## Chapter 8 Cereal Based Fermented Foods and Non-alcohol Beverages



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## 8.1 Introduction

Fermentation has a very long history of preserving food, perhaps 12,000 years old and is defined as the biochemical process of complex carbohydrate breakdown into simpler sugar by the action of bacteria and yeast. The enzyme produced by the selected microorganisms, either singularly or in combination alter the final product and make it desirable for human consumption. It is an inexpensive and low-energy process. It provides a natural way to destroy undesirable components and improves shelf-life, sensory, nutritional value of the product (Sanlier et al., 2019). Most bacterial fermentation produce lactic acid and yeast fermentation produce alcohol as secondary metabolites. During fermentation, various taste, aroma, and flavor active components are also produced, which are highly valued as they improve the overall acceptability of the final product. Indigenous fermented foods have been prepared for thousands of years and are strongly linked to cultures and traditions of people all over the world. The earliest record of fermentation appears back in 6000 BC, when people used the process in an artisan way to preserve milk, fish, and vegetables without knowing the role of microorganisms involved. Cereal fermentation was started with leavening wheat dough with yeast by ancient Egyptians in 3500 BC and indigenous souring cereal-legume batters were done by ancient Indians later in 500 AD (Nout et al., 2007). Since then, cereal fermented foods, in various forms has become a staple in diets of many population groups.

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Cereals are rich sources of carbohydrates, proteins, vitamins, minerals, and fiber, consumed as a primary source of energy by the people around the world. These are important substrates for fermented foods as they contain enough substrate for growth of microorganisms. However, nutritional, sensorial and functional quality of cereal-based products is inferior. The reason behind the inferiority is due to lack in lysine and presence of antinutritional compounds like phytic acid, tannins, and polyphenols. Several processing techniques, including fermentation has been implemented to ameliorate the acceptability of cereal-based products. Fermentation enhances the digestibility of cereal grains by assimilating nondigestible poly and oligosaccharides and providing optimum pH conditions for enzymatic degradation of antinutritional compounds. Fermentation also improves the nutritional quality by enhancing the production of lysine and B-group vitamins (Blandino et al., 2003). Cereal grains have recently come into focus for production of nonalcoholic fermented beverages due to the presence of natural sugars, antioxidants, vitamins, and other beneficial nutrients. Cereal-based beverages are mostly based on the grain suspension and depending upon the processing step involved, they are categorized either into fermented and non-fermented beverages or alcoholic and nonalcoholic beverages. Nonalcoholic beverages are mostly in the form of fermented functional beverages and stimulants like tea, coffee, cereal-based milk, soft drinks, or energy drinks. Fermented nonalcoholic cereal beverages have unique and specific health benefits depending upon the microbiological strain used for production.

Fermented foods and beverages prepared indigenously are remained as house culinary art or produced in small-scale industries. Therefore, there was no verified information of economical, nutritional, and quality control of traditional fermented foods. Rising awareness towards health benefits of functional foods in last 20 years necessitates commercialization of indigenous fermented foods and beverages to meet global demands. Other than the improved nutritional quality, cereal based fermented food possess gut-health promoting aspects, owing to the presence of edible beneficial microbes, also called probiotics: fermentable sugars (i.e. prebiotics), and group of microbe-derived hydrolytic enzymes, etc. Consequently, advance scientific works are being carried out to understand the manufacturing process and engineering interventions. This review focuses on the production processes of most common cereal-based fermented foods and nonalcoholic beverages highlighting the biochemical properties and commercialization of the final product. This will encourage food industries for large-scale production of the indigenous fermented foods to meet the growing market demand of functional foods.

# 8.2 Biochemical Process Involved in Cereal Grain Fermentation

Cereal fermentation involves conversion of complex organic compounds into energy through the metabolic process of microorganisms. The pioneer of fermentation process Louis Pasteur described fermentation as "a cascade of chemical events where

oxygen is transferred from one part of a sugar molecule to another, resulting in a highly oxidized product (CO<sub>2</sub>) and a highly reduced product (alcohol)". He succinctly defined the process of fermentation is "la vie sans l'air," or "life without air" (Alba-Lois & Segal-Kischinevzky, 2010). Furthermore, Traube discovered the involvement of protein-like substance enzymes which catalyze the process of fermentation. Later, Eduard Buchner in 1907 won Nobel prize in Chemistry by demonstrating sucrose fermentation and coined the term "zymase" to delineate the cellular enzyme which catalyzes the process of conversion of sugar to alcohol (Barnett & Lichtenthaler, 2001). The process of fermentation is diverse as different microorganism has different mechanism for the conversion of organic compounds into energy. Fermentation can also be described as a process of microbial cellular respiration or microbial glycolysis. Glycolysis is the sequence of chemical reactions where glucose converts into pyruvate. When oxygen is available, pyruvic acid enters aerobic glucose oxidization process, but lack of oxygen leads the pyruvic acid to follow two different pathways, depending on the type of cell. First is alcoholic fermentation pathway, where Pyruvate converts into alcohol and CO<sub>2</sub>, and second pathway is lactic acid fermentation where pyruvate transforms into lactate (Voet & Voet, 2004; Taillefer & Sparling, 2016). Lactic Acid Bacteria (LAB) is a group of gram-positive bacteria that converts carbohydrate into lactic acid at the end of fermentation cascades. Moreover, other common fermenting bacterial species are Leuconostoc, Streptococcus, Pediococcus, and Micrococcus. The common fermenting fungi species are Saccharomyces responsible for alcoholic fermentation in cereal grains (Blandino et al., 2003).

Natural fermentation in cereals results decrease in the carbohydrate content as well as the nondigestible poly and oligosaccharides, which improve the functional properties of cereal grains. Moreover, fermentation improve the protein quality by significantly increasing lysine content in lysine in cereal-based products. It was also found that fermentation improves the level of lysine, methionine, and tryptophan in corn meal (Nanson & Field, 1984). The pH of fermented food facilitates degradation of phytate which present in grain matrix combined with polyvalent cations such as iron, zinc, calcium, magnesium, and proteins. Thus, phytate degradation leads to availability of more soluble iron, zinc, and calcium in the final product. Fermentation process is known to increase shelf-life, taste, and aroma. During fermentation, several volatile compounds and organic acids are formed which are mostly responsible for improved sensory properties in the final product. Organic acids formed during fermentation of cereal grains are namely, lactic acid, acetic acid, butaric acid, formic acid, succinic acid etc. helps reducing the pH to below 4.0, making it difficult for some spoilage organisms to survive (Stanojević-Nikolić et al., 2016). Similarly, during yeast fermentation various alcohols are formed, viz. ethanol, n-propanol, isobutanol, amyl alcohol, isoamyl alcohol, etc. Aldehydes, ketones, and carbonyl compounds formed during fermentation are the basic flavour compounds in the final product (Karovičová & Kohajdova, 2007).

