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## Quantitative determination of active substances (preservatives) in *Piliostigma thonningii* and *Khaya ivorensis* leaves and subsequent transfer in treated dry-yam

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The local processors of dry-yams used varied quantities of *Piliostigma thonningii* (Abafe) and *Khaya ivorensis* (Agehu) leaves as preservatives in which unknown quantities of active substances (flavonoids and limonoids, respectively) from the leaves were transferred into the dry-yams. The quantity of active substances in *P. thonningii* and *K. ivorensis* leaves, respectively, and the amount transferred to dry-yam samples treated with varied quantities of leaves were investigated. The active substance A was of high quantity (43.3%) in D-AB-50-Y (yam treated with 50 g *P. thonningii* leaves) and the lowest (6.3%) in F-AB-10-Y (yam treated with 10 g *P. thonningii* leaves). The active substance E was of the highest quantity (81.2%) in CD-10-Y (yam treated with 10 g each of *P. thonningii* and *K. ivorensi* leaves) and of the lowest quantity (34.7%) in CF-20-Y (yam treated with 20 g each of *P. thonningii* and *K. ivorensi* leaves). Active substance A in *P. thonningii* and active substance E in *K. ivorensis* leaves were the most prominent active substances transferred to the dry-yam samples either when the leaves were used singly or in combination.

Key words: Quantitative, active substances, Piliostigma thonningii, Khaya ivorensis, dry-yam.

### INTRODUCTION

Yam (*Dioscorea* spp) is a widely distributed tuber crop in West Africa. More than 95% of the world's yams are produced in Africa with the remainder grown in the West Indies and part of Asia and South and Central America (Purseglove, 1988; 1991). Production of yam in Africa is largely confined to the "yam zones" comprising Cameroon, Nigeria, Benin, Togo, Ghana and Cote d'Ivore where approximately 90% of the world's production takes place (FAO, 2006). Nigeria alone accounts for considerably more than half of the world total production (Ihekoronye and Ngoddy, 1995). Yam is among the oldest recorded food crops ranked second after cassava in the supply of carbohydrates in West Africa (Nweke et al., 1991; FAO, 1997). It is a preferred staple crop that plays a prominent socio-cultural role in the lives of the people of sub-Saharan Africa (Andreas, 2003). Yam suffers high degree of post harvest spoilage due to high moisture content ranging between 65 - 85% of the weight of the tuber (Kordylas, 1990). Therefore, to overcome this pro-blem thereby making yam-based foods available all-year round, yam is processed using well established methods (Ige and Akintunde, 1981; Akissoe et al., 2001; Bricas et al., 1997). In some West African countries such as Nigeria and Republic of Benin, the age-long traditional

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method is still being used for processing of dry-yam. This involves peeling, slicing, blanching in hot water at 40 -60°C for 1-3 h), steeping (24 h) and sun-drying, into a product called "Gbodo" by the Yorubas of South-west Nigeria (Ona-yemi and Potter, 1974). Preliminary survey has shown that the local consumers have preference for the dry yams made by the Baruba/Baruten people of Kwara state who incidentally are the major producers of the yam (Babajide, 2005; 2007). This could be because they add either of *Piliostigma thonningii* (Abafe) or *Khaya* ivorensis (Agehu) leaves or their combination as local prese-rvatives during blanching of the yams (Babajide, 2005). P. thonningii (Schum) Milne-Rech is a member of the family Caesalpinioideae and is locally known as "Abafe" in Yoruba land of Nigeria Camel's foot tree and Monkey Bread (Jimoh and Oladimeji, 2005). The plant is a small, rounded deciduous tree, 3 - 5 m in height and grows on sandy soil in the bush. It has large two-lobed simple leaves and without thorns or spines. Akinpelu and Obuotor (2000) found that extract of P. thonningii stem bark exhibited bactericidal activity. Flavonoids, saponin, glycosides and anthraquinone (Jimoh and Oladimeii. 2005), some of these constituents have been reported to have inhibitory effects on some gram-negative bacterial such as Escherichia coli and Bacillus subtilis among others (Ibewuike et al., 1997). The C-methylflavonoid constituents were found to inhibit prostaglandin synthesis in vitro and have antibacterial activities against Staphylococcus aureus (Ibewuike et al., 1997).

