

Review Article FERMENTATION OF FRUITS AND VEGETABLES

Nasreena Sajjad¹, Akhtar Rasool², Abu Bakr Ahmad Fazili³ and Eijaz Ahmed Bhat^{4*}

 ¹Department of Biochemistry, University of Kashmir, Hazratbal, Jammu and Kashmir, India
² Department of Environmental Science, Osmania University, Hyderabad, Telangana, India
³ Colin Ratledge Center for Microbial Lipids, School of Agriculture Engineering and Food Science, Shandong University of Technology, Zibo 255049, China.
⁴Life science institute, Zhejiang University, Hangzhou, Zhejiang, 310058, P.R. China *Corresponding author email : eijazbhat05@gmail.com

Abstract

The fruits and vegetables are perishable food items owing to their high water activity and nutritive value. These conditions are basic and crucial in tropical and subtropical nations which support the development of decay causing microorganisms. Lactic acid fermentation expands timeframe of realistic usability of fruits and vegetables and furthermore improves a few advantageous properties, which include nutritive value and flavours, and less lethality. Fermented fruits and vegetables can be utilized as potential source of probiotics as they harbor a few lactic acid microscopic organisms, for example, *Lactobacillus plantarum, L. pentosus, L. brevis, L. acidophilus, L. fermentum, Leuconostoc fallax, and L. mesenteroides.* This review provides an overview on the current research prospects of Lactic acid fermentation of fruits and vegetables, lactic acid bacteria microbiota and spontaneous fermentation. It also provides an insight into traditionally used fermented fruits and vegetables in India.

Keywords: Fermentation, Fruits and Vegetables, Lactic Acid

Introduction

Foods undergo processing for various reasons. Removal of unwanted anti-nutritional components and enhancing their storage life takes place. Fermentation is one of the important food processing technology (Hassan et al., 2014). The indigenous fermented foods like curd, bread, cheese and wine have been made and eaten for years and they are related to culture particularly in rural areas. Fermentation is considerably efficient, energy preservation process that increases the shelf life and reduces the need of preservation technology (Capozzi et al., 2017). Fermented foods are in demand worldwide. It contributes to diet of many people. In Asia the fermented foods is common tradition. The fermented foods provide protein, minerals and various other nutrients. For example soy sauce is consumed worldwide. It is used as ingredient in various diets of Japan, China etc. About one billion litres are produced each year in Japan alone (Steinkraus, 1994). Gundruk a fermented vegetable product is essential for different Nepali communities especially in far flung areas. It is eaten as side dish and consumed as an appetiser in the bland, starchy diet. Annually gundruk in Nepal is approximately 2,000 tons. It provides minerals especially during the off-season when the diet consists primarily of starchy tubers and maize, which are low in minerals. In Africa the fermented cassava products (like gari and fufu) constitute part of their diet.

The various microbial and enzymatic processes which are responsible in the making of fermented foods was unknown for several years. There is potential for the use of micro-organisms towards meeting the growing world demand for food. Because of the tremendous importance of fermented fruits and vegetables in food preservation, it is necessary that the knowledge of their production is not lost. The introduction of western foods poses a threat to theses traditional foods (Battcock, 1998).

World Health Organization (WHO) recommends vegetables and fruits in daily diet to prevent various diseases like hypertension, coronary heart problems, and risk of strokes. People like to consume the foods and beverages which is fresh, nutritional, healthy and ready to eat or ready to drink (Prado et al., 2008). Lactic acid (LA) fermentation of vegetables and fruits is a common practice which is done to preserve the nutritional and sensory features of food items (Karovicova and Kohajdova, 2005). A variety of lactic acid bacteria (LAB) were isolated from different traditional fermented foods (Anandharaj and Sivasankari, 2013). Usually LAB like Lactobacillus plantarum, L. pentosus, L. brevis, L. fermentum, L. casei, Leuconostoc mesenteroides, and Pediococcus pentosaceus, are used in Asian fermented foods. Probiotic is a relatively new word meaning "for life" and it is commonly used to name the bacteria related with helpful effects for humans. Probiotics are basically live microbial feed such as Lactobacillus plantarum, L. casei, L. acidophilus, and Streptococcus lactis which are supplemented by food that benefit the host by maintaining intestinal balance (Tamang, 2009). Various researches have reported that addition of probiotics to food gives many health benefits like lowering of serum cholesterol, regulates gastrointestinal function, improved immune function and decrease risk of colon cancer (Berner and O'Donnell, 1998; Rafter, 2003; Anandharaj, Sivasankari et al., 2014).