## 8.3 Cereal-Based Fermented Foods

## 8.3.1 Rice-Based Fermented Foods

Rice is one of the most important cereal grain and staple food of South-Asian subcontinent. Major component of rice is starch making it suitable media for the growth of pervasive groups of microorganisms. Rice-based fermentation process involves two types of reaction, viz. acidic or alcoholic fermentation or both consecutively. The fermentation process includes pretreatment of rice grains to loosen the compact structure of starch and simultaneously release of antinutrient components out of the grain matrix. The pretreatments are done by soaking, grinding, and boiling. Sometimes, overnight soaking of rice in some food preparations activates various hydrolytic enzymes, help slack the dense starch by breaking the complex bonds. To complement the nutritional quality, some pulses are usually mixed with the rice to form the batter. During fermentation, formation of CO<sub>2</sub> in the batter makes the final product spongy. Lactobacillus and Bifidobacterium constitutes most of the LAB group which are commonly used for rice-based fermentation. Following indigenous rice-based fermented products are popular all over the world and produced commercially in various forms. Besides, some of other popular cereal-based fermented products and the microorganisms involved in their fermentation are given on Table 8.1.

#### 8.3.1.1 Idli, Dosa and Uttapam

*ldli* is a savory and spongy cake prepared from a thick batter of rice (*Oryza sativum*) and dehulled splitted black gram (Vigna mungo) and is very popular traditional fermented food of South Asia. Rice and black grams in a ratio of 3:1 is soaked separately for 6–8 h and then combinedly grind to make a thick batter after draining the soaked water. The batter is then fermented for 10–12 h at room temperature (about 30 °C) and the fermented batter is steamed inside a pan contains concave moulds to prepare starchy and spongy idli. It is mostly consumed during breakfast with sambhar, which is basically a lentil soup. Fermentation of batter is mediated by mixed culture of LAB including Lactobacillus fermenti, Lactobacillus lactis, Lactobacillus delbrueckii, Lactobacillus plantarum, Leuconostoc mesenteroides, Streptococcus faecalis and Pediococcus cerevisiae. The major physico-chemical changes such as decrease in pH and increase in the titrable acidity in idli batter is due to lactic acid fermentation. Bernard et al. (2021) observed remarkable drop in pH from 6.28 to 3.72 and increase in acidity from 0.24% to 0.92% during the period of 0-32 h of fermentation. They also concluded L. mesenteroides and S. faecalis strains are found to be responsible for leavening of the batter and lowering the pH. Increase in acidity of batter helps activity of yeasts by producing optimum pH for their growth. The yeast strains, viz. Torulopsis holmii, Torulopsis candida, Trichosporon pullulans, Geotrichum candidum, Candida fragilola, Candida tropicalis, Candida kefyr, Rhodotorula graminis, and Hansenula anomala have also been isolated from

Product	Substrates	Microorganisms	Nature of use	Regions of production
Adai	Rice with legumes	Pediococcus, Streptococcus, Leuconostoc	Breakfast/ snacks	India
Ambeli	Rice	Leuconostoc mesenteroids, Lactobacillus fermentum, Streptococcus faecalis	Breakfast meal	India
Anarshe	Rice	LAB	Breakfast/ snacks	India
Ang-kak	Red rice	Monascus purpureus	Dry red powder as food colorant	India
Appam	Rice	LAB	Breakfast	India
Atole	Maize	LAB	Porridge	Southern Mexico
Babru	Rice	LAB (Lactobacillus plantarum, Lactococcus lactis), yeasts such as Saccharomyces cerevisiae, Debaromycis sp.	Breakfast	India
Banku	Maize	LAB, molds	Dough	Ghana
Bhattejaanr	Rice	Hansenula anomala, Mucor rouxianus	Sweet and sour paste	India
Chakuli	Rice/black gram	LAB, mold	Breakfast	India
Chee-fan	Soybean, wheat	Mucor, Aspergillus glaucus	Cheese like product	China
Dalaki	Millets	Unknown	Thick porridge	Nigeria
Enduri Pitha	Rice and black gram	Lactobacillus fermentum	Spongy cake	India
Hamanatto	Wheat, soybeans	Streptococcus, Pediococcus and Aspergillus oryzae,	Flavoring compound	Japan
Ilambazi lokubilisa	Maize	LAB, yeasts, and molds	Porridge	Zimbabwe
Injera	Sorghum, maize or wheat	Candida guilliermondii	Bread like product	Ethiopia
Jalebi	Wheat, Bengal gram	Saccharomyces bayanus	Confection	India
Jamin-bang	Maize	Yeasts, bacteria	Spongy cake	Brazil
Kaanga- Kopuwai	Maize	Bacteria, yeasts	Soft, slimy eaten as vegetable	New Zealand
Khanomjeen	Rice	Lactobacillus, Streptococcus	Noodle	Thailand
Kichudok	Rice	Saccharomyces	Sponge cake	Korea
Kisra	Sorghum	Unknown	Staple food like bread	Sudan

 Table 8.1
 Most common indigenous cereal-based fermented foods

(continued)