K. ivorensis A. Juss. the family of Meliaceae is commonly referred to as African mahogany. Its other names include: "Agehu", "Aganho", "Ogwango", as called by three different Yoruba dialects. The stem barks of K. ivorensis are commonly used by the traditional medical practitioners and alcoholic beverage brewers in Ghana in preparing tonics for anaemia and appetizers (Samir et al., 2005). The species is reported to be found in all the timber producing areas of West Africa, Gabon (Laira, 2000). Samir et al. (2005) reported that the stem bark contained ten limonoids of angolensate, ring D-opened limonoids and menicanolides. These compounds had antifungal activity against pathogenic fungus such as Aspergillus flavus, Aspergillus fumigatus, Aspergillus *Candid*a albicans, niger, Microsporiun andonii. Trichoderms viride and Trichophyton metaprophytes (Adekunle et al., 2003). Varied guantities of P. thonningii (Abafe) and K. ivorensis (Agehu) leaves have been used, respectively, to treat (preserve) dry-yams by Babajide et al. (2008). The treated samples had lower microbial loads  $(>10 - 10^4 \text{ cfu/g})$  (total plate count, fungal count and staphylococcal count) compared to that of untreated sample  $(10^6 \text{ cfu/g})$  (Babajide et al., 2008).

The local processors of dry-yams used varied quantities of *P. thonningii* (Abafe) and *K. ivorensis* (Agehu) leaves as preservatives in which unknown varied quantities of active substances from the leaves were transferred into the dry yams. The quantity of active

substances (flavonoids) in *P. thonningii* and *K. ivorensis* leaves and the amount transferred to dry-yam samples treated with varied quantities of leaves were investigated in this research.

### MATERIALS AND METHODS

### **Raw materials**

White yam tubers (*Dioscorea esculenta*) were purchased from Odo-Oba market in Oyo, Nigeria. The leaves of *P. thonningii* (Abafe) and *K. ivorensis* (Agehu) were plucked from the herbal garden of the University of Agriculture, and Ogun-Osun River Basin Development Authority, Abeokuta, Nigeria, respectively. Both leaves were authenticated at the Forestry Research Institute of Nigeria, Jericho, Ibadan, Nigeria where voucher specimens have been deposited.

### Dry-yam (gbodo) processing

The processing of yam tubers to dried yam 'Gbodo' was carried out following the method described by Ige and Akintunde (1981) with some modification (Babajide, 2005; 2007). The yam tubers were peeled using sharp knife, washed in clean water and sliced to 2 - 3 cm thick to hasten drying. Sliced yam tubers (1.5 kg each) were blanched at 50°C for 2 h in a water bath (Clifton, England). Predetermined measurements of fresh and air-dried P. thonningii (Abafe) and K. ivorensis (Agehu) leaves were added singly and in combinations of (10, 20, 30, 40 and 50 g), respectively, at the blanching stage (Babajide and Atanda, 2008; Babajide et al., 2008). In all, 31 treatments including the control (untreated sample) were obtained. These consisting of: 5 levels of samples treated with fresh Abafe leaves (F-AB-Y), 5 levels of samples treated with dried Abafe leaves (D-AB-Y), 5 levels of samples treated with fresh Agehu leaves (F-AG-Y), 5 levels of samples treated with dried Agehu leaves (D-AG-Y), 5 levels of samples treated with combined fresh leaves (CF-Y), 5 levels of combined dried leaves (CD-Y) and the untreated sample (Table 1). After blanching, the yams were steeped in the same water for 24 h to become flabby, after which the water and leaves were drained for each sample and the yams were dried at 60°C in a LEEC cabinet dryer, the drying samples were weighed at intervals until a constant weight (average moisture content of 8%) was obtained for each sample at the 2nd day. The dried yam slices were packaged in woven polypropylene sacks and stored at ambient temperature  $(32 + 2^{\circ}C)$  prior to further analyses.

