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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

B.SC – MICROBIOLOGY

UNIT – II- FOOD FERMENTATION AND TECHNIQUES

History and scope of fermented foods

Fermentation is one of the oldest biotechnologies for the production of food products with desirable properties such as extended shelf-life and good organoleptic properties (Smid and Hugenholtz 2010). Finished fermented foods usually have an improved microbial stability and safety and some can be stored even at ambient temperatures. Furthermore, there are several examples of fermentation processes which lead to an increase in nutritional value or digestibility (Jägerstad et al. 2005) of food raw materials. Finally, food fermentation processes also deliver products with increased palatability for consumers. All these arguments have boosted the interest to explore natural food fermentation processes and more precisely to link the diversity of the community of fermenting microbes and their properties to the energetics of the process and to product quality.

From a biochemical point of view, fermentation is a metabolic process of deriving energy from organic compounds without the involvement of an exogenous oxidizing agent. Fermentation plays different roles in food processing. Major roles attributed to fermentation are: (1) Preservation of food through formation of inhibitory metabolites such as organic acid (lactic acid, acetic acid, formic acid, and propionic acid), ethanol, carbon dioxide, diacetyl, reutrin, bacteriocins, etc., often in combination with decrease of water activity (by drying or use of salt) (Gaggia et al. 2011); (2) improving food safety through inhibition of pathogens (Adams and Nicolaides 2008) or removal of toxic compounds (Ray and Panda 2007); (3) improving the nutritional value (Poutanen et al. 2009, van Boekel et al. 2010); and (4) organoleptic quality of the food (Lacroix et al. 2010, Sicard and Legras 2011).

The common groups of microorganisms involved in food fermentations are bacteria, yeasts and moulds. The most important bacteria in the fermentation of foods are the *Lactobacillaceae*, which have the ability to produce lactic acid from carbohydrates. Other important bacteria are the acetic acid producing *Acetobacter* (mainly from fermentation of fruits and vegetables) and *Bacillus* (from fermentation of legumes) species. The beneficial yeasts in terms of desirable food fermentation are from the *Saccharomyces* family, especially *S. cerevisiae*. Yeasts play an important role in the food industry as they produce enzymes that result in desirable biochemical reactions such as the production of wine, beer and ethanol, and leavening of bread (Sicard and Legras 2011). The lactic acid bacteria (LAB) are, however, the most commonly found microorganisms in fermented foods (Sengun and Karabiyikli 2011). Their crucial importance is associated with their physiological features such as substrate utilization, metabolic capabilities and probiotic properties. Their common occurrence in foods coupled with their long historical use contributes to their acceptance as GRAS (Generally Recognized As Safe) for human consumption (Silva et al. 2002). The various LAB have been isolated from different fermented foods. Their functions during or after food fermentation have gradually been elucidated. This chapter focuses briefly on the types of microorganisms involved in food fermentations, especially on the roles of LAB in fermented foods. In addition, the current research activities in the field of fermented foods are also discussed. The roles of other microorganisms such as yeasts and moulds in food fermentations have been reviewed briefly.

Microorganisms Involved in Food Fermentations

The most common groups of microorganisms involved in food fermentation are:

- Bacteria
- Yeasts
- Moulds

Bacteria

Several bacteria are present in foods, the majority of which are concerned with food spoilage, while some like *Clostridium* are the causative agent for production of toxin like botulin, causing botulism in man (Joshi et al 2006). As a result, the important role of bacteria in the food fermentations is often overlooked. Lactic acid bacteria like *Lactobacillus, Pediococcus, Streptococcus, Oenococcus,* etc. are the most important bacteria in fermented foods, followed by *Acetobacter* species, which oxidize alcohol to acetic acid. The acetic acid fermentation has been used extensively to produce fruit vinegars including cider vinegar (Joshi and Thakur 2000, Joshi and Sharma 2010). A third group of bacteria of significance in fermentation are the *Bacillus* species (*Bacillus subtilis*, *B. licheniformis* and *B. pumilus*), which bring about alkaline fermentation.

Bacillus subtilis is the dominant species causing the hydrolysis of protein to amino acids and peptides and releasing ammonia, which increases the alkalinity and makes the substrate unsuitable for the growth of spoilage organisms. Alkaline fermentations are more common with protein-rich foods such as soybeans and other legumes, although there are few examples utilizing plant seeds. For example, water melon seeds (ogiri in Nigeria) and sesame seeds (ogiri-saro in Sierra Leone) are the substrates for alkaline fermentation (Battcock and Azam Ali 2001).

Yeasts

Yeasts and yeast like fungi are widely distributed in nature. They are present in orchards and vineyards, in air and soil, and in the intestinal tract of animals. Like bacteria and moulds, yeasts can have beneficial and non- beneficial effects in food fermentations. Some of the yeasts like *Pichia* are viewed as spoilage of food products while those like *Candida* are utilized for the single cell protein production. The most beneficial yeast in terms of desirable food fermentations are from the *Saccharomyces* family, especially *S. cerevisiae* involved in bread making and alcohol in wine fermentations. *Saccharomyces cerevisiae* var. *ellipsoideus* is employed extensively in wine making (Joshi et al. 2011). *Schizosaccharomyces pombe* and *S. boulderi* are the dominant yeasts in the production of traditional fermented beverages, especially those derived from maize and millet (Battcock and Azam Ali 2001). *Saccharomyces cerevisiae* var. *carlbergenisis* is the yeast involved in beer production. *Schizosaccharomyces pombe* has been found to have capacity to degrade malic acid into ethanol and carbon dioxide, and has been used successfully to lower the acidity in the grape and plum musts (Vyas and Joshi 1988, Joshi et al. 1991). A number of yeasts like *Rhodotorula, Crptococcus* have capacity to produce pigment to be used as biocolur (Joshi et al. 2003).

Moulds

Moulds are also important organisms in food processing both as spoilers and preservers of foods. Many moulds have capacity to produce enzymes of commercial importance such as pectinase by *Aspergillus niger* (Joshi et al. 2006). Species of *Aspergillus* are involved in the production of citric acid from waste like apple pomace (Joshi et al. 2009, Joshi and Attri 2006). The *Aspergillus* species are often responsible for undesirable changes in foods causing spoilage. On the other hand, *Penicillum* species are associated with the ripening and flavour development in cheeses. While the species of *Ceratocystis* are involved in fruit flavour production, at the same time, *Penicillium* is the causal agent for production of toxin like patulin (Joshi et al. 2013).

2 History of Fermented Foods

Fermentation as a food processing technique can be traced back to thousands of years (Table 1). The history of fermented foods is lost in antiquity. It seems that the art of fermentation originated in the Indian Sub-continent, in the settlements that predate the great Indus Valley civilization (Prajapati and Nair 2003). The art of cheese making was developed as far back as 8000 yr ago in the fertile Crescent between Tigris and Euphrates rivers in Iraq, at a time when plants and animals were just being domesticated (Fox 1993). Later, alcoholic fermentations involved in wine making and brewing are thought to have been developed during the period 4000–2000 BCE by the Egyptians and Sumerians. The Egyptians also developed dough fermentations used in the production of leavened breads way back 4000–3500 BCE (Prajapati and Nair 2003). However, the scientific rationale behind fermentation started with the identification of microorganisms in 1665 by van Leeuwenhoek and Hooks (Gest 2004). Louis Pasteur revoked the “spontaneous generation theory” around 1859 AD by elegantly designed experimentation (Farley and Geison 1974). The role of a sole bacterium “bacterium” lactis (*Lactococuus lactis*), in fermented milk was shown around 1877 by Sir John Lister (Santer 2010). Fermentation, from the Latin word Fevere’ was defined by Louis Pasteur as “la vie sans l’air” (life without air). Coincidentally, this was the time of the industrial revolution in Europe which resulted in large scale migration of populations from villages to larger cities. There was therefore a dramatic shift from the food production for local communities to large scale food production, necessary to meet the requirements of expanding

Table 1. Milestones in the history of fermented foods.

Milestone	**Development/Location**
6000–4000 BCE	Dahi—Coagulated sour milk eaten as a food item in India
7000 BC	Cheese production in Iraq, following the domestication of animals
6000 BC	Wine making in the Near East
5000 BC	Nutritional and health value of fermented milk and beverages described
4000 BC	Egyptians discovered how to use yeasts to make leavened bread and wine
2000 BCE-1200 CE	Different types of fermented milks from different regions
1750 BCE	Sumerians fermented barley to beer
1500 BCE	Preparation of meat sausages by ancient Babylonians
500 BCE	Mouldy soyabean curd as antibiotic in China
300 BCE	Preservation of vegetables by fermentation by the Chinese
500–1000 CE	Development of cereal-legume based fermented foods
1276 CE	First whisky distillery established in Ireland
1500 CE	Fermentation of sauerkraut and yoghurt
1851 CE	Louis Pasteur developed pasteurization
1877 CE	*Bacterum lactis* (*Lactococcus lactis*) was shown in fermented milk by John Lister
1881 CE	Published literature on koji and sake brewing
1907 CE	Publication of book *Prolongation of Life* by Eli Metchnikoff describing therapeutic benefits of fermented milks

1900–1930 CE	Application of microbiology to fermentation, use of defined cultures
1928 CE	Discovery of nisin—antagonism of some lactococci to other LAB shown by Rogers and Whittier
1970 CE–present	Development of products containing probiotic cultures or friendly intestinal bacteria
1953 CE	Nisin marketed in UK and since approved for use in over 50 countries
1990 CE–Present	Deciphering of genetic code of various LAB isolated from fermented foods
2002 CE	First authoritative list of microorganisms to be used in dairy culture was released by IDF and EFFCA
2012 CE	The list of Microbial Food Cultures regarded as GRAS to be used in all types of food fermentations has been released by IDF and EFFCA

Source: Ross et al. (2002), Prajapati and Nair (2003), updated

and more distant markets. This in turn led to the development of large scale fermentation processes for commercial production of fermented foods and alcoholic beverages, with the most widely used microorganisms including yeast for the production of beer, wine and spirits, and LAB for a variety of dairy, vegetable and meat fermentations (Ross et al. 2002). Modern large scale production of fermented foods and beverages is dependant almost entirely on the use of defined strain starters, which have replaced the undefined strain mixture traditionally used for the manufacture of these products. This switch over to defined strains has meant that both culture performance and product quality and consistency have been dramatically improved, while it has also meant that a smaller number of strains are intensively used and relied upon by the food and beverage industries. This intensive use of specific starters has, however, some drawbacks and can lead to production problems resulting in unsatisfactory strain performance. In the case of lactococcal fermentations, bacteriophage proliferation can affect cheese starter performance (Klaenhammer and Fitzgerald 1994).

In 1928 CE, Rogers and Whittier discovered nisin produced by some LAB and demonstrated its antagonistic activity against other food-borne bacterial pathogens. In 2002, a complete list of microorganisms that can be used as safe microbial food culture in dairy industry has been released by the International Dairy Federation (IDF) (Mogensen et al. 2002a, 2002b). The “2002 IDF inventory” has become a *de facto* reference for food cultures in practical use. In 2002, an updated inventory of microorganisms (bacteria, fungi, filamentous fungi and yeasts) used in food fermentations covering a wide range of food matrices was prepared by the members of IDF Task force (Bourdichon et al. 2012).

3 Advantages of Food Fermentation

Food fermentations have been practiced for millennia resulting the existence of a tremendous variety of fermented foods ranging from those derived from cereals, fish and meat to those derived from milk and dairy products (Table 2). In each case, the fermentation process involves the oxidation of carbohydrates to generate a range of products which are principally organic acids, alcohol and CO_2 (Ray and Panda 2007). Such products have a preservative effect through limiting the growth of spoilage and/ or pathogenic flora in the food product (Dalié et al. 2010). In addition, a number of desirable products, which affect the quality of the food may be produced, including the flavour compounds diacetyl and acetaldehyde (Ross et al. 2002, Jacqes and Casergola 2008), as well as compounds which may have positive health implications such as vitamins, antioxidants and bioactive peptides (Hugenschmidt et al. 2010). When considering food fermentations (as distinct from alcoholic fermentations involving yeast), the LAB is primarily responsible for many of the microbial transformations found in the more common fermented food products (Table 2). This group is composed of a number of genera including *Lactoctoccus, Lactobacillus, Enterococcus, Streptococcus, Leuconostoc* and *Pediococcus,* and generally

Table 2. Representative examples of fermented foods.

Product	**Country**	**Microorganism(s)**	**Substrate**
Bread	International	*Saccharomyces cerevisiae*, other yeasts, lactic acid bacteria (LAB)	Wheat, rye, other grains
Cheese	International	LAB (*Lactobacillus lactis*, *Streptococccus thermophilus*, *Lb. shermanii*, *Lb. bulgaricus*), *Propionibacterium shermanii*, sometimes moulds (*Penicillium* spp.)	Milk
Fufu	West Africa	LAB, *Citrobacter freundii*, *Geotrichum* sp., *Candida* sp. and *Saccharomyces* sp.	Cassava root
Gari	West Africa	*Corynebacterium manihot*, yeasts, LAB (*Lb. plantarum*, *Streptococcus* spp.)	Cassava root
Idli	Southern India	LAB (*Leuconostoc mesenteroides*, *Enterococcus faecalis*), *Torulopsis*, *Candida*, *Trichosporon pullulans*	Rice and black gram
Injera	Ethiopia	Yeasts, some fungi including *Pullaria* sp., *Aspergillus* sp., *Penicillium* sp., *Rhodotorula* sp., *Hormodendrum* sp., *Candida* sp. and number of unidentified bacteria	Cereals such as teff and sorghum
Kefir	North Africa	LAB	Milk
Kenkey	Ghana	LAB (*Pediococcus cerevisiae*, *Leuconostoc mesenteroides*, and *Lc. fermentum*	Maize
Kimchi	Korea	LAB	Cabbage, vegetables, sometimes seafood, nuts
Nan	India	*Saccharomyces cerevisiae*, LAB	White wheat flour
Ogi	Nigeria, West Africa	Lactic bacteria *Cephalosporium*, *Fusarium*, *Aspergillus*, *Penicillium* spp., *Saccharomyces cerevisiae*, *Candida mycoderma*, *C. valida*, or *C. vini*	Maize
Olives	Mediterranean	*Lc. mesenteroides*, *Lb. plantarum*	Green olives
Pickles	International	*Pediococcus cerevisiae*, *Lb. plantarum*	Cucumber
Plara	Thailand	*Bacillus* sp., *Bacillus cerus*, *B. circulans*, *B. licheniformis*, *B. megaterium*, *B. pumilus* and *B. subtitils*	Fresh water and marine fish

Table 2. contd....