Spontaneous fermentation and lactic acid bacteria microbiota

The epiphytic microbial population of plants is generally affected by changes in physical and nutritional conditions (Lindow and Brandl, 2003). Each specific plant gives particular niche in terms of chemical composition, buffering capacity, competitive biota and natural antagonist compounds (Buckenhüskes, Jensen *et al.*, 1997). Moreover, temperature and harvesting conditions can also affect the microbiota. It can be inferred that every species of plant harbors a dominant and constant microbiota (Yang, Crowley *et al.*, 2001) Microbial population of raw vegetables and fruits ranges between 5.0 and 7.0 log cfu g^{-1} (Spurr Jr., 1994). Fruits usually get dominated by yeasts and fungi (Nyanga *et al.*, 2007). The species which belong to *Cryptococcus, Candida, Saccharomyces, Pichia, Hansenula, Debaromyces* and *Rh odotorula* genera are found to be dominating (Postmaster, 1997; Nielsen, 2007; Di Cagno, 2010). Moreover, Coagulase-positive staphylococci and other faecal bacteria can also occur in raw vegetables. They don't pose a serious threat to human health owing to their low density, inhibition by hostile environment and microbial competition unless certain circumstances takes place for example using highly contaminated water.

Lactic acid bacteria constitute small part (2.0-4.0 log cfu g⁻¹) of microbiota of raw vegetables and fruit. Heterofermentative and homo-fermentative species which belong to Leuconostoc, Lactobacillus, Weissella, Enterococcus and Pediococcus genera were identified that depends on the vegetables. Weissella cibaria/Weissella confusa and, especially, Lactobacillus plantarum were the common species. Raw vegetables and fruits are subjected to spontaneous lactic acid fermentation. when favourable conditions of anaerobiosis, water activity, salt concentration and temperature occur. Generally, Gram-negative bacteria are inhibited at the early stage of lactic acid fermentation. Back slopping (e.g., inoculation with a small amount of a previously and successfully fermented raw material) is easy method for induction of spontaneous fermentation. It is traditionally employed for sauerkraut fermentation to increase the dominance of the best adapted strains, that shorten the fermentation and reduce the risk of failure (Leroy and De Vuyst, 2004). The succession of hetero- and homofermentative lactic acid bacteria, together with or without yeasts leads to spontaneous fermentation of vegetables and fruits.

The microbiota of vegetables and fruits consist mainly of microbial agents which are responsible for the spontaneous lactic acid fermentation (Kim and Chun, 2005). The microbiota which were responsible for the spontaneous fermentation of kimchi was investigated on large scale (Park et al., 2010). Generally, Leuconostoc mesenteroides and Pediococcus pentosaceus induce the early stage of fermentation, and then the L. plantarum and Lactobacillus brevis or Lactobacillus maltaromicus and Lactobacillus bavaricus dominate that depends on the temperature of incubation (20-30 °C or 5-7 °C, respectively). Recently, metagenomic and metabolome approaches were employed to characterize the microbial community and related metabolites of kimchi during spontaneous fermentation (Jung et al., 2011). The number of unclassified phylotypic groups during early fermentation was greatest (ca. 97%), afterwards decreased as the process carried on. The genus Leuconostoc was the most abundant throughout fermentation but the abundance of Lactobacillus markedly increased after the first stage of fermentation.

