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Quantitative effect of 'abafe' (*Piliostigma thionnigii)* and 'agehu' (*Khaya ivorensis)* leaves on the microbial load of dry-yam 'gbodo'

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The quantitative effect of 'abafe' (*Piliostigma thionnigii)* and 'agehu' (*Khaya ivorensis)* leaves (fresh or dried; singly or combined) on the microbial load of dry-yam 'gbodo' was studied. The treated samples had lower microbial loads (>10 to 10^4 cfu/g) (total plate count, fungal count and staphylococcal count) compared to that of an untreated sample (10^6 cfu/g). Also, as the level of inclusion of leaves (especially fresh leaves) increased, the preservative effect of the leaves on dry-yam increased. Samples F-AB50-Y, F-AG40-Y and F-AG50-Y had the lowest staphylococcal count (>10 cfu/g). Sample CF50-Y had the lowest total plate count (5.1 x 10^2 cfu/g), fungal count (0.9 x 10^2 cfu/g) and staphylococcal count (>10 cfu/g) when compared with all the other treated samples. The most prominent microorganisms isolated from each dry-yam sample were *Staphylococcus aureus, Bacillus subtilis* and *Aspergillus flavus*. All the leave extracts suppressed growth of the isolated organisms. The combined form of the leave extracts (0.143 g/ml each) exhibited the strongest effect on the microorganisms.

Key words: Quantitative effect, abafe, agehu, microbial load, gbodo.

INTRODUCTION

Yam (*Dioscorea* spp.) is a widely distributed tuber crop in West Africa. More than 95% of the world's yam is produced in Africa with the remainder grown in the West Indies and part of Asia and South and Central America (Purseglove, 199, 1998). 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, 2001). 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 and is ranked second after cassava in the supply of carbohydrates in West Africa (Nweke et al., 1991). Yam is an important source of carbohydrate for many people of the sub-Saharan region, especially in the yam zones of West Africa (Akissoe et al., 2003).

Yam suffers a high degree of post-harvest loss due to its high moisture content, ranging between 65 - 85% of the weight of the tuber (Kordylas, 1990). Therefore, to overcome the high perishability of yam tubers due to its high moisture content and the seasonal nature of its production, yams are processed into flour using a well established traditional method (Ige and Akintunde, 1981; Bricas et al., 1997; Akissoe et al., 2001). In some West African countries such as Nigeria and the Republic of Benin, the age-old traditional method is still being used for processing of traditional dry-yam 'gbodo'. The dry-yam tubers/slices are processed by peeling, slicing, parboiling in hot water (40 – 60°C for 1 – 3 h), steeping (24 h) and sun-drying, into a product called 'gbodo' by the Yorubas of southwestern Nigeria (Onayemi and Potter, 1974). When 'gbodo' has been milled into flour, it is called 'elubo', which when stirred into boiling water, makes a thick paste known as 'amala' that is eaten with soup by the consumers (Akissoe et al., 2001).

A study carried out by Adisa (1998) showed that some of the organisms associated with the spoilage of dry-yam slices and flour include *Aspergillus flavus, Aspergillus fumigatus* and *Aspergillus niger*, while *Staphylococcus aureus, Bacillus* spp., *Proteus* spp., *Pseudomonas* spp., *A. niger and A. flavus* were identified from the dry-yams obtained from the traditional processors (Babajide and Atanda, 2008). The preservation of foods by drying is

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based upon the fact that micro-organisms and enzymes need water in order to be active (Jay, 1986). Therefore, control measures such as reducing water activity, adjusting pH, correcting storage temperatures, and additional factors such as modified atmosphere packaging, heat treatments or the addition of preservatives may be required to prevent food spoilage (Transter, 1994). Due to the humid tropical climate in sub-Sahara Africa, coupled with poor facilities and technical know-how, food preservation problems persist; thus resulting in a food crisis in the region (Adisa, 1998). Prices of dry-yam slices therefore fluctuate during the season as a result of a short shelf-life. It has become necessary to explore low cost and highly effective preservative methods for dry-yam 'gbodo'.