### High performance liquid chromatograph (HPLC) analysis

The materials used for HPLC analysis include: Ethanol solution 50%, HPLC grade acetonitrile, sodium dihydrogen orthophosphate salt (NaH<sub>2</sub>PO<sub>4</sub>), distilled water, ultrasonic bath (sonicator), test tubes, pipettes, volumetric flasks, chemical balance (SCALTEC SBC 31) pH meter (Orion, Japan), HPLC-Agilent (C-1310A), Hewlett Packard (G-1314A), Vacuum Degaser (G-1322A). All materials and equipment for HPLC analysis were used at the Drug and Quality Control Laboratory of the Lagos State University Teaching Hospital (LASUTH), Lagos, Nigeria.

### **Reagents preparation for HPLC analysis**

1. Phosphate Buffer: 0.025 M NaH<sub>2</sub>PO<sub>4</sub> at pH 2.5. 3.0 g of sodium hydrogen tetraoxophosphate (v) NaH<sub>2</sub>PO<sub>4</sub> of molecular weight, 120 g was weighed and dissolved in 1 dm<sup>3</sup> of distilled water. It was

**Table 1.** Code names for dry-yam samples treated with different proportions of *P. thonningii* (Abafe) and *K. ivorensis* (Agehu) leaves.

Sample name	Description
F-AB10-Y	Dry-yam treated with 10 g fresh Abafe leaves
F-AB20-Y	Dry-yam treated with 20 g fresh Abafe leaves
F-AB30-Y	Dry-yam treated with 30 g fresh Abafe leaves
F-AB40-Y	Dry-yam treated with 40 g fresh Abafe leaves
F-AB50-Y	Dry-yam treated with 50 g fresh Abafe leaves
D-AB10-Y	Dry-yam treated with 10 g dried Abafe leaves
D-AB20-Y	Dry-yam treated with 20 g dried Abafe leaves
D-AB30-Y	Dry-yam treated with 30 g dried Abafe leaves
D-AB40-Y	Dry-yam treated with 40 g dried Abafe leaves
D-AB50-Y	Dry-yam treated with 50 g dried Abafe leaves
F-AG10-Y	Dry-yam treated with 10 g of fresh Agehu leaves
F-AG20-Y	Dry-yam treated with 20 g of fresh Agehu leaves
F-AG30-Y	Dry-yam treated with 30 g of fresh Agehu leaves
F-AG40-Y	Dry-yam treated with 40 g of fresh Agehu leaves
F-AG50-Y	Dry-yam treated with 50 g of fresh Agehu leaves
D-AG10-Y	Dry-yam treated with 10 g of dried Agehu leaves
D-AG20-Y	Dry-yam treated with 20 g of dried Agehu leaves
D-AG30-Y	Dry-yam treated with 30 g of dried Agehu leaves
D-AG40-Y	Dry-yam treated with 40 g of dried Agehu leaves
D-AG50-Y	Dry-yam treated with 50 g of dried Agehu leaves
CF10-Y	Dry-yam treated with fresh10 g Abafe and 10 g Agehu leaves
CF20-Y	Dry-yam treated with fresh 20 g Abafe and 20 g Agehu leaves
CF30-Y	Dry-yam treated with fresh 30 g Abafe and 30 g Agehu leaves
CF40-Y	Dry-yam treated with fresh 40 g Abafe and 40 g Agehu leaves
CF50-Y	Dry-yam treated with fresh 50 g Abafe and 50 g Agehu leaves
CD10-Y	Dry-yam treated with dried 10 g Abafe and 10 g Agehu leaves
CD20-Y	Dry-yam treated with dried 10 g Abafe and 20 g Agehu leaves
CD30-Y	Dry-yam treated with dried 30 g Abafe and 30 g Agehu leaves
CD40-Y	Dry-yam treated with dried 40 g Abafe and 40 g Agehu leaves
CD50-Y	Dry-yam treated with dried 50 g Abafe and 50 g Agehu leaves

sonicated for 15 min for proper dissolution. The pH was adjusted to 2.5 with phosphoric acid using the pH meter.

2. Mobile phase: Acteonitrile (ACN): 0.025 M NaH\_2PO\_4 at pH 2.5 have % ratio of 25:75%.

### Chromatographic system

Column – hypersile octadecylsilane coated has a Flow rate: 0.8 min / ml. UV wavelength ( $\lambda$ ) = 210 nm. Stop time: 5 min. Injection volume: 20 µl (microlitre).