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Product	**Country**	**Microorganism(s)**	**Substrate**
Nam pla and Budu	Thailand	*Lentibacillus salicampi*, *Lentibacillus juripiscarius Lantibacillus halophilus*, *Halococcus thailandensis*	Marine fish
Pulque	Mexico	LAB (*Pediococcus parvulus*, *Lb. brevis*, *Lb. composti*, *Lb. parabuchneri*, and *Lb. plantarum*)	Juice of *Agave* species
Sausages	Southern and Central Europe USA	LAB (lactobacilli, pediococci), Catalase positive cocci (*Streptococcus carnosus*), sometimes yeasts and/or moulds	Mammalian meat, generally pork and/or beef, less often poultry
Sauerkraut	International	LAB (*Lc. mesenteroides*, *Lb. brevis*, *Lb. plantarum*, *Lb. curvatus*, *Lb. sake*	Cabbage
Tempeh	Indonesia, Surinam	*Rhizopus oligosporus*	Soybeans
Wara	North Africa	*Lactobacillus* sp., *Leuconostoc* sp., *Pediococcus* sp., *Lactococcus* sp., yeasts	Sodom apple plant or pawpaw leaves
Yog urt	International	*S. thermophilus*, *Lb. bulgaricus*	Milk, milk solids

produces lactic acid as their major end product. The lactic acid produced may be L (+) or, less frequently (–) or a mixture of both. It should be noted that D (–) lactic acid is not metabolized by humans and is not recommended for infants and young children. The LAB are strictly fermentative and lack functional heme-linked electron transport chains and a functional Krebs cycle, they obtain energy *via* substrate level phosphorylation (Montet et al. 2006). The most common members of the group which are exploited for food uses include lactococci for cheese manufacture, *Streptococcus salivarius* subsp. *thermophilus* for cheese and yogurt manufacture and various members of the *Lactobacillus* genus for a variety of cereals, dairy, meat and vegetable fermentations (Liu et al. 2011) (Table 2).

Members of the LAB can be sub-divided into two distinct groups (Table 3) based on their carbohydrate metabolism. The homo-fermentative group composing *Lactocococcus, Pediococcus, Enterococcus, Streptococcus* and some lactobacilli use the Embden-Meyerhof-Parnas pathway to convert 1 mol of glucose into 2 mol of lactate. In contrast, hetero-fermentative bacteria produce equi-molar amounts of lactate, CO_2 and ethanol from glucose using the hexose-monophosphate or pentose pathway (Caplice and Fitzgerald 1999, Montet et al. 2006, Di Cagno et al. 2013) (Fig. 1), and in so doing generate only half the energy of the homo-fermentative group. Members of this group include *Leuconostoc*, *Weissella* and some lactobacilli.

Table 3. Major lactic acid bacteria in fermented foods.

Homofermenter	***Facultative homofermenter***	***Obligate heterofermenter***
Enterococcus faecium	*Lactobacillus bavaricus*	*Lactobacillus brevis*
Lactobacillus acidophilus	*Lactobacillus casei (syn. Lb. rhamnosus)*	*Lactobacillus buchneri*
Lactobacillus delbrueckii	*Lactobacillus coryniformis*	*Lactobacillus cellobiosus*
Lactobacillus lactis	*Lactobacillus curvatus*	*Lactobacillus confusus*
Lactobacillus leichmannii	*Lactobacillus plantarum*	*Lactobacillus coprophilus*
Lactobacillus salivarius	*Lactobacillus sakei*	*Lactobacillus fermentum*
Pediococcus acidilactici	*Lactobacillus manihotivorans*	*Lactobacillus sanfrancisco*
Pediococcus cerevisiae		*Leuconostoc dextranicum*
Pediococcus damnosus		*Leuconostoc lactis*
Pediococcus pentosaceus		*Leuconostoc mesenteroides*
Streptococcus agalactiae		*Leuconostoc paramesenteroides*
Streptococcus bovis		
Streptococcus faecalis		
Streptococcus mutans		
Streptococcus salivarius		
Streptococcus thermophilus		

Source: Ray and Panda (2007)

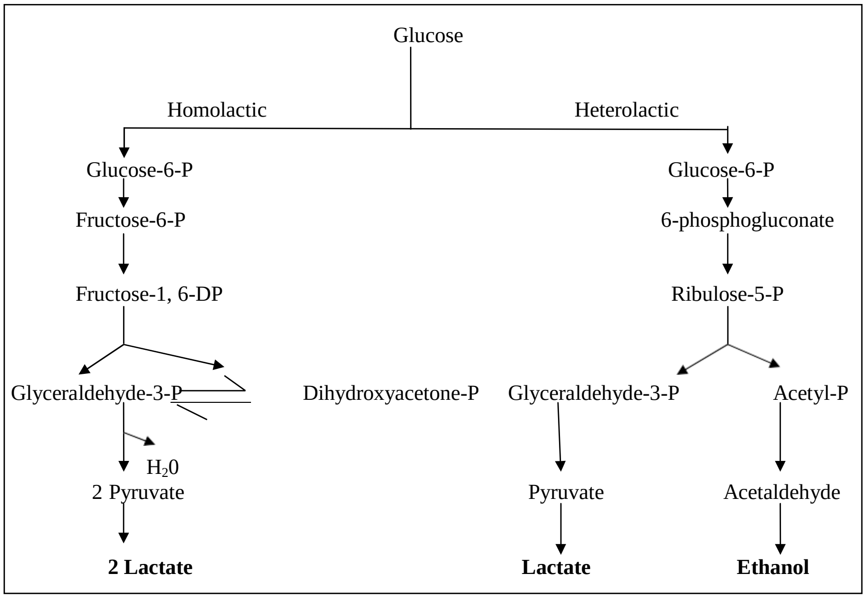


Fig. 1. Generalized scheme for the fermentation of glucose to lactic acid bacteria (Caplice and Fitzgerald 1999).

he metabolism of the disaccharide lactose is of primary importance in those LAB that are used in dairy fermentations (Shah 2007). Lactose may enter the cell using either a lactose carrier, lactose permease, followed by cleavage to glucose and galactose or *via* a phosphor-enolpyruvate- dependent phosphor-transferase (PTS) followed by cleavage to glucose and galactose-6-phosphate. Glucose is metabolized *via* glycolytic pathway, galactose *via* the Leloir pathway and galactose-6-phosphate *via* the tagatose 6-phosphate pathway. Most *Lactobacillus lactis* strains used as starters for dairy fermentations use the lactose PTS, the genes for which are plasmid located. Among some thermophilic LAB, only the glucose moiety of the sugar is metabolized and galactose is excreted into the medium, although mutants of *Streptococccus thermophilus* have been described, which metabolize galactose *via* the Leloir pathway (Caplice and Fitzerald 1999, Hansen 2002).

Citrate metabolism is important among *Lb. lactis* subsp. *lactis* (bv. *diacetylactis*) and *Leuconostoc mesenteroides* subsp. *cremoris* strains used in the dairy industry, as it results in excess pyruvate in the cell. The pyruvate may be converted *via* α-acetolactate to diacetyl, an important flavour and aroma component of butter and some other fermented milk products (Hansen 2002) (Fig. 2).

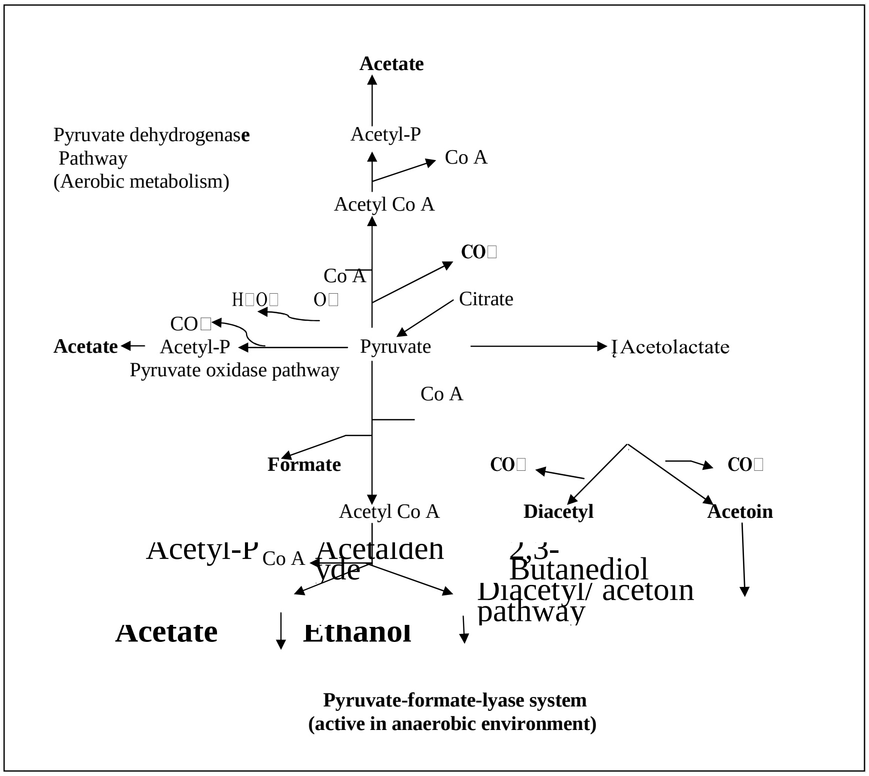


Fig. 2. Generalized scheme for the formation of important metabolic products from pyruvate in lactic acid bacteria (Caplice and Fitzgerald 1999, Hansen 2002).

Environmental parameters for fermentation process;

The six parameters are: 1. Preliminary Investigations 2. Scale Up 3. Culture Preservation 4. Feedstocks for Fermentation Processes 5. Fermenter Design and Operation 6. Downstream Processing.

1. Preliminary Investigations:

The starting point for a fermentation process is the discovery that a microorganism makes a useful product.

Such a discovery does not by itself constitute a sufficient basis for a successful industrial fermentation — the organism may grow poorly, the medium used may be expensive, and yields of the desired product may be very low.

These problems are overcome by improving the performance of the microorganism genetically and by providing it with the optimum environment for growth and product formation.

2. Scale Up:

When efficient growth and product formation by the organism and product recovery from the fermenter have been standardised on the pilot plant scale, and if it is found to be satisfactory, commercial production becomes feasible.

3. Culture Preservation:

A fungal strain that gives high yields of a valuable product is a precious asset. Such a strain devotes a substantial proportion of its carbon source to the formation of a metabolite in quantities which are unnecessary for survival. Repeated subculture is hence likely to result in the replacement of the high-yielding strain with variants that produce less of the desired metabolite and devote more of their resources to achieving higher growth rates.

Excessive subculture must, therefore, be avoided, and the material needed for initiating fermentations kept in a non-growing state. The ideal starting point for preparing cultures for preservation will be a master culture that has originated from a single uninucleate haploid cell and is hence genetically uniform, and which has been shown, by testing subcultures, to have the desired properties. From such a master culture a set of subcultures is prepared and from them material for preservation.

4. Feedstocks for Fermentation Processes:

For a successful fermentation, the fungus must behave in a consistent, predictable manner, giving similar growth rates and metabolite yields in successive fermentations. A crucial factor in achieving this is to provide a nutrient medium that contains utilizable sources of carbon, nitrogen and other essential elements in the appropriate amounts and ratios. This is easily done in the laboratory by making use of pure chemicals, but on an industrial scale this would be far too expensive. The selection of the right raw materials (feedstocks) for medium preparation is a key factor in the success of an industrial fermentation.

5. Fermenter Design and Operation:

Most industrial fermentations are carried out in stirred tank fermenters. A stirred tank fermenter (Fig. 11-1) is a cylindrical vessel designed to contain a volume of sterile medium that can be inoculated, aerated, stirred, monitored by sensing devices, heated or cooled, and sampled or fed with additional materials, all without bringing about contamination. It is constructed of high-grade stainless steel, to avoid corrosion by media or leaching of toxic metals into the medium.

6. Downstream Processing:

When a fermentation is complete it will contain cells (biomass) in a large volume of spent medium. In brewing, the spent medium is itself the desired product, and in single cell protein production—the biomass. Usually, however, the desired product is a minor component of the cells or broth.

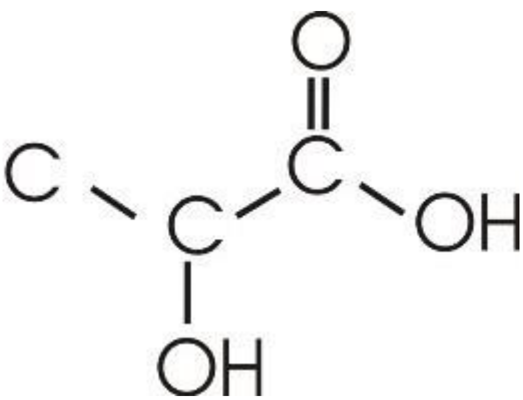
A liter of broth with biomass may, for example, contain only 1 g of a required enzyme or 10 g of an antibiotic. A small amount of product, hence, has to be separated from a large volume of waste material. A fermentation will be economic only if the downstream processing that accomplishes this is efficient.

A series of steps (unit operations) will be required in the concentration and purification of a product. Each unit operation involves expense in terms of equipment, manpower and energy or chemicals, and at each step there will be some loss of product. The number of steps hence, has to be kept to a minimum

CLASSIFICATION OF FERMENTATION PROCESSES FOR FERMENTED FOODS.