The increasing public perception of the effect of chemical preservatives in foods has stimulated interest in the development of natural antimicrobials and presservatives, and spoilage control measures for foods (Jay, 1986). Similarly, Babajide (2005) and Babajide et al. (2007) reported that local processors of traditional dry-yam in south-western Nigeria use local preservatives, that is, 'abafe' (*Piliostigma thionnigii)* and 'agehu' *(Khaya ivorensis)* leaves during yam parboiling to improve the storage life of dry-yam 'gbodo'. Previous research carried out on the extracts of plant leaves such as *P. thionnigii* (Ibewuike et al., 1997) and *K. ivorensis* (Adekunle et al., 2003 and Samir et al., 2005) have reported their bactericidal and fungicidal effects respectively. There were wide ranges between the quantities of local presservatives (leaves) added to varied quantities of yam by the processors (Babajide and Atanda, 2008). For instance, 0.36 – 2.70 kg of 'abafe' could be added to 40 – 135 kg of yam by dry-yam 'gbodo' processors (Babajide and Atanda, 2008). Thus, the effect of variation of quantities of 'abafe' and 'agehu' leaves (fresh or dried leaves; used singly or combined) on the microbial loads of 'gbodo' is the focus of this study.

MATERIALS AND METHODS

Raw materials

White yam tubers of the local variety 'ijedo' (*Dioscorea esculenta*) was purchased from the Odo-Oba market in Oyo, Nigeria. 'Abafe' (*P. thonningii*) and 'agehu' (*K. ivorensis*) leaves were plucked from the herbarium of the University of Agriculture, Abeokuta and Ogun Osun River Basin Development Authority, Abeokuta, 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 dry-yam 'gbodo' was carried out, as described by Babajide, (2005; 2007). Different measurements of fresh and dried 'abafe' and 'agehu' leaves (10, 20, 30, 40 and 50 g) were added respectively to 1.5 kg of yam slices (2 – 3 cm thick), either singly and in combinations, during parboiling in 1.3 litres of water. The fixed quantities of yam and water were obtained from the preliminary studies on processors' 'gbodo' carried out by Babajide and Atanda (2008). The different measurements of leaves were also obtained by varying the quantities - two levels backward and two levels forward - away from the approximate average quantity (30 g) obtained during the preliminary study (Babajide and Atanda, 2008). In all, 31 treatments, including the control untreated sample (without leaves), were performed. These consisted of: 5 levels of samples treated with fresh 'abafe' leaves, 5 levels of samples treated with dried 'abafe' leaves, 5 levels of samples treated with fresh 'agehu' leaves, 5 levels of samples treated with dried 'agehu' leaves, 5 levels of samples treated with fresh combined leaves, 5 levels of dried combined leaves and the untreated sample (Tables 1 - 6). After parboiling each sample at 50°C for 2 h, each sample was soaked in the parboiling water for 24 h with its leaves. The leaves and parboiling water for each sample were drained off before drying to constant moisture content. Each dry-yam sample was packaged in woven polypropylene sacks and stored at ambient temperature (32± 2°C) for further analyses.

Determination of moisture content

The moisture content was determined according to the method described in AOAC (2000).

Determination of pH

The pH was determined with a Jenway pH meter (Model 3015, Serial no. 1647, UK).

Microbiological analysis of treated and untreated dry-yam 'gbodo'

Aerobic plate counts were determined by diluting the samples serially and plating 1 ml aliquots on Nutrient agar (Oxoid), followed by incubation at 30°C for 48 h in a Gallenkamp plus II incubator (UK). The funagal count was determined by plating 1-ml aliquots of the samples on potato dextrose agar (PDA) (Oxoid) to which 0.01% chloramphenicol had been added to inhibit bacterial growth. The agar plates were incubated at 30°C for 48 h. Observed colonies were sub-cultured to obtain pure cultures that were subsequently isolated and identified using morphological characteristics, spore formation and the production of fruiting bodies (Barnett and Hunter, 1972; Raper and Fennel, 1973) after incubation for up to 5 - 7 days. Presumptive staphylococcal count was determined by inoculating mannitol salt agar (Oxoid) with the samples, followed by incubation at 32°C for 72 h. Pigmented colonies surrounded by bright yellow zones (halo) were counted. Confirmation of *S. aureus* was by positive coagulase tests (Franzier and Westhoff, 1988).

