

Full Length Research Paper

Nutritional compositions, fungi and aflatoxins detection in stored 'gbodo' (fermented *Dioscorea rotundata*) and 'elubo ogede' (fermented *Musa parasidiaca*) from South western Nigeria

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Inadequate storage facilities in Nigeria have led to yearly wastage of harvested agricultural food products thereby causing great economic loss to our farmers. The dried yam and plantain chips in storage usually lose their integrities and nutrients due to contamination from biodeteriorating and aflatoxigenic fungi. Aflatoxin detection, food values and mineral element compositions of locally made 'gbodo' (prepared from fermented yam, *Dioscorea rotundata*) and 'elubo ogede' (prepared from fermented plantain, *Musa parasidiaca*) stored for one and six months respectively were carried out using standard methods. Six different samples of each stored chips were collected and used for these investigations. Each set was carried out in triplicates using completely randomized design. A total number of 15 fungal species were isolated from stored 'gbodo' (GB) and 'elubo ogede' (EO) flour samples. These organisms include *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus japonicum*, *Aspergillus parasiticus*, *Aspergillus ochraceus*, *Aspergillus tamari*, *Aspergillus terreus*, *Fusarium sp*, *Mucor racemosus*, *Paccilomyces varioti*, *Penicillium sp.*, *Penicillium notatum*, *Rhizopus stolonifer*, *Rhizopus sp.* The presence of mycotoxins (aflatoxin B₁, B₂, G₁ and G) were detected in all the samples. Aflatoxin B₁ has the highest concentration: 32.33 µg/kg in 6 months old 'gbodo' (6MOG) and 25.17 µg/kg in (1MOG). Its concentration was however lower in 1 and 6 month old 'elubo ogede' (1MOEO and 6MOEO) in which 23.83 µg/kg and 15.0 µg/kg were detected respectively. The results showed that, with the exception of 1MOEO, with AFB₁ 15.0 µg/kg, the levels of aflatoxin contamination in all the stored chips exceeded the maximum AFB₁ residue limit permitted in most countries. Starch content of the samples was found to be higher in 1 MOG and EO with 73.06 and 75.89% respectively but, the sugar content was generally low. Moisture contents of stored GB and EO also varied from 10.51 to 14.36%. The flours were found to contain moderate amount of protein (7.73 to 9.19%). The ascorbic acid contents of 1MOG and 6MOG were 4.44 and 5.25 mg/100g while that of 1MOEO and 6MOEO were 4.85 and 6.46 mg/100g respectively. It was observed that both GB and EO contained adequate amount of mineral elements such as K, Mg, P, Ca and Na. These fermented flour also has trace amount of Mn, Fe, Cu and Zn. The results showed that 'gbodo' and 'elubo ogede' stored for over 6 months (without any preservatives) may be medically unsafe for consumption because of the contamination from aflatoxigenic and biodeteriorating fungi. All these observations were discussed in relation to the food safety of the fresh and stored traditional food investigated.

Key words: Traditional food, mycotoxins, white yam, plantain, food safety, storage chips.

INTRODUCTION

White yam (*Dioscorea rotundata*) and plantain (*Musa parasidiaca*) are undoubtedly major staple food for most parts of Africa. They are good sources of carbohydrates;

protein and dietary fibres (Jonathan and Olowolafe, 2001; Akissoe et al., 2003). They are important staple foods that contribute to the calories and sustenance of

economies in Africa. Because of lack of adequate storage facilities, yam tubers and plantain fruit are prone to gradual microbial and physiological deterioration, after short period of harvesting. This has therefore prompted the people in West Africa most especially Nigerian to devise a way of processing these food commodities to less perishable products such as fermented dried yam chips ('gbodo') and plantain chips 'elubo ogede' (Olorunda and Adelusola, 1997; Okigbo and Nwankamma, 2005). The yam or unripe plantain are partially peeled, sliced into pieces and parboiled. They are left inside water (used for parboiling) to undergo natural fermentation for 4 to 5 days and sun dried into chips known locally as 'gbodo and elubo ogede'. These could be milled and processed into flour (locally known as 'elubo'). It can now be reconstituted with hot water to form paste or dough known as 'amala' and 'kokonte' among the Yoruba and Ashante people of Nigeria and Ghana respectively.