The microbiota responsible for the spontaneous fermentation of various raw vegetables and fruits is of larger interest as tool to improve the microbial safety of fermented foods (Paramithiotis *et al.*, 2012). Biopreservation occur due to the synthesis of various antagonistic primary and secondary metabolites which include organic acids, carbon dioxide, ethanol, hydrogen peroxisde and diacetyl, antifungal

compounds (e.g., fatty acids, phenyllactic acid), bacteriocins and antibiotics (reutericyclin) (Fan and Hansen, 2012). Bacteriocins have attracted the interest due to their potential use as safe and natural food preservatives, among these compounds.

Notwithstanding the reliable value of the spontaneous fermentation to stabilize and preserve raw vegetables and fruits (e.g., cucumbers, onions, eggplants, red-beets, capers, lychee, cocoa beans), various factors are in favour of the use of selected starters. The risk of fermentation failure, the inadequate inhibition of spoilage and pathogen microorganisms, and the undesirable and not predictable variations of the sensory, nutritional and rheology properties are some of these factors. Contrarily to other fermented foods (e.g., cheeses, sausages and leavened baked goods), the use of starter cultures in vegetable and fruit fermentation is increased only recently (Molin, 2001; Montet *et al.*, 2006).

The use of starter cultures

Two major options may be used for the controlled lactic acid fermentation of vegetables and fruits: the use of autochthonous or allochthonous starters (Di Cagno, Cardinali *et al.*, 2010; Di Cagno, Surico *et al.*, 2011). Autochthonous starters are isolated from and re-used on the same raw matrix, apart from the geographical origin. Allochthonous starters means isolated from certain raw matrices but used to ferment different products. Mostly, commercial starters, that are used to ferment various vegetables and fruits, mostly coincide with the above definition of allochthonous strains. More than for other food matrices, this differentiation between starters is of fundamental importance for plant species, which represent completely different niches.

Fermentation of Fruits and Vegetables by LAB

Fermentation is considered as oldest technology to increase shelf life of the perishable foods. LA fermentation of cabbage to sauerkraut has studied extensively. With this fermentation of various vegetables has emerged, like cucumbers, beets, turnips, cauliflower, celery, radishes, and carrots (Roberts and Kidd, 2005). Sauerkraut, fermented cucumbers, and kimchi have been studied because of their commercial importance.

Traditional Fermented Fruits and Vegetables in India

Various perished foods are bioprocessed for storage and further consumption, in many parts of Himalayan regions of India. Gundruk, sinki, and khalpi are some examples of Lactic acid fermentation fermented vegetable product consumed by people of Nepal, Sikkim, and Bhutan. Lactobacillus brevis. L. plantarum, Pediococcus pentosaceus, P. acidilactici and Leuconostoc fallax are the common and dominant LAB which is involved in traditional fermented vegetables. Various LAB strains can also possess protective and functional properties that render them as potential candidates for use as starter culture for controlled and optimized production of fermented vegetable products (Tamang et al., 2009).

Khalpi

Khalpi or khalpi is a fermented cucumber (*Cucumis sativus* L.) product, usually eaten by the Brahmin Nepalis in Sikkim. In the whole of Himalayan region this is only reported fermented cucumber product (Yan *et al.*, 2008). Ripened cucumber is sliced and then sun dried for couple of

days. It is then kept in bamboo vessel. It is covered by dried leaves as to make it airtight. It undergoes fermentation naturally at room temperature for 3–5 days. After 5 days it becomes sour. Khalpi is consumed as pickle. Mustard oil, salt and powdered chilly is added to it. Khalpi is prepared in the months of September and October. Microorganisms isolated from *Khalpi include* L. plantarum, *L. brevis* and *Leuconostoc fallax* (Tamang *et al.*, 2009).