Preparation of leave extracts

Extracts of 'abafe' and 'agehu' leaves were prepared, using the method described by Kong et al. (2007). Aliquots of 10, 20, 30, 40 and 50 g of air-dried and pulverized leaves (singly and in combinations) were mixed into 250 ml of 75% (v/v) ethanol and left for 48 h in an enclosed flask with constant agitation. After filtration through Whatman no. 2 filter paper, the residue was re-extracted with an additional 100 ml of 75% ethanol for an additional 24 h and then filtered. The filtrates were subsequently concentrated in a rotary evaporator (type 349/2 Corning Ltd., Britain) at 60°C to 50 ml. The concentration of the extracts were recorded as grams per millilitre, based on the original weight of leaves.

Microbial inhibitory effect of leave extracts in inoculated agar medium

The antimicrobial activity of leave extracts were examined in tripli-

Table 1. Moisture content, pH and microbial load of 'gbodo' treated with fresh 'abafe' leaves.

Sample	Moisture content %	pH	Total plate count(cfu/g)	Fungal count (cfu/g)	Staphylococcal count(cfu/g)
Untreated	10.2[a]	5.73[a]	3.0 x 10^{6a}	1.0 x 10^{6a}	1.8 x 10^{6a}
F -AB10-Y	11.0[a]	5.89[a]	6.0 x 10^{3b}	6.0 x 10^{3b}	1.0 x 10^{3b}
F -AB20-Y	9.7[a]	5.84[a]	5.5 x 10^{3c}	4.0 x10^{3c}	1.0 x 10^{3b}
F -AB30-Y	10.0[a]	5.26[ab]	4.0 x 10^{3d}	3.0 x 10^{3d}	0.5 x 10^{3c}
F -AB40-Y	11.3[a]	5.36[ab]	3.0 x 10^{3e}	1.5 x 10^{3e}	0.5 x 10^{3c}
F -AB50-Y	10.8[a]	6.06[a]	1.0 x 10^{3f}	1.0 x10^{3f}	>10^{d}

Mean values with the same superscript letters in a column are not significantly different at P>0.05
Keys: Untreated= untreated dry-yam, 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'.

triplicate, using the method described by Kong et al. (2007). Mannitol salt agar was used for *Staphylococcus*, PDA for *A. flavus* and Nutrient agar was used for *B. subtilis* isolates. A 1 ml aliquot of each culture was placed in a sterile plate (Petri dish) and covered with 10 ml of sterile agar. The contents of the plate were mixed by gently swirling the plate before the agar solidified. Sterile plastic microcylinders (diameter: 5 mm, height: 10 mm) were set vertically on the agar. A 0.3 ml volume of each leaf extract was then aseptically pipetted into the microcylinder. The control comprised 0.3 ml of 75% ethanol alone. The agar plates were incubated at 32°C for 72 h (*S. aureus)* and at 30°C for 48 h (*A; flavus* and *B. subtilis*). The inhibitory effect was assessed by measuring with a vernier caliper the diameter of the inhibition zone (clear zone) around the microcylinder containing the extract.

Statistical analysis

All analyses were carried out in triplicate. Data obtained were subjected to analysis of variance (ANOVA) using SPSS version 10.0. Differences between the samples means were separated using the Least Significant Difference method.

RESULTS AND DISCUSSION

Effect of varied quantities of 'abafe' and 'agehu' leaves on the microbial load of 'gbodo'

Although uniform moisture content was ensured for each sample during processing, the variations in moisture content of the samples at the time of analysis were considered in this study, as this could affect the result on the microbial loads. The moisture content of all the treat-ed or untreated samples ranged between 9.6% (Tables 4 and 5) to 11.3% (Table 1), which were not significantly different (P>0.05) from each other and were still lower than minimum moisture content (13%) for dried food samples (Christensen and Kaumann, 1973). There is an indication that the leaves had no significant effect on the pH of the samples, as there was no significant difference (P>0.05) in the pH of both the treated (5.44 to 6.68) and the untreated (5.73) samples (Tables 1 to 6). This could therefore have no significant effect on the microbial load of the samples.

According to Adebajo et al. (1994), lower moisture content of food samples does not prevent the growth of microorganisms due to the favourable atmospheric conditions. In this study, there were significant differences (P<0.05) between the microbial loads (total plate count, fungal count and staphylococcal count) of the untreated dry-yam sample and all the treated samples (Tables 1 to 6). The untreated sample had high microbial loads (10^6 cfu/g) compared with the treated samples (>10 to 10^4 cfu/g). As the level of leave treatment increased, the microbial load decreased (Tables 1 to 6), except for the total plate count (2.4 x 10^2 cfu/g) of a sample treated with combined dried 'abafe' and 'agehu' leaves (CD40-Y), which is lower than that of the CD50-Y sample (1.0 x 10^3 cfu/g) (Table 6).

