



Enzymatic Reduction of Anti-nutritional Factors in Fermenting Soybeans by *Lactobacillus plantarum* Isolates from Fermenting Cereals

* Adeyemo, S.M. and Onilude, A.A.¹

ABSTRACT

Soybean is rich in dietary protein but contains some anti-nutritional factors (ANFs), including phytates, tannins, trypsin inhibitors and oligosaccharides. It is used with cereals in weaning foods to improve the protein content and supply essential amino acids. The objective of this work, therefore, was to use microorganisms, specifically *Lactobacillus plantarum* and the enzymes it produces to reduce anti-nutritional factors and improve the nutritional composition of such food blends. Nine strains of *Lactobacillus plantarum* isolated from spontaneously fermenting cereals, identified and characterised, were selected based on the abundant production of alpha-galactosidase for the fermentation of the legume. Samples were subjected to fermentation for 5 days and the reduction of anti-nutritional factors was monitored. Anti-nutritional factors and alpha-galactosidase were determined by UV-spectrophotometry. Data were analysed using ANOVA at $p = 0.05$. Fermentation reduced the tannin content in the raw samples from 1.93 to 0.12 mg/g. Phytate content reduced from 1.16 to 0.04 mg/g. The trypsin inhibitor and protease inhibitor also reduced from 1.20 to 0.010 and 1.2 to 0.020 respectively. The production of alpha-galactosidase by *L. plantarum* (1.8 unit/ml) enhanced the reduction while the nutritional composition of the food blend in which the soybean was added improved significantly. The use of alpha-galactosidase enzyme by *Lactobacillus plantarum* from local food sources is thus shown to reduce anti-nutritional factors in soybeans, which can be of benefit in enhancing the nutritional quality of cereal-legume gruels.

Keywords: Soybeans, enzymes, anti-nutrients, *Lactobacillus plantarum*, alpha-galactosidase.

Introduction

Lactic acid bacteria are a heterogeneous group of bacteria that are generally regarded as safe (GRAS). The use of these organisms in food products dates back to ancient time and they are used mainly because of their contribution to flavour, aroma and increased shelf life of fermented products (Nes *et al.*, 1996). Various members of this group are used commercially as starter cultures in the manufacture of foods including dairy products, fermented vegetables, fermented dough, alcoholic beverages, probiotics in animal feeds and meat products, lactic acid fermentation of sorghum and maize-based

cereals used as infant weaning foods (Wakil and Onilude, 2009).

Lactobacillus plantarum has been used for the preservation of food for increased shelf life and flavours to get the desired aroma in food (Daeshel, 2004). *L. plantarum* is one of the lactic acid producing bacteria that have been used for centuries for the preservation of human food. It is a simple, safe method that is still used in many undeveloped countries. In addition, scientists conducting different research stated that lactic acid fermentation, such as that used with *L. plantarum*, is the safest way to preserve food. It is one of the most versatile probiotics. *L. plantarum* has also been implicated in the lowering of anti-nutrients and unwanted materials in food

¹ Microbial Physiology and Biotechnology Unit,
Department of Microbiology, University of Ibadan.

* corresponding author: daystarzion@yahoo.com

during fermentation (Smid *et al.*, 2005; Ammor *et al.*, 2006).

L. plantarum has been implicated in the production of organic acids and aromatic compounds such as lactic acid, acetic acids, etc. (Monica *et al.*, 1999). The production of volatile compounds by the probiotic strain, *L. plantarum* in cereal-based media (oat, wheat, barley and malt) was investigated in the work of Salmeron *et al.* (2009) and co-workers which shows the anti-microbial properties of *L. plantarum*. *L. plantarum*, however, has over the years played a significant role in the reduction of anti-nutritional compounds and detoxification of harmful substances in food during fermentation due to its ability to produce enzymes in food. For example, linamarase enzyme in cassava production causes a detoxification in the linamarin while alpha-galactosidase enzyme is able to reduce the anti-nutrients in cereals during fermentation (Giraud *et al.*, 1992; Smid *et al.*, 2005; Ammor *et al.*, 2006).

Steinkraus (1995) pointed out that the traditional fermentation of foods by microorganisms serves several functions such as enrichment of food substrate biologically with proteins, essential amino acids, essential fatty acids and vitamins; this improves the digestibility and acceptability of foods, detoxification of toxic substances in foods, preservation of substantial amounts of food through production of anti-bacterial compounds such as lactic acid and acetic acid, a decrease in time and fuel requirement during cooking and enrichment of the diet through the development of flavours, aroma, taste, palatability and texture of such foods.

