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Evaluation of Different Strains of Yeast and Lactic Acid Bacteria for Nutritional Improvement of Jackfruit Waste Under Solid State Fermentation

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Abstract

The study on solid state fermentation of Jackfruit waste by different strains of yeast *viz., Saccharomyces cerevisiae, Saccharomyces boulardii*, *Candida utilis* and isolate *Saccharomyces* spp and lactic acid bacteria *viz., Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus brevis* and isolate *Lactobacillus* spp. were evaluated for the nutritional improvement. The results revealed that Jackfruit waste fermented by *S. boulardii* and lactic acid bacteria *L. acidophilus* recorded with lower pH (4.05 and 3.98) and crude fibre (21.46 and 21.75%) with highest titrable acidity (0.80 and 0.81%), crude protein (9.59 and 9.32%), crude fat (6.53 and 7.12%) and carbohydrate (75.31 and 74.05%), respectively. The results clearly indicated that the yeast strain *Saccharomyces boulardii* and LAB strain *Lactobacillus acidophilus* were more efficient in enhancement of nutrients of the jackfruit waste through solid state fermentation.

Keywords: Jackfruit waste, Yeast, Lactic acid bacteria, Solid state fermentation, Nutrient improvement

The role of fruit and vegetable wastes as a cheap source of nutrients capable of supplying adequate calories to livestock is very significant. Recycling of fruit and vegetable waste is one of the most important means of utilizing wastes into essential value added products required for human, animal and plant nutrition. Microbial technology is one which is available for recycling and processing of fruit and vegetable waste by different processes. Solid state fermentation technology has been identified as one of the less expensive means of increasing / enhancing the nutrients of fruits and vegetable wastes (Pandey, 2003). Jackfruit (*Artocarpus heterophyllus* L.) is an important under-utilized fruit of the tropics, native to the India and grows widely in the south western rain forest of India and is quite popular in Eastern and Southern India, being widely cultivated in states of Kerala, Karnataka, Andra Pradesh, Tamil Nadu, Maharashtra, Orissa, West Bengal, Goa, Assam, Andaman and Nicobar islands. In India, the total area under jackfruit is approximately 102,000 ha (Bose *et al.,* 2003). Nearly 60 per cent of the jackfruit is discarded after the fleshy parts are taken out. The jackfruit wastes, which consists of aerial part, skin, seed and heart, will be a feed resource with high organic matter (OM) digestibility (70-78%), with low crude protein (6 to 7% in DM) as reported by Kusmartono (2001). It is reported that the non-edible portion of the ripe jackfruit contains 83 per cent water, 8.23 per cent crude fibre, 1.81 per cent crude protein, 0.89 per cent crude fat, 0.54 per cent total ash and 5.68 per cent carbohydrates. This by-product of fruit waste could be a valuable material as animal feed by improving nutritional quality through solid state fermentation technology using different micro organisms. Thus, nearly 60 per cent of this waste could be recycled into a valuable product. The limitation in utilization of fresh jackfruit waste as animal feed is because of its high moisture and presently, huge quantity of this valuable byproduct waste is dumped in landfills, roadsides leading to environmental hazards. Hence, the study was under taken to improve the nutritional quality of the Jackfruit waste by different yeast and lactic acid bacteria under solid state fermentation technology. This approach would also help in proper utilization of jackfruit wastes which is otherwise discarded.

Material and Methods

The experiment was conducted at All India Coordinated Research Project, Post Harvest Engineering Technology, University of Agricultural Sciences,

GKVK, Bengaluru to evaluate the efficiency of different strains of yeast and lactic acid bacteria for the nutritional improvement of jackfruit waste under solid state fermentation. The experiment was conducted (Plate 1) under completely randomized design (CRD) with nine treatments and three replications.

Plate 1: Jackfruit waste used for fermentation studies

	Treatments
Yeast strains	T1 = Jackfruit waste only (Control)
	T2 = JF waste + *Saccharomyces cerevisiae,* UCD 522 (Yeast 1)
	T3 = JF waste + *Saccharomyces boulardii)* (Yeast 2)
	T4 = JF waste + *Candida utilis* (Yeast 3)
	T5 = JF waste + *Saccharomyces* spp. Isolate from jackfruit rind (Yeast 4)
LAB strains	T6 = JF waste + *Lactobacillus plantarum (*MTCC 6161) (LAB 1)
	T7 = JF waste + *Lactobacillus acidophilus* (MTCC 10307) (LAB 2)
	T8 = JF waste + *Lactobacillus brevis* (MTCC 1752) (LAB 3)
	T9 = JF waste + *Lactobacillus* spp. isolate from jackfruit rind (LAB 4)

Collection of Jackfruit Waste: Well matured and healthy jackfruits (Hard flesh variety) were collected from the Zonal Agricultural Research Station (ZARS) Farm, UAS, GKVK, Bengaluru for the experimentation. The jackfruits were cut, peeled and the edible bulbs are removed from the core rind. The jackfruit waste includes rind with arils and perigones used as a jackfruit waste for the experiment (Plate 1).