In Table 1, there were significant differences (P<0.05) in the total plate counts (1.0 x 10^3 to 6.0 x 10^3 cfu/g) and in fungal counts (1.0 x 10^3 to 6.0 x 10^3 cfu/g) of all the fresh 'abafe'-treated samples (F-AB-Y), respectively. There was no significant difference at P>0.05 in the staphylococcal count of the F-AB10-Y and F-AB20-Y samples (1.0 x 10^3 cfu/g), and of the F-AB30-Y and F-AB40-Y samples (0.5 x10^3 cfu/g), respectively, while the F-AB50-Y sample had less than 10 cfu/g of staphylococci (Table 1). There is an indication that fresh 'abafe' leaves had a greater effect on the staphylococci, as also previously shown by Ibewuike et al. (1997). As the level of treatment with fresh 'abafe' leaves increased in dry-yam, the microbial load was reduced, indicating it had both bactericidal and fungicidal effects.

In Table 2, there was no significant difference (P>0.05) in the total plate count of the dried 'abafe'-treated samples (D-AB10-Y and D-AB20-Y) (1.1 x 10^4 and 1.0 x 10^4 cfu/g), but they were significantly different (P<0.05) from that of the D-AB30-Y, D-AB40-Y and D-AB50-Y samples, which were significantly different (P<0.05) from each other (3.0 x 10^3 to 6.0 x 10^3 cfu/g). The fungal count of the dried 'abafe'-treated samples (D-AB-Y) were significantly different (P<0.05) from each other, except samples D-AB20-Y and D-AB30-Y, which were not significantly different (P>0.05) from each other (3.0 x 10^3 cfu/g) (Table

Table 2. Moisture content, pH and Microbial load of 'gbodo' treated with dried 'abafe' leaves.

Sample	Moisture content %	pH	Total plate Count (cfu/g)	Fungi count (cfu/g)	Staphylococci count (cfu/g)
Untreated	10.2^{a}	5.73^{a}	3.0×10^{6a}	1.0×10^{6a}	1.8×10^{6a}
D -AB10-Y	10.5^{a}	5.66^{a}	1.1×10^{4b}	3.5×10^{3b}	3.0×10^{3b}
D -AB20-Y	9.8^{a}	6.23^{a}	1.0×10^{4b}	3.0×10^{3c}	1.0×10^{3c}
D -AB30-Y	10.3^{a}	5.39^{ab}	6.0×10^{3c}	3.0×10^{3c}	0.5×10^{3d}
D -AB40-Y	9.7^{a}	5.55^{a}	4.0×10^{3d}	2.5×10^{3d}	0.5×10^{3d}
D -AB50-Y	10.0^{a}	5.93^{a}	3.0×10^{3e}	2.0×10^{3e}	0.5×10^{3d}

Mean values with the same superscript letters in a column are not significantly different at P>0.05.
Keys: Untreated= untreated dry-yam, 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'.

Table 3. Moisture content, pH and Microbial load of 'gbodo' treated with fresh 'agehu' leaves

Sample	Moisture content %	pH	Total plate count (cfu/g)	Fungi count (cfu/g)	Staphylococci Count (cfu/g)
Untreated	10.2^{a}	5.73^{a}	3.0×10^{6a}	1.0×10^{6a}	1.8×10^{6a}
F-AG10-Y	11.1^{a}	5.80^{a}	5.0×10^{3b}	1.0×10^{3b}	1.5×10^{3b}
F-AG20-Y	9.8^{a}	6.11^{a}	1.0×10^{3c}	1.0×10^{3b}	1.0×10^{3c}
F-AG30-Y	10.2^{a}	6.18^{a}	1.0×10^{3c}	0.5×10^{3c}	0.5×10^{3d}
F-AG40-Y	10.7^{a}	5.99^{a}	1.0×10^{3c}	0.5×10^{3c}	$>10^{e}$
F-AG50-Y	11.2^{a}	5.81^{a}	0.5×10^{3d}	0.5×10^{3c}	$>10^{e}$

Mean values followed by the same letters in the superscript in a column are not significantly different at P > 0.05.
Keys: Untreated = untreated dry-yam, 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'.