The reconstituted flour is a popular food among the Yorubas, Benins, Itsekiris and Hausa people of Nigeria (Akissoe et al., 2003, Abulude and Ojediran, 2006). Traditional food ('elubo') have been reported to be made up of 12.3% moisture and 80.6% carbohydrate, which make the food a good source of energy. Protein, fat and ash content have been reported to be 3.2, 0.3 and 2.0%, respectively (Okigbo and Nwankamma, 2005). On the other hand, the nutritional composition of plantain pulp, and in turn, flour, is diversely affected by natural ripening and processing (Adeniji and Tenkouano, 2008). Reports have shown that the quantity of total sugars in green and ripe plantain considerably increases during ripening from 1.3 to 17.3% in the pulp while starch concentration decrease from 83 to 66% (Akissoe et al., 2003).

Yam flour could be fortified with other flours such as plantain, soya and wheat, in order to improve its nutritional value (Abulude and Ojediran, 2006). Apart from making it as 'amala', plantain can also be used as an important raw material in feeding stuff formulation. Processing of fresh fruit into flour has been reported to have a number of advantages, including preservation, price stability, wider availability, and stimulation of agricultural production through market expansion (Akissoe et al., 2003). As important as these processed flour are to the natives, the problem of food contamination with aflatoxigenic fungi has received great deal of attention. The frequent incidence of these toxins in agricultural commodities has a potential negative impact. This is because harvest and post harvest techniques adequate for the prevention of mould growth are seldom practiced coupled with inadequate storage facilities (Jonathan and Olowolafe, 2001; Adebayo-Tayo et al., 2006). In spite of this, GB and EO and their flour may be contaminated with moulds and particularly toxigenic species such as

Aspergillus spp, *Mucor* sp, *Rhizopus* sp, and *Penicillium* sp. (Adisa, 1985; Adeyanju and Ikotun, 1988). These fungi may reduce nutrient contents of the food and produce mycotoxins that could cause serious health hazard to humans (Peraica et al., 1999; Adebayo-Tayo et al, 2006).

Intake of these aflatoxin-contaminated foods above the level considered to be safe may be harmful to human beings and other animals. Aflatoxins have been shown to be potent carcinogens, mutagens and teratogens (Peraica et al., 1999).

Among 18 different types of aflatoxins that have been identified, major ones are aflatoxin B₁, B₂, G₁ and G₂. Aflatoxin B₁ (AFB₁) exists predominantly in food products. Occurrence of aflatoxin B₁ has been identified in some food commodities such as dry yam chips, cassava flour, garri, maize flour etc, sold in African markets, with its concentration sometimes above the tolerance level (Okigbo and Nwankamma, 2005).

In this present study, attempts were made to determine food values, fungi associated with biodeterioration and aflatoxin contents of 'gbodo' and 'elubo ogede' that had been stored for one and six months.

MATERIALS AND METHODS

Sample collection and preparation for analysis

One and six months old 'gbodo' (fermented dried white yam; *D. rotundata* Poir) and 'elubo ogede' (fermented dried plantain *M. parasidiaca*) were purchased directly from farmers from Itamerin market Ibadan, Oyo State, Nigeria. Six different samples of each of these materials were purchased from different locations within the same market. They were taken to the Laboratory in polypropylene bags inside a closed cupboard before analyses. The chips were separately pounded with mortar and pestle and subsequently sieved through a 2 mm wire mesh. The samples were placed in dry containers at ambient temperature (30±2°C) and used for all the various experiments. The experiment for each set of the samples was carried out in triplicates in a completely randomized design.

Isolation of fungi, aflatoxin determination and other chemical analysis

Analyses carried out on the flours include fungi isolation, aflatoxin detection, proximate composition, determination of ascorbic acid and mineral element contents. All the experiments were carried out in triplicate, and the mean values taken. Fungi isolation was carried out by the method described by Jonathan and Olowolafe (2001). 1 g of each sample was suspended separately in 10 ml of sterile distilled water, and then further diluted to obtain a six-fold decimal dilution to 10⁶. 0.1 ml of each suspension was placed in 100.0 mm Petri dish containing sterile potato dextrose agar (PDA) in which 0.05 mg of streptomycin sulphate has been added to suppress bacterial growth. 0.1 ml of the same suspension was seeded into another Petri dish and overlaid with PDA. These were then incubated at 30±2°C for 7 days. The fungi that developed were purified by repeated sub-cultures. Identification of the isolated fungi were carried out using the descriptions of Alexopoulos et al. (1996).