Lactic Acid Fermentation

You may have not been aware that your muscle cells can ferment. **Fermentation** is the process of producing ATP in the absence of oxygen, through glycolysis alone. Recall that glycolysis breaks a glucose molecule into two pyruvate molecules, producing a net gain of two ATP and two NADH molecules. **Lactic acid fermentation** is the type of anaerobic respiration carried out by yogurt bacteria (*Lactobacillus* and others) and by your own muscle cells when you work them hard and fast.



Lactic acid, C3H6O3C3H6O3.

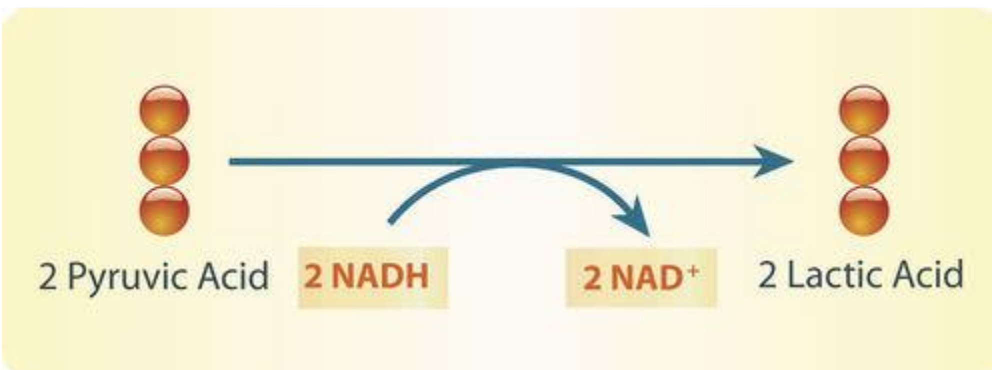
Lactic acid fermentation converts the 3-carbon pyruvate to the 3-carbon lactic acid (C3H6O3)(C3H6O3) (see figure below) and regenerates NAD++ in the process, allowing glycolysis to continue to make ATP in low-oxygen conditions. Since there is a limited supply of NAD++ available in any given cell, this electron acceptor must be regenerated to allow ATP production to continue. To achieve this, NADH donates its extra electrons to the pyruvate molecules, regenerating NAD++. Lactic acid is formed by the reduction of pyruvate.

C3H3O3(pyruvate)+NADH→C3H6O3(lactic

acid)+NAD+(15.3.1)(15.3.1)C3H3O3(pyruvate)+NADH→C3H6O3(lactic acid)+NAD+

actic acid fermentation converts pyruvate to lactic acid, and regenerates NAD++ from NADHNADH.

For *Lactobacillus* bacteria, the acid resulting from fermentation kills bacterial competitors in buttermilk, yogurt, and some cottage cheese. The benefits extend to humans who enjoy these foods, as well You may have notice this type of fermentation in your own muscles, because muscle fatigue and pain are associated with lactic acid. Lactic acid accumulates in your muscle cells as fermentation proceeds during times of strenuous exercise. During these times, your respiratory and cardiovascular systems cannot transport oxygen to your muscle cells, especially those in your legs, fast enough to maintain aerobic respiration. To allow the continuous production of some ATP, your muscle cells use lactic acid fermentation.



Lactic acid fermentation makes ATP in the absence of oxygen by converting glucose to lactic acid (through a pyruvate intermediate). Making lactic acid from pyruvate oxidizes NADH, regenerating NAD++ so that glycolysis can continue to make more ATP rapidly. Each circle represents a carbon atom.

Ethanol fermentation

Ethanol fermentation, also called **alcoholic fermentation**, is a biological process which converts sugars such as glucose, fructose, and sucrose into cellular energy, producing ethanol and carbon dioxide as by-products. Because yeasts perform this conversion in the absence of oxygen, alcoholic fermentation is considered an anaerobic process. It also takes place in some species of fish (including goldfish and carp) where (along with lactic acid fermentation) it provides energy when oxygen is scarce.[1]

Ethanol fermentation has many uses, including the production of alcoholic beverages, the production of ethanol fuel, and bread cooking.

The chemical equations below summarize the fermentation of sucrose ($C_{12}H_{22}O_{11}$) into ethanol (C_2H_5OH). Alcoholic fermentation converts one mole of glucose into two moles of ethanol and two moles of carbon dioxide, producing two moles of ATP in the process.

The overall chemical formula for alcoholic fermentation is:

$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$

Sucrose is a sugar composed of a glucose linked to a fructose. In the first step of alcoholic fermentation, the enzyme invertase cleaves the glycosidic linkage between the glucose and fructose molecules.

$C_{12}H_{22}O_{11} + H_2O + \text{invertase} \rightarrow 2\ C_6H_{12}O_6$

Next, each glucose molecule is broken down into two pyruvate molecules in a process known as glycolysis.[2] Glycolysis is summarized by the equation:

$C_6H_{12}O_6 + 2\ ADP + 2\ P_i + 2\ NAD^+ \rightarrow 2\ CH_3COCOO^- + 2\ ATP + 2\ NADH + 2\ H_2O + 2\ H^+$

CH_3COCOO^- is pyruvate, and P_i is inorganic phosphate. Finally, pyruvate is converted to ethanol and CO_2 in two steps, regenerating oxidized NAD+ needed for glycolysis:

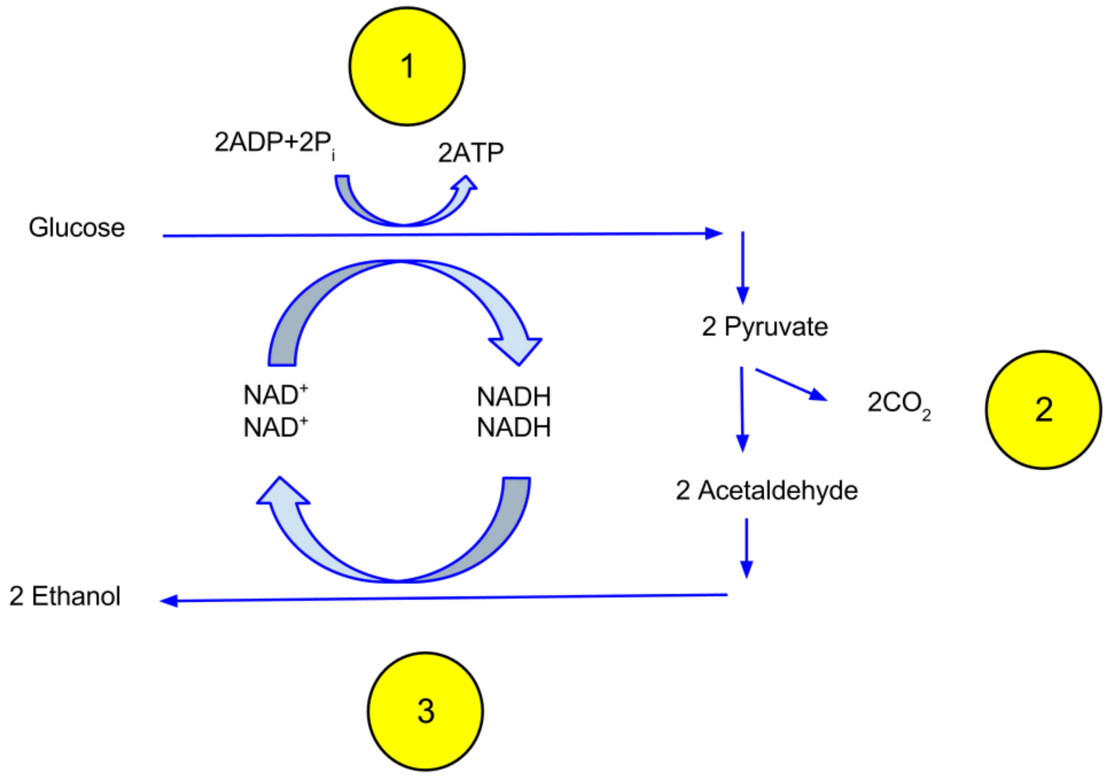
1. $CH_3COCOO^- + H^+ \rightarrow CH_3CHO + CO_2$

catalyzed by pyruvate decarboxylase

2. $CH_3CHO + NADH + H^+ \rightarrow C_2H_5OH + NAD^+$

This reaction is catalyzed by alcohol dehydrogenase (ADH1 in baker's yeast).[3]

As shown by the reaction equation, glycolysis causes the reduction of two molecules of NAD^+ to NADH. Two ADP molecules are also converted to two ATP and two water molecules via substrate-level phosphorylation.



Related processes

Fermentation of sugar to ethanol and CO_2 can also be done by *Zymomonas mobilis*, however the path is slightly different since formation of pyruvate does not happen by glycolysis but instead by the Entner–Doudoroff pathway. Other microorganisms can produce ethanol from sugars by fermentation but often only as a side product. Examples are[4]

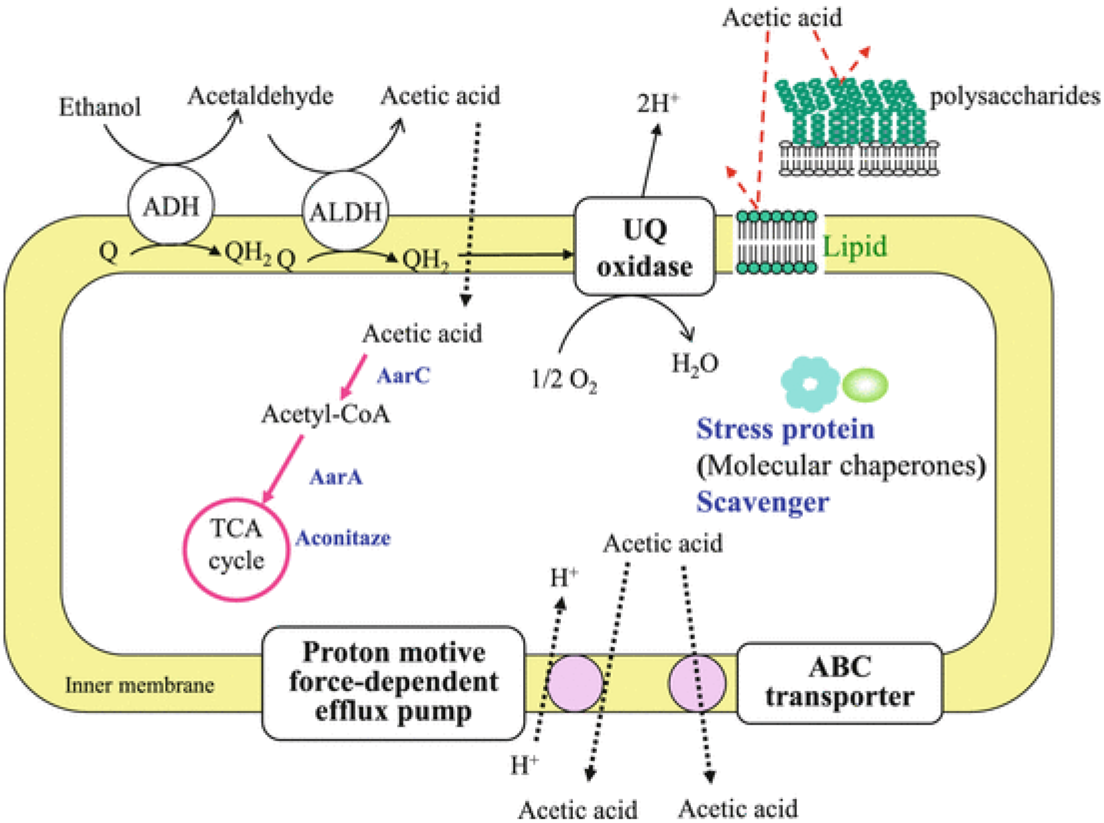
- Heterolactic acid fermentation in which Leuconostoc bacterias produce Lactate + Ethanol + CO_2
- Mixed acid fermentation where Escherichia produce ethanol mixed with lactate, acetate, succinate, formate, CO_2, and H_2
- 2,3-butanediol fermentation by Enterobacter producing ethanol, butanediol, lactate, formate, CO_2, and H_2

ACETIC ACID FERMENTATION PATHWAY

Acetic acid /əˈsiːtɪk/, systematically named **ethanoic acid** /ˌɛθəˈnoʊɪk/, is a colourless liquid organic compound with the chemical formula CH_3COOH (also written as CH_3CO_2H, $C_2H_4O_2$, or $HC_2H_3O_2$). When undiluted, it is sometimes called *glacial acetic acid*. Vinegar is no less than 4% acetic acid by volume, making acetic acid the main component of vinegar apart from water. Acetic acid has a distinctive sour taste and pungent smell. In addition to household vinegar, it is mainly produced as a precursor to polyvinyl acetate and cellulose acetate. It is classified as a weak acid since it only partially dissociates in solution, but concentrated acetic acid is corrosive and can attack the skin.

Acetic acid is the second simplest carboxylic acid (after formic acid). It consists of a methyl group attached to a carboxyl group. It is an important chemical reagent and industrial chemical, used primarily in the production of cellulose acetate for photographic film, polyvinyl acetate for wood glue, and synthetic fibres and fabrics. In households, diluted acetic acid is often used in descaling agents. In the food industry, acetic acid is controlled by the food additive code E260 as an acidity regulator and as a condiment. In biochemistry, the acetyl group, derived from acetic acid, is fundamental to all forms of life. When bound to coenzyme A, it is central to the metabolism of carbohydrates and fats.

The global demand for acetic acid is about 6.5 million metric tons per year (Mt/a), of which approximately 1.5 Mt/a is met by recycling; the remainder is manufactured from methanol.[9] Vinegar is mostly dilute acetic acid, often produced by fermentation and subsequent oxidation of ethanol.