2). There were significant differences (P<0.05) in the staphylococcal count of the D-AB10 and D-AB20-Y samples (1.0 $x10^3$ and 3.0 $x10^3$ cfu/g, respectively). There were no difference in the count as the leave treatment increased for D-AB30-Y, D-AB40-Y and D-AB50-Y, respectively (0.5 x 10^3 cfu/g) (Table 2). Comparing the results in Tables 1 and 2, samples treated with fresh 'abafe' leaves had a greater anti-microbial effect on 'gbodo' than the samples treated with dried 'abafe' leaves. It could be that the antimicrobial substances are more active in fresh 'abafe' leaves.

The total plate count of fresh 'agehu'-treated (F-AG-Y) samples were significantly different (P<0.05) from each other, except for the F-AG20-Y, F-AG30-Y and F-AG40-Y samples, which maintained the same value (1.0 x 10^3 cfu/g) (Table 3). The fungal count of samples F-AG10-Y and F-AG 20-Y were the same (1 x 10^3cfu/g), while those of F-AG30-Y to F-AG50-Y were also the same (0.5 x 10^3 cfu/g). There were significant differences (P<0.05) in the staphylococcal count of the F-AG-Y samples, except for that of the F-AG40-Y and F-AG50-Y samples, which were less than 10 cfu/g, respectively (Table 3). The reduction of fungal count caused by fresh 'agehu' agrees with the findings of Adekunle, (2003) and Samir et al. (2005) that 'agehu' leaves had a fungicidal effect. Fresh 'agehu'-treated samples were also capable of reducing the staphylococcal count. There is an indication that the rate at which the microbial load reduces becomes constant as the fresh 'agehu' treatment increases. Based on the results, perhaps that there is no need to go above 30 g of fresh 'agehu' leaves, especially when one has to take into consideration the potential effect of 'agehu' on the quality of the food.

In Table 4, the total plate count of samples treated with dried 'agehu' leaves, D-AG10-Y and D-AG20-Y, were not significantly different (P>0.05) from each other (1.6 $x10^4$ cfu/g and 1.5 $x10^4$ cfu/g, respectively). Likewise, the total plate count of D-AG40-Y and D-AG50-Y were the same (1.0 x 10^4 cfu/g). The fungal count of D-AG-Y samples ranged from 1.0 to 2.0 x 10^3 cfu/g, with the D-AG20-Y and D-AG30-Y samples having the same count (1.5 x 10^3 cfu/g), while D-AG40-Y and D-AG50-Y had the same count (1.0 $x10^3$ cfu/g) (Table 4). The staphylococcal count of D-AG-Y samples were significantly different (P<0.05) from each other, except for the D-AG30-Y and D-AG40-Y samples, which had the same count (4.5 x 10^3 cfu/g). There is an indication that the rate at which the total plate count and the fungal count reduces is constant for samples D-AG40-Y and D-AG50-Y. The results in Tables 3 and 4 showed that fresh 'agehu' leaves had a higher anti-microbial effect on treated samples than that of dried 'agehu' leaves.

The total plate count of samples treated with combined fresh 'abafe' and 'agehu' leaves (CF-Y) were significantly

Table 4. Moisture content, pH and Microbial load of 'gbodo' treated with dried 'agehu' leaves.

Sample	Moisture content %	pH	Total plate Count(cfu/g)	Fungi count (cfu/g)	Staphylococci Count (cfu/g)
Untreated	10.2^{a}	5.73^{b}	3.0×10^{6a}	1.0×10^{6a}	1.8×10^{6a}
D-AG10-Y	11.3^{a}	6.56^{a}	1.6×10^{4b}	2.0×10^{3b}	7.0×10^{3b}
D-AG20-Y	9.6^{a}	6.68^{a}	1.5×10^{4b}	1.5×10^{3c}	5.0×10^{3c}
D-AG30-Y	10.4^{a}	6.39^{ab}	1.3×10^{4bc}	1.5×10^{3c}	4.5×10^{3d}
D-AG40-Y	10.3^{a}	6.62^{a}	1.0×10^{4c}	1.0×10^{3d}	4.5×10^{3d}
D-AG50-Y	9.8^{a}	6.18^{ab}	1.0×10^{4c}	1.0×10^{3d}	1.0×10^{3e}

Mean values followed by the same letters in the superscript in a column are not significantly different at P > 0.05.
Keys: Untreated = untreated dry-yam, 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'.