Preparation of Jackfruit Waste for Experiment: Jackfruit waste includes rind, arils, perigones which are cut into small pieces (size-1 inches) and filled into the autoclavable polypropylene bags and samples were pasteurized at 90-100^{0}C for 10 minutes. After pasteurization, the jackfruit waste samples were transferred into polythene bags of 400 gauges for solid state fermentation process by different yeast and lactic acid bacterial strains (Plate 2).

Plate 2: Experiment set up for evaluation of yeast and lactic acid bacterial strains

Preparation of different Yeast Starter Cultures

Authenticated microbial cultures of yeast viz., *Saccharomyces cerevisiae* (UCD 522) (Yeast 1) *Saccharomyces boulardii* (Yeast 2), *Candida utilis* (Yeast 3) and isolate from jackfruit waste *Saccharomyces spp* (Yeast 4) were used. Purified and authenticated loop full of inoculums of different yeast cultures were transferred to conical flask containing Yeast Extract Peptone Dextrose broth (YEPDA). The probiotic yeast *S. boulardii* was inoculated in Sabourouds Hi-media broth. The inoculated flasks were kept for 2-3 days incubation at 28^{0}C. These broth cultures of yeast were inoculated at 3 per cent containing 10^7 cfu/ml to 400g of jackfruit waste contained in polythene bags under solid state fermentation.

Preparation of different LAB Starter Cultures

Authenticated microbial cultures of lactic acid bacteria *viz*., *Lactobacillus plantarum* (MTCC 6161), *L. acidophilus* (MTCC 10307), *L. brevis* (MTCC 1752) were obtained from Microbial Type Culture Collection Centre, Chandigarh, India in the form of lyophilized cultures and same were revived in the form of agar

based slant cultures and isolate from jackfruit waste *Lactobacillus* spp. were used. Loop full inoculum of purified and authenticated different lactic acid bacteria were transferred to conical flasks containing 100ml of MRS broth. The inoculated flasks were incubated for 2-3 days at 37^{0}C. These broth cultures were inoculated at 3 per cent containing 10^{7}cfu /ml to 400g of jackfruit waste contained in polythene bags under solid state fermentation.

Tray Drying and Grinding : After completion of 7 days of fermentation, the samples were subjected to drying in tray drier at 55 ^{0}C for 48 hours. Samples were spread uniformly in the tray for effective drying. The tray dried samples were grinded in mixer to get powder and subjected to biochemical analysis by following standard procedures.

Biochemical Analysis : The fermented selected fruit waste flours were subjected to proximate analysis for different nutrients such as moisture, protein, fat, fiber, ash and carbohydrate content. The biochemical analysis such as pH, TSS, titrable acidity was done by employing standard methods of AOAC (2005) and similarly, the proximate analysis of the samples were determined by standard procedures of AOAC methods (2005).

Results and Discussion

The results pertaining to changes in pH, total sugar and titrable acidity of fermented dried jackfruit waste by the influence of different Yeast and LAB strains is given in Table 1.

pH: The initial pH of the jackfruit waste was 5.16. After 7 days of fermentation by different Yeast and LAB strains, the change in pH of the fermented jackfruit waste varied from 3.98 to 4.40 between the strains. The highest reduction of pH (3.98) was observed by yeast strain *Saccharomyces cerevisiae* which was on par with *Saccharomyces boulardii* (4.05). The jackfruit waste fermented with LAB strain *Lactobacillus acidophilus* recorded lowest pH (3.99), which was on par with *Lactobacillus plantarum* (4.03). The decrease in the pH of fermented jackfruit waste by yeast *Saccharomyces cerevisiae* and lactic acid bacterial strain *Lactobacillus acidophilus* indicates one of the important parameter in maintaining the quality of the product. The decrease in pH is an indication of fermentation of the substrate and carbohydrate an organic compound is expected to yield organic acids such as lactic acid. The production of acid will increase the acidic content and thus reduction in pH value. Similar results were reported by Abalaka *et al*., (2011) in spent sorghum waste, the decrease in pH from 5.1 to 3.2 after fermentation by yeast.

