

# Technical note: Microorganisms associated with fermented fluted pumpkin seeds (*Telferia occidentalis*)

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## Keywords

Bacteria, Nigerian condiment, 'ogiri', starter organisms, yeasts.

## Introduction

Fluted pumpkin seeds (*Telferia occidentalis*) are edible seeds, obtained from green vegetables grown in many gardens in Nigeria. The seeds are sometimes fermented to produce 'ogiri', which is used as a condiment to flavour soups, or can be consumed when mixed with other ingredients in salads.

Although the chemical composition of the fermented and unfermented seeds is known (Achinewhu, 1983, 1986; Longe, Farinu & Fetuga, 1983), the microorganisms associated with the fermentation are not: these microorganisms, and their effects when isolated as starters to ferment the seeds, are the subjects for this study.

## Materials and methods

Freshly harvested fluted pumpkin seeds, with intact seed coats, were purchased from the local market and boiled (2 hr). The seed coats were removed by hand pressure, and the cotyledons ground to a paste with salt (1 g kg<sup>-1</sup>); then the paste was wrapped (150 g per pack) in blanched plantain leaves, and fermented for 5 days at 29–32°C.

### *Microbial analysis*

Duplicate samples (1 g) were serially diluted in 0.1% peptone water (Oxoid) containing 1% Tween 80 (Atlas) for total viable count. Samples (0.1 ml) were also plated onto plate count agar (PCA) at 37°C for 24 h (Harrigan & McCance, 1976); malt extract agar at 29–30°C for 7 days; and reinforced Clostridial agar (RCA, Oxoid) at 30°C for 48 h in an anaerobic jar (BBL Gas Pak). Only plates showing between 30 and 300 colonies were counted.

### *Enrichment and isolation*

Samples (5 g) were incubated with tryptone soya broth (TSB, Oxoid 10 ml) at 37°C, 24 h; then loops were streaked on tryptone soya agar (TSA, Oxoid) and incubated (37°C, 24 h). Duplicate 1 g samples were inoculated into Rogosa medium (Harrigan & McCance, 1976), incubated aerobically and anaerobically (29–32°C, 24 h) and subcultured onto Rogosa agar (RA) (Harrigan & McCance, 1976).

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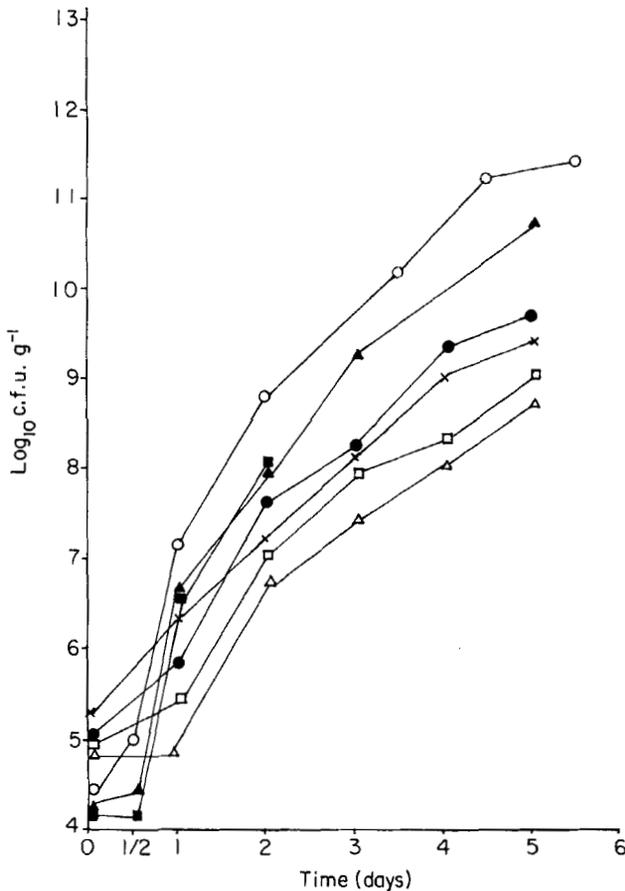
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### Characterization and identification of isolates

Representative colonies from all plates were selected morphologically and purified by repeated streaking, where necessary, on nutrient agar (NA) and TSA. When pure cultures were obtained, they were coded, transferred to NA slants, and stored in a refrigerator. The isolates were characterized according to Cowan & Steele (1965), Harrigan & McCance (1976) and Bergey (1974).

