

RESEARCH PAPER

Solid State Fermentation of Mango Peel and Mango Seed Waste by Different Yeasts and Bacteria for Nutritional Improvement

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Abstract

This study was under taken to improve the nutritional contents of mango peel and seed meal (*var.* Totapuri) by solid state fermentation, using different yeasts *viz.*, *Saccharomyces boulardii*, *S. cereviceae* (MTCC 170) and lactic acid bacteria *viz.*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*. The results revealed that the mango peel fermented by single inoculation with lactic acid bacteria, *L. plantarum* and yeasts *S. boulardii* showed more decrease in pH (3.21) and TSS (4.23 Brix) while combined inoculation significantly enhanced the protein (7.88%), fat (4.18), ash (5.74 %) and minerals Ca (0.70%), Mg (0.46%), K (1.30%), Fe (313ppm), Mn (45.80 ppm) over the control. Similarly, fermentation of mango seed meal by combined inoculation of *L. plantarum* and probiotic yeasts *S. boulardii* showed a significant enhancement of protein (13.01%) over the control (7.81%). The results showed that the strains *L. plantarum* and *S. boulardii* were more efficient in enhancing the nutrients in mango peel and mango seed wastes.

Keywords: Mango peel, Mango seed meal, yeasts, lactic acid bacteria, solid state fermentation

Fruit and vegetable processing generate huge quantity of waste and about 89% of the processed foods come from fruits and vegetables. Joshi and Bhutani (1995) have reported that about 9 MT of solid residues and 8 million gallons of waste water are produced annually by processing of fruit/vegetable in the processing industry. The waste is rich source of nutrients and their wastage in the form of different fruit wastes *viz.*, mango peel and kernels, banana peel and foliage, citrus pulp, pineapple waste, apple pomace and water melon waste have been evaluated as livestock feed. It was also found that fruit wastes, especially banana foliage and peels, mango peels and seed kernels, pineapple waste either fresh, dried or ensiled could serve as an excellent alternate resources for livestock

and poultry feed (Joshi and Attri, 2006; Beerh *et al.*, 1976). Apple pomace after fermentation with different species yeasts, followed by drying made the animal feed enriched with proteins, vitamins, minerals and fats and could be used for feeding animals (Joshi and Sandhu, 1996).

The mango fruit contains edible pulp upto 33-85% of the fresh fruit, while the peel and kernel about 7 – 24% and 9 – 40%, respectively on a fresh weight basis. As mango is a seasonal fruit, about 20% of the fruits are processed for different products such as puree, nectar, leather, pickles, canned slices, and chutney. During processing of mango, peel and kernel are generated as bi-products. Mango peels and seeds are rich in valuable bioactive compounds such as polyphenols,

carotenoids, dietary fibres, enzymes phytosterols and tocopherols, whereas peel extract exhibits potential antioxidant properties. Mango peels can be used fresh, dried or ensiled. Since mango peel is high in sugar content and is palatable to ruminants, it could be considered as energy feed for ruminants (Sruamsiri *et al.*, 2009). Because of their low protein content, addition of protein source is necessary to enhance the nutrient content by fermentation.

Microbial technology is one of the methods for recycling and processing of fruit and vegetable wastes for the product development by different processes (Anon, 2007). Solid state fermentation (SSF) has been considered recently as the cheapest and more environmental friendly, relative to submerged liquid fermentation (SLF) in the production of value added industrial products such as enzymes, biofuels and feeds (Pandey, 2003). SSF is the growth and/or cultivation of microorganisms under controlled conditions in the absence of free water for the production of desired products. The low availability of water reduces the possibility of contamination by bacteria and yeasts. Pandey (1992) have defined SSF as the fermentation involving solids in absence (or near absence) of free water. However, substrate must possess enough moisture to support the growth and metabolism of micro-organisms. Microbial processing of mango by-products reduces the waste disposal problems and also adds value to the product. Joshi and Attri (2006) reviewed that the solid state fermentation of apple pomace by *S. cereviceae* followed by drying, increased the nitrogen and fat

content in the fermented and dried apple pomace and thus, the same could be used as an animal feed.