Cereal and legumes which are also used as weaning foods also contain significant amounts of anti-nutrients, disaccharides and oligosaccharides. These further lower their nutritional quality. Legumes contain some natural toxicants which include tannins, phytic acid, protease and trypsin inhibitors, saponins, metal chelates, cyanogens, isoflavonoids, phytoalexins, flatus factors, etc (Pariza, 1996). Most cereals like sorghum and millet also contain some

appreciable amounts of phytate, tannins, protease and trypsin inhibitors and polyphenols. Some of these substances reduce the nutritional value of the food by interfering with mineral bioavailability and digestibility of proteins and carbohydrates (Salunkhe *et al.*, 1990; Haard, 1999; MacDonald *et al.*, 2012). Since legumes are usually consumed along with cereals in form of additives, or as protein source, proper processing of these food substances should therefore be encouraged to eliminate these anti-nutrients before they are consumed (Reddy and Pierson, 1994).

The content and quality of legume and cereal proteins is also improved by fermentation. Natural nutritive value and available lysine are also improved (Wakil and Onilude, 2009). Bacterial and yeast fermentations involving proteolytic activity are expected to increase the biological availability of essential amino acids and degrade carbohydrates and other unwanted substances.

Among the latest research on *L. plantarum* is that which has shown it to be highly effective in preventing soy-related allergies. In the work of Taylor *et al.* (2007) and Frias *et al.* (2008), soy seeds, flour, or meal was fermented using a variety of microorganisms.

Anti-nutrients have negative effects on the nutritional quality of the food in which they are present, and their attendant toxic effects. Hence, the need for their removal or reduction to certain safe level factors before being consumed by man or animals (Martin *et al.*, 1991; Horwitz and Latimer, 2008). They are present in many plant species, particularly legumes. The following are examples of the most widely studied anti-nutrients:

- **Protease** inhibitors which inhibit the activity of trypsin, chymotrypsin and other intestinal proteases. Their presence results in impaired growth and poor food utilization (Salunkhe *et al.*, 1990) and interference with digestion, causing pancreatic hypertrophy and metabolic disturbance of sulphur and amino acid utilization (Reddy and Pierson, 1994).

- **Tannins** are oligomers of flavan-3-ols and flavan-3, 4-diols. These compounds are concentrated in the bran fraction of legumes. Tannin-protein complexes may cause inactivation of digestive enzymes and reduce protein digestibility by interaction of protein substrate with ionisable iron (Salunkhe *et al.*, 1990).
- **Phytates** occur in several vegetable products. Their presence may affect bioavailability of minerals, solubility, functionality and digestibility of proteins and carbohydrates (Salunkhe *et al.*, 1990).

Several workers have shown that most methods of food processing such as cooking, roasting, dehulling and, most importantly, fermentation by Lactic Acid Bacteria reduce the anti-nutrients content of foods (Shalini, 2006; Buheloes, 2007). Fermentation technology has been employed over the years to reduce the anti-nutritional factors and oligosaccharides that are present in cereals and legumes used as weaning food. Adeyemi and Beckley (1986) reported that fermentation is able to solve some of the problems involved in the preparation of adequate weaning food for infants. It also reduces the phytic acid content by the action of enzyme phytases (produced during fermentation) which catalyses the conversion of phytate to inorganic orthophosphate. Tannin, protease and trypsin inhibitor content has also been reportedly reduced by fermentation (Haard, 1999; MacDonald *et al.*, 2012).

The growth-inhibiting effect of feeding pre-treated and modified soybeans to humans and young animals reduces the factors mentioned above and they become less detrimental to health. It has also been shown that soybean treatments improved growth performance and improved the nutritional quality of the foods in which they are added (Shalini, 2006; Buheloes, 2007).

Materials and Methods

Fermentation of soybeans with *L. plantarum* isolates

Two batches each of one gram, two grams and

three grams each of the raw, cooked and roasted soybeans were milled into powder with waring blender. It was sieved to pass through a 0.5 mm diameter sieve. The samples were weighed in triplicates into screw capped bottles. 10 ml sterile distilled water was added to it for the sample to become a paste. 1 ml each of the standardised inocula of *L. plantarum* was added separately to the two batches and allowed to ferment for 5 days. Samples were taken for anti-nutritional content determination every 24 h. A control was set up for the samples with additional 1 ml of sterile distilled water in the samples without the organisms.