Table 5. Moisture content, pH and Microbial load of 'gbodo' treated with fresh 'abafe' and 'agehu' leaves.

Sample	Moisture content %	pH	Total plate count (cfu/g)	Fungi count (cfu/g)	Staphylococci count (cfu/g)
Untreated	10.2^{a}	5.73^{a}	3.0×10^{6a}	1.0×10^{6a}	1.8×10^{6a}
CF10-Y	10.7^{a}	5.55^{a}	1.5×10^{4b}	2.5×10^{3b}	3.0×10^{2b}
CF20-Y	10.5^{a}	5.74^{a}	6.2×10^{3c}	2.0×10^{3c}	2.8×10^{2bc}
CF30-Y	11.1^{a}	5.51^{a}	3.8×10^{3d}	2.1×10^{2d}	2.4×10^{2c}
CF40-Y	10.2^{a}	5.77^{a}	8.3×10^{2e}	1.0×10^{2e}	6.0×10^{d}
CF50-Y	9.6^{a}	5.68^{a}	5.1×10^{2f}	0.9×10^{2e}	$>10^{e}$

Mean value followed by the same letters in a column are not significantly different at (P>0.05)
Key: Untreated=untreated dry-yam, 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.

different (P<0.05) from each other (5.1×10^{2} to 1.5×10^{4} cfu/g) (Table 5). The fungal count (0.9×10^{2} to 2.5×10^{3} cfu/g) and the staphylococcal count (>10 to 3.0×10^{2} cfu/g) of CF-Y samples were significantly different (P<0.05) from each other, except the fungal count of the CF40-Y and CF50-Y samples, which were 1.0×10^{2} cfu/g and 0.9×10^{2} cfu/g, respectively.

In Table 6, there were significant differences (P<0.05) in the total plate count (2.4×10^{2} to 1.1×10^{4} cfu/g), fungal count (2.7×10^{3} to 4.5×10^{3} cfu/g) and staphylococcal count (2.6×10^{3} to 4.8×10^{3} cfu/g) of all the samples treated with combined dried 'abafe' and 'agehu' leaves (CD-Y samples). Comparing the results of Tables 5 and 6, the microbial loads of CF-Y samples were lower than those of CD-Y samples; thus indicating that the fresh combined leaves had higher anti-microbial effect than the dried leaves.

The most prominent microorganisms isolated from each 'gbodo' sample, especially from the untreated sample, were *S. aureus, B. subtilis* and *A. flavus.*

Microbial inhibitory effect of leaves' extracts in inoculated agar medium

In Table 7, all the leave extracts exhibited some degree of growth inhibition, as indicated by the expansion of the clear zone where the microorganisms failed to grow. The size of the clear zone beyond the cylinder diameter (5 mm) indicated the degree of microbial inhibition. There were significant differences (P<0.05) in the growth inhibition of all the organisms by the leave extracts and, as the concentration of the leave extract increased, the degree of inhibition increased for each leave extract and for the combined forms of the leave extracts. An 'abafe' leave extract concentration of 0.143 g/ml gave the highest inhibition for *S. aureus*, while an 'agehu' leave. extract concentration of 0.143 g/ml gave the highest inhibition for *A. flavus*. It was also observed that there was no significant difference (P>0.05) in the inhibitory zones of 'agehu' leave extracts at concentrations of 0.114 and 0.143 g/ml, respectively, on the *S. aureus*, and the inhibitory effect was lower compared to the 'abafe' leave extract. Of all the leave extracts, the combined form of the extracts ('abafe':'agehu') had the highest inhibitory effect (18.1 to 18.7 mm) against the three test organisms. The relatively high R value (Table 7) suggests that the concentration of leave extracts had a critical influence on the overall microbial inhibition of the leave extracts, whether used singly or in combined form. The results in Table 7 are in accordance with the findings of Ibewuike et

Table 6. Moisture content, pH and Microbial load of 'gbodo' treated with dried 'abafe' and 'agehu' leaves

Sample	Moisture content %	pH	Total plate count (cfu/g)	Fungi count (cfu/g)	Staphylococci count (cfu/g)
Untreated	10.2[a]	5.73[a]	3.0×10^{6a}	1.0×10^{6a}	1.8×10^{6a}
CD10-Y	10.6[a]	5.44[a]	1.1×10^{4b}	4.5×10^{3b}	4.8×10^{3b}
CD20-Y	9.8[a]	5.59[a]	1.5×10^{3c}	4.0×10^{3c}	4.4×10^{3bc}
CD30-Y	10.0[a]	5.51[a]	1.3×10^{3cd}	3.5×10^{3d}	3.8×10^{3c}
CD40-Y	11.3[a]	5.77[a]	2.4×10^{2e}	3.8×10^{3cd}	3.4×10^{3cd}
CD50-Y	10.1[a]	5.68[a]	1.0×10^{3d}	2.7×10^{3e}	2.6×10^{3d}

Mean value followed by the same letters in a column are not significantly different at (P>0.05)
Key: Untreated=untreated dry-yam, 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.