### Extraction of the active substances in Abafe, Agehu leaves and samples

Twenty grams (20 g) of the dry-milled leaves were extracted in 200 ml of 50% ethanol to give extracts of Abafe (AB) and Agehu (AG) leaves as controls, respectively. Similarly, 20 g of untreated dry-yam flour was extracted in 200 ml of 50% ethanol to give untreated dry-yam control. Five grams of each milled dry-yam samples

treated with leaves (singly or combined) was dissolved in 50 ml of 50% ethanol and shaken for two hours.

### Sample preparation for HPLC analysis

Dilution of 1:10 from 10 mg/ml of leaves extracts and untreated yam were made with the mobile phase mixed and sonicated for 10 min to give a solution of 1 mg/ml concentration. Dilution of 1:10 from 100 mg/ml of treated yams were made with the mobile phase, mixed and sonicated for 10 min to give a solution concentration of 10 mg/ml.

### Quantitative determination of active substances

The quantity of each active substance that corresponds to the peaks observed was calculated as follows:

Quantity of active substances (%) = (Peak Area of treated dry-yam active substance x Concentration factor)/ (Peak Area of control active substance) x 100

Peaks	Area (mAU)	Height (%)	Retention time (min)
А	742.21	53.38	1.33
В	710.98	57.38	1.54
С	428.87	39.72	1.76
D	1853.01	32.32	2.01
Е	308.11	23.41	1.41
F	439.13	20.62	1.52
G	420.43	14.26	2.08
Н	184.58	5.71	3.07
I	516.08	27.70	1.39
J	75.91	4.86	1.80

**Table 2.** Areas, heights and retention times of observed peaks

A = Peak due to active substance A in Abafe leaves, B = Peak due to active substance B in Abafe leaves, C = Peak due to active substance C in Abafe leaves, D = Peak due to active substance D in Abafe leaves, E = Peak due to active substance E in Agehu leaves, F = Peak due to active substance F in Agehu leaves, G = Peak due to active substance G in Agehu leaves, H = Peak due to active substance H in Agehu leaves, I = Peak due to active substance I in untreated dry-yam, J = Peak due to active substance J in untreated dry-yam and (mAU) = micro atomic unit.

Where, Concentration factor = 0.1.

### **RESULTS AND DISCUSSION**

# Quantities of active components of *P. thonningii* (Abafe) and *K. ivorensis* (Agehu) leaves and its subsequent transfer to treated dry-yam samples

When the chromatograms of the controls that is, *P. thonningii* (Abafe) and *K. ivorensis* (Agehu) leaves and the untreated dry-yam were compared with those of the treated dry-yam slices, similar peaks appearing in the chromatograms of the control and those of the treated yam slices showed evidence for the transfer of a particular active substance from the leaves to the treated dry-yam samples.

In *P. thonningii* (Abafe) leaves, the four peaks of the active substances observed were named A, B, C and D while E, F, G and H represent the four peaks observed for *K. ivorensis* leaves. I and J represent the two peaks observed in untreated dry-yam (Table 2). The peaks (I and J) due to the untreated dry-yam were noted to be different from those of the treated dry-yam samples.

The chromatograms of *P. thonningii* leaves and that of the *P. thonningii* treated dry-yam samples were compared (Table 3), this revealed that peak due to active substance A is the most prominent in the fresh and dried *P. thonningii* treated dry-yam samples. Active substances A occurred in relatively large quantities in D-AB50-Y (43.3%), F-AB50-Y (31.4%) and F-AB20-Y (31.2%) and in small quantities in F-AB10-Y (6.3%) and D-AB10-Y (8.9%).

Peak due to active substance B was the least prominent as it occurred in only D-AB30-Y (16%) and D-AB40-Y (17.7%). Therefore, active substances A and B

from the *P. thonningii* leaves were those mainly transferred into the treated dry-yam samples and was said to be principally responsible for the antimicrobial property of the *P. thonningii* leaves. There was no transfer of active substances C and D from *P. thonningii* leaves to all the Abafe treated dry-yam samples.

