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Microbial Studies on Laboratory Fermentation of *Iregi* – A Potential Fermented Food Condiment from *Delonix regia* (Boj. ex Hook.) Raf

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ABSTRACT

Bacterial strains isolated from fermenting boiled cotyledons of *Delonix regia* in the laboratory production of *iregi* were characterised as *Bacillus cereus* var. *mycoides* (21), *B. licheniformis* (37), *B. megaterium* (10), *B. pumilus* (24), *B. subtilis* (20), *Staphylococcus aureus* (8), *S. epidermidis* (2) and *S. saprophyticus* (4). The aerobic mesophilic bacterial flora of the simulated fermenting *iregi* mash were dominated by *Bacillus* species (1.6×10^4 cfu g^{-1}), followed by enterobacteria (2.3×10^2 cfu g^{-1}), lactic acid bacteria (2.1×10^2 cfu g^{-1}) and *Staphylococcus* species (1.3×10^2 cfu g^{-1}), however, in the laboratory-fermented mash the aerobic mesophilic bacterial flora of *iregi* were *Bacillus* species (1.2×10^5 cfu g^{-1}), lactic acid bacteria (3.6×10^2 cfu g^{-1}), enterobacteria (1.1×10^2 cfu g^{-1}) and *Staphylococcus* species (1.7×10^1 cfu g^{-1}). The total aerobic mesophilic bacterial counts declined during the last three days of the fermentation but the *Bacillus* counts increased throughout the fermentation period. The pH of the fermenting mash was between 7.3 and 8.7 while the optimal temperature for fermentation was 45-46°C. Physical observation of the fermented mash showed that it was dark brown in appearance with a creamish mucilaginous slime moulding the fermented cotyledons together.

Keywords: bacterial flora, ethnic foods, fermented food culinary products, human nutrition, plant foods
Abbreviations: cfu, colony forming units

INTRODUCTION

Alkaline-fermented foods constitute a group of less-known food products that are widely consumed in Southeast Asia and African countries, and can be made from different raw ingredients. For instance, Japanese *natto*, Thai *thua-nao*, and *kinema* are made from cooked soybeans, *dawadawa* from African locust beans (*Parkia biglobosa*), *ogiri* from melon seeds (*Citrilus vulgaris*), *ugba* from African oil beans (*Penthacletra macrophyla*), *kawal* from fresh *legale* leaves, *owoh* from cotton seeds (*Gossypium hirsitum*), *okpehe* from mesquite (*Prosopis africana*), *pidan* from fresh poultry eggs and *aisa* from *Albizia saman* (Odunfa 1981; Obeta 1982; Ogbadu and Okagbue 1988; Sanni and Ogbonna 1991; Wang and Fung 1996; Ogunshe *et al*. 2006, 2007). These alkaline-fermented foods, also known as fermented food condiments in Nigeria and many other North and West African countries are popular strong smelling fermented food culinary products which give a pleasant aroma to soups, sauces and other prepared dishes. In Africa, many proteinaceous oily seeds are fermented to produce food condiments. According to Odunfa (1981), most leguminous species are little used as fermented condiments yet, and many of them are almost unknown to science in the area of food fermentation; similarly, there have been no documented reports on fermentation of boiled cotyledons of *Delonix regia* in the production of a food condiment. This study was therefore carried out to determine the possibility of laboratory fermentation of boiled cotyledons of *D. regia* in the production of a Nigerian indigenous food condiment known as *iregi*.

MATERIALS AND METHODS

Sample collection

Harvested pods of *D. regia* were picked from within the University of Ibadan campus between January and March from about 21-year old *D. regia* trees. The pods were split open and the seeds (**Fig. 1A**) directly removed from the pods by hand.

Laboratory method of fermentation

One kilogram of harvested and dried seeds of *D. regia* per fermentation were washed with tap water, followed by heat treatment. The seeds were measured directly into a pressure cooker containing tap water or a stainless bowl placed in an autoclave and boiled (autoclaved) at 121°C for about 50–60 min.. The boiled and swollen cotyledons (**Fig. 1B**) were then dehulled manually by pressing in between fingers. The boiled cotyledons were rinsed with sterile cold water and the seed coats decanted along with the water, leaving the clean cotyledons, which were re-boiled for another 30-45 min. The cotyledons were later drained and cooled to about 35°C before wrapping with different leaves, which had been cleaned with warm sterile distilled water. The wrapped cotyledons were separately packed in nylon and incubated between 37 and 50°C for 6 days to produce fermented *iregi* mash, i.e. sticky mucilaginous brownish strong-smelling mash.

