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RESEARCH PAPER

Salt Tolerant Microorganisms in Fermented Raw Jackfruit and Standardization of a Method for Improved Preservation

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Abstract

People in the south-west coast of India preserve raw jackfruit kernels in the brine solution by natural fermentation. Traditionally, high salt concentration brine is utilized to maintain texture and avoid contamination; the product is then, stored and consumed until the next fruiting season. Study was conducted to identify salt-tolerant microbiota in traditionally fermented jackfruit. Given priority to the texture, taste and odour of the fermented kernels, attempt was made to prevent excess use of salt by standardizing brine solution and at the same time inhibiting undesirable yeasts and moulds in the fermented product. Study revealed the presence of salt tolerant lactic acid bacteria, *Leuconostoc* and *Lactobacillus* spp. and yeasts belonging to genera *Saccharomyces* and *Geotrichum*. Population of moulds and yeasts reduced significantly at 12 % salt concentration compared to 5 % and 10 %. Texture, odour and overall acceptability of the fermented kernels were recorded as the best at 12 % brine solution though it revealed saltish taste.

Keywords: Raw jackfruit, traditional fermentation, salt, lactic acid bacteria

Jackfruit *(Artocarpus heterophyllus)* is a seasonal fruit which is consumed by the people in the west-peninsular India in various traditional ways. The fresh ripened fruits are relished only in mid-summer and cannot be preserved as such unless canned in sugar syrup. Sometimes the ripe fruit kernels are mashed with sugar, dried to thin strips under the sun and consumed as chewy toffees. Raw jackfruits are available in the south-west coast of India from February to April and are cooked like any other green vegetables. Fermentation of fruits and vegetables is one of the ancient methods that helped people to keep-up with their nutrition during the off-season (Battcock and Azam-Ali, 1998). Recently, it has been reported (Barker, 2016) that consumption of raw jackfruit on daily basis can reduce external insulin administration in early diabetic individuals. The fermented kernels can be added to the daily diet of patients with constipation as it can be a natural laxative. The traditionally brined kernels are soaked in water for 2 h to drain out excess salt and then used for preparing chips or flour coated fries. Brining of raw jackfruit is still practiced in the coastal villages of India. It has now become a delicacy and is consumed as additives in coconut curries prepared with chickpea, cowpea and beans. It is also added in red-meat preparations as well. It is low in cholesterol and a good source of fibres (Che-Othman *et al*., 2012). Though rich in carbohydrates, it is considered as a low-calorie food with good antioxidant activity (Sharma *et al*., 2013). Considering salt content as one of the preservation tool in the product, study was carried out to enumerate and identify the salt-tolerant microbiota involved in the traditional fermentation process. Giving priority to the texture, taste and odour of brined jackfruit kernels, attempt

was made to standardize brine concentration so as to avoid excess use of salt but, prevent contamination by moulds and undesirable yeasts.

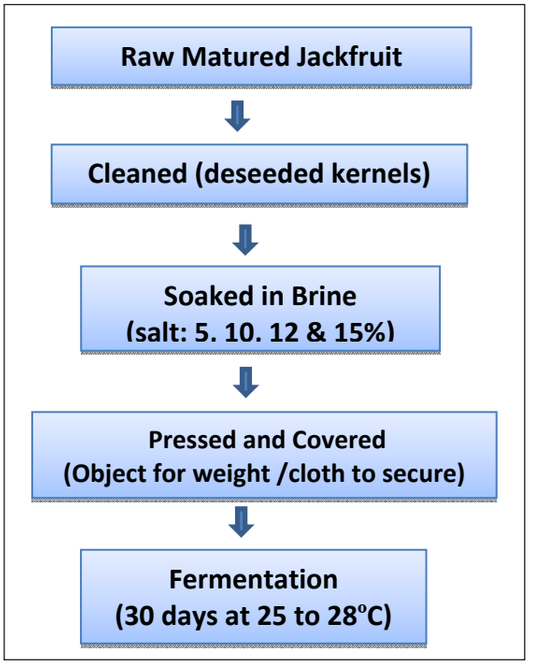
MATERIALS AND METHODS

Substrate (kernels) Preparation

Kernels were cleaned and de-seeded under aseptic conditions from a healthy and matured raw jackfruit. To standardize concentration of brine, deseeded kernels (500 g) were mixed thoroughly with table salt at different levels (viz. 0, 5, 10, 12 and 15 % w/w) and transferred to sterile glass jars. A control was maintained with zero salt content. To each jar, 500 mL of potable water (boiled and cooled) was added and mixed.

Fermentation

Traditional fermentation method was used in the study (Flow chart-1).



Flow chart I: Raw Jackfruit Fermentation

Clean granite tile was placed on the substrate and a heavy weight object was kept to submerge the kernels completely into the brine solution. Mouth of the jar was tied with four layers of muslin cloth and incubated for fermentation in a cool place (25-28 °C) for 30 days.