Acetic acid
Ethanol
Acetaldehyde
Acetic acid
2H+
polysaccharides
ADH
ALDH
Q
QH2
Q
QH2
UQ oxidase
Lipid
Acetic acid
1/2 O2
H2O
AarC
Acetyl-CoA
Stress protein
(Molecular chaperones)
Scavenger
AarA
TCA cycle
Aconitaze
Acetic acid
H+
Proton motive force-dependent efflux pump
ABC transporter
Inner membrane
H+
Acetic acid
Acetic acid

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

B.SC – MICROBIOLOGY

UNIT – II- FOOD FERMENTATION AND TECHNIQUES

Brewing; beer production process

- Brewing is the process of production of malt beverages. Beers, ale and lagers are the main malt beverages produced by a method called brewing. Brewing is a complex fermentation process. It differs from other industrial fermentation because flavor, aroma, clarity, color, foam production, foam stability and percentage of alcohol are the factors associated with finished product.

Steps involved in beer production are:

1. Malting:

- Beer is produced from barley grains.
- Barley grains are first cleaned and then soaked in water for about 2 days. Then excess water is drained away and the barley are incubated for 4-5 days to allow germination
- The germination steps allow the formation of highly active α-amylase, β-amylase and proteases enzymes as well as various flavor and color components
  - **Malt adjuncts:**
    - Barley contains considerable amount of protein. So, if only barley are used for beer production, the final beer will be dark and unstable. Therefore, protein present in malt should be diluted by adding additional starch or sugary materials.
    - Such sugary or starchy materials are called malt adjuncts and includes dextrose sugar syrup.

2. Kilning:

- The germinated seed are then killed by slow heating at 80° This process is called kilning.
- The kilning temperature must not harm amylase enzyme. Furthermore, if kilning temperature is higher, darker will be the beer produced.

3. Mailing:

- The dried barley grains are then crushed between rollers to produced coarse powder called grist

4. Mashing:

- Grist is mixed with warm water and the resulting materials is maintained at 65°C for about 1 hour.
- In doing so, starch is hydrolyzed by amylase enzyme to produce single sugar, maltose, dextrose etc. similarly, protein is hydrolyzed by proteolytic enzymes into small fragments and amino acids.
- The degree of enzymatic hydrolysis is strongly depends on pH and temperature. β-amylase has optimum activity at temperature 57-65°C whereas α-amylase has optimum activity at temperature 70-75°

- The liquid obtained by mashing is called wort. The husks and other grains residue as well as precipitated proteins are removed filtration.

5. Boiling of wort:

- The filtrate is then bolied with stirring for 2-3 hours and hop flowers are added at various interval during boiling.
- Reasons for boiling of wort:
  - For extraction of hop flavor from hop flower
  - Boiling coagulate remaining protein and partially hydrolyze protein and help in removal of protein
  - Boiling inactivates enzymes that were active during mashing, otherwise causes caramelization of sugar
  - Boiling also sterilize and concentrate the wort

6. Hops:

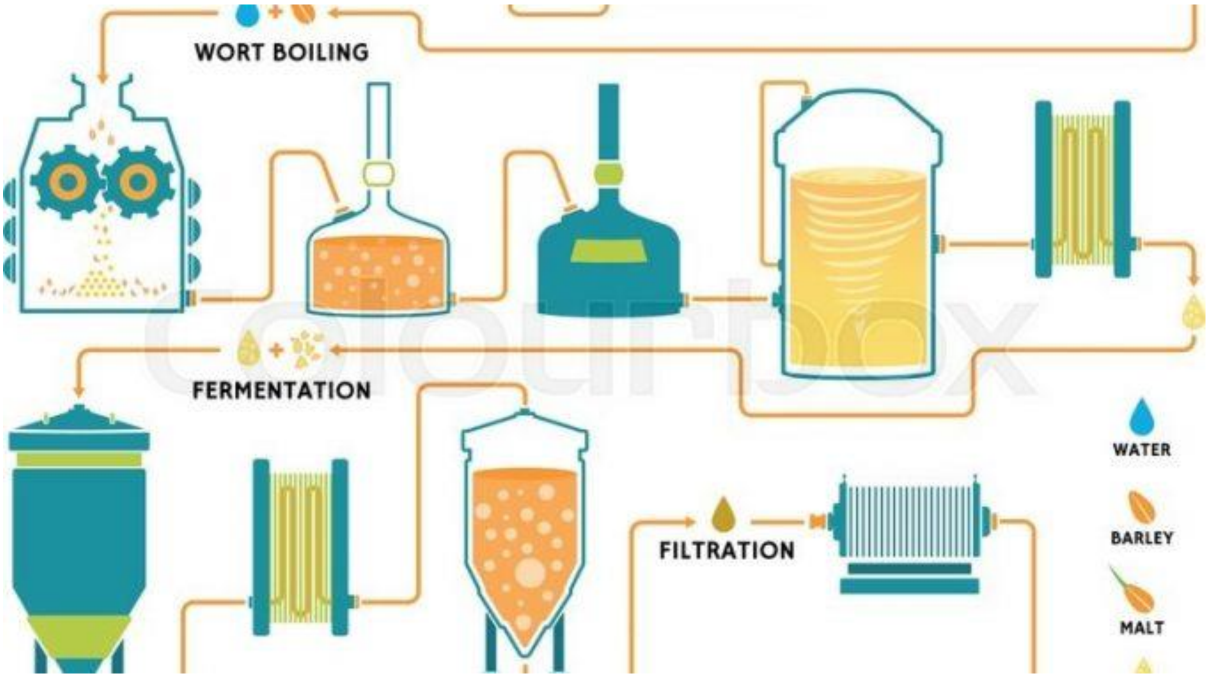
- Hops are dried female flower of hop plant Humulus lupulus. Approximately one quarter pound of hop flower is added per barrel of beer and up to 2 pound per barrel of ale.
- Advantages of hop addition in beer are;
  - Provide beer with its pungent and aromatic character
  - Provide tannin which helps in coagulation of remaining protein
  - Contains α-resin and β-resin which gives bitter flavor as well as preservative action against gram Positive bacteria
  - Contains pectin which is responsible for foam characteristic of beer

7. Fermentation:

- Beer production utilize strain of Saccharomyces carlsbergens and S. varum which are bottom yeast and S. cerevisiae which is a top yeast.
- Yeast cells for inoculation are usually recover from previous fermentation tank by treatment with phosphoric acid, tartaric acid or ammonium persulphate to reduce the pH and removed considerable bacterial contamination.
- Fermentation is usually carried out at 3-4 °C but it may range from 3- 14° Fermentation usually completes in 14 days.
- During fermentation yeast converts sugar mainly into ethanol and CO2 plus some amount of glycerol and acetic acid.
- For fermentation open tank fermenter can be used however closed fermenter tank is preferred, so that CO2 liberated during fermentation can be collected for later carbonation step.
- CO2 evolution is maximum by fifth day of fermentation, there is no evolution of CO2 by 7-9 days because yeast cells become inactive and flocculate.
- Most beer contains 3.5-5% alcohol.

8. Finishing, Ageing, Maturation and Carbonation:

- The young and green beer is stored in vat at 0°C for several weeks to several months. During this period, precipitation of protein, yeast, resin and other undesirable substances take place and beer become clear.
- Ester and other compounds are also produced during ageing which gives taste and aroma.
- After ageing, the beer is carbonated by carbondioxide of 0.45-0.52%.
- The beer is then cooled, clarified, filtered and packed in bottles, barrels and cans



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Wine

- Wine is a kind of undistilled alcoholic beverage mainly prepared from fruit juice. (mainly from grapes).
- The process of preparation of wine is known as **vinification** and the branch of science that deals with study of wine is known as **enology** (American) or oenology (British).
- There are different types of wine on different basis.
- Besides fruit and berries, non-toxic plants (flowers) etc can also be used for wine production.
- Since, basic constituent of wine is alcohol, other substrates are also added in it.
- It contains 3-22% of alcohol.

Color	Red(pigmented) White (not pigmented)
Sugar content	Sweet wine – some sugars are left during fermentation and recovery Dry wine- all sugars fermented into alcohol
Alcohol content	Table wine- 3-10% alcohol Fortified wine- 19-22% alcohol (since yeast cannot accumulate late alcohol, whisky is added) Dessert wine = Fortified sweet wine with 22% alcohol
Carbonation or CO_2 content	Still wine: no CO_2 is produced during fermentation. Sparkling wine: with CO_2 production. E.g. champagne

Microbiology: Grape juice (27% sugar) is fermented by various strains of *Saccharomyces cerevisiae.*

Wine production:

- Wine is basically the transformation of sugars of grapes of yeast under anaerobic condition into ethanol, CO_2 and small amounts of byproducts such as D-glucose.

- **Step I: Harvesting of fruits:**
  - Appropriate variety of fruits and berries are harvested.
  - They must contain high amount of fermentable sugars.
  - Grapes usually contain 5-25% total soluble sugar (Total soluble sugar).
- **Step II: Crushing and extraction:**
  - Thus, obtained fruits are crushed and extracted mechanically.
  - This process releases juice and a little bit pigment.
  - The whole mass is known as Must.
  - For white wine preparation, the skin is removed. The harvested fruits are de-steamed for white wine preparation which is not required for red wine preparation.
  - In case of red wine, the steam gives vegetable aroma due to presence of 2 methoxy-3-isopropyl pyrazine.
  - Color is also extracted from steam.
  - In case of red wine, the Must should be fermented.

- **Step III: Optimization:**
    - The must is optimized for two parameters, TSS and pH.
    - The TSS is generally optimized between 17-22% and pH in between 3-4, depending on yeast strains to be used.
    - KNS (potassium metabisulphite) may or may not be added at this stage which is an antimicrobial compound against *Acetobacter* spp. and competitive yeast.
    - It also acts as anti-oxidant and antifungal agent.
- **Step IV: Primary fermentation:**
    - The optimized Must is inoculated with 2-10% of inoculum and fermentation is carried out under optimum temperature.
    - Red wine preparation= 22-27^{o}C for 3-5 days
    - White wine preparation= 10-21^{o}C for 7-14 days
    - During the fermentation, the content is mixed twice a day by punching the floating skin for proper aeration.
    - It also helps in color extraction.
    - This fermentation allows rapid multiplication of yeast cell as well as sugar fermentation to ethanol, when the TSS is decreased nearly about 9-10% then primary fermentation is terminated.
- **Step V: Pressing:**
    - The skin of must is taken out and pressed in order to release juice and alcohol.
    - The liquid is again transferred into tank.
    - In case of white wine, pressing is carried out before fermentation.
    - During pressing color of fruits and berries is extracted.
- **Step VI: Heat and cold sterilization:**
    - The main aim of this technique is to remove the tartarate crystals (wine diamonds or wine crystals).
    - In cold sterilization method, the fermented must is cooled to nearly freezing and kept for one to two weeks.
    - During this period, the crystals gets separated or stirred in the wall of fermenter and clear liquid is collected on secondary fermented tank.
    - In heat stabilization technique, it is gently heated in between 50-60^{o}C for an hour and kept overnight.
    - The proteins get decanted.
    - The clear contents are pumped out and remaining turbid substance adsorbed on to bentonite.
- **Step VII: Secondary fermentation:**
    - It is carried out in stainless steel or oak barrel or concrete tank lined with plastic.
    - The stabilized, sterilized wine is now kept at 15-20^{o}C for 3-6 months under strict anaerobic condition usually in case of sweet wine, the fermentation is terminated when sugar content is reduced to 4-6%.
    - During secondary fermentation, aroma is developed.
    - The aroma in wine is categorized into 3 types:
        - Primary aroma —-> contributed by fruits or berries
        - Secondary aroma —–> developed during secondary fermentation

        - Tertiary aroma ——-> developed during bottled ageing
    - The aroma compound may be volatile or non-volatile.
    - It is developed due to chemical reactions among acids (malic acid, citric acid etc), sugars, alcohols and phenolic compounds.
    - The main compound responsible for aroma is methoxyparazine , monoterpenes, nor-isoprenoids, thiols, esters etc. among which ester is the principal one.
    - Esters are produced by reaction between alcohols and acids which is very slow.
    - It takes nearly one year for secondary fermentation
    - Before secondary fermentation malo-lactic fermentation occurs.
    - Malic acid (sharp sour) —–Lactic acid bacteria (LAB)—-> Lactic acid
- **Step VIII: Laboratory testing:**
    - After secondary fermentation, certain laboratory tests are conducted which includes bricks reading, bricks pH, titrable acidity, residual sugars, free or available sulfur, total sulfur, volatile acidity and alcohol percentage.
- **Step IX: Blending and fining:**
    - It is the most crucial to produce good quality of wine giving special taste and aroma.
    - In blending process, spices, extracts of aromatic plants, essential oils, fruit juices and other things are added in appropriate proportion.
    - Blending is kept trade secret in winery (wine industry).
    - In fining process, tannins and microscopic particles are removed in order to make clear wine.
    - For this purpose, wine is treated with gelatin, potassium caseinate, egg albumin, lysozymes, skimmed milk powder etc. or it is filtered through membrane filter or diatomaceous earth cellulose filter.
    - Finally, wine is clarified in order to remove pectin which is achieved with the use of pectinase enzyme.
- **Step X**: **Preservation:**
    - Pasteurization technique and use of KMS (Potassium metabisulphite) are mainly used for preservation.
    - It kills sugar utilizing micro-organisms.
- **Step XI: Bottling:**
    - Finally, wine is aseptically filled in bottle and bottle is corked, which is usually made with oak.
    - Finally, the outside cork is sealed.
    - The bottled wine can be directly consumed or preserved.

Distillation of wine:

- These are the alcoholic beverages/drinks obtained by the distillation of wine or fermented cereals.
- It may be aged or unaged (i.e. the distilled liquor).
- Distilled liquor is commonly called spirits.
- They consist of more than 40% ethanol.