Sinki

Sinki, an ethnic fermented radish tap root food, is prepared by pit fermentation. Pit fermentation is different kind of biopreservation of foods by LA fermentation in the Sikkim Himalayas. For preparation of sinki, a pit is dug in a dry place. It is cleaned, then plastered with mud and warmed by burning. Ashes are removed, and then pit is lined with bamboo sheaths and paddy straw. Radish tap roots are wilted for couple of days, crushed, dipped in lukewarm water, squeezed, and then tightly pressed into the pit, covered with dry leaves and weighted down by heavy planks or stones. The top of the pit is covered with mud and then allowed to ferment for 22-30 days. After fermentation is over, freshly formed sinki is removed, sliced into small pieces and sun dried for 2-3 days. It is then stored at room temperature for future consumption (Yan et al., 2008). Pit fermentation is common in the South Pacific and Ethiopia for preservation of breadfruit, taro, banana, and cassava. Sinki fermentation is carried out by various LAB including L. plantarum, L. brevis, L. casei, and Leuconostoc fallax (Tamang et al., 2005).

Gundruk

Gundruk is a nonsalted, fermented, and acidic vegetable product traditional to the Himalayas. It is formed from fermentation of fresh leaves of local vegetables known as rayosag (Brassica rapa subsp. campestris var. cuneifolia), mustard leaves (Brassica juncea (L.) Czern), cauliflower leaves (Brassica oleracea L. var. botrytis L.), and cabbages (Brassica sp.). They are allowed to wilt for 1-2 days. Then the wilted leaves are crushed and pressed into a container or earthen pot, made airtight and fermented naturally for about 15-22 days. After the process of fermentation is over, products are removed. After that they are sun-dried for 2-4 days. Gundruk is taken as pickle or soup and bear resemblance to other fermented vegetables like kimchi of Korea, sauerkraut of Germany, and sunki of Japan (Yan, 2008). The predominant microflora of Gundruk includes various LAB such as L. fermentum, L. plantarum, L. casei, L. casei subsp. pseudoplantarum, and Pediococcus pentosaceus (Yan et al., 2008).

Inziangsang

The people of north eastern areas like Nagaland and Manipur consume Inziangsang, traditional fermented leafy vegetable product prepared from mustard leaves and similar to gundruk (Yan *et al.*, 2008). Mustard leaves, locally called hangam (*Brassica juncea* L. Czern), are collected, crushed, and soaked in warm water. Then the excess water is squeezed and pressed into the container. It is made airtight to maintain the anaerobic condition. The container is kept at temperature (20°C- 30°C) and allowed to ferment for 7–10 days. It is sun-dried for 4-5 days and stored in a closed container for a year or more at room temperature for further consumption. The people of Nagaland consume inziangsang as a soup time

with steamed rice. This fermentation is also done by LAB which includes *L. plantarum*, *L. brevis* and Pediococcus (Tamang *et al.*, 2005; Tamang *et al.*, 2009)

Goyang

Goyang, an important fermented vegetable foodstuff consumed in Sikkim and Nepal. Leaves of magane-saag (Cardamine macrophylla Willd.), which belong to family Brassicaceae, are collected and washed. Then they are sliced and dried. They are then pressed tightly into bamboo baskets lined with two to three layers of leaves of fig plants. The tops of the baskets are then covered with fig plant leaves and fermented naturally at room temperature (15°C–25°C) for 25–30 days. *L. plantarum, L. brevis, Lactococcus lactis,Enterococcus faecium*, and *Pediococcus pentosaceus*, yeasts Candida spp., were LAB isolated from goyang (Tamang, 2007).