Table 7. Antimicrobial activity of 'abafe' and 'agehu' leave extracts at various concentration in inoculated agar medium.

Local preservatives	Leave extract Con. (g/ml)	Diameter of inhibition zone (mm)[a]			
		Staphylococcus aureus	***Bacillus subtilis***	***Aspergillus flavus***	**mean**
Control	**0**	**5.0**	**5.0**	**5.0**	**5.0**
'Abafe'	0.029	7.9e	7.2e	7.1e	7.4e
	0.057	10.4d	9.6d	8.8d	9.6d
	0.086	13.2c	12.2c	10.9c	12.1c
	0.114	16.7b	15.4b	12.7b	14.9d
	0.143	18.2a	17.8ª	15.3ª	17.1ª
'Agehu'	0.029	7.2d	7.5e	8.7e	7.8e
	0.057	9.3c	8.9d	10.3d	9.5d
	0.086	11.5b	10.8c	13.3c	11.9c
	0.114	12.4a	13.4b	15.7b	13.8b
	0.143	13.1a	15.2a	17.9a	15.2a
'Abafe' :'Agehu'	0.029 : 0.029	8.1e	8.6e	8.5e	8.4e
	0.057 : 0.057	10.7d	11.2d	12.3d	11.4d
	0.086 : 0.086	13.2c	14.3c	14.8c	14.1c
	0.114 : 0.114	15.8b	16.5b	16.9b	16.4b
	0.143 : 0.143	18.5a	18.1a	18.7a	18.4a
k_1 (0.029)[b]	7.87				
k_2 (0.057)	10.17				
k_3 (0.086)	12.70				
k_4 (0.114)	15.03				
k_5 (0.143)	16.90				
R value[c]	9.03				

[a]Within a column, for the same leave extract, means with different letters are significantly different (P<0.05).
[b]The k_i value for each leave extract was calculated as the average of the three mean inhibition zones corresponding to each extract concentration: 0.029, 0.057, 0.086, 0.114 or 0.143 g/ml. e.g: average of 7.4, 7.8 and 8.4 is 7.87.
[c]The R value for each leave extract represents the difference between the minimum and the maximum k_i values

al. (1997), that is, the extracts of *P. thonningii* were found to inhibit prostaglandin synthesis *in vitro* and have anti-bacterial activities against *S. aureus*. *K. ivorensis* was reported to have antifungi activity, especially against *A. flavus, A. fumigatus, A. niger*, *Candida albicans*, *Micro-sporiun andonii, Trichoderms viride* and *Trichophyton metaprophytes* (Adekunle et al., 2003). However, results obtained in this study indicated that the fresh 'agehu' leaves had both bactericidal and fungicidal effects on 'gbodo' (Tables 3 and 4).

Conclusion

The moisture contents and pH of the treated and untreated samples were not significantly different from each other and would therefore not be expected to have had a significant effect on the microbial load. As the level of inclusion of leaves increased, the preservative effect of the leaves on dry-yam 'gbodo' increased. Sample CF50-Y (containing 50 g each of the respective leaves) had the lowest total plate count (5.1 x 10^2 cfu/g), fungal count (0.9 x 10^2 cfu/g) and staphylococcal count (>10 cfu/g) when compared with all the other treated samples. The most prominent microorganisms isolated from each dry-yam sample, especially from the untreated sample, were *S. aureus, B. subtilis* and *A. flavus*. All the leave extracts suppressed the growth of these isolated organisms, with the combined form of the leave extracts, at a concentration of 0.143 g/ml each, exhibiting the strongest inhibittory effect on the microorganisms. This investigation should be extended to study the safety level of inclusion of these local preservatives ('abafe' and 'agehu' leaves) in dry-yam 'gbodo'.

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