Determination of Aflatoxin B₁, B₂, G₁ and G₂ levels in the samples were carried out using thin-layer chromatography (TLC) method of Anon (1976). Quantification of the levels of aflatoxin was done by

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Table 1. Occurrence of fungi from 'gbodo' and 'elubo ogede' flour samples.

| Fungi | Sources of the isolates |
|--------------------------------|-------------------------|
| <i>Aspergillus flavus</i> | 1MOEO,1MOG,6MOEO,6MOG |
| <i>Aspergillus fumigatus</i> | 6MOG,6MOEO |
| <i>Aspergillus niger</i> | 1MOEO,1MOG,6MOEO,6MOG |
| <i>Aspergillus japonicum</i> | 1MOG,6MOEO,6MOG |
| <i>Aspergillus parasiticus</i> | 1MOG,6MOEO,6MOG |
| <i>Aspergillus ochraceus</i> | 1MOG,6MOG |
| <i>Aspergillus tamari</i> | 6MOEO,1MOEO |
| <i>Aspergillus terreus</i> | 6MOG |
| <i>Fusarium</i> sp | 1MOEO,1MOG |
| <i>Mucor racemosus</i> | 1MOEO,1MOG |
| <i>Paccilomyces varioti</i> | 1MOEO,,6MOEO,6MOG |
| <i>Penicillium</i> sp. | 1MOEO,6MOG,6MOEO |
| <i>Penicillium notatum</i> | 1MOE |
| <i>Rhizopus stolonifer</i> | 1MOEO,1MOG,6MOEO |
| <i>Rhizopus</i> sp. | 1MOEO,1MOG,6MOEO,6MOG |

1MOG: One month old 'gbodo'; 6MOG: Six months old 'gbodo'; 1MOEO: One month old 'elubo ogede'; 6MOEO: Six months old 'elubo ogede'.

Table 2. Aflatoxin levels ($\mu\text{g}/\text{kg}$) in 'gbodo' and 'elubo ogede' flour samples.

| SAMPLE | AFB ₁ | AFB ₂ | AFG ₁ | AFG ₂ | Total AFs |
|--------|--------------------|--------------------|--------------------|--------------------|---------------------|
| 6MOG | 32.33 ^a | 26.17 ^a | 19.17 ^a | 18.67 ^a | 96.34 ^a |
| 1MOG | 25.17 ^b | 23.00 ^b | 15.67 ^b | 14.00 ^b | 77.84 ^b |
| 6MOEO | 23.83 ^b | 18.67 ^c | 13.17 ^c | 9.50 ^c | 65.17 ^{bc} |
| 1MOEO | 15.00 ^c | 11.00 ^d | 6.50 ^d | 5.17 ^d | 37.67 ^d |

Values are means of three experiments ($n = 3$). Values followed by the same superscript along the same column are not significantly different using Duncan's multiple range test ($P < 0.05$). (1MOG: One month old 'gbodo'; 6MOG: Six months old 'gbodo'; 1MOEO: One month old 'elubo ogede'; 6MOEO: Six months old 'elubo ogede') AFB₁: Aflatoxin B₁; AFB₂: Aflatoxin B₂; AFG₁: Aflatoxin G₁; AFG₂: Aflatoxin G₂.

comparing the extract in fluorescent spots with standard aflatoxin spots under long wave UV light (365 nm) (Singh et al., 1991). Proximate composition of the samples was carried out by methods described by AOAC (1990). Protein was determined by the micro-Kjeldahl method and was obtained by multiplying kjeldahl nitrogen by the factor 6.25. Moisture content was obtained by heating 10.0 g portions of each of the samples to a constant weight in a hot air-circulating Gallenkamp oven at 105°C for 24 h (Onyeike et al., 2008). Ash was determined by incineration in a pre-heated muffle furnace at 600°C for 2 h, fat by soxhlet extraction, Starch and total sugar contents were determined using a colorimetric method by Dubois et al. (1956). Total dietary fibre content was determined by the enzymatic gravimetric method described by Prosky et al., (1985).