Simulated traditional method of fermentation

Dried seeds of *D. regia* were fermented as described in the laboratory fermentation except that the boiled cotyledons were washed with various water samples obtained from traditional producers of *iru*, a most commonly consumed traditionally fermented indigenous condiment of Nigeria.

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Fig. 1 (**A**) *Delonix regia* seeds. (**B**) Boiled cotyledons of *D. regia*. (**C**) Fermented cotyledons of *D. regia* as *iregi*.

Isolation and characterisation of bacterial species

Bacterial species were isolated from the fermenting mash on appropriate culture media nutrient agar (NA), cystein lactose electrolyte defficient agar (CLED), mannitol salt agar (MSA), potato dextrose agar (PDA) and plate count agar (PCA). Characterisation of the bacterial isolates were according to appropriate taxonomic tools (Seeley and Van Denmark 1972). Biochemical characteristics of the isolates were as described earlier (Edwards and Ewing 1972; Holding and Colle 1972; Bailey and Scott 1974; Harrigan and McCance 1976; Paick 1980; Vera and Power 1980).

RESULTS

The total aerobic and anaerobic plate counts of fermenting mash are recorded in **Table 1**.

The pH of the fermenting mash increased gradually from 7.3 at the start of fermentation (day 0) to 7.8 by day 3 before dropping to 7.6 on day 4 and finally increasing to 8.7 by the end of fermentation. The optimal temperature for the fermentation was 45-46°C.

One hundred and twenty six Gram-positive bacterial strains isolated from the fermenting cotyledons of *D. regia* into *iregi* were characterised as *Bacillus cereus* var. *mycoides* (21), *B. licheniformis* (37), *B. megaterium* (10), *B. pumilus* (24), *B. subtilis* (20), *Staphylococcus aureus* (8), *S. epidermidis* (2) and *S. saprophyticus* (4). *Bacillus* species occured most consistently and predominated the laboratory fermentation of *D. regia* into *iregi*, with the production of a strong ammonia-like aroma, characteristic of leguminous-based fermented condiments.

The physical observation of the fermented mash was dark brown in appearance with creamish mucilaginous slime moulding the fermented cotyledons together (**Fig. 1C**), while cotyledons wrapped with cocoyam leaves gave the best characteristic physical parameters similar to other Nigerian indigenous fermented condiments. *Iregi pete* is the softer type of the condiment in which cotyledons are completely fermented into pasty form, due to prolong the fermentation period.

DISCUSSION

Condiments give a pleasant aroma to soups and sauces in many countries especially in Africa and India where protein calorie malnutrition is a major problem (Sarker *et al*. 1993). They also have great potential as key protein, fatty acid and good sources of gross energy (Mbajunwa 1995), therefore, condiments are basic ingredients for food supplementation and their socio-economic importance cannot be over emphasised.

The Royal Poinciana, *Delonix regia* (family Fabaceae, Syn. *Caesalpinia regia*; Common names: flamboyant, royal poinciana, flame tree, peacock flower, flamboyant), is a tropical or subtropical flowering plant which has been described as the most colourful tree in the world. Native to Madagascar, it is also found in India, where it is referred to as the Gulmohar. The tree's vivid red/orange flowers and bright green foliage in any case make it an exceptionally striking sight. In addition to its ornamental value, it is also a useful shade tree in tropical conditions, because it usually grows to a modest height (typically around 5 m, though it can reach as high as 12 m) but spreads widely, and its dense foliage provides full shade (Du Puy 1998). The seed pods are dark brown and can be up to 60 cm long and 5 cm wide; the individual seeds, however, are small, weighing around 0.4 g on average. Seeds are relatively loosely attached in lateral grooves inside the legume. When mature, the legumes split into 2 parts lengthwise and are dark brown to black (Little and Wadsworth 1964) The seed pods of Royal Poincianas are used in the Caribbean as a percussion instrument known as the shak-shak or maraca (Dy Puy 1998). According to Little and Wadsworth (1964), Menninger (1962) and Webb *et al*. (1984), the legume (seed pod) is edible. However, no documented reports of fermentation of *D. regia* into a food condiment have been found.