### Fermentation of fluted pumpkin seeds using pure isolates as starters

Pumpkin seed paste portions (50 g) were autoclaved (20 min, 121°C), inoculated with cell suspension (1 ml) as single starters, as equal pairs, or as all four isolates, and thoroughly mixed. They were then transferred to sterile aluminium foil packets and incubated (29–32°C, 5 days). Total viable counts, pH and total titratable acidity (TTA) of all samples were measured every 24 h (AOAC, 1984).

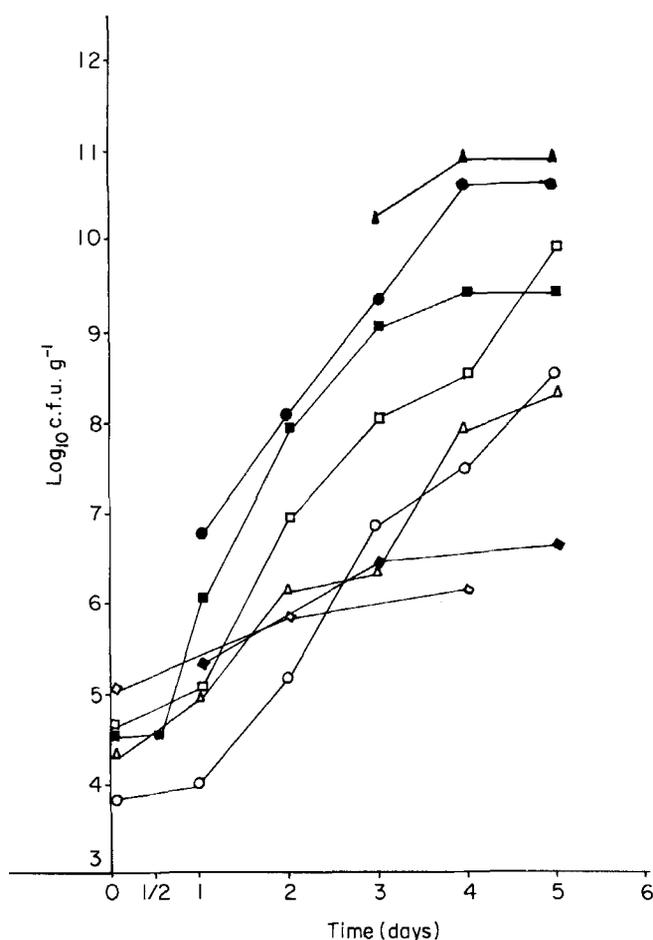


**Figure 1.** Changes in total viable counts of naturally fermented fluted pumpkin seeds and from pumpkin seed fermented by combination of isolates: ○ = Natural fermentation; × = Yeast+Bacillus sp.; △ = Bacillus+Pseudomonas sp.; □ = Yeast+Pseudomonas sp.; ● = Combination of Bacillus, Yeast, Pseudomonas and Streptococcus; ▲ = *S. aureus*; ■ = *E. coli*.

## Results and discussion

In the natural fermentation, counts of total and individual aerobic organisms, including yeasts, increased in the typical form from  $6.9 \times 10^4$  to  $1.7 \times 10^{11}$  c.f.u.  $g^{-1}$  after 5 days (Figs 1 and 2). Anaerobic counts increased from only  $2.4 \times 10^5$  after 48 h to  $2.8 \times 10^6$  c.f.u.  $g^{-1}$ , and were only streptococci (Table 1 and Fig. 2). The initial aerobic, and overall anaerobic counts are similar to those reported by Odunfa (1981a) for fermenting melon seeds; although final aerobic counts obtained in this study ( $10^{11}$ ) are much higher.

Yeasts occur in many fermented products, e.g. in 'puto', a fermented, steamed rice cake (Tongananta & Orillo, 1983), maize (Akinrele, 1970; Moss, Mpuchane & Murphy, 1984), castor seeds (Onunkwo, 1982) and melon seeds (Ogundana, 1980). Growth is probably facilitated by the acid pH, which decreased steadily from 5.6 to 4.5, as occurs



**Figure 2.** Changes in viable counts of organisms from natural and inoculated fermentation of fluted pumpkin seeds: ▲ = *Pseudomonas* sp. from natural fermentation; ● = Yeast sp. from natural fermentation; ■ = *Bacillus* sp. from natural fermentation; ◆ = *Streptococcus* sp. from natural fermentation; △ = *Pseudomonas* sp. from inoculated fermentation; ○ = Yeast sp. from inoculated fermentation; □ = *Bacillus* sp. from inoculated fermentation; ◇ = *Streptococcus* sp. from inoculated fermentation.