Mineral analysis was performed according to the method described by AOAC (1990). Samples for analyses were prepared by weighing 1 g sample into a Pyrex glass conical flask and then introducing 10 ml of concentrated nitric acid into the flask by means of a straight pipette. 5 ml of perchloric acid was also added and the mixture was heated on an electro thermal heater for about 20 min

until a clear digest was obtained.

The digest was allowed to cool at room temperature and then diluted to 50 ml with distilled water and afterward filtered into a plastic vial for AAS analysis. Mineral analysis was carried out on the extracts using atomic absorption spectrophotometer (Bulk Scientific model 210 VGP). Vitamin C was determined by indophenols titration method (AOAC, 1990).

ANALYSIS OF DATA

The data generated from these investigations were subjected to analysis of variance (ANOVA). The test of significance were carried out using Duncan's multiple range tests (DMRT).

RESULTS AND DISCUSSION

A total number of 15 fungal species were isolated from stored 'gbodo' and 'elubo ogede' flour samples. The sources of the isolates according to the substrates and their period of storage were represented on Table 1. The isolated fungi were *A. flavus*, *A. fumigatus*, *A. niger*, *A. japonicum*, *A. parasiticus*, *A. ochraceus*, *A. tamari*, *A. terreus*, *Fusarium* sp, *M. racemosus*, *P. varioti*, *Penicillium* sp, *P. notatum*, *R. stolonifer*, *Rhizopus* sp (Table 1). *A. flavus*, *A. niger* and *Rhizopus* sp were isolated from all the flour samples while *A. japonicum* and *A. parasiticus* were isolated from 1MOG, 6MOEO and 6MOG respectively. *Fusarium* sp and *M. racemosus* were isolated from 1MOEO and 1MOG respectively. It was also observed that *P. notatum* was isolated from one month old 'elubo ogede' only while *A. terreus* was isolated from only 6 months old 'gbodo'.

The biodeteriorating and aflatoxigenic fungal species that colonized 'gbodo' (GB) and 'elubo ogede' (EO) flours must have been present in the atmosphere in form of spores after fermentation, during the sun drying and storage of the yam and plantation chips. The fungi could have been introduced during exposure and direct contact of these agricultural products in the market. (Ekundayo, 1986; Aboaba and Amisike, 1991; Okigbo, 2003). *Aspergillus* species are the common fungi isolated in this study. The predominance of *Aspergillus* spp in these stored food products may be the factors responsible for the high level of aflatoxin detected in them.

The results of the analysis of GB and EO flour samples for mycotoxins shows the presence of aflatoxin B₁ (AFB₁), aflatoxin B₂ (AFB₂), aflatoxin G₁ (AFG₁), and aflatoxin G₂ (AFG₂) in all the four flour samples. The concentrations of each aflatoxin and total aflatoxin detected in all the 4 AFB₁ samples were shown on Table 2. High concentration of AFB₁ was detected in all the samples. The highest level of AFB₁ (32.33 $\mu\text{g}/\text{kg}$), representing 33.6% of the total aflatoxin was detected in 6MOG. This was followed with a statistically different value of 25.17 $\mu\text{g}/\text{kg}$, ($P < 0.05$), representing 32.3% of the total aflatoxin detected in 1MOG. Likewise, 23.83 $\mu\text{g}/\text{kg}$ of AFB₁ was found in 6MOEO. This represents 36.6% of the total aflatoxin detected in 6MOEO.

Table 3. Food values of 'gbodo' and 'elubo ogede' flour samples.

| Parameters | 6MOG | 1MOG | 6MOEO | 1MOEO |
|-------------------------|--------------------|--------------------|--------------------|--------------------|
| Moisture content (%) | 14.36 ^a | 13.29 ^b | 12.21 ^c | 10.51 ^d |
| Crude protein (%) | 7.73 ^c | 7.30 ^c | 9.19 ^{ab} | 8.90 ^a |
| Crude fibre (%) | 1.45 ^c | 1.30 ^d | 1.98 ^a | 1.54 ^b |
| Ash (%) | 0.94 ^c | 0.63 ^d | 2.33 ^a | 1.41 ^b |
| Starch (%) | 69.75 ^c | 73.06 ^b | 71.95 ^b | 75.89 ^a |
| Sugar (%) | 5.74 ^a | 4.43 ^b | 2.36 ^c | 1.74 ^c |
| Ascorbic acid (mg/100g) | 5.25 ^b | 4.44 ^b | 6.46 ^a | 4.85 ^b |