The traditional preparation of *iregi* in the laboratory simulated the same process of locust beans seeds fermenta-

Table 1 pH values and viable mesophilic counts (cfu g^{-1}) of the simulated and laboratory fermented *D. regia* cotyledons into *iregi*.

Time (Days)	pH	*Bacillus* spp.[a]	*Staphylococcus* spp.[a]	Acid bacteria[a]	Enterobacteria[a]
Simulated fermentation					
1	7.3	$1.4 \times 10^1 \pm 0.4$	$1.0 \times 10^1 \pm 0.0$	-	$2.3 \times 10^1 \pm 0.2$
2	7.5	$2.1 \times 10^1 \pm 0.3$	$1.4 \times 10^1 \pm 0.1$	$1.1 \times 10^1 \pm 0.1$	$3.3 \times 10^2 \pm 0.3$
3	7.8	$3.3 \times 10^3 \pm 0.3$	$1.2 \times 10^1 \pm 0.1$	$2.6 \times 10^1 \pm 0.1$	$2.2 \times 10^1 \pm 0.6$
4	7.6	$3.7 \times 10^3 \pm 0.3$	$3.0 \times 10^1 \pm 0.2$	$2.1 \times 10^1 \pm 0.1$	$1.3 \times 10^3 \pm 0.1$
5	8.1	$1.7 \times 10^4 \pm 0.1$	$1.2 \times 10^1 \pm 0.2$	$1.7 \times 10^1 \pm 0.3$	$2.2 \times 10^3 \pm 0.1$
6*	8.7	$1.1 \times 10^5 \pm 0.1$	$1.0 \times 10^1 \pm 0.0$	$1.0 \times 10^1 \pm 0.0$	$1.8 \times 10^3 \pm 0.2$
Laboratory fermentation					
1	7.3	$1.0 \times 10^1 \pm 0.0$	-	-	$1.0 \times 10^1 \pm 0.0$
2	7.5	$2.0 \times 10^1 \pm 0.1$	$1.5 \times 10^1 \pm 0.0$	$1.1 \times 10^1 \pm 0.1$	$1.5 \times 10^2 \pm 0.0$
3	7.8	$3.0 \times 10^3 \pm 0.1$	$2.0 \times 10^2 \pm 0.1$	$2.2 \times 10^2 \pm 0.1$	$1.1 \times 10^2 \pm 0.1$
4	7.6	$2.1 \times 10^4 \pm 0.1$	$2.0 \times 10^1 \pm 0.2$	$3.3 \times 10^2 \pm 0.5$	$2.5 \times 10^1 \pm 0.1$
5	8.1	$1.4 \times 10^5 \pm 0.2$	$1.9 \times 10^1 \pm 0.3$	$1.6 \times 10^1 \pm 0.0$	$2.3 \times 10^1 \pm 0.2$
6*	8.7	$1.2 \times 10^6 \pm 0.2$	$4.9 \times 10^1 \pm 0.3$	$1.1 \times 10^1 \pm 0.0$	$1.4 \times 10^1 \pm 0.1$

* *iregi pete* (a softer mash of *iregi*)
[a] bacterial counts in colony forming units (cfu g^{-1})

tion to *iru* which is known to be a traditional family art, practiced in homes in a crude manner like other Nigerian traditional fermented foods. The increase in pH of the fermenting mash of *iregi* from 6.0 to 8.0 agrees with some earlier reports, which all recorded a slightly alkaline to alkaline pH in fermented food condiments from vegetable proteins (Hesseltine 1965; Odunfa 1985; Baird-Parker 1994; Tamang 1998; Ogunshe *et al*. 2006) may be due to an abundant inrease of NH_3 during the later stages of the fermentation, leading to alkalinity by the hydrolysis of protein (Whitaker 1978), or due to protease and deaminase enzymes produced by *Bacillus* isolates (Hesseltine and Wang 1967). The gradual development of ammonical odour is in agreement with the the increased pH changes from around neutral to alkaline pH range (Barimala 1994). In alkaline-fermented foods, the protein of the raw materials is broken down into amino acids and peptides; ammonia is released during the fermentation, raising the pH of the final products and giving the food a strong ammoniacal or pungent smell, which tends to mellow during cooking applications.