Isolation and Identification of Microorganisms

Enumeration and isolation was carried out after 30 days of fermentation by sequential dilution and standard plate count technique. The selective media used were Martin's rose Bengal agar (MRBA) to isolate moulds (Martin, 1950). Malt extracts agar (MEA) was used to isolate yeasts (Skaar and Stenwig, 1996). Tetracycline was added to MEA to eliminate bacteria. Man Rogosa and Sharpe's agar (MRS) was used to isolate lactic acid bacteria (LAB) (De Man *et al.,* 1960). Cycloheximide was added to MRS to eliminate the growth of yeasts (Amoa-Awua *et al.,* 2007). The plates were incubated at 32 °C for 48 h.

Identification was done based on morphology and biochemical tests (Bisen *et al.,* 2012). The morphology included colony types and growth habit (aerobic or facultative); Gram staining; cell shape, arrangement and size (micrometry); presence of spores/endospores and capsulation. The biochemical tests included carbohydrate utilization (glucose, sucrose, lactose and galactose), acid/gas production (with Durham's tube), pH determination, MRVP tests, catalase activity, exopolysaccharide (EPS) production and antibiotic (vancomycin-50µg.mL^{-1}) resistance (Zdolec *et al.,* 2011).

Sensory Evaluation

Six panelists with expertise in evaluation of fermented foods were considered for sensory evaluation of the fermented product. The kernels were rinsed in hot water and evaluated directly for texture, taste, odour and overall acceptability using 9- Point Hedonic Scale (Lawless and Heymann, 2010)

RESULTS AND DISCUSSION

Microbial Profile, pH and Sensory Evaluation

The control (0 % salt) jar displayed white mould on the surface of the fermenting bottles. These kernels

in the later stages showed black spots. Strong alcohol odour and sloppy-soft texture of the kernels indicated that raw jackfruit required salt for desired fermentation and preservation (Viander *et al.,* 2003). Jackfruit is a rich in source of carbohydrates, hence results in natural fermentation by yeast giving alcohol odour (Kumoro *et al.,* 2012). The jar with 5 % salt showed significantly higher population of yeasts (72.67×103 mL^{-1}), LAB (275.3×103 mL^{-1}) and mould (6.67×103 mL^{-1}) compared to the treatments with 10-15 % salt. Moulds are considered as contaminants in the fermentation process. Its growth in sauerkraut fermentation at low salt concentration has been reported by Viander *et al.* (2003).

Sensory evaluation of raw jackfruit kernels at 5 % salt resulted in soft texture with mild alcohol odour. Fermentation at 10 % salt concentration substantially reduced the population of moulds and considerably maintained population of yeasts and Lactic acid Bacteria (LAB). Similar experiment was tried by Romero-Gil *et al.* (2013) in fermentation of olives at high salt concentration and recorded good survival of yeasts and *Lactobacillus plantarum*. The population of yeasts and LAB at 12 % salt concentration was significantly less compared to 5 and 10% salt (Table 1). However, sensory evaluation with 12% salt showed better acceptance of the kernel texture and odour compared to that fermented with 10% salt. The kernels have the tendency to remain rigid in texture as it is fermented with relatively high salt concentration. It is established that, salting directs the subsequent course of the fermentation, limiting the amount of pectinolytic and proteolytic hydrolysis, thereby controlling the softening as well as preventing putrefaction of the fermenting product (Steinkraus, 1992 and 1997). Change in pH is also an indication of acidic flavour and odour of the fermented kernels. Furthermore, brining at 12 % controlled the growth of moulds significantly (4.0×10^2 mL^{-1}). Salt favours growth of some beneficial organisms thereby, inhibiting the growth of undesirable spoilage bacteria and moulds naturally present in the foods (Doyle *et al*., 2001). The results of the microbial population, pH and sensory evaluation are shown in the Table 1 along with the comments made by the panel of judges.

Table 1: Microbial profile and product variation at different salt concentration

Salt % (w/w)	Population 30 DAF (Cfu mL^{-1})			pH	Sensory Evaluation (Mean values) (9 pt. Hedonic Scale)				Remarks
	Yeasts ($\times10^3$)	LAB ($\times10^3$)	Molds ($\times10^2$)		Texture	Odour	Taste	Overall Acceptance	
0	183.7	217.0	179.3	6.4	1.50	1.33	1.17	1.33	Soft, sloppy and high alcoholic-odour
5	72.67	275.3	66.70	5.8	4.67	4.17	5.50	4.50	Soft, moderate alcoholic and mild acidic-odour
10	46.33	190.7	12.00	5.5	7.50	7.33	6.17	6.50	Tender, firm, salted, mild alcoholic and acidic-odour
12	03.00	101.3	04.00	5.2	7.67	8.33	6.08	**8.17**	Tender, firm, crunchy and salted, acidic-odour
15	01.33	28.67	01.30	6.1	8.33	8.67	5.00	5.33	Tender, firm, crunchy, mild acidic-odour, brackish (too salted)
F test	*	*	*		*	*	*	*	
CD at 5%	8.15	11.25	16.10	—	0.63	0.68	0.80	0.58	—
S.Em. ±	2.58	3.57	5.10		0.22	0.23	0.28	0.20	

Note: Statistical analyses by one way ANOVA (CRD). Variables differ significantly - (*****); LAB- lactic acid bacteria; DAF-days after fermentation; Cfu- colony forming units /mL of brine solution. Sensory evaluation by 9 -Point Hedonic Scale.