Mango kernel is a good source of starch and fat. A preliminary study showed that the seed forms 20 to 60% of the whole fruit weight, depending on the variety of mango and the kernel inside the seed, which represents from 45 to 75% of the whole seed (Maisuthisakul and Gordon, 2009). Mango seed consists of a tenacious coat enclosing the kernel. The seed content of different varieties of mangoes ranges from 9 to 23% of the fruit weight (Palaniswamy *et al.*, 1974) and the kernel content of the seed ranges from 45.7 to 72.8% (Hemavathy *et al.*, 1988). Currently, these by-products namely, mango peels and kernels are not utilized for any commercial purpose and it is discarded as a waste causing environmental pollution. Therefore, the present study was undertaken to improve the nutritional contents of mango peel and seed meal by solid state fermentation using yeasts and lactic acid bacteria, and to utilize the same as a source of animal feed supplement.

MATERIAL AND METHODS

Collection of mango peel and kernel waste: Industrial processed mango peel and kernel wastes (variety: Totapuri) available in plenty during the season (April – August) at Mother Dairy (National Dairy Development Board) near Whitefield, Bengaluru was collected. The processed raw mango peel and kernel seed wastes were collected from the mother dairy processing plant and stored in a deep freezer for the experimental studies (Plate I).



Plate I: Raw mango peel and seed kernel

The experiment consisted of two parts *viz.*, solid state fermentation of mango peel and mango seed meal with seven treatments with three replications each as detailed here:

Details of the different treatments used in the study

Mango peel	Mango seed meal
Treatments	Treatments
T1 – Mango peel waste only	T1 Control (mango seed meal only)
T2 – Mango peel + <i>L. acidophilus</i>	T2 Mango seed meal + <i>S. boulardii</i>
T3- Mango peel + <i>L. plantarum</i>	T3 Mango seed meal + <i>L. acidophilus</i>
T4 - Mango peel + <i>S. boulardii</i>	T4 Mango seed meal + <i>L. plantarum</i>
T5- Mango peel + <i>L. acidophilus</i> + <i>S. boulardii</i> ,	T5 Mango seed meal + <i>L. plantarum</i> + <i>S. boulardii</i>
T6- Mango peel + <i>L. plantarum</i> + <i>S. boulardii</i>	T6 Mango seed meal + <i>S. boulardii</i> + <i>L. acidophilus</i>
T7 - Mango peel + <i>S. cereviceae</i> (MTCC 170)	T7 Mango seed meal + <i>S. boulardii</i> + <i>L. acidophylus</i> + <i>L. plantarum</i>

Microbial cultures used

Authenticated microbial cultures of lactic acid bacteria *viz.*, lactic acid bacteria, *L. plantarum* MTCC 6161, *L. acidophilus* MTCC 10307 and yeasts *S. cereviceae* MTCC 170 were obtained from Microbial Type Culture Collection Centre, Chandigarh, India in the form of lyophilized cultures and the same were revived in the form of agar based slant cultures. Probiotic yeasts, *S. boulardii* was isolated from the commercially available sachets from local medical stores using Sabourauds culture media. These cultures were purified and characterized based on morphological and biochemical tests, and were maintained in respective agar culture media in the form of agar slant culture.

Preparation of probiotic yeasts starter culture: Purified and authenticated culture of probiotic yeasts *S. boulardii* was inoculated into conical flask containing Sabourauds Hi-media broth. The inoculated flasks were incubated at 28°C for 48 h. About 500g each of the mango peel and mango seed meal held in polythene bags were inoculated with 5%

of broth culture of probiotic yeasts containing 10⁷cfu/ml to initiate the solid state fermentation process.