Determination of tannin

One gram of each sample was weighed into a beaker. Each was soaked with solvent mixture (80 ml of acetone and 20 ml of glacial acetic acid) for 5 h to extract tannin. The samples were filtered through a double layer filter paper to obtain the filtrates which were stored for further use. A standard solution of tannic acid was prepared ranging from 10 ppm to 30 ppm. The absorbances of the standard solution as well as that of the filtrates were read at 500 nm on a Spectronic 20, England spectrophotometer (AOAC, 1990).

Determination of phytates

Two grams of each sample was weighed into a 250 ml conical flask. 100 ml of 2% hydrochloric acid was used to soak each sample in a conical flask for 3 h. This was filtered through a double layer of hardened filter paper Whatman No. 3. 50 ml of each filtrate was placed in 250 ml beaker and 107 ml of distilled water was added in each case. 10 ml of 0.3% ammonium thiocyanate solution was added into each solution as indicator. This was titrated with standard iron (III) chloride solution, which contained 0.00195 g iron per ml. The end point is slightly brownish yellow, which persisted for 5 m. The percentage phytates was calculated using the formula:

$$\% \text{ Phytates} = \frac{X \times 1.19 \times 100}{0.00195}$$

Where X = Titre value (AOAC, 1990).

Determination of trypsin inhibitors

Two batches each of the samples (0.2 g each) were weighed into a screw capped centrifuge tube. 10 ml of 0.1M phosphate buffer was added and shaken vigorously. The contents were left at 25°C for 1 h on a UDY 60 shaker, England. The suspension obtained was centrifuged at 5000 rpm for 5 min and filtered through Whatman No. 42 filter paper. The volume of each was adjusted to 2 ml with phosphate buffer. The test tubes were placed in a water bath, maintained at 37°C. 6 ml of 5% Trichloroacetic Acid (TCA) solution was added to one of the tubes to serve as a blank. 2 ml of casein solution was added to all the tubes, which was previously kept at 37°C. These were incubated for 20 min. The reaction was stopped after 20 min by adding 6 ml of TCA solution to the experimental tubes and shaken. The reaction was left for 1 h at room temperature after which it was filtered through Whatman No. 42 filter paper. Absorbance of filtrate from sample and trypsin standard solutions was read at 380 nm on a Spectronic 20, England spectrophotometer. The trypsin inhibitor in mg/g sample was calculated using the formula:

Trypsin mg/g =

$$\frac{A_{STD} - A_{sample}}{19 \times \text{sample wt in g}} \times \text{Dilution factor} \times 1000$$

(AOAC, 1990)

Determination of protease inhibitor

Egg albumin 2% solution and 0.1% solution of Bromelain, both in pH 7 phosphate buffer, were prepared. 5 ml of the egg albumin substrate and 1ml of the Bromelain enzyme was incubated at 55°C for 10 min. 5 ml 10% TCA was added to stop the reaction. The precipitate was filtered off with Whatman No. 1 filter paper and the absorbance of the filtrate was measured at 280 nm on the Atomic Absorption Spectrophotometer (AAS) labelled (Ai). The entire procedure was repeated but incubating with the enzyme and substrate mixture, i.e. 1 ml of the extract of the material for protease inhibitor determination labelled (As). The absorbance of the filtrate was measured at 280 nm. This was denoted Ai.

$$\% \text{ Protease Inhibitor} = \frac{A_s - A_i \times 100}{A_s}$$

Where A_s = Absorbance of sample

A_i = Absorbance of blank/initial

(Cuatrecasas and Anfisen, 1991)

Assay of alpha-galactosidase and characterization

Alpha-galactosidase activity was determined using the method of Mital *et al.* (1973) as modified by Hassan and Durr (1974). The assay medium consisted of 200 μ l of 100 mM sodium acetate buffer, pH 5.0, 2.5 ml of 2 mM PNP-alpha Gal solution and 0.5 ml of enzyme preparation. The assay was carried out for 15 min at 50°C and stopped by the addition of 1ml of 0.5 M sodium carbonate. The amount of Para-nitrophenyl- α -D-galactopyranoside (PNP- α -G) released was determined at 410 nm by taking the absorbance. Undiluted mixture treated in the same way was used to set the spectrophotometer to zero.

*One unit of enzyme activity was defined as the number of macromolecules of p-nitrophenyl liberated from PNP-G per ml of cells as read from a standard curve.