Titrable Acidity: The titrable acidity varied from 0.60 to 0.81 per cent between the Yeast and LAB strains after 7 days of fermentation (Table 1). The jackfruit waste fermented by yeast *Saccharomyces cerevisiae* recorded maximum titrable acidity (0.80%), followed by *S. boulardii* (0.75%) *C. utilis* (0.75%) and least titrable acidity (0.60%) was recorded by the isolate yeast.

The jackfruit waste fermented with LAB strain *Lactobacillus acidophilus* (LAB 2) recorded highest titrable acidity (0.81%), which was on par with isolate LAB (LAB4) (0.76%). These results partially related with the investigations of Ogunsua (1980), who reported that after five days of fermentation of cassava tuber, the titrable acidity was increased during the course of fermentation.

Total Sugar: The changes in total sugar varied from 0.51 to 0.85 per cent after 7 days of fermentation by different Yeast and LAB strains (Table 1). Among Yeast strains, the lowest total sugar (0.51%) was observed in the jackfruit waste fermented by *Saccharomyces boulardii*. The yeast strain *Candida utilis* showed higher total sugar content (0.68%), indicates that low efficiency in the utilization of total sugar during solid state fermentation.

Among LAB strains, all the LAB strains showed higher total sugar content except *Lactobacillus acidophilus* (0.67%) which indicates the higher efficiency in utilization of total sugar during solid state fermentation of jackfruit waste than other LAB strains. The jackfruit waste fermented by yeast strain *Saccharomyces boulardii* recorded more reduction of total sugar (0.51%) and was on par with *Saccharomyces cerevisiae* (0.57%). Similar results were reported by Joshi and Sandhu (1996) in apple pomace by

TABLE 1

Changes in pH, titrable acidity, and total sugars of the fermented dried jackfruit waste as influenced by different strains of Yeast and Lactic acid bacteria

Strains	Treatments	pH	Titrable acidity (%)	Total sugars (%)
	T_1=JF waste only	5.16 ^{a}	0.71 ^{ab}	0.73 ^{abc}
	T_2 = JF waste + *Saccharomyces cereviciae*,	3.98 ^{e}	0.80 ^{a}	0.57 ^{de}
	T_3 = JF waste + *Saccharomyces boulardii,*	4.05 ^{cd}	0.75 ^{ab}	0.51 ^{e}
Yeast strains	T_4 = JF waste + *Candida utilis,*	4.31 ^{bcd}	0.75 ^{ab}	0.68 ^{bcd}
	T_5 = JF waste + *Saccharomyces* spp. (Isolate from JF waste)	4.40 ^{b}	0.60 ^{c}	0.65 ^{cde}
	T_6 = JF waste + *Lactobacillus plantarum*	4.03 ^{cd}	0.68 ^{bc}	0.70 ^{bcd}
	T_7 = JF waste + *Lactobacillus acidophilus*	3.99 ^{d}	0.81 ^{a}	0.67 ^{bcd}
LAB strains	T_8 = JF waste + *Lactobacillus brevis,*	4.24 ^{bcd}	0.67 ^{bc}	0.81 ^{ab}
	T_9 = JF waste + *Lactobacillus* spp. (Isolate from JF waste)	4.32 ^{bc}	0.76 ^{ab}	0.85 ^{a}
	S.Em ±	0.10	0.03	0.05
	CD (at 5 %)	0.32	0.10	0.14

* Fermentation period 7 days

Saccharomyces spp. and *Candida* spp. Where there was reduction of total sugar during solid state fermentation. Results revealed the higher fermentation efficiency in jackfruit waste treated with *Saccharomyces boulardii* and *Lactobacillus acidophilus*.

The results pertaining to crude fibre, crude fat and crude protein content of fermented jackfruit waste by the influence of different Yeast and LAB strains is presented in Table 2.

Crude Fibre: The crude fibre of jackfruit waste in the control was 22.02 per cent. After seven days of solid state fermentation, the crude fibre varied from 21.46 to 21.99% between the Yeast and LAB strains. Among Yeast strains, the highest crude fibre (21.93%) was obtained in the jackfruit waste fermented by *Saccharomyces cerevisiae* and least crude fibre (21.46%) was obtained in *Saccharomyces boulardii.* Among LAB strains, the highest crude fibre (21.99%) was obtained in the jackfruit waste fermented by *Lactobacillus plantarum* and least crude fibre (21.75%) was observed in *Lactobacillus acidophilus.* The results of crude fibre of fermented jackfruit was not showed non significant with the enhancement of crude fibre by yeast and LAB strains. However, crude fibre reduction was more in the yeast fermentation compared to LAB fermentation. This may be attributed to the fact that during fermentation, carbohydrates including cellulose, pectin, lignocelluloses and starch are broken down by fermenting micro-organisms there by reducing the fibre content (Raimbult and Tewe, 2001). Similarly, these results support the work of Oboh and Akindahunsi (2003) who reported that fermentation of cassava waste by *Saccharomyces cerevisiae* resulted in non significant changes in the crude fibre contents of the cassava fermented products.