Table 2: Morphology and biochemical characterization of salt tolerant microorganisms isolated from fermented raw jackfruit

Organisms Identified	Morphology							
	Growth/Colony type		Cell type					
Moulds (5-10% salt)								
A*spergillus* sp.	White cottony, surface mycelia later showing black aerial spores. Sclerotia globose shape (D: 450- 675µm)		Conidia globose to subglobose shaped, D: 3.5- 4.5µm, conidial heads (D: 250-360 µm) radiated wide, Sterigmata biseriated.					
Yeasts (5-12% salt)								
1. *Saccharomyces* sp.	Moist, opaque, circular, convex colonies. No pseudohyphae or spores.		Ellipsoidal or oval shape, single, D: 2.6-3.25µm and budding type (budding cells elongated to D:5.2-7.0 µm)					
2. *Geotrichum* sp.	Initially sticky circular colonies, later changes to white powdery mould (spores)		Barrel shape, D: 4.6-5.2 µm, hyaline & filamentous hyphae, non-budding, spores observed in later stages.					
Bacteria	Morphology		Biochemical tests					
(5-15% salt)	Growth /Colony type	Cell type and Gram staining	Catalase activity	MRVP tests	EPS	Lactose use		Spores & Capsule
						Gas	pH	
1. *Lactobacillus* sp.	Small, circular, smooth, opaque and raised colonies on isolation agar.	Single rods, some slightly curved. Gram-positive. Size- (5.5×1.5 µm)	–	–	–	–	5.2	Non-capsulated spores absent
2. *Leuconostoc* sp.	Spindle colonies partially inserted into the isolation agar. Mucilaginous substance observed in later stages.	Diplococci or short chain cocci. Gram positive. Size- (0.8×1.2 µm)	–	–	+	+	5.4	Non-capsulated but slime detected. Spores absent

Note: Tests-Positive (+) and Negative (-), D- diameter, MRVP- Methyl Red Voges Proskauer, EPS- Exopolysaccharides. Characterization was done only of salt tolerant microbes.

Isolation and Identification of Microorganisms

The microscopic observations of isolates from MRS-cycloheximide plates showed presence of lactic acid bacteria (Table 2). Carbon utilization mainly lactose broth tests revealed acid production with some species displaying gas accumulation in the Durham's tube. Cell growth in lactose broth was observed at the bottom of the tube indicating facultative anaerobic or microaerophillic nature. Lactic acid bacteria producing EPS were resistant to vancomycin. The results (Table 2) were compared with Bergey's manual of determinative bacteriology and characterized as *Leuconostoc* and *Lactobacillus* spp. (Bergey and Holt, 1994). Similar observations were reported by Kim *et al.* (2000) with *kimchi* fermentation, a traditional Korean fermented food prepared with salt and vegetables like Chinese cabbage, radish and cucumber. They demonstrated that lactic acid bacteria, like *Leuconostoc* sp. were involved in fermentation and could grow even at 10 % salt concentration. Yeast cells isolated from fermented products were single and ellipsoidal in shape with budding nature. Chiou *et al.* (1999) isolated yeasts as well as LAB from the salt fermented soybean '*Miso*' (Japanese seasoning sauce). The contaminated jars (0 and 5% salt) showed growth of mycelial (cottony) as well as pseudomycelial moulds. Microscopic observations reveled the black spores producing moulds as *Aspergillus* sp. while the white pseudomycelial yeasts on the surface of the fermented product were identified as *Geotrichum* sp. The main aim was to maintain tenderness and firmness of kernels with maximum elimination of moulds and alcohol odour. Fermentation of fully matured raw jackfruit at 12%

salt concentration (w/w) ensured good texture and firmness to the kernels, simultaneously kept a check on mould population, which could cause blackening of the fermented kernels in the later stages of storage. The product received good overall acceptability from the judges at 12%. At 15%, though fermented kernels maintained its texture, it suppressed the overall microbiota significantly and resulted in highly salted product.

CONCLUSION

Due to overproduction during the season and poor keeping quality, most of the fruits produced are used as fodder or dumped in the farm pits as manure. Preserving raw jackfruit with 10-12% brine solution could keep a check on moulds; prevent an excessive use of salt and at the same time maintain quality of the product. The method standardized in this study could be used to preserve raw jackfruit from the household to the industrial scale.

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