Results and Discussion

Table 1 shows the reduction of anti-nutrients in soybean during pre-treatment and fermentation. Soybeans contain ANFs like tannin, phytates, trypsin and protease inhibitor. It was pre-treated by cooking and roasting. It was observed that roasting reduced the different anti-nutritional factors better than other pre-treatment methods. The raw soybeans contain 1.93 mg/g tannin; it was reduced to 1.12 mg/g by cooking for 1 h while roasting reduced it to 0.49 mg/g. Roasting also reduced the phytates content from 1.6 to 0.25 mg/g, trypsin inhibitor from 1.20 to 0.025 mg/g and protease inhibitor from 1.20 to 0.03 mg/g. Cooking reduced the anti-nutritional factors but not as much as roasting. There was a significant difference between the pre-treatment methods. This agrees with the work of Livingstone *et al.* (1990).

Fermentation also reduced the anti-nutritional factors in the samples. Fermentation reduced the tannin content in the raw sample from 1.93 to 0.12 mg/g. Phytates content reduced from 1.16 to 0.04 mg/g. The trypsin inhibitor and protease inhibitor reduced from 1.20 to 0.010 mg/g and 1.2 to 0.020 mg/g respectively. The roasted sample was chosen for further work using *L. plantarum* isolates for all the pre-treatment methods.

Table 2 shows the production of alpha-galactosidase enzyme by the *L. plantarum* isolate used for the fermentation of the soybeans. The organisms chosen were labelled *LV1, *LV2 and *LV3 because they were the ones that produced the enzymes in abundance (1.8 unit/ml). The production of enzymes enhanced the hydrolysis of the soybeans during fermentation as well as the reduction of the anti-nutritional factors. This agrees with the work of Taylor *et al.* (2007).

Table 1: Anti-nutritional factors (mg/g) in the milled soybeans after the pre-treatment of the samples and fermentation for 5 days

A.N.F.	Raw (Day 0) (Before fermentation)	Cooked	Roasted	Fermented with <i>L. plantarum</i> isolates (Day 5)
Tannin	1.93 ± 0.19 ^a	1.12 ± 0.02 ^c	0.49 ± 0.12 ^d	0.120 ± 0.05 ^e
Phytate	1.16 ± 0.05 ^a	0.28 ± 0.02 ^c	0.25 ± 0.03 ^d	0.047 ± 0.03 ^e
Trypsin inhibitor	1.20 ± 0.12 ^a	0.05 ± 0.05 ^c	0.02 ± 0.25 ^d	0.010 ± 0.02 ^e
Protease inhibitor	1.20 ± 0.02 ^a	0.05 ± 0.05 ^c	0.03 ± 0.03 ^d	0.020 ± 0.03 ^e

* Each value is a mean of duplicate determinations with Standard Error

Table 2: Production of α-Galactosidase enzyme (Unit/ml) by selected *L. plantarum* isolates

Isolate code	Conc. (units/ml)
<i>L. plantarum</i> TV 1	*1.114 ± 0.020
<i>L. plantarum</i> TV 2	1.102 ± 0.003
<i>L. plantarum</i> TV 3	1.108 ± 0.025
* <i>L. plantarum</i> Lv1	1.818 ± 0.002
* <i>L. plantarum</i> Lv2	1.820 ± 0.025
* <i>L. plantarum</i> Lv3	1.805 ± 0.010
<i>L. plantarum</i> Co1	1.217 ± 0.020
<i>L. plantarum</i> Co2	1.212 ± 0.005
<i>L. plantarum</i> Co3	1.202 ± 0.003

*All value recorded are means of replicate determination +SE

* LV1, LV2, LV3 – *L. plantarum* selected for fermentation.

Table 3 shows the nutritional analysis of soybean samples before and after fermentation with *L. plantarum*. It was observed that the nutritional composition of the food blends (the soybean was mixed with *ogi* in ratio 1:3 to prepare weaning food

for children) improved significantly after soybean was added to it because the anti-nutritional factors had reduced. This agrees with the work of Ado *et al.* (1995) on the improvement of weaning foods by the addition of soyflour.

From the result shown in Tables 1 – 3, it can be observed that fermentation with *L. plantarum* isolates reduced the anti-nutritional factors in the food blend. Also, the different pre-treatment methods affected the anti-nutritional factors. Roasting the soybeans reduced all the antinutritional factors significantly than cooking of the sample; this is shown when compared with the raw soybean samples. This was why the roasted sample was chosen for the composition of the food blend.