Crude Fat: The initial crude fat in the jackfruit waste was 6.30 per cent. After seven days of fermentation by different Yeast and LAB strains, the changes in crude fat varied from 5.42 to 7.12 per cent between the strains. The lowest crude fat (5.69%) was observed in the jackfruit waste fermented by isolate yeast *Saccharomyces* spp. which was on par with *Saccharomyces cerevisiae* (5.83%) the yeast strain *Saccharomyces boulardii* showed higher crude fat

TABLE 2

Changes in crude fibre, crude fat and crude protein of the fermented dried jackfruit waste as influenced by different Yeast and Lactic acid bacterial strains

Strains	Treatments	Crude fibre (%)	Crude fat (%)	Crude protein (%)
	T_1=JF waste only	22.02 ^{a}	6.30 ^{c}	8.28 ^{a}
	T_2 = JF waste + *Saccharomyces cereviciae*,	21.93 ^{a}	5.83 ^{de}	9.14 ^{e}
	T_3 = JF waste + *Saccharomyces boulardii,*	21.46 ^{b}	6.53 ^{bc}	9.59 ^{f}
Yeast strains	T_4 = JF waste + *Candida utilis,*	21.64 ^{b}	6.21 ^{cd}	9.06 ^{de}
	T_5 = JF waste + *Saccharomyces* spp. (Isolate from JF waste)	21.67 ^{c}	5.69 ^{e}	8.77 ^{cd}
	T_6 = JF waste + *Lactobacillus plantarum*	21.99 ^{ab}	6.16 ^{cd}	8.68 ^{bc}
	T_7 = JF waste + *Lactobacillus acidophilus*	21.75 ^{ab}	7.12 ^{a}	9.32 ^{ef}
LAB strains	T_8 = JF waste + *Lactobacillus brevis*	21.81 ^{b}	6.84 ^{ab}	8.40 ^{ab}
	T_9 = JF waste + *Lactobacillus* spp. (Isolate from JF waste)	21.85 ^{a}	5.42 e	8.15 ^{a}
	S.Em±	0.19	0.14	0.11
	CD (at 5%)	0.58	0.41	0.32

***** Fermentation period 7 days

content (6.53%). The LAB strains showed significant difference in crude fat content of jackfruit waste. Among LAB strains, least crude fat (5.42%) was obtained in the jackfruit waste fermented by isolate LAB (LAB 4). Highest crude fat (7.12%) was obtained in the jackfruit waste fermented by *Lactobacillus acidophilus* (LAB 2). The increase in fat content of the jackfruit waste may be attributed to the fact that the micro organisms degrade the jackfruit waste containing sugars and carbohydrates by fermentation activity as well as includes microbial biomass (Odekotum, 2000).

Crude Protein: The initial crude protein in the jackfruit waste was 8.28 per cent. After seven days of fermentation by different Yeast and LAB strains, the changes in crude protein varied from 8.15 to 9.59 per cent. The crude protein content increased in the jackfruit waste after fermentation by all the yeast and LAB strains. It is an indicative of the carbohydrates and sugars in the substrate which was utilized by all the strains of yeast and LAB. However, the yeast strain *S. boulardii* is more efficient in utilizing crude protein (9.59%) compared to other strains (Table 2). Similarly, among LAB strains, the strain *L. acidophilus* was more efficient in utilization of carbohydrates and sugars from the substrate to convert more enhancement of protein (9.32%). The ability of carbohydrate degradation is more by yeast strains than LAB strains but not significant. Similar results were reported in potato starch residue by Lei *et al.* (2012) that the potato starch residue fermented by *Candida tropicalis and S. fubuligera* increased the protein content to 16.1 per cent which provides good feedstuff for ruminants. Similarly, Joshi and Sandhu (1996) reported that the apple pomace fermented by *Saccharomyces* and *candida* under solid-state fermentation (SSF) increases the crude protein to 3 times more than the uncontrolled one. Iyayi and Losel (2001) reported that cassava peels waste fermented by *Saccharomyces cerevisiae* increased the protein content from 5.60 to 16.74 per cent. The increase in protein content could be attributed to the ability of microorganisms to secrete some extra cellular enzymes capable of degrading cellulolytic materials

during fermentation and also could be attributed to increase in microbial nitrogen during fermentation due to increased production of single cell proteins (Ekpe *et al*., 2007).