Product	Substrates	Microorganisms	Nature of use	Regions of production
Koko	Maize	LAB (Lactobacillus platarum, L. brevis), yeasts (Saccharomyces cerevisiae, Candida mycoderma), Enterobacter clocae, Acinetobacter	Porridge	Ghana
Lao-chao	Rice	Rhizopus oryzae, R. chinensis, Chlamydomucor oryzae, Saccharomycopsis	Spongy buns	China, Indonesia
Mahewu	Maize	Streptococcus lactis	Porridge	South Africa
Mantou	Wheat	Saccharomyces	Steamed cake	China
Mutwiwa	Maize	LAB, bacteria and moulds	Porridge	Zimbawe
Naan	Wheat	Saccharomyces cerevisiae, LAB	Flattened bread	India, Pakistan, Afghanistan, Iran
Puto	Rice	Leuconostoc mesenteroides, Strepromyces faecalis, yeasts	Solid paste	Philippines
Soy sauce	Wheat and soybean	Aspergillus oryzae or A. soyae, Zygosaccharomyces rouxi, LAB	Liquid seasoning	Japan, China, Taiwan
Selroti	Rice	LAB (Leuconostoc mesenteroids, Lactobacillus curvatus), Enterococcus faecium, Pediococcus pentosaceus, Saccharomyces cerevisiae, Saccharomyces kluyveri, Debaryomyces hansenii, Pichia burtonii and Zygosaccharomyces rouxii	Spongy and ring-shaped rice bread	India
Sierra rice	Rice	Aspergillus flavus, A. candidus, Bacillus subtilis	Brownish- yellow rice for lunch/ dinner	Ecuador
Sour rice	Rice	LAB (Lactobacillus bulgaricus, Lactobacillus casei), Sacchaeromyces sp. and Pediococcus acidilactici, Streptococcus faecalis, Streptococcus thermophilus, Macrobacterium flavum	Cooked rice as staple food	India
Sez	Rice	Saccharolytic and ethanol producing microbes	Cooked rice with seasonings	India
Taotjo	Wheat/rice and soybean	Aspergillus oryzae	Condiment	India
Uji	Maize. Sorghum, millet	Leuconostoc mesenteriodes, Lactobacillus platarum	Porridge	Kenia, Uganda, Tanganyika
Vada	Rice and black gram	Pediococcus, Streptococcus, Leuconostoc	Fried spongy snacks	India

Table 8.1 (continued)

fermented *idli* batter (Shaikh et al., 2021). LAB and yeasts work synergistically in the fermented *idli* batter and enhance the nutritional value and produce desirable texture and flavor. The aroma and flavour attributes of idli mainly depends on the production of organic compounds arise from both the raw materials and fermentation process. Major flavour compounds arise from rice and black gram are ketones (ethanone, pentanone and butanones) and polyunsaturated fatty acids, respectively (Agrawal et al., 2000). With reference to the nutritional attributes, fermentation enhances the level of soluble solids, essential amino acids, viz. lysine, cystine and metheionine, non-protein nitrogen, soluble vitamins (folate, vitamin A, B1, B2 and B12) content in the batter and reduces antinutrient phytic acid content making *idlis* more functional.

Dosa and uttapam batters are similar except the proportion of raw material used and particle size during grinding. The ratio of rice and black gram is about 4:1 and the soaked rice used are ground more finely to prepare *dosa* and *uttapam* batter. Instead of steaming, the batter is spread and heated with a little oil, over a flat pan. *Dosa* is spread over the heated pan as a thin layer, while *uttapam* is spread as a thick layer with some vegetable toppings to prepare the final product as pancakes. Like *idli* batter, the *dosa* and *uttapam* batters are also fermented by a mix culture of LAB and yeasts increasing the acidity and pH. Minor yeast strains found in *dosa* batter are belonging to *Saccharomyces cerevisiae*, *Debaryomyces hansenii*, and *Trichosporon beigelli*.

With the growing demands for low-calorie functional breakfast and snack foods, active research works have been conducted to replace rice in *idli*, *dosa* and *uttapam* batters with other grains, viz. brown rice, pearl millets, amaranth, finger millets and buck wheat. Though incorporation of these functional cereals improves the nutritional attributes greatly, it decreased the palatability, texture, and overall acceptability of the product (Rani et al., 2019; Dhillon et al., 2020; Kumari et al., 2020). Therefore, to maintain balance between the nutritional and sensory attributes, researchers are more inclined towards partial replacement of rice with other cereals. Increasing priority towards convenience have resulted in the development of readyto-cook foods and instant food premixes of the fermented foods. Shelf-life enhancement and instantization are two major processes for commercialization of the fermented batter. Use of combined thermal and electron beam radiation of the fermented batter and packaging of the treated batter in oxygen barrier multi-layered packaging film for increasing shelf-life of *idli* and *dosa* batter are highly studied area in the last decade (Mulmule et al., 2017; Gaikwad et al., 2020). Accelerating the process of fermentation using selected starter culture is another aspect for commercial production of fermented batter (Chelliah et al., 2017).

#### 8.3.1.2 Dhokla

*Dhokla* is a soft and spongy rice cake popularly consumed as breakfast in Western India. It is made from a batter of polished white rice and Bengal gram (*Cicer arietinuum* L.). Rice and Bengal gram are soaked separately for 5–10 h, then drained to ground into a fine batter. The batter is then mixed with salt (approximately 1% w/w)

and fermented at room temperature (30-32 °C) for 12-14 h. Additional seasonings and chopped green leafy vegetables are often added to the fermented batter and steamed over a flat pan for 10–15 min. The product is then cooled and cut in the shape of diamond and seasoned with heated oil, mustard seeds, and curry leaves (Murrava koenigii). Sponginess and volume in the batter is resulted due to the production of CO<sub>2</sub> by Yeasts like Torulopsis sp., Candida sp., Tpullulans. LABs, viz. L. fermentum, Leuconostoc mesenteroids, Pichia silvicola, streptococcus faecalis, etc. produce lactic acid and acetoin in the batter during fermentation, which impart sour taste and a pleasant flavour (Aidoo et al., 2006; Ray et al., 2016). Like other fermented foods, the biological value and net protein utilization of *dhokla* are significantly improved due to fermentation. Antinutritional compounds like tannins, phytic acid, total biogenic amines, trypsin inhibitor and haemagglutinating activities reduced because of fermentation (Sharma et al., 2018). It can be used as supplementary diet for children suffering from malnutrition and patients with digestive disorders (Desai & Salunkhe, 2018). Industrial manufacturing of *dhokla* depends largely on the scale of manufacturing, location of production, climatic conditions. Due to increased demand towards convenient foods, manufacturers mostly produce instant *dhokla* mix by drying the fermented batter. To improve the functional property of *dhokla*, several researchers have studied the nutritional and organoleptic properties of dhokla after incorporation of soybean, ragi, gareden cress seeds, okra, and pumpkin flour (Lohekar & Arya, 2014; Ray et al., 2017). Incorporation of the functional ingredients improves the nutritional parameters, viz. antioxidant properties, carotene content, folic acid and vitamin C, but reduced the sensory values significantly. Therefore, dhokla can be prepared with incorporating optimum ration of rice, Bengal gram and other functional ingredient to maintain balance between the nutritional and sensory parameters.

