \frac{100X}{100 - Y} \quad (\text{Otusanya, 1994}).^{10}$$

Where x is the weight of infected tissue and Y is percent weight loss.

### Data analysis

Analysis of variance was carried out and Pearson's correlation test analysis. Means were separated with Tukey's Studentized Range (HSD) test.

## Results

### Infection and weight loss assessment

Infection and weight loss were significantly different among the market samples, as presented in Table 1. Significant differences in infection are as follows: Kuto 65.48% > Osiele 57.7% > Lafenwa 47.82%. Weight loss in the Kuto tubers which was 65.42% was significantly higher than in those of Osiele 34.01%. Lafenwa tubers weight loss of 48.62% was not significantly different from that of Kuto or Osiele.

### Phenol and mineral content in tubers

Calcium and magnesium were not significantly different in the tubers from the 3 markets as shown in Table 2. The range of calcium content was 15.60 to 16.96mg/100gdm. The range of magnesium

was 29.25 to 29.87mg/100gdm. Tuber K varied significantly as follows: 69.58 (Lafenwa)>66.75/62.64mg/100gdm (Kuto/Osiele). N varied significantly as follows: 1241.7 (Lafenwa)>1214.3 (Kuto)>1169mg/100gdm (Osiele). Sulphur varied significantly as follows: 914.67 (Osiele)>890.33 (Lafenwa)>876.67mg/100gdm (Kuto). Tuber phosphorus varied significantly as follows: 72.39 (Osiele)>71.63 (Kuto)>68.92 (Lafenwa). Tuber iron varied significantly as follows: 1.11/1.05 (Osiele/Kuto)> 0.96 (Lafenwa) mg/100gdm. Phenol content also varied significantly as follows: 306.98 (Lafenwa) > 270.93 (Osiele)>179.79 (Kuto) mg/100gdm.

**Proximate content in tubers**

None of the proximate components was significantly different in the tubers sampled from the three markets as shown in Table 3. Tuber dry matter range was 86.72 to 88.85% (Table 3). Range of ash was 3.74 to 3.84% dm. Fat content range was 0.40 to 0.43% dm. Crude fibre was 6.21 to 6.55% dm. Crude protein was 9.08 to 9.30% dm. Carbohydrate was 80.02 to 80.68% dm. Calcium which is a mineral is in the last column in Table 3 to indicate its value relative to that of phenol. Mean value of calcium relative to the mean value of phenol is 6.38%.

**Correlation of assessed parameters**

Correlation of all parameters was carried out, but only those of proximate content, mineral content and infection are presented in Table 4. There were significant correlations between tuber Fe and P (r =0.8988), Fe and N (r=-0.9112), N and P (r=-0.8907), S and N (r=-0.7199). Correlation between calcium and infection was negative but not significant (r =-0.0006). K had negative but not significant correlation with infection also (r =-0.0465). Phosphorus and Nitrogen also had negative correlation with infection but which were not significant also, they are r=-0.0370 and r =-0.0059 respectively. Carbohydrate correlated significantly with dry matter, crude fibre, crude protein and ash (r= -0.7039, -0.9879, -0.8789 and -0.7317 respectively). Dry matter correlated positively and significantly with crude fibre and crude protein (r=0.6979 and 0.7347). Fat correlated significantly with ash (r=0.7158). Ash correlated significantly with crude fibre (r=0.7545). Crude protein correlated significantly with crude fibre (r=0.8735). There were correlations which were negative but were not significant between dry matter and infection (r = -0.3945) and between fat and infection (r=-0.1331).

**Table 1** Infection and weight loss in *Solanum tuberosum* tubers from markets in Abeokuta Township, incubated with *Botryodiplodia theobromae* for 5 days

Market source of <i>Solanum tuberosum</i>	Infection (%)	Weight loss (%)
Kuto	57.70b	34.01b
Osiele	47.82c	48.62ba
Lafenwa	65.48a	65.42a

Means in any column followed by a common letter are not significantly different at P<0.05 (Tukey's test)

**Table 2** Minerals (mg/100gdm) and phenol (mg/100gdm) content of *Solanum tuberosum* tubers sourced from markets in Abeokuta town

Market source of <i>solanum tuberosum</i>	Phenol	Calcium	Mg	K	S	N	P	Fe
Kuto	179.79c	15.75a	29.87a	66.75b	876.67c	1214.3b	71.63b	1.05a
Osiele	270.93b	16.96a	29.48a	62.64b	914.67a	1169.0c	72.39a	1.11a
Lafenwa	306.98a	15.60a	29.25a	69.58a	890.33b	1241.7a	68.92c	0.96b