Preparation of probiotic LAB starter culture: Loop full of inoculum of purified and authenticated probiotic lactic acid bacteria *L. acidophilus*, *L. plantarum* were transferred to the conical flasks containing 100 ml of Manns Rogasa Sharp broth (MRS). Inoculated flasks were incubated for 48 h at 37°C. These broth cultures of LAB were inoculated at 5% containing 10⁷cfu/ml to 500 g of mango peel and mango seed meal contained in polythene bags to initiate solid state fermentation.

Microbial processing of selected fruit wastes for nutritional improvement: The experiment was conducted to study the influence of different microbial cultures *viz.*, probiotic yeasts *S. boulardii* and lactic acid bacteria: *L. acidophilus*, *L. plantarum* for fermentation of mango peel and mango seed meal for the nutritional improvement under solid state fermentation.



Plate II: Solid state fermentation of mango peel

Preparation of mango peel waste for solid state fermentation: The industrially processed raw mango peel of 1kg weight with a moisture content of about 60 – 70% (Plate II) was placed in autoclavable polythene bags of 400 gauge and subjected to heat treatment at 115°C under autoclave for removal / reduction of anti nutritional factors. After the heat processing, the bags were inoculated with 5% microbial broth cultures containing 10⁷cfu/ml of inoculums by sealing in completely air tight polythene bags. The sealed bags were incubated at 37°C for 6 days.

Preparation of mango seed meal waste for solid state fermentation: The industrially processed raw mango seed kernels were washed with water and dried under the sun for 24 hours. The sun dried kernels were decorticated manually to collect the seeds. The seeds were then, tray dried for 48 hours at 55°C. The dried seeds were subjected for grinding followed by sieving to get the powdered mango seed meal. A 500 g seed meal was placed in autoclavable polythene bags of 400 gauge, moisture was adjusted to 70% and subjected for pasteurization at 115°C in an autoclave for the removal of antinutritional factors. After pasteurization, the bags were inoculated with microbial broth cultures containing 10⁷cfu/ml at 5% inoculums and the bags were sealed using a sealing machine. The sealed bags were kept for incubation for 6 days at 34°C.

Tray drying: After completion of 6 days of fermentation, the samples were spread uniformly in a tray drier at 50°C for 48 h for drying.

Grinding: Fermented dried mango peel and seed meal were ground in a mixer/grinder to get flour and the same was sieved in BIS 18 mesh sieve. The sieved flour of different treatments were packed in polyethylene bags of 400 gauge and stored at room temperature.

Biochemical analysis: The fermented selected fruit waste flours were subjected to proximate analysis for different chemical characteristic such as moisture, protein, fat, fiber, ash and carbohydrate and mineral viz., calcium, phosphorous, potassium, zinc, magnesium, iron. The biochemical analysis such as pH, TSS, titrable acidity was done by employing standard methods of AOAC (2005). Similarly, the proximate analysis of the samples were determined by the standard procedures of AOAC (2005).

Determination of phosphorus and magnesium

The phosphorus and magnesium contents of the samples were estimated using a Flame Photometer. Ash obtained from the ignition of mango peel waste was digested in 6N HCl, evaporated over a water bath and dissolved using a little quantity of 6N HCl and

the volume was made up to 100 ml. This ash solution was used for estimating the minerals content.

Determination of calcium, iron and zinc

The iron, calcium and zinc contents of mango waste samples were estimated using Atomic Absorption Spectrometer (AAS). Ash obtained from the ignition of fruit waste sample was digested in 6N HCl evaporated over water bath and dissolved using little quantity of 6N HCl and the volume was made up to 100ml. This ash solution was used for estimating ions of above three elements with AAS.

RESULTS AND DISCUSSION

The raw processed mango peel and seed meal were fermented by different lactic acid bacteria and yeasts by solid state fermentation. The changes in pH, TSS and titrable acidity of the fermented mango peel as influenced by different lactic acid bacteria and yeasts are presented in Table 1.