The result of this work agrees with that of Ammor *et al.* (2006) and Wakil and Onilude (2009) on the reduction of anti-nutritional factors in food during fermentation. Anti-microbial properties which lower the pathogenic organisms in food are also

produced during fermentation. This is another attribute of *L. plantarum* that is desirable in weaning foods (Taylor *et al.*, 2007; Salmeron *et al.*, 2009).

Conclusion

Results obtained in this work revealed that *L. plantarum* reduced the anti-nutritional factors in

soybeans significantly, which can be useful in producing a good soya-based weaning food. The reduction of ANFs in soybeans by *L. plantarum* during fermentation is thus a desirable quality of the organisms that can be harnessed in the formulation of good weaning diets for infants.

Table 3: Nutritional analysis of milled soybean samples before and after fermentation with *L. plantarum*

Parameters	Raw	Roasted	Cooked	Roasted and fermented with <i>L. plantarum</i>
Moisture content %	*11.2 ± 0.02	8.7 ± 0.14	48.8 ± 0.20	42.1 ± 0.20
Protein %	30.5 ± 0.25	31.8 ± 0.10	17.3 ± 0.10	33.2 ± 0.20
Ether extract (Fat) %	22.1 ± 0.10	23.4 ± 0.25	12.9 ± 0.03	14.1 ± 0.20
Ash %	4.3 ± 0.02	4.5 ± 0.03	2.7 ± 0.25	1.3 ± 0.02
Crude fibre %	2.9 ± 0.05	3.1 ± 0.03	1.8 ± 0.22	1.2 ± 0.10
Carbohydrate (by difference) %	29.0 ± 0.02	28.5 ± 0.02	16.5 ± 0.14	8.1 ± 0.11
Ascorbic Acid (mg/100 g)	18.3 ± 0.03	6.5 ± 0.05	12.8 ± 0.50	16.3 ± 0.14
Reducing sugar %	1.1 ± 0.02	0.4 ± 0.05	0.5 ± 0.50	0.8 ± 0.15
Total sugar %	2.4 ± 0.10	1.2 ± 0.02	1.5 ± 0.10	1.8 ± 0.14
Thiamine (mg/100 g)	1.0 ± 0.10	0.4 ± 0.25	0.5 ± 0.05	1.8 ± 0.33
Riboflavin (mg/100 g)	0.5 ± 0.05	0.2 ± 0.25	0.3 ± 0.50	0.8 ± 0.33
Niacin (mg/100 g)	2.2 ± 0.25	1.6 ± 0.25	1.2 ± 0.14	2.7 ± 0.13
Ca ⁺⁺ (mg/100 g)	175 ± 0.05	170 ± 0.33	145 ± 0.03	180 ± 0.30
Fe ⁺⁺ (mg/100 g)	5.5 ± 0.02	4.3 ± 0.33	4.0 ± 0.33	4.9 ± 0.30
P ₀₄ ⁺⁺ (mg/100 g)	280 ± 0.10	275 ± 0.33	220 ± 0.25	285 ± 0.25

*All value recorded are means of replicate determination ±SE

Fermentation of the legume through microbial activity led to the hydrolysis and the reduction of ANF. These reduced with fermentation time which could be due to the breakdown and degradation of the anti-nutrients into smaller units by the action of the enzymes mobilized during the fermentation period and the activity of alpha-galactosidases.

The nutritional composition of the blend also increased with fermentation time. This further justifies the use of cereal and legume-based gruels

as weaning foods for infants. The problem of preparing adequate weaning foods for infants can thus be overcome by making use of modified forms of such readily available local raw materials.