**Table 1.** Characterization of microorganisms isolated from fluted pumpkin seeds fermented in the laboratory

	Organism						
	A	B	C	D	E	F	G
Gram reaction	+	+	-	+	+	-	+
Shape (rods or spheres)	R	S	R	S	S	R	S
Spore	+	-	-	-	-	-	-
Motility	+	-	+	-	-	+	-
Catalase test	+	+	+	+	+	+	-
Oxidase test	-	-	-	-	-	+	-
Growth aerobically	+	+	+	+	+	+	+
Growth anaerobically	+	-	-	+	-	-	+
Glucose acid	+	+	+	-	+	+	+
O/F	O	F	O/F	O/F	O/F	O	F
Glucose	A	AG	AG	A	A	A	A
Xylose	A	-	-	-	-	-	-
Maltose	A	A	A	A	-	-	A
Arabinose	A	A	A	A		-	-
Mannitol (aerobic)	A	A	A	A		-	-
Mannitol (anaerobic)	-	A	-	-		-	-
Indole test	-	-	+	-	NA	NA	NA
Coagulase test	-	+	-	-	NA	-	-
Methyl red test	-	-	+	+	NA	-	NA
Voges-Proskauer test	+	-	-	-	NA	-	NA
NH <sub>3</sub> from arginine	NA	-	NA	-	NA	+	+
Citrate	+	-	-	-	NA	-	-
Starch hydrolysis	+	-	NA	NA	NA	NA	NA
Gelatin liquefaction	+	-	NA	NA	NA	+	NA
Lecithinase	-	-	NA	NA	NA	NA	NA
Probable identity	<i>Bacillus</i> spp.	<i>Staph.</i> <i>aureus</i>	<i>E. coli</i>	<i>Staph.</i> spp.	<i>Yeast</i>	<i>Pseudo-</i> <i>monas</i> spp.	<i>Strep.</i> spp.

+ = positive; - = negative; R = Rods; S = Spheres; O/F = Oxidation/fermentation; NA = Not applicable; A = Acid; AG = Acid and gas.

in starch-based materials such as cassava and maize (Akinrele, 1970), and is the optimum range for yeast. In contrast, Odunfa (1981a), Steinkraus *et al.* (1983) and Achinewhu (1983, 1986) reported pH increased in fermenting melon, soybeans and fluted pumpkin seeds respectively. Other organisms isolated from fermenting pumpkin seeds on PCA and TSA included *Bacillus* sp. *Staphylococcus albus*, *E. coli* and *Pseudomonas* sp. (Table 1). *Bacillus* sp. are associated with soy fermentation (natto) (Sakurai, 1960), oil bean seeds (Obeta, 1983) and locust bean seeds (Odunfa, 1981b; Campbell-Platt, 1984). The high counts of *Staphylococcus* sp. are probably the result of the traditional hand wrapping in leaves for fermentation, and is of public health concern; although this particular product is usually added to soups during cooking as a flavouring agent.

All the isolates inoculated into fluted pumpkin pastes increased in numbers during subsequent fermentation. The initial counts were slightly higher than those obtained

from samples fermented by the traditional method (Fig. 1), but the final counts were similar, except for *Pseudomonas* sp., which were much lower. Inoculation with combinations of isolates did not increase counts more than with single isolates (Fig. 2).

The failure to record higher counts after 5 days in samples inoculated with pure isolates, and to obtain fermentation might be caused by heat inactivation of growth factors on autoclaving, thus slowing initial growth, for at the end of the 5-day period, the organisms were still actively growing. However, since one aim was to shorten the fermentation period, tests were not extended.

The pH change with pure cultures was different from that in the natural fermentation; pH increased in all except those inoculated with yeast and streptococcus. None of these inocula gave the characteristic odour of fermented pumpkin seeds, and none, singly or in combination, fermented the seeds.

In conclusion, although six genera of microorganisms were isolated from fermented pumpkin seeds, none could ferment them; so other organisms might be involved. Further investigation is in progress.

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