Values followed by the same superscript in the same column are not significantly different using Duncan's multiple range test ($P < 0.05$). (1MOG: One month old 'gbodo'; 6MOG: Six months old 'gbodo'; 1MOEO: One month old 'elubo ogede'; 6MOEO: Six months old 'elubo ogede').

Also, concentration of 15.00 $\mu\text{g}/\text{kg}$ of AFB_1 was detected in 1MOEO. This value represents 39.8% of the total aflatoxin found in 1MOEO. For aflatoxin B_2 (AFB_2), fairly high concentrations of 26.17, 23.00, 18.67 and 11.00 $\mu\text{g}/\text{kg}$ respectively were detected for 6MOG, 1MOG, 6MOEO and 1 MOEO. Total aflatoxin ($\text{AFB}_1 + \text{AFB}_2 + \text{AFG}_1 + \text{AFG}_2$) detected in all our samples exceeded 20 ppb. The total highest concentration of aflatoxins (96.34 $\mu\text{g}/\text{kg}$) (AFB_1 , AFB_2 , AFG_1 and AFG_2) was detected in 6MOG. This are followed in order by 1MOG, 6MOEO and 1MOEO with 77.84, 65.17 and 37.67 $\mu\text{g}/\text{kg}$ respectively ($P < 0.05$). These results showed that, with the exception of 1MOEO (with 15 AFB_1), the levels of aflatoxin contamination in all the samples tested exceeded the maximum AFB_1 residue limit of 20 $\mu\text{g}/\text{kg}$ permitted in Nigerian foods (Bankole et al., 2004). The maximum aflatoxin B_1 concentrations allowed for human consumption ranged from 5 to 50 ppb. This level varies from country to country. Most countries limit aflatoxin in food to 20 $\mu\text{g}/\text{kg}$ (Bankole et al, 2004). The high number of fungi and adequate moisture which is present for their growth may be the major factor for the high level of aflatoxin detected in the flour samples. This result is similar to the observation of Adebayo-Tayo et al. (2006) on bush mango.

Results of proximate compositions of 'gbodo' and 'elubo ogede' (Table 3) showed variations in food values of 1 and 6 months flour samples. The moisture content of 14.4, 13.3, 12.21 and 10.51% were recorded for 6MOG, 1MOG, 6MOEO and 1 MOEO respectively. The moisture contents of 6 months 'gbodo' and 'elubo ogede' were surprisingly observed to be higher than that of 1 month flour samples. This may be due to high relative humidity obtained during the storage period. The same reason may be adduced for slight variations (7.7 to 12.30%) reported as the moisture content of unfortified yam flour (Akingbala et al., 1995; Jimoh and Olatidoye, 2009) and 1.26 to 2.5% reported for plantain (Agbagba) flour (Adeniji and Tenkouano, 2008). This finding indicates the

tendency of the chips to grow mould because higher moisture content encourages the growth of micro-organisms (Abulude and Ojediran, 2006). Prolonged storage therefore should be discouraged because it could lead to the infestation of the chips by aflatoxigenic fungi.

The percentage (%) compositions of starch were higher in 'elubo ogede' than 'gbodo'. The values of 75.69 and 71.95% were recorded for 1MOEO and 6MOEO respectively. In 'gbodo' samples 73.06 and 69.75% were observed for 1MOG and 6MOG respectively. The starch contents were observed to be reducing with storage period. The depletion may be linked to the usage of this important food by the contaminating fungi, (Akingbala et al., 2005). As shown in Table 3, significant variations were also observed in all other foods ($P < 0.05$). These include crude protein, ash and crude fibre. Crude fat was however not detected in our samples. High crude protein composition of 9.19 and 8.90% were detected in 6MOEO and 1MOEO respectively while 7.73 and 7.30% 6MOG and 1MOG respectively. The highest sugar concentration (5.74%) was found in 6MOG, this was followed in order by 1MOG, 6MOEO and 1MOEO. These chips have sugar values of 4.43, 2.36 and 1.74% respectively. Six months old 'gbodo' (6MOG) has the greatest content of ascorbic acid (6.46 mg/100g). This was followed by 6 MOEO with the value of 5.25 mg/100g. The lowest concentration of ascorbic acid (4.44 mg/100g) was found in 1MOG. The sugar level of this stored chips are considerably low. This may be due to the fact that the colonizing fungi would have utilized sugars in these stored products ('gbodo' and 'elubo ogede') as their energy source. (Jonathan et al., 2009).