#### 8.3.1.3 Miso

Miso is a traditional Japanese seasoning paste produced by fermenting the mixture of rice, soybeans, and salt. Preparation of miso involves two successive stages of fermentation. In the first stage, rice is soaked and steamed for complete conversion of raw starch ( $\beta$ -starch) to gelatinized starch ( $\alpha$ -starch) and then inoculated with strains of *Aspergillus oryzae*. The mold rice is called 'koji' serves as a source of enzyme for catalyzing the second stage fermentation. In the meantime, whole soybeans are soaked and cooked to gelatinize the starch. In the second stage, koji, cooked soybeans and salt are ground together to form a thick paste and inoculated with the miso from previous fermentation. The mixture is then kept for fermentation 42–48 h at a temperature 28–32 °C to produce the final product as *miso* (Kusumoto et al., 2021). Based on the proportion of soybean used for preparation, miso can be of three types, viz. white, red and dark miso, respectively. Although indigenous *miso* preparation was done in small batches in Japanese households, commercialized production has more recently been superseded to meet global demand for consistent,

high quality and safe food. During industrial production, temperature and humidity control and use of commercial strain of microorganism reduced the fermentation time of miso. In addition, pasteurization process, use of suitable packaging material and invention of smart packaging increased the shelf-life of commercially produced miso (Allwood et al., 2021). Barley, legumes, nuts, functional seeds, mushrooms can be used as koji substrates during miso preparation to replace rice (Shurtleff & Aoyagi, 2018).

## 8.3.2 Wheat-Based Fermented Products

Wheat is an important source of carbohydrate, protein, vitamins, and minerals. It contains gluten protein, which is responsible for most of the viscoelastic properties of wheat flour doughs and is the main factor dictating the use of a wheat for making spongy fermented products. Starch is the major component of wheat flour which helps in the water absorption and fermentation time requirements of bread-making doughs and crumb textural properties of the final products. Following are some of the popular fermented products made from wheat and their commercial aspects of production.

#### 8.3.2.1 Bread

Bread is a popular fermented product produced from wheat dough by baking process. Bread dough is usually leavened by naturally occurring microbes (e.g., sourdough), or yeast (Saccharomyces cerevisiae), or high-pressure aeration which creates the gas bubbles that fluffs up during baking. It is believed that ancient Egyptians first fermented wheat dough and baked them in clay ovens during 3500 BC (Özdemir & Altıner, 2021). Preparation steps of bread involves mixing of flour, water and yeast to form a visco-elastic dough, allowing the yeast to fermentation at 24-28 °C for 2-4 h to an aerated mass and then baking the structure inside a high temperature oven at 220 °C for 20-25 min. The fermentation in bread dough is primarily done by bakers' yeast or Saccharomyces cerevisiae, which is a top fermenting yeast. Growth of the yeast is optimum at pH range of 4-5 and at temperature of 25–28 °C (Narendranath & Power, 2005). The kneading process of dough introduces oxygen which converts the accumulated sugars to pyruvate by the yeast cells through aerobic glycolytic pathway. Later, the dough started anaerobic fermentation due to production of  $CO_2$  in glucose metabolism (Heitmann et al., 2018).  $CO_2$  production primarily makes the fermented dough into a spongy and elastic structure by entrapped in the gluten matrix. Remixing of the dough facilitates additional CO<sub>2</sub> production and growth of LAB to produce organic acids, which lowers the pH of the dough and give acidic pleasant aroma and flavour during baking of bread.

Since the beginning of modern civilization, bread is manufactured commercially as it is a staple food for Middle East, Central Asia, North Africa, Europe, and in America. Temperature and relative humidity (RH) are two important parameters in industrial bread baking as it affects yeast growth and fermentative capacity. Followed by fermentation, the dough mass is cut into loaf-sized portions and proofing is done for 30-60 min at 35-42 °C and RH of 70-80% prior to baking. The proofed loaves are baked in high temperature ovens in five stages, viz. (a) enzyme inactivation zone  $(30-60 \ ^{\circ}C)$ , (b) yeast inactivation zone  $(55-60 \ ^{\circ}C)$ , (c) starch gelatinization zone (55 °C to <90 °C); (d) water evaporation (>100 °C); and (v) Maillard browning and aroma formation (>200 °C) (Erkmen & Bozoglu, 2016). Several aroma and flavouractive compounds are formed during baking and the process is called Maillard browning. Major compounds are organic acids, ethyl esters, alcohols, aldehydes, ketones, sulfur-containing compounds, maltol, isomaltol, melanoidin, furan derivatives, lactones, pyrazines, pyridines, and pyrroles (Birch et al., 2014). Sever heating condition produces certain pigments responsible for brown crust colour formation. Although vitamin B and niacin content increases due to bread dough fermentation, baking at high temperature reduces some nutritional quality of bread due to loss of some amino acids and reducing sugars in Maillard browning reactions.

#### 8.3.2.2 Soy Sauce

Soy sauce is a traditional condiment made from wheat and soybean blend, used as a key seasoning in Asian countries. Soy sauce was originated as chiang-yu in China over 2500 years ago and popular as shovu in Japan, kunjang in Korea, tovo in the Philippines and kicap in Indonesia and Malaysia (Yokotsuka, 1993). Soy sauce has a salty and unique umami taste due to the phytochemicals present in it, which also helps in reduction of total and low-density lipoprotein cholesterol (Larkin et al., 2009). Manufacturing of soy sauce involves two primary fermentation process, a solid-state fermentation process and a long-term liquid-state fermentation process. Cooked soybeans are mixed with coarse wheat flour keeping initial moisture content of 55% (w/w) and the mixture is allowed to ferment by Aspergillus mold (A. oryzae or A. sojae) for 3 days at 25-35 °C. Aspergillus mold produce enzyme that break down the complex molecules of wheat and soybean, which helps in the development of halophilic bacteria, LABs, bacillus species and yeasts (Zygosaccharomyces rouxii, T. halophilus and Candida species). The fermented mixture is called as koji is then immersed in a brine solution with 18-20% salt content in the ratio of 1:3 (w/v). The brine solution containing koji is called as moromi, which is left to ferment for 1 month to 4 years of maturation to finally produce soy sauce (Sassi et al., 2021). The detailed flowchart of soy sauce manufacturing is given in Fig. 8.1. After the long-term maturation, the soy sauce is then filtered, pasteurized, and bottled (Harada et al., 2017; Det-Udom et al., 2019). Raw soy sauce is usually heat pasteurized at 70-80 °C for 30 min or can be flash pasteurized at 115 °C or higher for 3-5 s to increase the shelf life and stability. The characteristics taste and aroma of soy sauce is generated due to the enzymatic activities of yeasts and