Table 1: Changes in pH, TSS and titrable acidity of the fermented mango peel waste as influenced by different yeasts and lactic acid bacteria

Treatment	pH	TSS (°Brix)	Titrable acidity (%)
T1 – Mango peel waste only	3.42	7.33	1.62
T2 – Mango peel + <i>L acidophilus</i>	3.21	7.33	2.01
T3–Mango peel + <i>L plantarum</i>	2.63	6.30	3.73
T4 – Mango peel + <i>S.boulardii</i>	3.36	5.23	3.25
T5–Mango peel + <i>L. acidophilus</i> + <i>S. boulardii</i>	3.17	4.70	3.86
T6– Mango peel + <i>L. plantarum</i> + <i>S. boulardii</i>	3.21	5.00	2.91
T7 –Mango peel + <i>S. cereviceae</i> (MTCC 170)	3.21	4.83	3.43
CD@ 5%	0.10	0.46	0.21
SEM±0.05	0.03	0.15	0.07

The results revealed that the pH ranged from 2.63 – 3.42 between the treatments, pH of mango peel without fermentation was 3.42, when fermented by lactic acid bacteria *L. plantarum* and *S. boulardii*

showed a significant decrease in pH 2.63 compared to other treatments, indicating that pH of a culture may change in response to metabolic activities. The reason might be due to production of organic acids such as citric, acetic or lactic acid which are known to causes to decrease the pH. The slight variation in pH reduction between treatments by yeasts and bacteria could be due to the fermentation potential of the microorganisms. There was a decrease in pH in the fermentation by different microorganisms. Similar findings have been reported by Raimbault and Tewe (2001) in mango peel.

The reduction of TSS varied from 7.33 to 4.23°Brix among the inoculated treatments. The result indicated that *S. boulardii* utilized more sugar than *L. acidophilus* during fermentation time (Table 1).

Upon completion of yeasts and lactic acid fermentation of mango peel, production of titrable acidity in terms of lactic acid was more important in the fermented products. The mango peel fermented by combined inoculation with probiotic yeasts and *L. acidophilus* resulted in a significant increase in the titrable acidity (3.86%) (Table 1). Fermentation is known to cause a decrease in pH with a simultaneous increase in titrable acidity.

The proximate composition of the mango peel fermented by different yeasts, bacterial cultures and unfermented mango peel is presented in Table 2. Mango peel fermented by *L. plantarum* showed higher enhancement of nutrients in comparison with the control and other treatments (Table 2). However, combined inoculation of *L. plantarum* and *S. boulardii* significantly enhanced the protein (7.88%) and fat (4.18%) content over un-inoculated control (4.89% and 1.78%, respectively). The increase in protein and fat content of the fermented mango peel sample may be attributed to the fact that the microorganisms degrade the substrate as well synthesize compounds like proteins as well as the contribution of microbial biomass (Odetokum, 2000). The results indicated that the strain *L. plantarum* and probiotic yeasts, *S. boulardii* were more efficient in enhancing the nutrients of fermented mango peel waste than others. The results are in agreement with the findings of Ojokoh (2007), who found that the fermented mango peel had an increased in protein content and decrease in pH, fiber, tannin and phytate contents. Further, the fiber content decreased in the fermented sample (8.12%) compared to unfermented sample (13.1%). This affected the carbohydrate content (calculated by difference) in which there were no significant

Table 2: Changes in proximate composition of the fermented mango peel waste as influenced by different yeasts and lactic acid bacteria