Table 4 revealed that peak due to active substance E of K. ivorensi leaves was the most prominent in the Agehu leaves as it occurred in all but five of the K. ivorensi treated dry-yam samples (D-AG10-Y, D-AG30-Y, D-AG40-Y, D-AG50-Y and F-AG50Y). Active substance E however, occurred in large quantities in DAG-20-Y (74.7%), F-AG40-Y (66.0%) and F-AG10-Y (55.6%). Peaks due to active substances G and H occurred only in F-AG50-Y as 13.1 and 7.2%, respectively (Table 4). Active substance F was absent in all the K. ivorensi treated dry-yam samples and there was no transfer of active substance to samples D-AG10-Y, D-AG30-Y, D-AG40-Y and D-AG50-Y. This could be the reason why dried K. ivorensi leaves had less anti-microbial effect on dry-yam samples (Babajide and Atanda, 2008; Babajide et al., 2008).

In Table 5, peaks due to active substance in *P. thonningii* and Agehu leaves (that is, A and E, respectively) were the must prominent in the combined (*P. thonningii* and *K. ivorensi* leaves) treated dry-yam samples. Peak due to active substances B from *P. thonningii* leaves was only present in CF20-Y (9.5%). Peak due to active substance A from the *P. thonningii* leaves failed to occur in CF20-Y to CF50-Y treated dry-yam samples. Peak due to active substances E from the *K. ivorensi* leaves occurred in large quantities in all the combined (*P. thonningii* and *K. ivorensi* leaves) treated dry-yam samples. It showed high values especially in CD10-Y (81.4%), CF30-Y (74.4%), CF40-Y (79.4%), and CF50-Y (73.3%). Active substance H was only present

Samples	Peak observed	Quantity of active substance A (%)	Quantity of active substance B (%)	Quantity of active substance C (%)	Quantity of active substance D (%)
AB control	A, B, C and D	100	100	100	100
F-AB10-Y	А	6.3	-	-	-
F-AB20-Y	А	31.2	-	-	-
F-AB30-Y	А	27.5	-	-	-
F-AB40-Y	А	9.8	-	-	-
F-AB50-Y	А	31.4	-	-	-
D-AB10-Y	А	8.9	-	-	-
D-AB20-Y	А	15.1	-	-	-
D-AB30-Y	А, В	25.0	16	-	-
D-AB40-Y	А, В	26.9	17.7	-	-
D-AB50-Y	A	43.3	-	-	-

Table 3. Percentage active substances in Abafe treated dry-yam samples.

F-AB10-Y= dry-yam treated with 10 g fresh Abafe, F-AB20-Y= dry-yam treated with 20 g fresh Abafe, F-AB30-Y= dry-yam treated with 30 g fresh Abafe, F-AB40-Y= dry-yam treated with 40 g fresh Abafe. F-AB50-Y= dry-yam treated with 50 g fresh Abafe. D-AB10-Y= dry-yam treated with 10 g dried Abafe, D-AB20-Y= dry-yam treated with 20 g dried Abafe, D-AB30-Y= dry-yam treated with 30 g dried Abafe, D-AB40-Y= dry-yam treated with 50 g dried Abafe, D-AB50-Y= dry-yam treated with 50 g dried Abafe.

Table 4. Percentage active substances in Agehu treated dry-yam samples.

Samples	Peak observed	Quantity of active substance E (%)	Quantity of active substance F (%)	Quantity of active substance G (%)	Quantity of active substance H (%)
AG control	E,F, G and H	100	100	100	100
F-AG10-Y	E	55.6	-	-	-
F-AG20-Y	E	41.9	-	-	-
F-AG30-Y	E	49.9	-	-	-
F-AG40-Y	E	66.0	-	-	-
F-AG50-Y	G, H	-	-	13.1	7.2
D-AG10-Y	-	-	-	-	-
D-AG20-Y	Е	74.7	-	-	-
D-AG30-Y	-	-	-	-	-
D-AG40-Y	-	-	-	-	-
D-AG50-Y	-	-	-	-	-

F-AG10-Y = dry-yam treated with 10 g of fresh Agehu, F-AG20-Y = dry-yam treated with 20 g of fresh Agehu, F-AG30-Y = dry-yam treated with 30 g of fresh Agehu, F-AG40-Y = dry-yam treated with 40 g of fresh Agehu, F-AG50-Y = dry-yam treated with 50 g of fresh Agehu. D-AG10-Y = dry-yam treated with 10 g of dried Agehu, D-AG20-Y = dry-yam treated with 20 g of dried Agehu, D-AG30-Y = dry-yam treated with 30 g of dried Agehu, D-AG30-Y = dry-yam treated with 30 g of dried Agehu, D-AG30-Y = dry-yam treated with 30 g of dried Agehu, D-AG30-Y = dry-yam treated with 50 g of dried Agehu.

in CD50-Y as 4.5% apart from active substance A and E. Peaks due to active substances C, D, F and G do not occur in all the dry - yam samples treated with combine leaves.