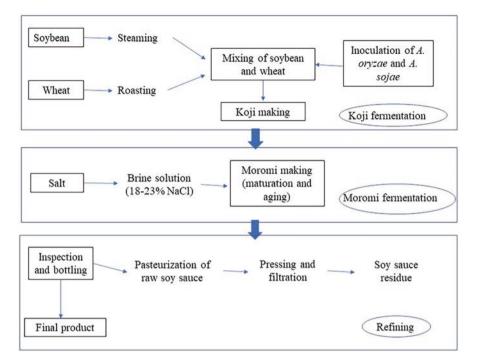


Fig. 8.1 Process flow chart of soy sauce manufacturing involving two stage fermentation

some osmophilic LABs. Major odor active compounds found in soy sauce are Methional, ethyl lactate, furfuryl alcohol, furanone, propanal etc. The pH of soy sauce is generally 4.6–4.8 and typical salt concentration is about 17–19%. High salt concentration in soy sauce may be a concern hypertension, health driven industries are trying to reduce the sodium concentration by maintaining the salt percentage as salt less than 16% can result in the development of putrefactive species during fermentation (Devanthi et al., 2018).

## 8.3.2.3 Kishk

*Kishk* is a popular dried fermented product of Egypt and middle east made from sour milk and parboiled wheat (bulgar) mixture. The raw materials required for *kishk* preparation are parboiled wheat flour, *laban zeer* (Tamime & O'connor, 1995). Wheat is slowly boiled in a large, covered cooking pans followed by cooling and drying to produce bulgar or *belila*. The dried and hard bulgar is then coarsely ground and mixed with *laban zeer* to form a homogenous paste, called *hamma*. *Laban zeeer* is the remaining by-product obtained from squeezing the sour milk through a skin bag. The *hamma* is then fermented for 24 h and then kneaded by hand and divided into small dough portions and dried in the open air to form the final product, called *kishk* (Morcos et al., 1973). Sometimes, cumin and pepper are added as seasoning

to the *laban zeer* for enhancing the flavour. *Kishk* is rich in various nutrients, including many vitamins and growth factors. The microorganisms responsible for fermentation of *kishk* are *Lactobacillus plantarum*, *Lactobacillus casei*, Lactobacillus *brevis*, Lactobacillus delbrueckii subsp. bulgaricus, and *Bacillus subtilis* (Abd El-Ghani et al., 2014). To facilitates consistent industrial production of *kishk*, the modern manufacturing process uses a combination of starter culture containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* at 45 °C for fermentation, which leads to more rapid acid production, and suppresses the growth of other pathogenic bacteria (Abou-Zeid, 2016). The functionality of kishk can be improved by incorporation of barley, pearl millets and quinoa instead of bulgar in optimized proportions to get highly nutritious product without compromising the sensory quality.

#### 8.3.2.4 Tarhana

Tarhana is a traditional Turkish fermented food prepared from wheat flour, yoghurt and different vegetables, followed by for fermentation from 1 to 7 days (Daglioğlu, 2000). Fermented tarhana mixture is usually dried to a moisture content of 6-10% for longer shelf life. The dried *tarhana* mixture is then reconstituted and consumed as soup at lunch and dinner. Tarhana is produced by simultaneous LAB and yeast fermentation. During fermentation, complex molecules like carbohydrates, protein and fat are break down to lower molecules and organic acids constituents by microorganisms, which gives acidic taste to the product. LAB involved in tarhana fermentation are Streptococcus thermophilus, Lactococcus lactis, Lactococcus diacetylactis, Lactobacillus bulgaricus, Lactobacillus acidophilus, Leuconostoc cremoris, Lactobacillus casei, responsible for lowering the pH to 3.3-5.0. The yeast, Saccharomyces cerevisiae gives the final product the body, taste and flavour (Sengun et al., 2009). Traditionally, tarhana was prepared in households by the way that have been learned from their ancestors, but to meet increasing demand for consistent, convenient and safe food, it is started manufactured in the commercial plants. In industries, there are two methods for tarhana production, viz. straight method and sourdough method. In straight method, the ingredients are combined by kneading at 50 rpm for 15 min, spread to a depth of 1–1.5 cm, kept for fermentation at 35 °C for 5 days, dried at 55 °C for 28 h to obtain moisture content less than 10%, ground to particle size <800 µm and finally sieved (Ozdemir et al., 2007). Sourdough method is done in three stages given in Fig. 8.2. Although there are various packaging materials are available for storage of fermented dough to read-to-use in the households, it is difficult to avoid moisture absorption during storage, resulting unwanted growth of microbes like E. coli O157:H7, S. aureus, S. typhimurium and B. cereus. Therefore, irradiation of the fermented dough is emerging method adopted by industries to reduce the risk of microbiological encroachment during the storage of tarhana (TaŞoğulları & ŞimŞek, 2020).

Tarhana soup is highly flavored and nutritious and can be easily digested. The nutritional importance of tarhana is remarkable due to improvement of basic amino

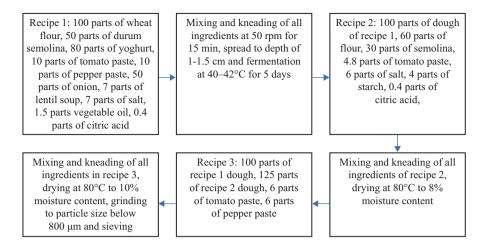


Fig. 8.2 The flow chart of commercial tarhana production using sourdough method. (Daglioğlu, 2000)

acid content of cereal and proteolytic activity by adding the dairy protein and fermentation, respectively. Increase in water soluble vitamins were reported to increase during fermentation, while drying after fermentation had a adverse effect. Tarhana is a good source of total minerals, viz. Ca, Mg, and K with favorable bioavailibities due to reduction of phytic acid, which is an antinutritional compound binds minerals and proteins, altering alters their solubility, functionality, digestibility, and absorption. Several researchers have replaced the wheat flour with other cereals like buck wheat, barley, quinoa, legumes, wheat germ, or wheat bran to raise the nutritional value of tarhana (Erkan et al., 2006; Bilgiçli, 2009; Demir, 2014).

## 8.3.3 Maize-Based Fermented Products

Maize is considered as one of the principal staple foods and has the highest global production among all the cereal grains. Maize kernel consists of three major parts, viz. the endosperm, the germ and the pericarp. The endosperm predominantly consists of starch (70–73%), which makes maize as a good base for fermentation. The fat and protein content of germ are high are around 33% and 19%, respectively makes the fermented product highly nutritious. Fermentation of maize usually consists of four common steps, viz. cleaning, soaking overnight to soften the kernel, grinding and fermentation. The fermentation process depends on temperature, pH, the quantity of inoculum and types of microorganism used. Following are some of the popular maize-based fermented products and their commercial aspects of production.