A gradual, slight increase in temperature was observed during the fermentation period, which shows that from a thermal view point that fermentation of *D. regia* seeds into *iregi* is an exothermic process in which heat evolved. The heat liberated by the microorganisms involved in fermentatiom may be responsible for the rise in the temperature of the fermenting mash, therefore making the fermenting process a putrefactive one. This temperature rise agrees with previous documented works on the fermentation of leguminous proteins (Oyewole and Odunfa 1990; Sanni *et al*. 2000; Ogunshe *et al*. 2006). The physical observation of the fermented *iregi* mash was dark brown in appearance with creamish mucilaginous slime, moulding the fermented cotyledons together, while the fermented *iregi* mash with cocoyam leaves gave the most accepted product when compared to pawpaw, banana and almond leaves.

In this study, *Bacillus* species were the most predominant species and produced the highest amoniacal smell characteristic of typical indigenous fermented food condiments. Several fermented products rely on the participation of various *Bacillus* species, including *Bacillus natto* and *B. subtilis*. Most alkaline fermentations are achieved spontaneously by mixed bacteria cultures, principally dominated by *Bacillus subtilis*. In other cases, pure cultures can be used. Often, the finished products are of a very indigenous character and exhibit sensory properties resulting from unique flora and processing technologies applied in small scale, home-based fermentations (Tamang 1998). Fermentation with *B. natto* and *B. subtilis* can produce very characteristic aromas in fermented products such as *natto* and *dawadawa* (also referred to as *daddawa*). Other African fermentations in which *Bacillus* spp. play a role include the production of Nigerian *ugba* (fermented African oil bean), Nigerian *ogiri* (fermented watermelon seeds) and *ogiri-saro* (sesame seed, pumpkin or castor oil seed fermentation) from Sierra Leone. Similarly, in the study of Sanni *et al*. (2000) the bacterial isolates from Nigerian fermented condiments, obtained from retail markets located in Southwestern Nigeria were identified as *B. subtilis* (33%), *B. pumilus* (19%), *B. licheniformis* (22%), *B. brevis* (9%), *B. megaterium* (12%) and *B. polymyxa* (5%).

A comparison was made between the traditional and laboratory methods of fermentation of *D. regia* into *iregi* food condiment, and the results obtained were similar except for the presence of more Gram-negative bacterial flora in the simulated traditional method. In comparism, more isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Proteus mirabilis* were isolated in addition to the *Bacillus* and *Staphylococcus* species in the simulated traditionally fermented *iregi* samples. It is, however, important to note that the water samples used in the traditional fermentation were obtained from the traditional producers of indigenous condiments. The water samples were mostly total and faecal coliform positive; and high recovery rates of Gram-negative bacterial flora were obtained from the water samples. It can then be confirmed that the water samples used in the traditional preparation of the indigenous fermented condiments were a major source of the Gram-negative bacteria species usually associated with the indigenous fermented condiments. Similarly, the most commonly encountered pathogens in African fermented food condiments include *Bacillus cereus*, *E. coli*, *Salmonella* sp., *Staphylococcus aureus*, *Vibrio* cholerae, *Aeromonas*, *Klebsiella*, *Campylobacter* and *Shigella* sp. (Ogbadu and Okagbue 1988; Ogunshe *et al*. 2006, 2007; Ogunshe and Olasugba 2008).

The fermented *iregi* has a similar aroma to other condiments such as *iru* and *okpehe*. Based on organoleptic parameters, the fermented condiment, *iregi* was highly acceptable as alternative condiment to *iru*, *ogiri* and *okpehe*. This preliminary study indicates that boiled cotyledons of *Delonix regia* can be fermented into an indigenous food condiment such as *iregi*. Further studies to determine the biochemical and nutritional compositions of the fermented product are on-going in our laboratories.

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