- There are various types of distilled liquor. The primary types are:
- Whisky (Barley and others) —–> aged
- Brandy (Wine distillation) ——> aged
- Rum (fermented molasses) ——> aged
- Vodka (fermented cereals) ——> not aged
- Gin (distillation of fermented cereals) ——> unaged but flavored

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Introduction

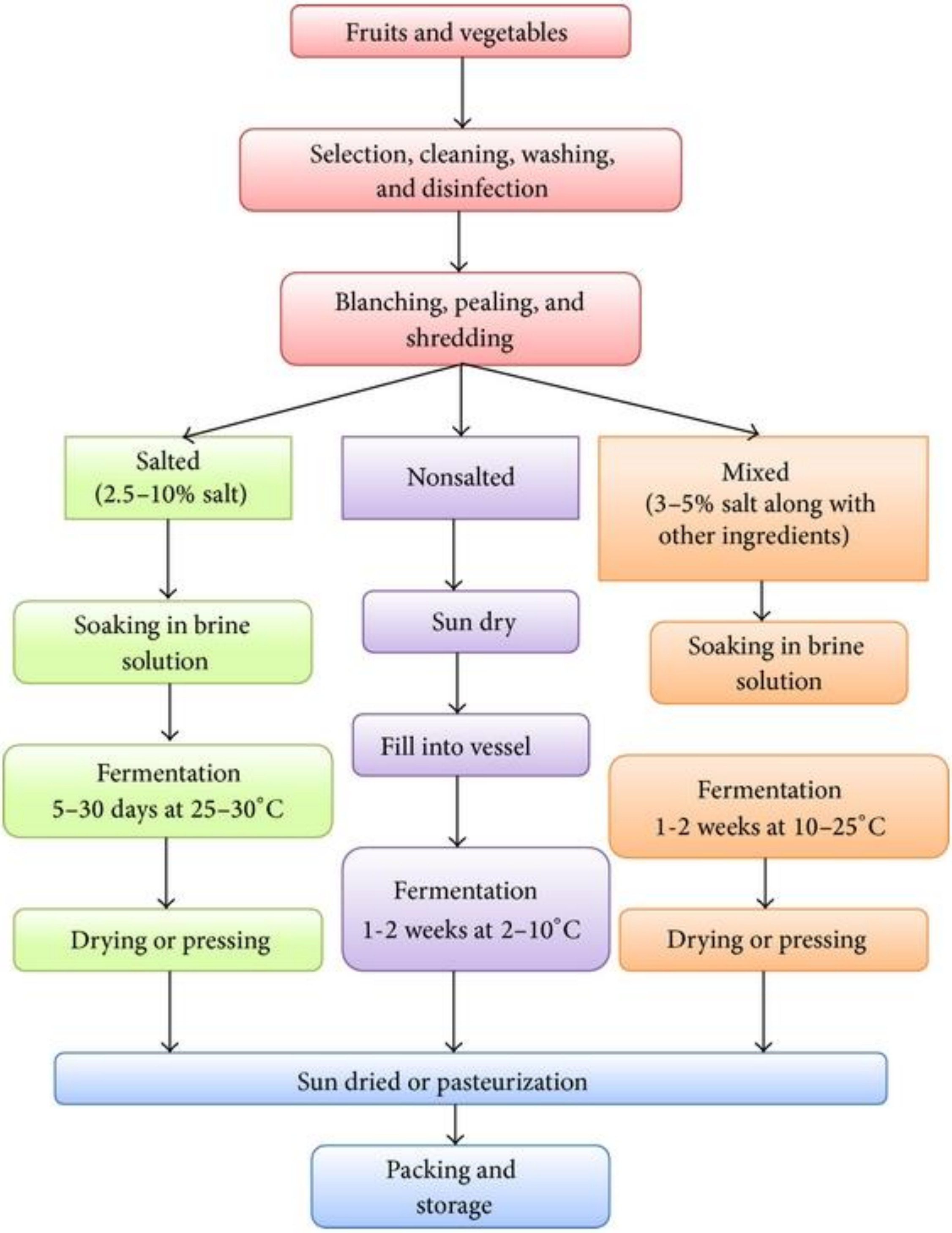
Fermented foods and beverages have heterogeneity of traditions and cultural preferences found in the different geographical areas, where they are produced. Fermentation has enabled our ancestors in temperate and cooler regions to survive during the winter season and those in the tropics to survive drought periods. Fermentation is a slow decomposition process of organic substances induced by microorganisms or enzymes that essentially convert carbohydrates to alcohols or organic acids [1]. In many instances, production methods of different traditional fermented foods were unknown and passed down to subsequent generations as family traditions. Drying and salting are common fermentation practices in the oldest methods of food preservation. Fermentation processes are believed to have been developed in order to preserve fruits and vegetables for times of scarcity by preserving the food by organic acid and alcohols, impart desirable flavour, texture to foods, reduce toxicity, and decrease cooking time [2].

World Health Organization (WHO) and Food and Agriculture Organization (FAO) recommended intake of a specific dose of vegetable and fruits in daily food to prevent chronic pathologies such as hypertension, coronary heart problems, and risk of strokes. The consumers tend to prefer the foods and beverages which is fresh, highly nutritional, health promoting and ready to eat or ready to drink [3]. Lactic acid (LA) fermentation of vegetables and fruits is a common practice to maintain and improve the nutritional and sensory features of food commodities [4–6]. A great number of potential lactic acid bacteria (LAB) were isolated from various traditional naturally fermented foods [7]. Asian traditional fermented foods are generally fermented by LAB such as *Lactobacillus plantarum*, *L. pentosus*, *L. brevis*, *L. fermentum*, *L. casei*, *Leuconostoc mesenteroides*, *L. kimchi*, *L. fallax*, *Weissella confusa*, *W. koreenis*, *W. cibaria*, and *Pediococcus pentosaceus*, which are considered as the probiotic source of the food practice. Availability of certain specific nutrients such as vitamins, minerals, and acidic nature of fruits and vegetables provides conducible medium for fermentation by LAB.

Probiotic is a relatively new word meaning “for life” and it is generally used to name the bacteria associated with beneficial effects for humans [8, 9]. Probiotics are defined as live microbial feed such as *Lactobacillus plantarum*, *L. casei*, *L. acidophilus*, and *Streptococcus lactis* which are supplemented by food that beneficially affect the host by improving its intestinal balance [10]. Several studies have shown that supplementation of probiotics to food provides several health benefits such as reduction of serum cholesterol, improved gastrointestinal function, enhanced immune system, and lower risk of colon cancer [11–15]. This review provides an overview on the current research prospects of LA fermentation of fruits and vegetables with regard to human nutrition and health.

2. *Fermentation of Fruits and Vegetables by LAB*

Shelf life of the perishable food can be improved by fermentation which is considered as the oldest technology compared to the refrigeration. Fermentation is one of the oldest processing techniques to extend the shelf life of perishable food and was particularly important before refrigeration. LA fermentation of cabbage to produce sauerkraut has been widely studied for many years [16, 17]. Basic outline of the fruit and vegetable fermentation is given in Figure 1. With the popularity and success of sauerkraut, fermentation of many other vegetables has emerged, such as cucumbers, beets, turnips, cauliflower, celery, radishes, and carrots [18] (Table 1).


Fruits and vegetables
Selection, cleaning, washing, and disinfection
Blanching, pealing, and shredding
Salted (2.5–10% salt)
Nonsalted
Mixed (3–5% salt along with other ingredients)
Soaking in brine solution
Sun dry
Soaking in brine solution
Fill into vessel
Fermentation 5–30 days at 25–30°C
Fermentation 1-2 weeks at 10–25°C
Fermentation 1-2 weeks at 2–10°C
Drying or pressing
Drying or pressing
Sun dried or pasteurization
Packing and storage

Depending on the type of raw materials in final fermented products, vegetable fermentation is characterized accordingly. Sauerkraut, fermented cucumbers, and kimchi are the most studied lactic acid fermented vegetables mainly due to their commercial importance. Canning or freezing is often too expensive method in food preservation which cannot be affordable by millions of world's economically deprived people and lactic acid fermentation [19].

Fermented fruits and vegetables (Table 2) have an important role in feeding the world's population on every continent today [20, 21]. They play an important role in preservation, production of wholesome nutritious foods in a wide variety of flavours, aromas, and textures which enrich the human diet and remove antinutritional factors to make the food safe to eat [4]. Fermentation serves many benefits, which include food security, improved nutrition, and better social well-being of the people living in marginalized and vulnerable society [22]. Fermentation-based industries are an important source of income and employment in Asia, Africa, and Latin America [23]. Fermentation of fruits and vegetables can occur "spontaneously" by the natural lactic bacterial surface microflora, such as *Lactobacillus* spp., *Leuconostoc* spp., and *Pediococcus* spp.; however, the use of starter culture such as *L. plantarum*, *L. rhamnosus*, *L. gasseri*, and *L. acidophilus* provides consistency and reliability of performance [24].

Fruits and vegetables are exclusive sources of water-soluble vitamins C and B-complex, provitamin A, phytosterols, dietary fibres, minerals, and phytochemicals for the human diet [25]. Vegetables have low sugar content but are rich in minerals and vitamins and have neutral pH and thus provide a natural medium for LA fermentation [26]. LA fermentation enhances the organoleptic and nutritional quality of the fermented fruits and vegetables and retains the nutrients and coloured pigments [27]. LA fermentation of vegetable products applied as a preservation method for the production of finished and half-finished products is considered as an important technology and is further investigated because of the growing amount of raw materials processed in the food industry [22], and these foods are well suited to promoting the positive health image of probiotics [28]. The consumption of LA fermented fruits and vegetables helps to enhance human nutrition in several ways such as the attainment of balanced nutrition, providing vitamins, minerals, and carbohydrates, and preventing several diseases such as diarrhoea and cirrhosis of liver because of probiotic properties [29]. Some of the fermented fruits and vegetables contain coloured pigments such as flavonoids, lycopene, anthocyanin, β-carotene, and glucosinolates, which act as antioxidants in the body by scavenging harmful free radicals implicated in degenerative diseases like cancer, arthritis, and ageing [30]. Lactic acid fermentation of vegetables has an industrial significance only for cucumbers, cabbages, and olives [22]. In Italy, the industrial production of fermented vegetables is limited to sauerkrauts and table olives [31].

According to Kim et al. the Chinese cabbage, cabbage, tomato, carrot, and spinach provide relatively higher fermentability than other vegetables (okra and gourds) because they have more fermentable saccharides [32]. The most reported fermented fruits and vegetables are categorized as follows.(i)Root vegetables: carrots, turnips, beetroot, radishes, celeriac, and sweet potato [72].(ii)Vegetable fruits: cucumbers, olives, tomatoes, peppers, okra, and green peas [27].(iii)Vegetables juices: carrot, turnips, tomato pulp, onion, sweet potato, beet, and horseradish [75].(iv)Fruits: apples, pears, immature mangoes, immature palms, lemons, and fruit pulps such as banana [22].

3. Traditional Fermented Fruits and Vegetables in India

In eastern Himalayan regions of India a wide range of fermented vegetable products are prepared for bioprocessing the perishable vegetable for storage and further consumption [33]. Lactic acid fermentation vegetables such as gundruk, sinki, and khalpi are fermented vegetable product of Nepal, Sikkim, and Bhutan. *Lactobacillus brevis*, *L. plantarum*, *Pediococcus pentosaceus*, *P. acidilactici*, and *Leuconostoc fallax* are the predominant LAB involved in ethnic fermented vegetables. Predominant functional LAB strains associated with the ethnic fermented tender bamboo shoot products, mesu, soidon, soibum, and soijim of the Himalayas, were identified as *L. brevis*, *L. plantarum*, *L. curvatus*, *P. pentosaceus*, *L. mesenteroides* subsp. *mesenteroides*, *L. fallax*, *L. lactis*, *L. citreum*, and *Enterococcus durans* [33]. Some of the LAB strains may also possess protective and functional properties that render them as interesting candidates for use as starter culture(s) for controlled and optimized production of fermented vegetable products [34].

Gundruk

Gundruk is a nonsalted, fermented, and acidic vegetable product indigenous to the Himalayas. During fermentation of gundruk, 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.) are wilted for 1-2 days. Wilted leaves are crushed mildly and pressed into a container or earthen pot, made airtight and fermented naturally for about 15–22 days. After desirable fermentation, products are removed and sun-dried for 2–4 days. Gundruk is consumed as pickle or soup and has some resemblance with other fermented acidic vegetable products such as kimchi of Korea, sauerkraut of Germany, and sunki of Japan. The predominant microflora of Gundruk includes various LAB such as *L. fermentum*, *L. plantarum*, *L. casei*, *L. casei* subsp. *pseudoplantarum*, and *Pediococcus pentosaceus* .

Sinki

Sinki, an indigenous fermented radish tap root food, is traditionally prepared by pit fermentation, which is a unique type of biopreservation of foods by LA fermentation in the Sikkim Himalayas. For sinki production, a pit was dug with 2-3 ft diameter in a dry place. The pit is cleaned, plastered with mud, and warmed by burning. After removing the ashes, the pit is lined with bamboo sheaths and paddy straw. Radish tap roots are wilted for 2-3 days, crushed, dipped in lukewarm water, squeezed, and pressed tightly into the pit, covered with dry leaves and weighted down by heavy planks or stones. The top of the pit is plastered with mud and left to ferment for 22–30 days. After fermentation, fresh sinki is removed, cut into small pieces, sun-dried for 2-3 days, and stored at room temperature for future consumption. Pit fermentation has been practiced 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* .

Khalpi

Khalpi or khalpi is a fermented cucumber (*Cucumis sativus* L.) product, commonly consumed by the Brahmin Nepalis in Sikkim. It is the only reported fermented cucumber product in the entire Himalayan region. Ripened cucumber is cut into suitable pieces and sun-dried for 2 days, and then put into a bamboo vessel and made airtight by covering with dried leaves. It is fermented naturally at room temperature for 3–5 days. Fermentation after 5 days makes the product sour in taste. Khalpi is consumed as pickle by adding mustard oil, salt, and powdered chilies. Khalpi is prepared in the months of September and October. Microorganisms isolated from Khalpi include *L. plantarum*, *L. brevis,* and *Leuconostoc fallax* .