The results pertaining to ash, carbohydrates and moisture content of fermented jackfruit waste by the influence of different Yeast and LAB strains is presented in Table 3.

Ash: In the present study, the ash content (6.18%) was more in the jackfruit waste fermented by *Saccharomyces cerevisiae* followed by isolate yeast *Saccharomyces* spp. 5.88%) while *Saccharomyces boulardii* showed least ash content (5.06%). Among LAB strains, the ash content (6.10%) was more in the jackfruit waste fermented by *Lactobacillus brevis* and least ash content (5.41%) by *L. acidophilus*. Both yeast and LAB strains showed significant difference in ash content of jackfruit waste, higher ash content in fermented jackfruit waste with yeast and LAB compared to uninoculated showed improvement in the mineral content of the waste due to fermentation activity. Similar results were earlier reported by Joshi and Sandhu (1996) that solid-state fermentation (SSF) of dried apple pomace by *Saccharomyces* and *Candida* were found to increase the ash content of the product. Similarly, Onifade *et al.,* 2004 also reported that fermentation increases the ash content of the samples.

Carbohydrates: The carbohydrate content varied between yeast and LAB fermentation of jackfruit waste. Among yeast strains, the lowest carbohydrate content (72.37%) was observed in the jackfruit waste fermented by isolate yeast (Yeast 4), the yeast strain *Saccharomyces boulardii* (Yeast 2) showed higher carbohydrate content (75.31%). Among LAB strains, the highest carbohydrate content (74.66%) was obtained in the jackfruit waste fermented by *Lactobacillus plantarum* and least carbohydrate content (72.56%) was obtained in the jackfruit waste fermented by isolate LAB (LAB 4). Both yeast and LAB strains showed non significant difference in carbohydrate content of jackfruit waste. This could be attributed to the soluble carbohydrates which are essential substrates for the

TABLE 3

Changes in ash, carbohydrates and moisture of the fermented dried jackfruit waste as influenced by different Yeast and Lactic acid bacterial strains

Strains	Treatments	Ash (%)	Carbohydrates (%)	Moisture (%)
Control	T_1=JF waste only	4.98 ^{a}	72.77 ^{b}	5.83 ^{ab}
	T_2 = JF waste + *Saccharomyces cereviciae*	6.18 ^{d}	73.99 ^{c}	5.53 ^{bcd}
	T_3 = JF waste + *Saccharomyces boulardii*	5.06 ^{a}	75.31 ^{cde}	5.25 ^{d}
Yeast strains	T_4 = JF waste + *Candida utilis,*	5.61 ^{bc}	74.32 ^{bc}	5.90 ^{ab}
	T_5 = JF waste + *Saccharomyces* spp. (Isolate from JF waste)	5.88 ^{bcd}	72.37 ^{a}	6.00 ^{a}
	T_6 = JF waste + *Lactobacillus plantarum*	5.65 ^{bcd}	74.66 ^{cd}	5.73 ^{abc}
	T_7 = JF waste + *Lactobacillus acidophilus,*	5.41 ^{ab}	74.05 ^{c}	5.34 ^{cd}
LAB strains	T_8 = JF waste + *Lactobacillus brevis*	6.10 ^{cd}	74.08 ^{c}	5.33 ^{cd}
	T_9 = JF waste + *Lactobacillus* spp. (Isolate from JF waste)	5.81 ^{bcd}	72.56 ^{a}	6.04 ^{a}
	S.Em±	0.16	0.17	0.14
	CD (at 5%)	0.49	0.50	0.42

***** Fermentation period 7 days

growth of yeast and lactic acid bacteria for proper fermentation (Mc Donald *et al.,* 1991).

Moisture: The moisture content in the fermented JFW did not vary too much. However, the lowest moisture content (5.25%) was observed in the jackfruit waste fermented by yeast *Saccharomyces boulardii*, the isolate yeast strain showed higher moisture content (6.00%) followed by *Candida utilis* (5.90%). Among LAB strains, the least moisture content (5.33%) was obtained in the jackfruit waste fermented by *Lactobacillus brevis* which was on par with *Lactobacillus acidophilus* (5.34%). The moisture content variation in fermented jackfruit waste may be attributed to drying process and depends upon the moisture content present in substrate.

It can be concluded the Yeast strain *Saccharomyces boulardii* and LAB strain *Lactobacillus acidophilus* were found to be more efficient in enhancement of major nutrient contents of the jackfruit waste through solid state fermentation.

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