#### 8.3.3.1 Ogi

Ogi is a traditional fermented food popular in Western Africa and Nigeria is a sour starch cake made from fermentation of maize, sorghum or millets flour. Ogi is usually consumed as a smooth porridge by boiling the sour cake at the time of breakfast. Considering the health benefits of ogi, it is considered as an important weaning food for infants in West Africa. Traditionally *ogi* is prepared by steeping the grain in an earthen pot for 1-3 days, allowing it for spontaneous fermentation at  $28 \pm 2$  °C. After softening of the grain, it is milling into a smooth paste. The paste is then sieved to remove the unwanted bran, germ and hull and the filtrate is allowed to undergo a secondary fermentation for about 24-72 h in order to develop its characteristic sour taste (Adisa & Enujiugha, 2020). LABs, yeast and molds are responsible for fermentation of maize grain to produce *ogi*. Lactobacillus plantarum is the dominant bacteria in ogi and other bacteria such as Corvnebacterium, B. suhtilis, Klebsiella oxytoca, Staphylococcus aureus hydrolyze the corn starch to make the grain soften. In the secondary fermentation, yeasts of the Saccharomyces and Candida species also contribute to flavour development (Adegoke & Babalola, 1988). Indigenous production involves unskilled producers who doesn't follow good manufacturing practices and cleanliness, resulting contamination from unwanted microorganisms during fermentation. In addition, to overcome the limitations of labor-intensive and time-consuming traditional process, commercial manufacturing of ogi has been started to meet the increasing demand day by day. Flowchart for industrial production of ogi is illustrated in Fig. 8.3. The nutritional value of ogi, specifically aminoacids and is reduced by 20-50% as that of the original cereal grains used because of the loss of aleurone layer and germ during wet milling and filtration. To compensate the losses, lysine and methionine excreting mutants of Lactobacillus and yeasts have been used to fortify ogi by several researchers (Odunfa & Oyewole, 1998).

#### 8.3.3.2 Kenky

*Kenkey* is a popular maize sourdough mostly consumed in Ghana. It is usually eaten in form of dumplings or porridge depending upon the final preparation method. In the first type of *kenkey* production, maize grains are soaked for 1–2 days at room temperature and then wet-milling of the hydrolyzed grain is done after draining the water. The milled maize meal is kept for solid state fermentation to produce stiff sourdough, which can be consumed in form of dumplings. In the second type of *kenkey* preparation, filtration of the chaff from the soaked maize meal is done to give it a smooth texture. Then the meal is kept for overnight fermentation followed by discarding the water to get a wet mash, which is used for porridge making (Ganguly et al., 2021). *Kenkey* fermentation is mostly regulated by obligatively heterofermentative LABs, viz. *Lactobacillus fermentum* and *L. reuteri*. Yeasts and mould flora, viz. *Candida, Saccharomyces, Penicillium, Trichosporon, Kluyveromyces and* 

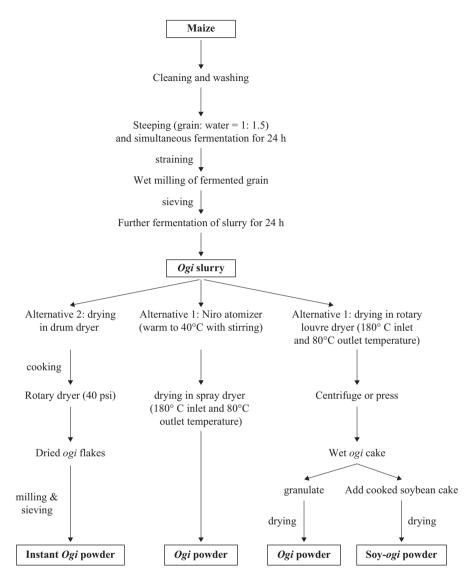


Fig. 8.3 Flowchart of industrial ogi production

*Debaryomyces* were also reported to form flavour compounds in the final product (Ekpa et al., 2019).

Several aroma compounds and organic acids are formed during kenkey production, which are responsible for the pleasant flavour and sour taste of the final product. Aroma compounds such as propanol, acetoine, Butanediol and organic acids such as propanoic acid, butanoic acid, pentanoic acid, hexanoic acid, heptanoic acid, benzanoic acid, a furan, two phenolic compounds and an alkene were isolated from GC/MS analysis of fermented kenkey dough by Annan et al. (2003). Indigenous preparation of *kenkey* sourdough dumplings is labor intensive and time-consuming takes usually 4–6 days. For commercial production, accelerated fermentation, dry-milled maize and pure starter cultures have been used to increase the productivity and consistency of the final product (Nout et al., 1995).

## 8.4 Millet-Based Fermented Products

## 8.4.1 Injera

*Injera* is an Ethiopian traditional pancake made from ancient grain teff. It can also be made from other grains like sorghum, barley, or finger millet. Sometimes people add some rice flour to make *injera* white. Gebrekidan and Belainesh (1981) described *injera* as circular, soft, spongy and resilient pan cake with uniformly spaced honeycomb-like 'eyes' on the top. Teff grains are dehulled and milled into flour and one part of flour is mixed with two parts of water in a clay container called "bohaka" to form a batter. The batter is then mixed with 16% seed culture or "ersho" and fermented for 2–3 days. Ersho is leftover fluid saved from previously fermented batter used as seed culture (Neela & Fanta, 2020). After fermentation, the batter is thinned down slightly and poured on to a lightly greased pan in form of a thin circular cake. The pan temperature during injera baking usually reached up to 200 °C and baking time is about 2-3 min (Adem & Ambie, 2017). Yeasts responsible for fermentation present in the seed culture or ersho are Pichia fermentans, Pichia occidentalis, Candida humilis, Saccharomyces cerevisiae, and Kazachstania bulderi (Tadesse et al., 2019). Some of the researchers reported the presence of LABs namely Pediococcus pentosaceus, Lactobacillus fermentum, Lactococcus piscium, Lactococcus plantarum, Pediococcus acidilactici, Leuconostoc mesenteroides etc. (Desiye & Abegaz, 2013; Desiye et al., 2017). Injera has slightly sour flavour due to fermentation and rich in nutrients, mostly calcium and iron. Fermentation time and viscosity of batter are to important parameter for final quality of injera.