Inziangsang

In Northeast India, especially the people of Nagaland and Manipur consume Inziangsang, traditional fermented leafy vegetable product prepared from mustard leaves and similar to gundruk. Preparation process of inziangsang is like of gundruk. Mustard leaves, locally called hangam (*Brassica juncea* L. Czern), are collected, crushed, and soaked in warm water. Leaves are squeezed to remove excess water and pressed into the container and made airtight to maintain the anaerobic condition. The container is kept at ambient temperature (20°C–30°C) and allowed to ferment for 7–10 days. Like gundruk, freshly prepared inziangsang is sun-dried for 4-5 days and stored in a closed container for a year or more at room temperature for future consumption. Nagaland people consume inziangsang as a soup time with steamed rice. In resident meal, the fermented extract of *ziang dui* is used as a condiment. This fermentation is also supported by set of LAB which includes *L. plantarum*, *L. brevis*, and *Pediococcus*.

Soidon

Soidonis a widespread fermented product of Manipur prepared from the tip of mature bamboo shoots. Main source of fermentation is the tips or apical meristems of mature bamboo shoots (*Bambusa tulda*, *Dendrocalamus giganteus*, and *Melocanna bambusoides*). Outer casings and lower portions of the bamboo shoots were removed and whole tips are submerged in water in an earthen pot. The sour liquid (soijim) of a previous batch is added as starter in 1 : 1 dilution, and the preparation is covered. Fermentation was carried out for 3–7 days at room temperature. Leaves of *Garcinia pedunculata* Roxb. (family: Guttiferae), locally called heibungin in Manipuri language, may be added in the fermenting vessel during fermentation to enhance the flavor of soidon. After 3–7 days, soidon is removed 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.

Goyang

Goyang, a prominent traditional fermented vegetable foodstuff of the Sikkim and Nepal, leafs of *magane-saag* (*Cardamine macrophylla* Willd.), belonging to the family Brassicaceae, are collected, washed, cut into pieces, and then squeezed to drain off excess water and are tightly pressed 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.

Traditional Fermented Fruits and Vegetables in Other Asian Countries

Kimchi

Kimchi is a Korean traditional fermented vegetable made from Chinese cabbage (beachu), radish, green onion, red pepper powder, garlic, ginger, and fermented seafood (jeotgal), which is traditionally made at home and served as a side dish at meals. Kimchi is a generic term indicating a group of traditional LA fermented vegetables in Korea. The major raw materials (oriental cabbage or radish) are salted after prebrining, blended with various spices (red pepper, garlic, green onion, ginger, etc.) and other minor ingredients (seasonings, salted sea foods, fruits and vegetables, cereals, fish, and meats, etc.), and then fermented at low temperature (2–5°C). Kimchi fermentation is temperature-dependent process. It ripens in one week at 15°C and took three days at 25°C. But low temperature is preferred in kimchifermentation to prevent production of strong acid, overripening, and extended period of optimum taste. Kimchi is characterised particularly by its sour, sweet, and carbonated taste and differs in flavour from sauerkrautand pickles that are popular fermented vegetables [44]. The classical identification of bacterial isolates from kimchi revealed that *Leuconostoc mesenteroides* and *Lactobacillus plantarum* were the predominant species. Several results suggested that LAB contributing to kimchi fermentation include *L. mesenteroides*, *L. citreum*, *L. gasicomitatum*, *Lactobacillus brevis*, *L. curvatus*, *L. plantarum*, *L. sakei*, *L. lactis*, *P. pentosaceus*, *W. confusa*, and *W. koreensis*. Some important species thought to be responsible for kimchi fermentation are *Leuconostoc mesenteroides*, *L. pseudomesenteroides*, and *L. lactis*, as the pH gradually falls to 4.0 .

Kimchi contains various health-promoting components, including *β*-carotene, chlorophyll, vitamin C, and dietary fibre. In addition, antimutagen, antioxidation, and angiotensin-converting enzyme inhibition activities of kimchi are thought to protect against disease. Bacteria isolated from kimchi produce beneficial enzymes, such as dextransucrase and alcohol/acetaldehyde dehydrogenase. Because of these beneficial properties, kimchi was nominated as one of the world’s healthiest foods in a 2006 issue of Health Magazine. Optimum taste of kimchiis attained when the pH and acidity reach approximately 4.0–4.5 and 0.5-0.6, respectively. Vitamin C content is maximal at this point.

Sauerkraut

Sauerkraut means sour cabbage. In sauerkraut fermentation, fresh cabbage is shredded and mixed with 2.3–3.0% salt before allowing for natural fermentation. Sauerkrautproduction typically relies on a sequential microbial process that involves heterofermentative and homofermentative LAB, generally involving *Leuconostoc* spp. in the initial phase and *Lactobacillus* spp. and *Pediococcus* spp. in the subsequent phases. The pH of final product varies from 3.5 to 3.8 . At this pH, the cabbage or other vegetables will be preserved for a long period of time. Sauerkraut brine is an important byproduct of the cabbage fermentation industry and can be used as a substance for the production of carotenoids by *Rhodotorula rubra* or for *β*-glucosidase production by *Candida wickerhamii* for commercial applications.

Paocai

The most favored customary tableware of Chinese is Paocai, a lactic acid fermented vegetable with saltish palate. In certain places of China, the surplus vegetables such as cabbage, celery, cucumber, and radish were retained during superfluous season. Usually Paocai is served as an accompaniment with the chief meal and occasionally used as a Nipple. Paocai is a type of pickle, varies in terms of taste and method of preparation in different areas. Taiwanese paocai has crunchy texture and tangy taste, which is made with many kinds of vegetables, spices, and other ingredients by anaerobic fermentation in a special container. Paocai fermentation is initiated by various microorganisms presented in the raw materials, and LAB become the dominate bacterial finally. *Lactobacillus pentosus*, *L. plantarum*, *L. brevis*, *L. lactis*, *L. fermentum*, and *Leuconostoc mesenteroides* are the LAB isolated from paocai .

4.4. Yan-Dong-Gua

In Taiwan, the extensively used customary fermented nutriment is Yan-dong-gua, prepared using wax gourd. Harvested wax gourd is washed and sliced into little pieces, dried in sunlight, combined with salt, sugar, and fermented soybeans, and layered in a bucket. Usually, minor mass of Mijiu (Taiwanese rice wine) is mixed in the earlier stage of fermentation and the bucket was sealed. The time of fermentation process is for one month, but it may be elongated even more than two months. Yan-dong-gua is usually used as a seasoning for fish, pork, meatballs, and various other foods. *Weissella cibaria* and *W. Paramesenteroides* are the bacteria responsible for fermentation.

Tempoyak

Tempoyak is a traditional Malaysian fermented condiment made from the pulp of the durian fruit (*Durio zibethinus*). Salt is sometimes added to proceed fermentation at ambient temperature. Seeded durian is mixed with small amount of salt and left to ferment at ambient temperature in a tightly closed container for 4–7 days. The acidity of tempoyak was reported as approximately 2.8 to 3.6%. The sour taste of tempoyak is attributed to the acid produced by lactic acid bacteria (LAB) during fermentation. LAB were the predominant microorganisms including *Lactobacillus brevis*, *L. mali*, *L. fermentum*, *L. durianis*, *Leuconostoc mesenteroides*, and an unidentified *Lactobacillus* sp..

Sayur Asin

Sayur asin is a fermented mustard cabbage leaf food product of Indonesia. A similar product, hum choy, is produced in China and other South East Asian countries. Mustard cabbage leaves (*Brassica juncea* var. rugosa) are wilted, rubbed, or squeezed with 2.5%–5% salt. Liquid from boiled rice is added to provide fermentable carbohydrates to ensure that sufficient acid is produced during the fermentation. Fermentation was characterized by a sequential growth of the lactic acid bacteria, *Leuconostoc mesenteroides*, *Lactobacillus confusus*, *Lactobacillus curvatus*, *Pediococcus pentosaceus*, and *Lactobacillus plantarum*. Starch degrading species of *Bacillus*, *Staphylococcus*, and *Corynebacterium* exhibited limited growth during the first day of fermentation. The yeasts, *Candida sake* and *Candida guilliermondii*, contributed to the fermentation.

Salam Juice

Shalgam juice is prepared from the mixture of turnips, black carrot bulgur (broken wheat) flour, salt, and water by lactic acid fermentation. Shalgam is widely used in Turkey. Shalgam juices were prepared by two methods for commercial production, which are the traditional and direct methods. Traditional method has two stages of fermentation that includes sour-dough fermentation (first fermentation) and carrot fermentation (second fermentation). The direct method has only second fermentation. The shalgam juice fermentation was mainly carried out by LAB that belong to the genera *Lactobacillus*, *Leuconostoc*, and *Pediococcus* . The LAB species predominantly include *Lactobacillus plantarum*, *L. brevis*, *L. paracasei*, *L. buchneri*, and *Pediococcus pentosaceus*.

Yan-Taozih

Yan-taozih (pickled peaches) is a popular pickled fruit in China and Taiwan. Fresh peaches (*Prunus persica*) are mixed with 5%–10% salt and then shaken gently until water exudes from the peaches. The peaches are then washed and mixed with 5%–10% sugar and 1%-2% pickled plums. All of the ingredients are mixed well and then allowed to ferment at low temperature (6–10°C) for 1 day. Chen et al. isolated *Leuconostoc mesenteroides, L. lactis, Weissella cibaria, W. paramesenteroides, W. minor, Enterococcus faecalis,* and *Lactobacillus brevis* from Yan-taozih.

Pobuzihi Nozawana-zuke is a low-salt pickle prepared by using field mustard, locally called Nozawana (*Brassica campestris* var. rapa), a leafy turnip plant. It is majorly consumed by Japanese people. The pickle is manufactured by lactic acid fermentation after adding various inorganic salts and red pepper powder containing spicy components to nozawana. The fermentation is achieved by various plant-derived genera of lactic acid bacteria (LAB), including *Lactobacillus* and *Leuconostoc.* These LAB contribute to generating the sensory properties of Nozawana zuke and preventing its contamination from disadvantageous bacteria by producing organic acids. The fermentation was carried out by *Lactobacillus curvatus*..

Pobuzihi is a widely used traditional fermented food prepared with cummingcordia in Taiwan. Two types of Pobuzihi are mainly available that can be easily differentiated from the appearance of the final products. Caked or granular pobuzihi is prepared by boiling cummingcordia (*Cordia dichotoma* Forst. f.) for several minutes and mixing it with salt. The caked pobuzihi is prepared by filling up the boiled cummingcordia into containers and after cooling removed from the containers. Chen et al. isolated novel *Lactobacillus pobuzihii*, *L. plantarum*, *Weissella cibaria*, *W. paramesenteroides*, and *Pediococcus pentosaceus* from fermented pobuzihi.

Nozawana-Zuke

4.11. Yan-Jiang

Yan-jiangis a traditional fermented ginger widely used in Taiwan. It is prepared by two methods, such as with addition of plums and without addition of plums. The ginger (*Zingiber officinale* Roscoe) was washed, shredded, mixed with salt (NaCl), and layered in a bucket for 2–6 h. After the exuded water is removed, the ginger is mixed with sugar, and pickled plums are added only in method P. Salt and sugar are added to a final concentration of approximately 30–60 g kg^{-1}. Fermentation usually continues for 3–5 days at low temperature (6–10°C), but some producers maintain a fermentation time of 1 week or even longer. Initial fermentation was carried out by *Lactobacillus sakei* and *Lactococcus lactis* subsp. *Lactis* and this species are replaced by *Weissella cibaria* and *L. plantarum* at the final stages of fermentation.

4.12. Yan-Tsai-Shin

Yan-tsai-shin is a fermented Broccoli (*Brassica oleracea*) stem, which is belonging to cabbage family. It is widely used in Taiwan. Harvested broccoli is washed, peeled, cut, mixed with salt (NaCl), and filled in a bucket for approximately 6 h. After the exuded water is removed, fermented broccoli is mixed with various ingredients, including sugar, soy sauce, and sesame oil. Some producers also add rice wine or sliced hot pepper to obtain a unique flavour. The ingredients were mixed well and then fermented at low temperature (6–10°C) for 1 day. The most common bacterial species include *Weissella paramesenteroides*, *W. cibaria*, *W. minor*, *Leuconostoc Mesenteroides*, *Lactobacillus Plantarum, and Enterococcus sulphurous*.

. Jiang-Gua

Jiang-guais a popular traditional fermented cucumber in Taiwan that can be served as a side dish or a seasoning. Harvested cucumbers (*Cucumis sativus* L.) are washed, cut, mixed with salt (NaCl), layered in a bucket, and then sealed with heavy stones on the cover. This process usually continues for 4-5 h, but some producers maintain a longer processing time. After the exuded water has been drained off, the cucumbers are mixed with sugar and vinegar. In addition, soy sauce is added optionally depending on the recipe. Fermentation usually continues for at least 1 day at low temperature (6–10°C). Fermentation depends upon *Weissella cibaria*, *W. hellenica*, *L. Plantarum*, *Leuconostoc lactis*, and *Enterococcus casseliflavus*.

Other Fermented Vegetables and Fruits

Pickles from various vegetables and fruits such as mango (*Mangifera indica* L.) and amla (*Emblica officinalis* L.) are dietary supplements and used for culinary purposes in several parts of the world. Pickling of cucumber is made in Africa, Asia, Europe, and Latin America. Khalpi is a cucumber pickle popular during summer months in Nepal. Although, a variety of methods are used, placing the cucumbers in 5% salt brine is a satisfactory method. The cucumbers absorb salt until there is equilibrium between the salt in the cucumbers and the brine (about 3% salt in the brine). When the pH attains at about 4.7–5.7, the brine is inoculated with either *L. plantarum* or *Pediococcus pentosaceus* or a combination of these organisms for a total cell count of 1–4 billion cells/gallon of brined cucumbers. The final product has an acidity of 0.6–1.0% (as LA) and a pH of 3.4–3.6 in about two weeks, depending upon the temperature. Similarly, sweet potato lacto-pickles may serve as an additional source of pickle with usual beneficial probiotic properties .