### Conclusion

Generally, active substances A from *P. thonningii* leaves and E from *K. ivorensis* leaves were most prominent in the treated dry-yam samples and was said to have the most contributory effect towards the antimicrobial activity of the leaves. It can be established that there were transfer of active substances from the *P. thonningii* and *K. ivorensi* leaves during blanching into the dry-yam samples. The active substance A was of high quantity (43.3%) in D-AB-50-Y (yam treated with 50 g *P. thonningii* leaves) and the lowest (6.3%) in F-AB-10-Y (yam treated with 10 g *P. thonningii* leaves). The active substance E was of the highest quantity (81.2%) in CD-10-Y (yam treated with 10 g each of *P. thonningii* and *K. ivorensi* leaves) and of the lowest quantity (34.7%) in CF-20-Y (yam treated with 20 g each of *P. thonningii* and *k. ivorensi* leaves). Active substance A in *P. thonningii* and active substance E in *K. ivorensi* leaves were the most prominent active substances transferred to the dry-yam samples either when the leaves were used singly or in combination.

Peak observed	Quantity of active substances A (%)	Quantity of active substances B (%)	Quantity of active substances C (%)	Quantity of active substances D (%)	Quantity of active substances E (%)	Quantity of active substances F (%)	Quantity of active substances G (%)	Quantity of active substances H (%)
A, B, C,D, E,F,G and H	100	100	100	100	100	100	100	100
A, E	9.5	-	-	-	64.6	-	-	-
B, E	-	9.5	-	-	34.7	-	-	-
A, E	11.9	-	-	-	74.4	-	-	-
E	-	-	-	-	79.4	-	-	-
E	-	-	-	-	73.3	-	-	-
A, E	10.7	-	-	-	81.4	-	-	-
A, E	6.3	-	-	-	50.1	-	-	-
A, E	8.9	-	-	-	49.7	-	-	-
A, E	8.4	-	-	-	54.8	-	-	-
CD50 – Y	A, E,H	10.5	-	-	-	58.6	-	4.5

Table 5. Percentage active substances in Abafe and Agehu treated dry-yam samples.

CF10-Y = dry-yam treated with fresh10 g Abafe and 10 g Agehu CF20-Y = dry-yam treated with fresh 20 g Abafe and 20 g Agehu, CF30-Y = dry-yam treated with fresh 30 g Abafe and 30 g Agehu, CF40-Y = dry-yam treated with fresh 40 g Abafe and 40 g Agehu, CF50-Y = dry-yam treated with 50 g Abafe and 50 g Agehu fresh leaves. CD10-Y = dry-yam treated with dried 10 g Abafe and 20 g Agehu, CD20-Y = dry-yam treated with dried 30 g Agehu, CD20-Y = dry-yam treated with dried 40 g Abafe and 20 g Agehu, CD30-Y = dry-yam treated with dried 30 g Abafe and 30 g Agehu, CD40-Y = dry-yam treated with dried 40 g Abafe and 40 g Agehu, CD30-Y = dry-yam treated with dried 30 g Abafe and 30 g Agehu, CD40-Y = dry-yam treated with dried 40 g Abafe and 40 g Agehu.

There is need to study the safety level of inclusion of these local preservatives (*P. thonningii* Abafe and *K. ivorensi* Agehu leaves) in dry-yam 'gbodo'. Toxicological studies such as acute toxicity, chronic toxicity and maximum tolerated dose should be conducted to determine the level of toxicity of these active substances (flavonoids and limonoids) and to know which particular levels of active substances achieve the best result in terms of antimicrobial property and safety for human consumptions.

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