## 8.4.2 Kisra

*Kisra* is like *injera* and mostly consumed in Sudan and some parts of Arabian Gulf and Iraq (Blandino et al., 2003). The Kisra dough is made from sorghum and pearl millet flour by adding water followed by fermentation. Traditionally fermentation was initiated by adding seed culture from previous batch of dough. The fermented dough is then rolled into thin sheets and then dried for few hours to store it for a year. The *kisra* sheets are then baked as unleavened bread on a hot steel plate (150–160 °C) for 30–40 s in a process called as "aowasa" (Ibnouf, 2012). *Kisra* is usually consumed with certain types of stew prepared from vegetables and meat. Microorganisms responsible for *kisra* fermentation were studied by some researchers and the main stains isolated are *C. intermedia*, *C. krusei*, *Debrayomyces hansenii*, *Enterococcus faecium*, *L. amylovorus*, *L. brevis*, *L. confusus*, *L. fermentum*, *Pichia kudriavzevii* (Adebo, 2020). Nutritional benefits of kisra bread were studied extensively and reported that fermentation increased riboflavin content of the dough and significantly decreased thiamine content, however there was no significant effect on mineral content (Mahgoub et al., 1999).

## 8.5 Cereal-Based Non-alcoholic Beverages

#### 8.5.1 Cereal-Based Sour Milk

Cereal-based sour milk are prepared by fermentation of cereal-based milk substitutes like rice milk and oat milk. Rice and oat milk are prepared by soaking the grains in water for hydration followed by grinding and filtration. Fermentation is carried by LABs yoghurt like product, which is then diluted and seasoned with cumin seeds and black pepper to produce cereal-based sour milk. Cereal-based milks and yoghurts are now popular in market, however cereal-based non-dairy fermented beverages are still under research for optimization of starter culture, sensory attributes and storability. Beneficial microbes like *Streptococcus thermophiles*, *Lactobacillus acidophilus*, *Lactobacillus reuteri and Bifidobacterium bifidum* etc. has been tested for their use production of cereal-based fermented beverages and the final products are proven to be of great nutritional interest, specifically for gut health (Bernat et al., 2015; Atwaa et al., 2020). Table 8.2 summarizes some of the popular cereal-based beverages consumed by various parts of the world.

## 8.5.2 Boza

Boza is a traditional nonfermented beverage popular in Turkey and Bulgaria. It is a thick homogeneous suspension, sweet to sour in taste, indigenously made from maize, wheat and rice flours in the ratio of 2:1:1, respectively. The grain flours are mixed with five times (w/v) of water and boiled for 1 h with continuous stirring. The slurry is cooled and diluted with 2.5 times with water and sugar (20% w/v). The slurry is kept for fermentation at 15–25 °C for 24 h using the boza from previous batch (Hancioğlu & Karapinar, 1997). Two types of fermentation are involved in boza production, viz. yeast fermentation produces carbon dioxide bubbles and increases the volume and LAB fermentation produces sour taste (Arici &

Product	Substrates	Microorganisms	Regions of production
Borde	Maize, sorghum or millet	LAB (Lactobacillus brevis, Lactobacillus viridescens), Weissella confusa, Pediococcus pentosaceus and P. pentosaceus subsp. intermedius	Ethiopia
Braga	Millet	Lactobacillus plantarum, Lactobacillus fermentum, and Lactobacillus delbrueckii	Romania
Busa	Rice or millet	Lactobacillus, Saccharomyces	Syria, Egypt, Turkestan
Darassum	Millet	Unknown	Mongolia
Haria	Rice	LAB, yeast and molds	India
Mangisi	Millet	lactic acid bacteria and yeast and molds	Zimbawe
Munkoyo	Kaffir corn, millet or maize in addition to roots of munkoyo	Streptococcaceae, Leuconostocaceae, Enterobacteriaceae, Lactabacillales, Bacillaceae and Aeromonadaceae	Africa
Tchang	Millets	Yeast (Saccharomycopsis fibuligera, Saccharomyces cerevisiae), Molds (Mucor cicinelloides, Rhizopus chinensis, R. stolonifer var. lyococcus), Lactobacillus sp.	India
Tobwa	Maize	LAB	Zimbawe
Uji	Maize, millet, sorghum or cassava flours	LAB (Lactobacillus plantarum, Lactobacillus paracasei ssp. paracasei, Lactobacillus fermentum, Lactobacillus buchneri), Pediococcus acidilactici	Sub-Saharan Africa
Yu	Rice	Unknown	India
Yosa	Oat	LAB or bifidobacteria	Finland and Scandinavia

Table 8.2 Common indigenous cereal-based fermented non-alcoholic beverages

Daglioglu, 2002). *Lactobacillus* and *Leuconostoc* genera, *Saccharomyces cerevisiae*, *Streptococcus spp.*, *Micrococcus spp.* are common microorganisms isolated from traditionally fermented boza. Total titratable acidity by means of lactic acid is about 0.2–0.5% in sweet boza and 0.5–1.0% in sour boza (Gotcheva et al., 2001). Indigenously fermented boza cann't be stored for more than 2 days due to uncontrolled growth of microbes making the product sour and unacceptable. Therefore, industrial boza production uses standard starter culture and controlled environment for consistent product quality. Sugar or saccharine is added before bottling of boza as significant decrease in pH, viscosity, free amino nitrogen content and dry matter during fermentation.

## 8.5.3 Torani/Kanji

Torani is a traditional non-alcoholic probiotic beverage popular in Eastern states of India (Odisha and West Bengal), consumed for improved gut health and electrolyte balance in the body. Torani is made from rice, water and curd taste mostly like sour rice. It is also called as kanji in North India, prepared by boiling the torani with vegetables and spices (Sahoo et al., 2017). To make torani, cooked rice is soaked overnight in a clay pot for fermentation. After fermentation, the soaked water is drained and mixed with some curd and seasoned with heated oil, crackled mustard seeds and curry leaves. The fermented rice is also called as *pakhala* used as a staple food by indigenous people of Odisha (Ray & Swain, 2013). The fermented rice water is a store house of various beneficial microbes, which produces short-chain fatty acids play important role in improving gut health. The production of *pakahala*, torani and kanji is mostly indigenous; however, in last decade researchers have shown interest in studying the commercial feasibility of torani, owing to the health benefits. Though not much scientific literature available regarding the microbiological characterization of torani, preliminary research suggested that pakahla and torani contains several yeasts (Saccharomyces spp.) and lactobacilli, which bring typical aroma and sourness to the final product.