Different varieties of onions (*Allium cepa*) such as sweet, white and yellow storage were used for LA fermentation. White and yellow storage onions are typically used for processing due to their high solid content, so they were chosen for fermentation. Sweet onions are a spring/summer variety with low solids and mild flavour and are often consumed fresh.

Sweet cherry (*Prunus avium* L.) is one of the most popular of temperate fruits. Italy, together with United States, Iran, and Turkey, is one of the main world producers of sweet cherries.

The fermentation of beetroot and carrot juices, with addition of brewer's yeast autolysate, was also carried out by various workers like Rankin et al. A mixture of beetroot and carrot juices with brewer's yeast autolysate (fermented bio product) has optimum proportions of pigments, vitamins, and minerals. This balanced material represents a valuable product as far as nutrition and health are concerned [74]. Red beets were evaluated as a potential substrate for the production of probiotic beet juice by four species of lactic acid bacteria (*Lactobacillus acidophilus*, *L. casei*, *L. delbrueckii*, and *L. plantarum*).

Spontaneous cauliflower fermentation is commonly encountered in many countries with local variations depending mainly upon tradition and availability of raw materials. *L. plantarum* and *Leuconostoc mesenteroides* were isolated from the cauliflower fermentation [19].

The consumption of LA fermented vegetable juices (lacto-juice) has increased in many countries. Lacto-juices are produced mainly from cabbage, red beet, carrot, celery, and tomato [4]. They can be produced by either of the following procedures:(i)usual way of vegetable fermentation and then processed by pressing the juice (manufacture from sauerkraut);(ii)fermentation of vegetable mash or juice.

There are three types of lactic fermentation of vegetable juices:(i)spontaneous fermentation by natural microflora;(ii)fermentation by starter cultures that are added into raw materials;(iii)fermentation of heat-treated materials by starter cultures.

During the manufacture of lacto-juices, the pressed juice can be pasteurized at first and consecutively it is inoculated by a culture of selected LAB at a concentration varying from 2×10^5 to 5×10^6 CFU/mL [4, 75]. For fermentation of juices of highest quality, it is imperative to use commercially supplied starter cultures such as *L. plantarum*, *L. bavaricus*, *L. xylosus*, *L. bifidus*, and *L. brevis*. The criteria used for finding out suitability of a strain are as follows [76]:(i)the rate and total production of LA, change in pH, loss of nutritionally important substances;(ii)decrease in nitrate concentration and production of biogenic amines (BAs);(iii)ability of substrate to accept a starter culture;(iv)type of metabolism and ability of culture to create desirable sensory properties of fermented products.

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6. Probiotic Microorganisms

6.1. Lactic Acid Bacteria

The genus *Lactobacillus* is a heterogeneous group of LAB with important application in food and feed fermentation. *Lactobacilli* are used as probiotics inoculants and as starters in fermented food [77]. The genus *Lactobacillus* is Gram-positive organisms which produce lactic acid by fermentation which belongs to the large group of LAB. Other genera such as *Lactococcus*, *Enterococcus*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Leuconostoc*, and *Lactobacillus* are also considered in LAB group due to lactic acid production ability [78].

The genus *Lactobacillus* is a heterogeneous group of LAB with important implications in food and feed fermentation. *Lactobacilli* are currently used as probiotics, silage inoculants, and as starters in fermented food [77]. The genus *Lactobacillus* belongs to the large group of LAB, which are all Gram-positive organisms which produce lactic acid by fermentation. Genera of LAB include, among others, *Lactococcus*, *Enterococcus*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Leuconostoc*, and *Lactobacillus* [78]. *Lactobacillus* is rod shaped, often organized in chain belonging to a large group within a family Lactobacillaceae. They grow well in anaerobic condition and strictly fermentative in nature. *Lactobacillus* is generally divided into two groups depending on the ability of the sugar fermentation: homofermentative species, converting sugars mostly into lactic acid and heterofermentative species, converting sugars into lactic acid, acetic acid and CO_2. LAB can influence the flavour of fermented foods in a variety of ways. During fermentation, lactic acid is produced due to the metabolism of sugars. As a result, the sweetness tastes will likely decrease as sourness increases [76].

Lactobacilli prefer relatively acidic conditions ranges from pH 5.5 to 6.5 due to the main catabolite as lactic acid. It can be found in a wide ranges of ecological niches such as plant, animal, raw milks, and in insects [79]. Due to the wide verity in habitat *Lactobacillus* possess a wide range of metabolites versatility in the LAB group. It has been used for food preservation, starter for dairy products, fermented vegetables, fish, and sausages as well as silage inoculants for decades. Lactobacillus is proposed as potential probiotics due to its potential therapeutic and prophylactic attributes. *L. paracasei*, *L. rhamnosus*, and *L. casei* belong to the group of lactobacillus which are commonly found in food and feed as well as common inhabitants of the animal/human gastrointestinal tract (GIT) [80]. *L. plantarum* is considered a food-grade microorganism because of its long and documented history of safe use in fermented foods [81]. *L. fermentum*, one of the best-known species of this group, has been isolated from vegetable and dairy fermentation [77, 80, 82].

The *Weissella* species are Gram-positive, catalase negative, non-spore-forming, heterofermentative, nonmotile, irregular, or coccoid rod-shaped organisms [83]. Members of the genus *Weissella* have been isolated from a variety of sources, such as fresh vegetables and fermented silage [84–86]. The genus *Weissella* encompasses a phylogenetically coherent group of lactic acid bacteria and includes eight *Leuconostoc*-like species, including *Weissella confuse* (formerly *Lactobacillus confuses*), *W. minor* (formerly *Lactobacillus minor*), *W. kandleri* (formerly *Lactobacillus kandleri*), *W. halotolerans* (formerly *Lactobacillus halotolerans*), *W. viridescens* (formerly *Lactobacillus viridescens*), *W. paramesenteroides* (formerly *Leuconostoc paramesenteroides*), and *W. hellenica* [83].

6.2. Definition and Mechanism of Action of Probiotics

According to the Food and Agriculture Organization (FAO) Probiotics are defined as “living microorganisms which, when administrated in adequate amounts, confer health benefit on the host”. Many studies supported that maintenance of health gut microflora provides protection against gastrointestinal disorder including gastrointestinal infections and inflammatory bowel diseases. On the other hand, probiotics can be used as an alternative to the use of antibiotics in the treatment of enteric infection or to reduce the symptoms of antibiotic associated diarrhea [87]. Probiotic bacterial cultures support the growth of intestinal microbiota, by suppressing potentially harmful bacteria and reinforce the body’s natural defence mechanisms. Currently, much evidence exists on the positive effects of probiotics on human health [77, 88–91].

6.3. Selection and Application of Probiotics

Lactobacilli are the most extensively studied and widely used probiotics within the LAB. Most *Lactobacillus* strains belong to the *L. acidophilus* group. *L. paracasei*, *L. plantarum*, *L. reuteri*, and *L. salivarius*, which represent the respective phylogenetic groups, are known to contain probiotic strains. In order for a probiotic to be of benefit to human health, it must fulfil several criteria (Figure 2). It must survive passage through the upper GIT and reach its site of action alive, and it must be able to function in the gut environment. The functional requirements of probiotics include tolerance to human gastric juice and bile, adherence to epithelial surfaces, persistence in the human GIT, immune stimulation, antagonistic activity toward intestinal pathogens (such as *Helicobacter pylori*, *Salmonella* spp., *Listeria monocytogenes*, and *Clostridium difficile*), and the capacity to stabilize and modulate the intestinal microbiota [88–92].

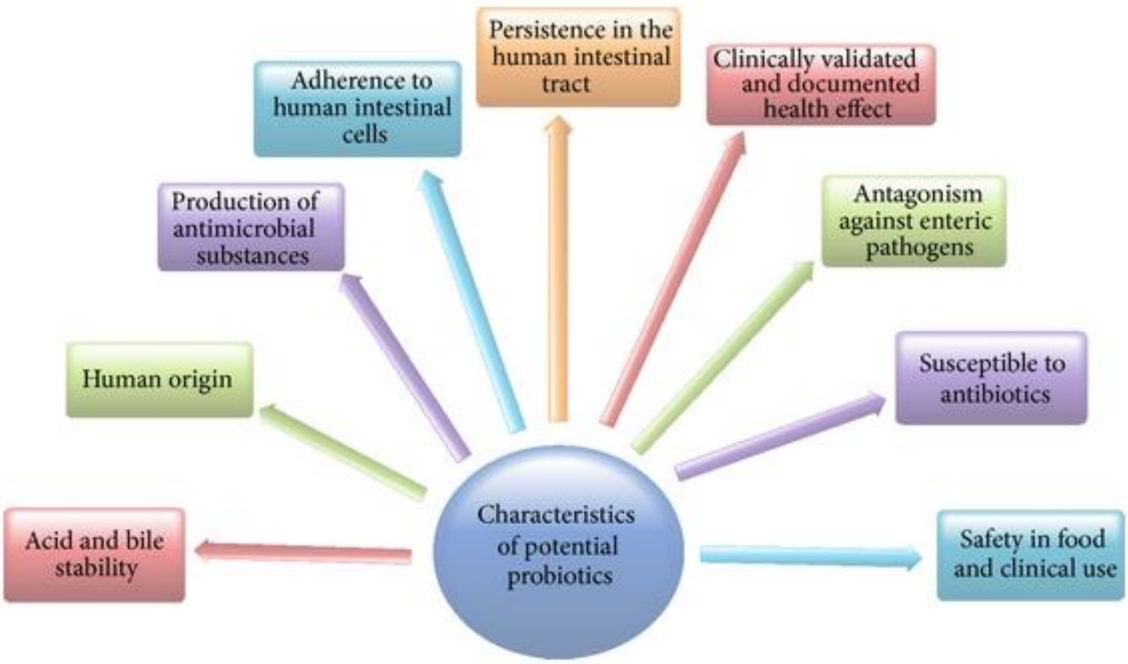


Figure 2

Basic characteristics of selection of a probiotic strains.

7. Raw Materials Pretreatments

Pretreatments can promote growth of lactic flora that can be used depending on the fruit or vegetable to be fermented. Washing fruits and vegetables prior to fermentation reduces the initial microbial count, thus favouring the development of lactic flora [93]. Vegetables are also macerated with pectinolytic enzymes [75] to allow for their homogenization prior to lactic fermentation, mainly for the production of cocktails and juices [4]. Many vegetables contain glycosides that hamper efficient fermentation [94]. For LA fermentation of tomatoes, choosing very ripe fruit is recommended, since the high solanin content of unripe fruit might inhibit the growth of LAB.

8. Role of Ingredients Used in Fermentations of Fruits and Vegetables

8.1. Addition of Salt

LA fermentation of fruits and vegetables is mostly carried out in a salted medium [95]. Salting is done by adding common dry salt (NaCl) with high water content or by soaking in brine solution. The optimum salt concentration depends on the type of vegetables or fruits [96]. Substituting NaCl by KCl up to 50% in the preparation of *kimchi* from cabbage did not affect the sensory qualities (saltiness, bitterness, sourness, hotness, and texture). The main role of salt is to promote the growth of LAB over spoilage bacteria and to inhibit potential pectinolytic and proteolytic enzymes that can cause vegetable softening and further putrefaction. Salt induces plasmolysis in the plant cells and the appearance of a liquid phase, which creates anaerobic conditions around the submerged product. Anaerobic conditions are more effective in the finely cut and shredded cut material.

8.2. Ingredients Favouring Bacterial Growth

Some ingredients when added to LA fermented vegetables or fruits seem to enhance the development of lactic flora. They have three major roles:(i)they are a source of nutrients such as sugars, vitamins, and minerals which initiate fermentation;(ii)they add desirable aroma, flavour, and taste to the fermented product;(iii)they help in combating the spoilage bacteria by lowering the pH.

For some vegetables with low nutrient contents, such as turnip and cucumber, the addition of sugar promotes bacterial growth, thereby accelerating fermentation. In Spanish-style olive fermentation, olives have undergone alkaline treatment to eliminate their bitterness, followed by repeated washings. They are then replaced with glucose on sucrose to improve LA fermentation [71]. Whey is often recommended for use in traditional LA vegetable fermentation processes as it has high lactose content, which is a potential energy substrate for LAB. It also supplies minerals salts and vitamins necessary for the lactic flora metabolism.

8.3. Ingredients with Antiseptic Properties

Spices or aromatic herbs are added to most of the lactic fruits and vegetable fermentation to improve the flavour of the end products [21]. Certain spices, mainly garlic, cloves, juniper berries, and red chillies help to inhibit the growth of spoilage bacteria [22]. There are many sulphur compounds with antibacterial properties in garlic which must be combined with other vegetables at ratios not higher than 150 g/kg of vegetables. Chemical preservatives such ascorbic on benzoic acid salts are sometimes used in industrial production of LA fermented *sauerkraut*, olives, or cucumbers [69]. The role of essential spice oils such as thyme, sage, lemon, and dill is to inhibit the growth during fermentations of olives [70]. Mustard seed contains allyl isothiocyanate, a volatile aromatic compound with antibacterial and antifungal properties, which inhibits the growth of yeast (*Saccharomyces cerevisiae*) and promotes growth of LAB [69, 70].

8.4. Ingredients Modifying the pH and Buffers Effect of Brines

To promote the growth of LAB over yeasts, moulds and other pathogenic or unwanted bacterial strains, acids, or buffer systems (acid + acid salts) are often added to the fermentation medium. During the fermentation of fruits and vegetables with high fermentable sugar contents, the fermentation medium has to be buffered to slow down acidification, thus allowing the LAB to consume all the sugars. An acetic acid + calcium acetate buffer system has been reported to improve the LA cucumber fermentation process.

9. Beneficial Effect of Fermented Fruits and Vegetables

9.1. Enhancing Food Quality and Safety

Nutritional quality of food can be enhanced by fermentation, which may improve the digestibility and beneficial components of fermented food. The raw materials have increased the level of vitamin and mineral content compared to its initial content. Several antimicrobial compounds such as organic acids, hydrogen peroxide, diacetyls, and bacteriocins are produced during the fermentation process, which impacts unrequited bacterial growth and on the other hand increases the shelf life of the food.