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References

- Adeyemi, I.A. and Beckley, O. (1986). Effect of period of maize fermentation and souring on chemical properties and amylograph pasting viscosities of *ogi*. *Cereal Science* 4: 353 – 360.
- Ado, K., Lykins, O. and Cotton, C. (1995). Indigenous fermentation and soy fortification: Effects on protein quality and carbohydrate digestibility of a traditional Ghanaian corn meal. *Food Chemistry* 57: 377 – 380.
- Ammor, S., Tauveron, G., Dufour, E. and Chevallier, I. (2006). Antimicrobial activity of Lactic acid bacteria against spoilage and pathogenic bacteria isolated from the same meat small-scale facility. I-screening and characterisation of the antimicrobial compounds. *Food Control* 17: 454 – 465.
- Buheloes, A., Knetcher, N. and Pitcher, B. (2007). Correlation between lactose absorption and mutations of the lactase-phosphohydrolase. *Brazilian Journal of Medical and Biological Research* 8: 7 – 19.
- Daeshel, M.A. (2004). Strains of *Lactobacillus plantarum* found in foods from different cultures. *African Journal of Food and Nutritional Sciences* 49: 112 – 115.
- Frias, E.D., Juana, M.P. and James, S.P. (2008). Overcoming allergic reactions to soy. *Journal of Agriculture and Food Chemistry* 22: 111 – 119.
- Giraud, E., Gosselm, L. and Raimbault, M. (1992). Degradation of cassava linamarin by lactic acid bacteria. *Biotechnology Letters* 14: 593 – 598.
- Haard, N.F. (1999). Cereals: Rational for fermentation In: *Fermented Cereals – A Global Perspective*. FAO, Agricultural Sciences Bulletin. No. 138 FAO/UN, Rome, Italy.
- Hassan, N. and Durr, I.F. (1973). Utilization of lactose, glucose and galactose by a mixed culture of lactic acid bacteria isolated from milk. *Applied and Environmental Journal* 32: 951 – 958.
- Horwitz, W. and Latimer, G. Jr, editors. (2008). *Official Methods of Analysis of AOAC*. AOAC INTERNATIONAL. 20th ed. Gaithersburg, Marcel Dekker, Inc.
- Livingstone, A.S., Feng, J.J. and Malleshi, N.G. (1993). Development and nutritional quality evaluation of weaning foods based on malted, popped and roller dried wheat and chickpea. *International Journal of Food Science and Technology* 28: 35 – 43.
- MacDonald, A., Evans, S., Cochrane, B. and Wildgoose, J. (2012). Weaning infants with phenylketonuria: A review. *Journal of Human Nutrition and Dietetics* 25: 103 – 110.
- Martin P., Shalini, O.O. and Werry, K.T. (1991). Evaluation of the LAB isolated from faeces of breast-fed infants. *African Journal of Biotechnology* 6: 939 – 949.
- Mital, R., Worthington and Larry, R.B.C. (1973). Alpha-galactosidase activity of fungi on intestinal gas-forming peanut oligosaccharide. *Journal of Agriculture Food and Chemistry* 22: 816 – 914.
- Monica, H., Luis, A., Garcia and Mario Diaz. (1999). Organic acids in cider. *Journal of Biotechnology* 5: 229 – 232.
- Nes, I.F., Diep, D.B., Hanvastein, L.S., Bruberg, M.B., Eijsink, V. and Holo, H. (1996). Biosynthesis of bacteriocins in lactic acid bacteria. *Antonie van Leeuwenhoek* 70: 113 – 128.
- Pariza, M.W. (1996). Toxic substances. In Birch, G. (ed). *Food Chemistry* Marcel Dekker, New York, pp. 825 – 840.
- Reddy, N.R. and Pierson, M.D. (1994). Reduction in antinutritional and toxic components in plant foods by fermentation. *Food Research International* 27: 281 – 285.
- Salmeron, I., Fucinos, P., Charalampopoulos, D. and Pandiella, S.S. (2009). Volatile compounds produced by the probiotic strain *Lactobacillus plantarum* NCIMB 8826 in cereal-based substrates. *Food Chemistry* 117: 265 – 271.
- Salunkhe, D.K., Chavan, J.K. and Kaden, S.S. (1990). *Dietary Tannins: Consequences and Remedies*. Boca Roton, FL, CRC Press, pp. 67 – 75.
- Shalini, J.P. (2006). Reducing by-product formation in L-lactic acid fermentation by *Rhizopus oryzae*. *Journal of Industrial Microbiology and Biotechnology* 56: 117 – 123.
- Smid, E.J., Enckevort, A., Wegkamp, J., Boekhorst, D., Molenaar, J., Hugenholtz, R.J. and Siezen, R.J. (2005). Metabolic models for rational improvement of lactic acid bacteria as cell factories. *Journal of Applied Microbiology* 98: 1326 – 1331.
- Steinkraus, K.H., (ed.) (1995). *Handbook of Indigenous Fermented Foods*. New York, Marcel Dekker, Inc.
- Taylor, F., John, R. and Mitchell, D. (2007). *The Wonder of Probiotics*. St. Martin's Press, New York, NY, pp. 203 – 245.
- Wakil, S.M. and Onilude, A.A. (2009). Microbiological and chemical changes during production of malted and fermented cereal-legume weaning foods. *Advances in Food Sciences* 31: 139 – 145.