Soidon

It is mainly consumed in Manipur. It is fermented product prepared from the tip of mature bamboo shoots. Major source is the tips or apical meristems of mature bamboo shoots (Bambusa tulda, Dendrocalamus giganteus, and Melocanna bambusoides). Whole portion of tips are submerged in water in an earthen pot. The sour liquid (soijim) of a previous batch is added as starter, and the preparation is then covered. Fermentation is carried out upto 7 days at room temperature. Leaves of Garcinia pedunculata Roxb. (family: Guttiferae), locally named as heibungin in Manipuri language, can be added in the fermenting vessel during the process of fermentation to increase the flavor of soidon. After 3-7 days, soidon is taken out from the pot and stored in a closed container at room temperature for a year. L. brevis, Leuconostoc fallax, and Lactococcus lactis take part in fermentation (Tamang 2007, Tamang 2009).

Conclusion

Essential comprehension about the connection between nourishment, helpful microorganisms, and health of the individual is imperative to enhance the nature of food and furthermore for counteractive action of several maladies. The measure of nourishment fixings and additives in fermented foods, for example, sugar, salt, and monosodium glutamate, should fit in with the acknowledged guidelines set up by the controls of target markets. Blended fermentation with high inconstancy ought to be supplanted by unadulterated culture accomplish huge scale production. Lactic acid to fermentation without any doubt speaks to the least demanding and the most reasonable path for expanding the everyday utilization of fresh like vegetables and fruits. Regularly lactic acid fermentation occurs immediately following protocols of manufacture, which are firmly connected to the way of life and customs of overall nations. New molecular ways to deal with composition of the microbiota and to choose autochthonous starters focused for various vegetables and fruits must be urged to get new bits of knowledge and to permit controlled fermentation processes as it was done for other fermented foods (cheeses, sausages, leavened baked goods). Lactic acid bacteria custom-made to the different inherent and extraneous environmental conditions totally abuse the capability of vegetables and fruits, which upgrades the cleanliness, tangible, dietary and shelf life properties.

Declaration

Ethics approval and consent to participate, This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used in the current study are available from the corresponding author by request.

Competing interest

The authors have no personal or financial conflicts of interest associated with this work.

References

- Anandharaj, M. and Sivasankari, B. (2013). Isolation of potential probiotic Lactobacillus strains from human milk. International Journal of Research in Pharmacy and Life Sciences, 1(1): 26-29.
- Anandharaj, M. *et al.* (2014). Effects of probiotics, prebiotics, and synbiotics on hypercholesterolemia: a review. Chinese Journal of Biology 2014.
- Battcock, M. (1998). Fermented fruits and vegetables: a global perspective, Food & Agriculture Org.
- Berner, L.A. and O'Donnell, J.A. (1998). Functional foods and health claims legislation: applications to dairy foods. International Dairy Journal, 8(5-6): 355-362.
- Buckenhüskes, H. *et al.* (1997). Fermented vegetables, ASM Press, Washington DC: 595-609.
- Capozzi, V.; Fragasso, M.; Romaniello, R.; Berbegal, C.; Russo, P. and Spano, G. (2017). Spontaneous Food Fermentations and Potential Risks for Human Health. Fermentation, 3(4): 49.
- Di Cagno, R. *et al.* (2010). Comparison of phenotypic (Biolog System) and genotypic (random amplified polymorphic DNA-polymerase chain reaction, RAPD-PCR, and amplified fragment length polymorphism, AFLP) methods for typing Lactobacillus plantarum isolates from raw vegetables and fruits. International journal of food microbiology, 143(3): 246-253.
- Di Cagno, R. *et al.* (2011). Exploitation of sweet cherry (*Prunus avium* L.) puree added of stem infusion through fermentation by selected autochthonous lactic acid bacteria. Food microbiology, 28(5): 900-909.
- Fan, L. and Hansen, L.T. (2012). Fermentation and biopreservation of plant based foods with lactic acid bacteria. Handbook of Plant Based Fermented Food and Beverage Technology, 2nd Edition, CRC Press, Boca Raton: 35-48.
- Hasan, M.N.; Sultan, M.Z. and Mar-E-Um, M. (2014). Significance of Fermented Food in Nutrition and Food Science J. Sci. Res., 6(2): 373-386
- Jung, J.Y. *et al.* (2011). Metagenomic analysis of kimchi, a traditional Korean fermented food. Applied and environmental microbiology, 77(7): 2264-2274.