Treatments	M (%)	Protein (%)	Fat (%)	Ash (%)	Crude Fibre (%)	Carbohydrates (%)	Energy (K. Cal /100g)
T1- Mango peel waste only	5.38	4.89	1.78	3.31	13.10	71.54	321.74
T2 – Mango peel + <i>L. acidophilus</i>	5.41	5.19	2.13	3.46	9.85	73.96	335.77
T3– Mango peel + <i>L. plantarum</i>	5.42	5.17	1.90	3.40	8.05	76.06	342.02
T4– Mango peel + <i>S. boulardii</i> ,	6.26	7.49	4.06	5.08	8.60	68.51	340.54
T5–Mango peel + <i>L. acidophilus</i> + <i>S. boulardii</i>	5.65	7.45	4.25	5.14	9.15	68.36	341.49
T6– Mango peel + <i>L. plantarum</i> + <i>S. boulardii</i>	4.84	7.88	4.18	5.74	8.12	69.24	346.10
T7 – Mango peel + <i>S. cereviceae</i> (MTCC 170)	4.57	4.00	4.09	5.39	8.90	73.05	345.01
CD 5%	0.80	0.44	0.39	0.46	0.63		
SEM±0.05	0.26	0.14	0.13	0.15	0.21		

M = Moisture

differences. Odetokum (2000) reported that increase in carbohydrate content during fermentation may be due to a reduction in the fiber content and increase in both reducing sugars and total soluble sugars. This may also be attributed to the fact that during fermentation, complex carbohydrate including cellulose, pectin, lignocellulose and starch are broken down by fermenting microorganisms, thereby reducing the fiber content (Raimbault and Tewe, 2001).

The result of mineral composition revealed that mango peel fermented by combined inoculation of *L. plantarum* and *S. boulardii* significantly enhanced minerals namely, Ca (0.70%), Mg (0.46%) and K

(1.30%) over un-inoculated control as well as other treatments (Table 3). However, manganese and iron contents were reduced in the combined fermentation by *L. plantarum* and *S. boulardii*. The combined fermentation treatment was found to be rich in some essential minerals which perform various functions in the body (Akindahunsi *et al.*, 1999). The increase or decrease in the mineral component could be attributed to the fact that when dry matter is reduced the relative concentration of the components disaffected.

Mango seed /stones represents 20–60% of the fruit weight depending upon the variety. The ground mango seeds can be called as mango seed meal.

Table 3: Changes in mineral contents of fermented mango peel as influenced by different yeasts and lactic acid bacteria

Treatments	Ca (%)	Mg (%)	K (%)	Fe (ppm)	Mn (ppm)
T1 – Mango peel waste only,	0.42	0.30	0.73	230	32.60
T2 – Mango peel + <i>L. acidophilus</i>	0.42	0.28	0.81	250	26.90
T3– Mango peel + <i>L. plantarum</i>	0.53	0.39	0.71	230	28.20
T4– Mango peel + <i>S. boulardii</i>	0.71	0.30	1.28	293	44.10
T5– Mango peel + <i>L. acidophilus</i> + <i>S. boulardii</i>	0.70	0.43	1.30	313	45.80
T6– Mango peel + <i>L. plantarum</i> + <i>S. boulardii</i>	0.67	0.46	1.29	211	37.50
T7– Mango peel + <i>S. cereviceae</i> (MTCC 170)	0.58	0.38	1.26	258	40.00
CD@ 5%	0.098	0.100	0.052	41.66	3.69
SEM±0.05	0.032	0.033	0.017	13.73	1.21

Table 4: Changes in biochemical characteristics and other parameters of fermented mango seed meal as influenced by different yeasts and lactic acid bacteria