Means in any column followed by a common letter are not significantly different at P<0.05 (Tukey's test)

**Table 3** Proximate content (% drymatter) and phenol (mg/100g dry matter) in tubers of *Solanum tuberosum* from markets within Abeokuta

Market source of <i>solanum tuberosum</i>	Dry matter	Ash	Fat	Crude fibre	Crude protein	Carbohydrate	Phenol	Calcium
Kuto	86.72a	3.84a	0.43a	6.49a	9.15a	80.09a	179.79c	15.75a
Osiele	88.73a	3.78a	0.42a	6.21a	9.08a	80.68a	269.93b	16.96a
Lafenwa	88.85a	3.74a	0.40a	6.55a	9.30a	80.02a	306.98a	15.60a

**Table 4** Correlations of proximate contents, minerals and infection

Fe and P	r = 0.8988**
Fe and N	r = -0.9112**
P and N	r = -0.8907**
S and N	r = -0.7199*
Ca and infection	r = -0.00059ns
K and infection	r = -0.0465ns
N and infection	r = -0.0059ns
P and infection	r = -0.0370ns
Carbohydrate (CHO) and dry matter	r = -0.7039*
CHO and crude protein	r = -0.8789**
CHO and crude fibre	r = -0.9879**
CHO and ash	r = -0.7317*
Dry matter and crude fibre	r = 0.6979*
Dry matter and crude protein	r = 0.7347*
Fat and ash	r = 0.7158*
Crude fibre and ash	r = 0.7545*
Crude fibre and crude protein	r = 0.8735**
Dry matter and infection	r = -0.3945ns
Fat and infection	r = -0.1331ns

\*, \*\* P = 0.05, 0.01; N = 9; ns: Not significant

## Discussion

*Solanum tuberosum* tubers sourced for this study were discarded twice because of rots. Tubers were sourced from the three markets a third time and the experiment shortened to 14 days only. Proximate components level were appreciable as crude protein was over 9% dm and crude fibre over 6% dm and ash over 3% dm. The calorie component carbohydrate was numerically higher than all others with an average of over 80% dm. Relative to other components fat was low, with an average of below 0.5% dm. The proximate components of tubers from the three markets were not significantly different from one another. Mineral content varied significantly in tubers from the three markets. Potassium and Nitrogen were highest in tubers from Lafenwa, but Sulphur and Phosphorus were highest in the Osiele market tubers. Lafenwa market tubers were lowest in phosphorus and iron. Magnesium and Calcium were not significantly different in tubers from the three markets. Mean calcium content was over 16mg/100gdm. Infection and weight loss which were each an average of over 45% are quite high for a period of 14 days incubation with *Botryodiplodia theobromae*, a major pathogen of Irish potatoes. Infection decreased with increase in phenol content. Phenol content decreased thus: 306.98mg/100gdm (Lafenwa) > 270.93mg/100gdm (Osiele) > 179.79mg/100gdm (Kuto), whereas infection increased thus: Lafenwa (47.82%) < Osiele (57.7%) < Kuto (65.48%). Phenol and phenolics have been reported to be directly involved in increased resistance to infection in crop plants.<sup>11</sup> Calcium on the other hand is implicated in the synthesis of phenol.<sup>12</sup> There was no correlation between calcium and phenol in this study. But there were negative

correlations between four mineral elements and infection which were not significant in this study. They are Ca and infection (r = -0.0006), N and infection (r = -0.0059), Phosphorus and infection (r = -0.0370) as well as potassium and infection (r = -0.0465). Application of calcium carbonate and NPK fertilizer reduced infection and weight loss by *Botryodiplodia theobromae* after long term storage, in two improved varieties of *Dioscorea* species (*D. rotundata* TDr 131 and *D. alata* TDa 92-2).<sup>13</sup> Even potato (*Solanum tuberosum*) yield has been increased with increased calcium.<sup>14</sup> Research is necessary in Nigeria, to vary these four mineral components one after the other in different proportions to ascertain optimal levels commensurate to lowest infection to *B. theobromae* and other rot pathogens, as well as to correlate various levels of these minerals with phenol or phenolic compounds in the tubers. This will standardize tuber storage and export quality for *S. tuberosum* in the major areas of production (Plateau region/state and the middle belt) in Nigeria.

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## Conflicts of interest

The authors declared there are no conflicts of interest.

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