- Karovicova, J. and Kohajdova, Z. (2005). Lactic acidfermented vegetable juices-palatable and wholesome foods. Chem. Pap, 59: 143-148.
- Kim, M. and Chun, J. (2005). Bacterial community structure in kimchi, a Korean fermented vegetable food, as revealed by 16S rRNA gene analysis. International journal of food microbiology, 103(1): 91-96.
- Leroy, F. and De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends in Food Science & Technology, 15(2): 67-78.
- Lindow, S.E. and Brandl, M.T. (2003). Microbiology of the phyllosphere. Applied and environmental microbiology, 69(4): 1875-1883.
- Molin, G. (2001). Probiotics in foods not containing milk or milk constituents, with special reference to Lactobacillus plantarum 299v. The American journal of clinical nutrition, 73(2): 380s-385s.
- Montet, D. *et al.* (2006). "Microbial technology of fermented vegetables."
- Nielsen, D.S. *et al.* (2007). The microbiology of Ghanaian cocoa fermentations analysed using culture-dependent and culture-independent methods. International journal of food microbiology, 114(2): 168-186.
- Nyanga, L.K. *et al.* (2007). Yeasts and lactic acid bacteria microbiota from masau (*Ziziphus mauritiana*) fruits and their fermented fruit pulp in Zimbabwe. International journal of food microbiology 120(1): 159-166.
- Paramithiotis, S. *et al.* (2012). Fate of *Listeria monocytogenes* and *Salmonella typhimurium* during spontaneous cauliflower fermentation. Food Control, 27(1): 178-183.
- Park, J.M. *et al.* (2010). Identification of the lactic acid bacteria in kimchi according to initial and over-ripened fermentation using PCR and 16S rRNA gene sequence analysis. Food Science and Biotechnology 19(2): 541-546.
- Postmaster, A. *et al.* (1997). Enumeration and identity of microorganisms isolated from the surface of banana fruits at three developmental stages. Scientia Horticulturae, 69(3-4): 189-197.
- Prado, F.C. *et al.* (2008). Trends in non-dairy probiotic beverages. Food Research International, 41(2): 111-123.
- Rafter, J. (2003). Probiotics and colon cancer. Best Practice & Research Clinical Gastroenterology, 17(5): 849-859.
- Roberts, J. and Kidd, D. (2005). Lactic acid fermentation of onions. LWT-Food Science and Technology 38(2): 185-190.
- Spurr Jr, H. (1994). The microbial ecology of fruit and vegetable surfaces: its relationship to postharvest biocontrol. Biological control of postharvest diseases-theory and practice.
- Steinkraus, K.H. (1994). Nutritional significance of fermented foods. Food Research International, 27(3): 259-267.

- Tamang, J.P. (2009). Himalayan fermented foods: microbiology, nutrition, and ethnic values, CRC Press.
- Tamang, J.P. *et al.* (2005). Identification of predominant lactic acid bacteria isolated from traditionally fermented vegetable products of the Eastern Himalayas. International journal of food microbiology 105(3): 347-356.
- Tamang, J.P. et al. (2009). Functional properties of lactic acid bacteria isolated from ethnic fermented vegetables

of the Himalayas. International journal of food microbiology, 135(1): 28-33.

- Yan, P.-M. *et al.* (2008). Effect of inoculating lactic acid bacteria starter cultures on the nitrite concentration of fermenting Chinese paocai. Food Control, 19(1): 50-55.
- Yang, C.-H. *et al.* (2001). Microbial phyllosphere populations are more complex than previously realized. Proceedings of the National Academy of Sciences, 98(7): 3889-3894.