Treatments	pH	TSS	Acidity (%CA)	Moisture (%)	Protein (%)	Fat (%)	Fibre (%)	Ash (%)	Carbohydrates (g/100g)	Energy (K.Cal/100g)
T1 Control (mango seed only)	5.06	1.1	0.84	4.64	7.81	7.75	2.65	2.45	74.70	399.79
T2 Mango seed + <i>S. boulardii</i>	4.84	0.5	1.80	5.15	9.41	4.40	2.60	2.35	76.09	381.60
T3 Mango seed + <i>L. acidophilus</i>	5.00	1.0	0.98	5.64	8.42	6.27	2.60	2.40	74.67	388.79
T4 Mango seed + <i>L. plantarum</i>	4.80	0.5	1.91	5.17	12.12	4.57	2.40	2.30	73.44	383.37
T5 Mango seed + <i>L. plantarum</i> + <i>S. boulardii</i>	4.82	0.5	2.01	6.64	13.01	4.50	2.10	2.30	71.04	378.34
T6 Mango seed+ <i>S. boulardii</i> + <i>L. acidophylus</i>	4.90	0.5	1.10	5.08	12.67	5.73	2.58	2.40	71.54	388.41
T7 Mango seed+ <i>S. boulardii</i> + <i>L. acidophilus</i> + <i>L. plantarum</i>	4.90	0.5	0.98	6.64	10.90	4.84	2.52	2.40	72.70	377.96
CD@ 5%	0.12	0.05	0.23	0.34	0.86	0.39	0.31	0.32		
SEM±0.05	0.06	0.01	0.08	0.14	0.28	0.13	0.10	0.10		

Different yeasts (*S. boulardii* and *S. cereviceae*) and lactic acid bacteria (*L. plantarum* and *L. acidophilus*) were evaluated for the fermentation of mango seed meal for nutritional improvement. The changes in pH, TSS and titrable acidity of the fermented mango seed meal as influenced by different lactic acid bacteria and yeasts under solid state fermentation is presented in Table 4. The results revealed that the pH range was 5.06 – 4.80 between the treatments while, pH of seed meal without fermentation was 5.06, when fermented by lactic acid bacteria *L. plantarum* and *S. boulardii* showed slight decrease in pH (4.80), however, fermented mango seed meal showed decrease in pH indicating that change in response metabolic activities. The slight variation in pH reduction by yeasts and bacteria was due to the fermentation potential of the microorganisms. Similar results have reported by Raimbault and Tewe (2001) in mango peel.

Fermentation of mango seed meal by yeasts and lactic acid bacteria resulted in slight decreasing of TSS content (Table 4). The reduction of TSS content varied from 1.1 to 0.5° brix between inoculated treatments. The results indicated that *S. boulardii* utilized more sugar while *L. acidophilus* utilized less sugar. The mango seed meal fermented by combined inoculation with probiotic yeasts *S. boulardii* and *L. plantarum* resulted in a significant increase in the titrable acidity (2.01%) (Table 4). Fermentation is known to cause decrease in pH with a simultaneous increase in titrable acidity in several fermented products.

The proximate composition of the fermented mango seed meal by different yeasts and bacteria is shown in Table 4. Mango seed meal fermented by combined inoculation with *L. plantarum* and *S. boulardii* showed significant enhancement of protein (13.01%) over un-inoculated (7.81%). Further, mango seed meal fermented by different yeasts and lactic acid bacteria enhanced protein over un-inoculated control. There was however, a reduction in fat from 7.75 to 4.5%, fibre from 2.65 to 2.10% and a slight reduction in ash from 2.45 to 2.30%. The increase in moisture content could be due to the addition of water to the mango

waste prior to fermentation as observed by Ojokoh (2007). The increase in protein of the fermented mango seed meal was in agreement of Fadare and Ajaiyeoba (2008) findings. The high protein content could be attributed to the ability of microorganisms to synthesis and secrete some extra cellular enzymes capable of degrading cellulolytic materials during fermentation (Oseni and Ekperigin, 2007). The increase in protein level could also be attributed to increase in microbial nitrogen during fermentation due to increased production of single cell proteins (Ekpe *et al.*, 2007).

CONCLUSION

Mango peel and mango seed wastes have nutritional benefits which can further be enhanced by solid state fermentation using yeasts *S. boulardii* and bacteria *L. plantarum*. Fermentation helps to increase the protein and mineral contents in the mango peel and mango seed meal, thus improving its nutritional quality. There was also reduction in the fiber and fat which is good for the body. Hence, it could be concluded that fermented mango peel and seed meal could serve as a good source of animal feed supplement.

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