## 8.5.4 Kvass

Kvass is a cereal-based nonalcoholic refreshment drink, prepared from fermented barley, rye malt, rye flour. It achieved high commercial success and popularly manufactured in Russia, Poland, Latvia and Lithuania (Basinskiene & Cizeikiene, 2020). Traditionally, Kvass is made by cutting stale rye bread into small pieces followed by adding hot water (1:3 w/v) with continuous stirring for 1 h. Sugar, yeast and raisins are then added to the extracted slurry and kept for fermentation at 28 °C for 24 h. Following fermentation, the sediments are filtered to get the kvass. The microorganism responsible for fermentation are LABs and Saccharomyces cerevisiae, symbiotically produce the characteristic sour taste and flavor. Industrial kvass is produced in a slightly modified way, where the pre-extruded whole rye flour is hydrolyzed by  $\alpha$ -amylase, amyloglucosidase, and  $\beta$ -xylanolytic enzymes at 100 °C for 10 min. After cooling, starter cultures (LAB, yeast) are added and kept for fermentation at 30–37 °C for 48–60 h. The fermented slurry is mixed with sugar and caramelized malt and filtered to get kvass, which is usually bottled and stored at refrigerator temperature (8 °C) (Dlusskaya et al., 2008). As a result of microbial fermentation complex starch is converted to oligosaccharides, simpler sugars viz. maltose, glucose, fructose, xylose and proteins are converted to amino acids. Other metabolites formed are lactic acid, acetic acid, ethanol, minerals, and vitamins (Lidums et al., 2017). The volatile compounds formed during kvass production are esters, alcohols (<1%), organic acids, aldehydes and ketones, mostly responsible for the flavor of the final product (Lidums et al., 2015). Kvass helps in eliminating digestive disorders and increases the bioavailability of minerals. It doesn't require any heat treatment after fermentation, thus contains viable yeast and LAB cells over  $10^7$  cfu/mL making the product probiotic (Basinskiene et al., 2016).

## 8.5.5 Togwa

Togwa is a traditional non-alcoholic beverage consumed as weaning food in rural villages of East Africa. Raw materials used for togwa preparation are flour of maize and germinated finger millet flour, which are source of starch and amylase, respectively (Mashau et al., 2021). At times, as a source of starch, sorghum flour and cassava flour are also used. For preparation of togwa, suspension of maize flour is heated with stirring up to 80 °C to form a gel paste and cooled to about 50 °C, then the germinated finger millet flour is added to the warm porridge kept at the same temperature for 20 min. After addition of finger millet flour, the gel of maize flour is converted to a viscous liquid, which is then fermented for about 15 h (Kitabatake et al., 2003). After fermentation, the viscous paste is diluted to make a non-alcoholic drink. Togwa has opaque and brownish colour due to presence of hull of finger millet and maize and has a sweet and sour taste. Microorganisms responsible for togwa fermentation were reported to be Lactobacillus species, Issatchenkia orientalis and Saccharomyes cerevisiae. Unhygienic production process of togwa and poor shelf life led to declination of popularity amongst the Tanzanian people. Use of selective culture, good manufacturing process and proper packaging could improve the industrial value of togwa manufacturing.

## 8.5.6 Mahewu

Mahewu is a Southern African and Zimbabwean non-alcoholic beverage made from maize fermentation (Olusanya et al., 2021). It is prepared by mixing one part of maize meal with nine parts of boiling water and cooking for about 10–15 min. Cooking results maize starch gelatinization and swelling of the starch granules making the amylase leaching and increase in the viscosity of porridge. The gelatinized mixture is then allowed to cool, and wheat flour (5% w/w of maize flour) is added as a starter or inoculum source (Fadahunsi & Soremekun, 2017). The porridge is then kept for spontaneous fermentation for 36 h at room temperature (20–30 °C) with stirring at the beginning. The fermented *Mahewu* can be stored in a cool place for 20–25 days (Simango, 2002). The major microflora involved in the *Mahewu* fermentation is *Lactococcus lactis* subsp. *Lactis*, however other researchers have also tried *Lactobacillus delbruckii*, *L. bulgaricus* and some yeasts to produce acceptable mahewu at room temperature (Mashau et al., 2021). *Mahewu* is

reported to address childhood diarrhea challenges by enhancing the microbial balance in the gut. Moreover, Mahewu is now considered as a important functional food as it helps enhancing the immune system, and bioavailability of nutrients (Olusanya et al., 2021).

#### 8.6 Commercialization Status and Future Prospects

The demand for fermented food has become significant in modern society as it possesses great combination of very good sensory as well as nutritional attributes. Optimization of fermented products for commercial production and scaling up the process is important to obtain consistent quality. As fermentation process is dynamic in nature, the knowledge of involvement of microorganisms and their mode of functioning directed food scientists to use specific strains as starter culture to get desirable characteristics under controlled conditions. In addition, efficient use of novel food process methods, viz. co-culturing, molecular tools, genetic engineering, recombinant DNA technology and accelerated fermentation helps design and development of specific starter cultures which can perform better than the natural one in a controlled environment. For maintaining safety during fermentation and to increase the shelf life of the final product without hampering the nutritional value, large scale food industries are keen to implement advance thermal and non-thermal techniques, viz., ohmic heating, pulse electric field heating, microwave processing, gamma irradiation, UV light disinfection, etc. FDA (Food and Drug Administration, USA) has already approved the use of cobalt-60, cesium-137 and UV light for use in food applications. For safe production of the cereal-based fermented foods, small and medium-scale industries should implement good manufacturing practices (GMP) and hazard analysis critical control point (HACCP) principles.

Fermentation technology needs evolution as manufacturing process of many indigenous cereal-based fermented foods is yet to be optimized at commercial scale. For higher yield, the areas of modern fermenters, automation of the process and sensor development are important future aspects. Biochemical changes in the cereal starch during fermentation using microbial enzymes is another important future prospect for manufacturing of high quality and consistent product. Further research is needed in the area of supplementation or fortification of cereal-based fermented foods with protein isolated from plant sources and feasibility of the process in commercial scale. Moreover, to improve the functionality of cereal-based beverages, use of probiotic microbes and their safe delivery into the colon through nano emulsions are extensively being researched. Future studies should concentrate on developing novel cereal-based fermented foods that are fortified with underutilized millets and indigenous herbs targeting improved health benefits.

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