Results of the ascorbic acid determination indicated that GB and EO have an appreciable amount of this vitamin. Therefore, eating fermented yam and plantain flour (that have not been contaminated with bio-deteriorating fungi) could be a good source of vitamin C. Results of mineral element determination showed the presence of nine mineral elements in various concentrations. These minerals were potassium, calcium, magnesium, phosphorus, zinc, iron, copper, manganese and sodium are summarized in Table 4. In all the 4 samples, potassium, phosphorus, calcium and magnesium showed high levels of concentration. Their concentrations were however observed to be higher in the two plantain flour samples than those of the yam flour samples. The concentration of potassium, K, in plantain flour samples were 18.16 $\mu\text{g}/100\text{g}$ 6MOEO and 11.50 $\mu\text{g}/100\text{g}$ in 1MOEO, whereas concentrations of 8.26 and 6.45 $\mu\text{g}/100\text{g}$ were observed in 6MOG and 1MOG, respectively. The concentration of magnesium in EO is significantly higher in 1 month than 6 months with 9.49 to 13.18 $\mu\text{g}/100\text{g}$ respectively.

Comparison of the samples (based on their period of storage) showed that, with the exception of magnesium, sodium, and manganese in GB and EO, 6 months stored samples had higher concentrations of minerals than 1 month-stored samples. All the micronutrients (copper,

Table 4. Mineral element compositions of 'gbodo' and elubo ogede' ($\mu\text{g}/100\text{g}$) flour samples.

| Mineral elements | 6MOG | 1MOG | 6MOEO | 1MOEO |
|------------------|-------------------|-------------------|--------------------|--------------------|
| Calcium | 4.17 ^b | 3.23 ^c | 5.30 ^a | 4.36 ^b |
| Magnesium | 3.99 ^c | 2.45 ^d | 9.49 ^a | 13.18 ^b |
| Potassium | 8.26 ^c | 6.45 ^d | 18.16 ^a | 11.50 ^b |
| Sodium | 1.48 ^b | 1.98 ^a | 1.97 ^a | 0.87 ^c |
| Manganese | .027 ^c | .033 ^c | .13 ^a | .80 ^b |
| Iron | 1.03 ^b | 1.02 ^b | 2.45 ^a | .76 ^c |
| Copper | .007 ^a | .003 ^c | .006 ^b | .005 ^b |
| Zinc | .023 ^a | .008 ^c | .014 ^b | .007 ^c |
| Phosphorus | 5.55 ^b | 5.26 ^b | 7.59 ^a | 7.01 ^a |

Values are means of three (n = 3) separate experiments. Mean values followed by the same superscript along the same column are not significantly different using Duncan's multiple range test ($P < 0.05$). (1MOG: One month old 'gbodo'; 6MOG: Six months old 'gbodo'; 1MOEO: One month old 'elubo ogede'; 6MOEO: Six months old 'elubo ogede'.

manganese and zinc) have concentrations below 0.25 $\mu\text{g}/100\text{g}$ in all the four flour samples. The presence of important mineral elements inside 'gbodo' and 'elubo ogede' shows that they are nutritious food.

Conclusion

It was observed from these studies that the longer the storage time, the higher the concentration of aflatoxin produced. Proper storage methods therefore needed to be ensured in the preparation of 'gbodo' and 'elubo ogede' to avoid mould growth. Long storage period, before processing to flour, for consumption should also be discouraged. If these food products will be kept for a long time, the storage in humid environment must be discouraged. These materials must be placed in dry environment (with very low relative humidity) in order to limit the tendency of the growth of aflatoxigenic fungi which could produce mycotoxins after a long storage period.

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