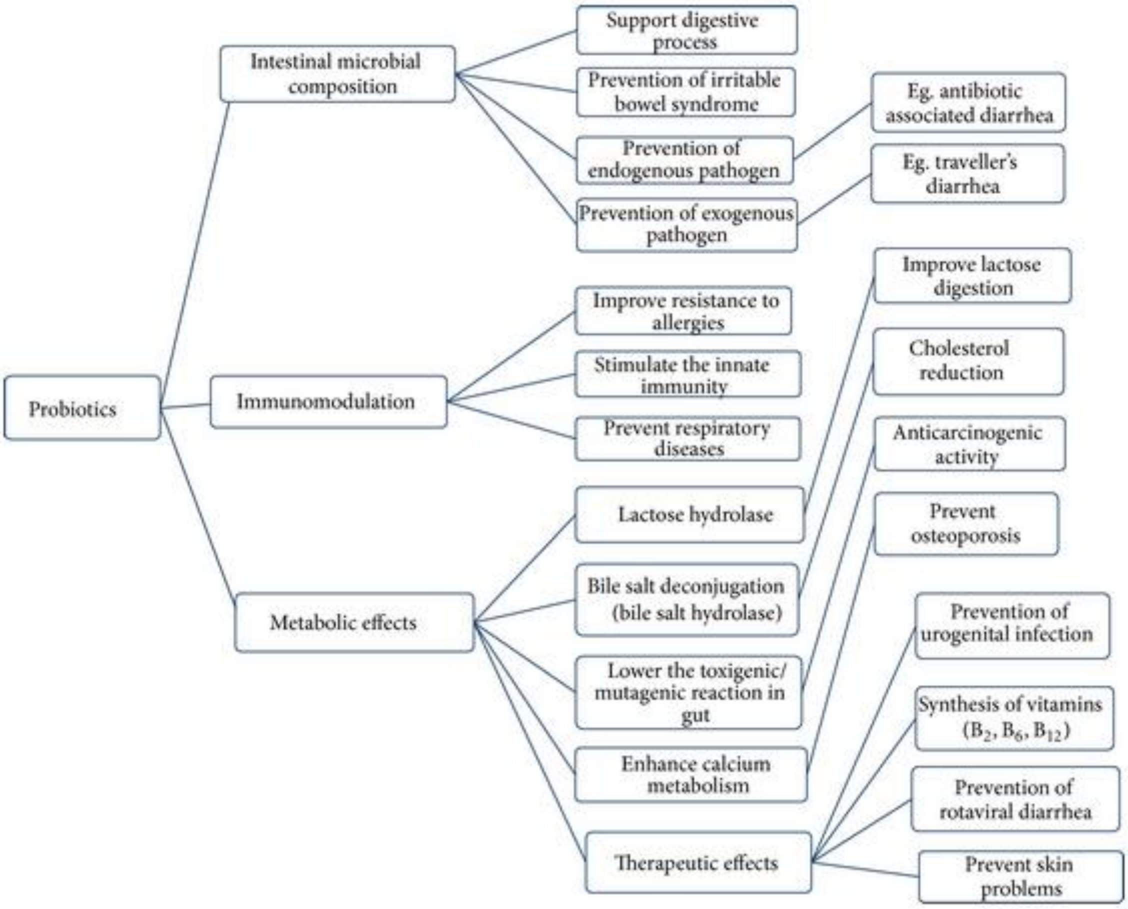
Lactic acid content of fermented food product may enhance the utilization of calcium, phosphorus, and iron and also increase adsorption of iron and vitamin D. Fermented foods have a variety of enzymes and each enzyme can play a different role in increasing food quality. Lactase in fermented food product degrades the lactose into galactose. Galactose is an important constituent of cerebroside that can promote brain development in infants. Similarly proteinases produced by LAB can break down the casein into small digestible molecules. Fermented foods are rich in globular fats which can be easily digested.

9.2. Removal of Antinutrient Compounds

Most of the fruits and vegetables contain toxins and antinutritional compounds. These can be removed or detoxified by the action of microorganisms during fermentation process. Plant foods contain a series of compounds, collectively referred to as antinutrients, which generally interfere with the assimilation of some nutrients and in some cases may even confer toxic or undesirable physiological effects. Such antinutrients include oxalate, protease, and α-amylase inhibitors, lectins, condensed tannins, and phytic acid. Numerous processing and cooking methods have been shown to possibly reduce the amount of these antinutrients and hence their adverse effects. It has been concluded that the way food is prepared and cooked is equally important as the identity of the food itself. Research is currently focused on identifying the antinutrient effect of several constituents rather than studying their fate during lactic acid fermentation.

9.3. Improving the Health Benefits of Humans

Several researchers have described the beneficial effects of LAB. This can modify the intestinal microbiota positively and prevent the colonization of other enteric pathogens. LAB strains also improve the digestive functions, enhance the immune system, reduce the risk of colorectal cancer, control the serum cholesterol levels, and eliminate the unrequired antinutritional compounds present in food materials. The overall health benefits of LAB are elucidated in Figure 3.



9.4. Biopreservation

Nowadays, consumers are particularly aware of the health concerns regarding food additives; the health benefits of “natural” and “traditional” foods, processed with no added chemical preservatives, are becoming more and more attractive. Chemical additives have generally been used to combat-specific microorganisms. In the case of fermented foods, lactic acid bacteria (LAB) have been essential for these millennia. LAB play a defining role in the preservation and microbial safety of fermented foods, thus promoting the microbial stability of the final products of fermentation. Protection of foods is due to the production of organic acids, carbon dioxide, ethanol, hydrogen peroxide, and diacetyl antifungal compounds such as fatty acids or phenyllactic acid, bacteriocins, and antibiotics such as reutericyclin [97].

The term “bacteriocin” was coined in 1953 to define colicin produced by *Escherichia coli*. Like LAB, also bacteriocins have been consumed for millennia by mankind as products of LAB and, for this reason, they may be considered as natural food ingredients. As reported by Cotter et al. “bacteriocins can be used to confer a rudimentary form of innate immunity to foodstuffs.” Bacteriocins are ribosomally synthesised, extracellularly released low molecular-mass peptides or proteins (usually 30–60 amino acids), which have a bactericidal or bacteriostatic effect on other bacteria, either in the same species (narrow spectrum) or across genera (broad spectrum) [97–99]. Bacteriocin production has been found in numerous species of bacteria, among which, due to their “generally recognized as safe” (GRAS) status, LAB have attracted great interest in terms of food safety. In fact, LAB bacteriocins enjoy a food grade and this offers food scientists the possibility of allowing the development of desirable flora in fermented foods or preventing the development of specific unwanted (spoilage and pathogenic) bacteria in both fermented and nonfermented foods by using a broad- and narrow-host-range bacteriocins, respectively.

Regarding the application of bacteriocin-producing starter strains in food fermentation, the major problem is related to the in situ antimicrobial efficacy that can be negatively influenced by various factors, such as the binding of bacteriocins to food components (fat or protein particles) and food additives (e.g., triglyceride oils), inactivation by proteases or other inhibitors, changes in solubility and charge, and changes in the cell envelope of the target bacteria [97, 100]. The most recent food application of bacteriocins encompasses their binding to polymeric packaging, a technology referred to as active packaging. Bacteriocins have generally a cationic character and easily interact with Gram-positive bacteria that have a high content of anionic lipids in the membrane determining the formation of pores [97].

Modern Techniques Used for Analyzing Microflora of Fermented Fruits and Vegetables

In addition to traditional methods (microscopy, plate count, etc.), several modern techniques like RAPD- (Random Amplified Polymorphic DNA-) PCR (Polymerase Chain Reaction), species-specific PCR, multiplex PCR, 16s rDNA sequencing, gradient gel electrophoresis, RFLPs (Restriction Fragment Length Polymorphism), and cluster analysis of TTGE (Temporal Temperature Gradient Electrophoresis) are employed to isolate and characterize different type of LAB strains of fermented fruits and vegetables [101]. RFLPs and 16s rDNA were employed to isolate and characterize lactic acid bacteria from dochi (fermented black beans) and suan-tsai (fermented mustard), a traditional fermented food in Taiwan [102]. The isolated strains are *L. plantarum*, *Salmonella enterica*, *E. coli*, *P. pentosaceus*, *Tetragenococcus halophilus*, *Bacillus licheniformis*, and so on. Tamang [10] isolated 269 strains of LAB from gundruk, sinki, inziangsang (a fermented leafy vegetable), and Khalpi samples and studied the phenotypic characteristics of these strains by genotyping using RAPD-PCR, repetitive element PCR, and species-specific PCR techniques. The major representatives of LAB involved in these fermentations were *L. plantarum*, *L. brevis*, *P. acidilactici*, and *L. fallax*. RAPD-PCR and gradient gel electrophoresis were used to isolate *L. plantarum* strains from ben saalga, a traditional fermented gruel from *Burkina Faso*. MALDI-TOF mass spectrometry and DGGE analysis were also used to analyze the fermented vegetable samples [103]. Characterization of LAB isolates by using MALDI-TOF MS fingerprinting revealed genetic variability within highly heterogeneous species. Previous research investigated the genetic diversity of LAB isolates associated with the production of fermented Almagro eggplants using a combination of randomly amplified polymorphic DNA (RAPD) and pulsed-field gel electrophoresis (PFGE) [104].

11. Research Prospects and Future Applications

Even though it has been broadly verified that dairy fermented products are the best matrices for delivering probiotics, there is growing evidence of the possibility of obtaining probiotic foods from nondairy matrices. Several raw materials (such as cereals, fruits, and vegetables) have recently been investigated to determine their suitability for designing new, nondairy probiotic foods [115]. Generally existing probiotics belong to the genus *Lactobacillus*. However, few strains are commercially obtainable for probiotic function (Table 1). Gene technology and relative genomics will play a role in rapid searching and developing new strains, with gene sequencing allowing for an increased thoughtful of mechanisms and the functionality of probiotics .

FERMENTATION STARTER CULTURE:

A **fermentation starter** (called simply **starter** within the corresponding context, sometimes called a **mother**[1]) is a preparation to assist the beginning of the fermentation process in preparation of various foods and alcoholic drinks. Food groups where they are used include breads, especially sourdough bread, and cheese. A **starter culture** is a microbiological culture which actually performs fermentation. These starters usually consist of a cultivation medium, such as grains, seeds, or nutrient liquids that have been well colonized by the microorganisms used for the fermentation.

These starters are formed using a specific cultivation medium and a specific mix of fungal and bacterial strains.[2][3]

Typical microorganisms used in starters include various bacteria and fungi (yeasts and molds): *Rhizopus*, *Aspergillus*, *Mucor*, *Amylomyces*, *Endomycopsis*, *Saccharomyces*, *Hansenula anomala*, *Lactobacillus*, *Acetobacter*, etc. Various national cultures have various active ingredients in starters, and often involve mixed microflora.[2]

Industrial starters include various enzymes, in addition to microflora.[2]

National name

In descriptions of national cuisines, fermentation starters may be referred to by their national names:

- **Qū** (simplified: 曲; traditional: 麴, also romanized as **chu**)
    - **Jiuqu** (simplified Chinese: 酒曲; traditional Chinese: 酒麴; pinyin: *jiǔ qū*): the starter used for making Chinese alcoholic beverages

- ◦ **Laomian** (simplified Chinese: 老面; traditional Chinese: 老麵; pinyin: *lǎomiàn*; lit. 'old dough' pinyin: *mianfei*; lit. 'dough fat'): Chinese sourdough starter commonly used in Northern Chinese cuisine, the sourness of the starter is commonly quenched with sodium carbonate prior to use.[4]

- **Nuruk**
- **Meju**
- **Koji**
- **Ragi tapai** (Indonesia and Malaysia)
- **Bakhar, ranu, marchaar (murcha), Virjan** (India)
- **Bubod**, **tapay**, **budbud** (Philippines)
- **Luk paeng** (Thai: ลูกแป้ง) (Thailand)
- **Levain** (France)
- **Bread *zakvaska*** (закваска, sourdough) (Russia, Ukraine) or **zakwas** (Poland)
- **Opara** (опара), (Russia), a starter based on yeast

Ingredients of bread:

- **Flour** is the bulking ingredient of bread, it forms the structure of the product. It contains **gluten**. Gluten helps to form an elastic stretchy dough.
- **Yeast** is a raising agent. Yeast produces gases to make the bread rise. Because it is living, correct conditions are needed for growth - food, warmth, time and moisture.
- **Salt** is required to bring out flavour in the bread. This ingredient is used in small quantities. Too much of this ingredient will stop the yeast from growing.

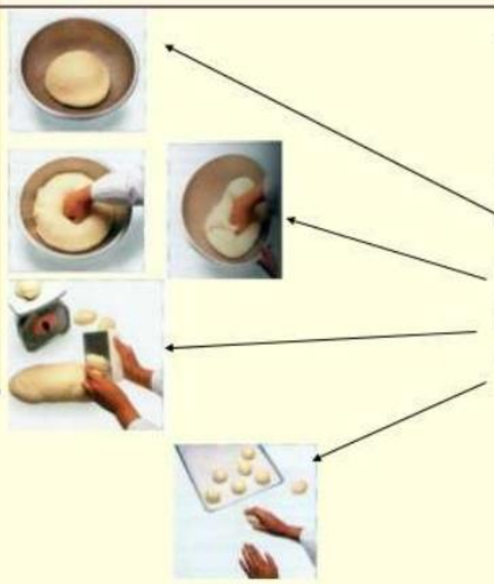
Production of Breads

- involves growth of *Saccharomyces cerevisiae* (baker's yeast) under aerobic conditions
  - maximizes CO_2 production, which leavens bread
- other microbes used to make special breads (e.g., sourdough bread)
- can be spoiled by *Bacillus* species that produce ropiness

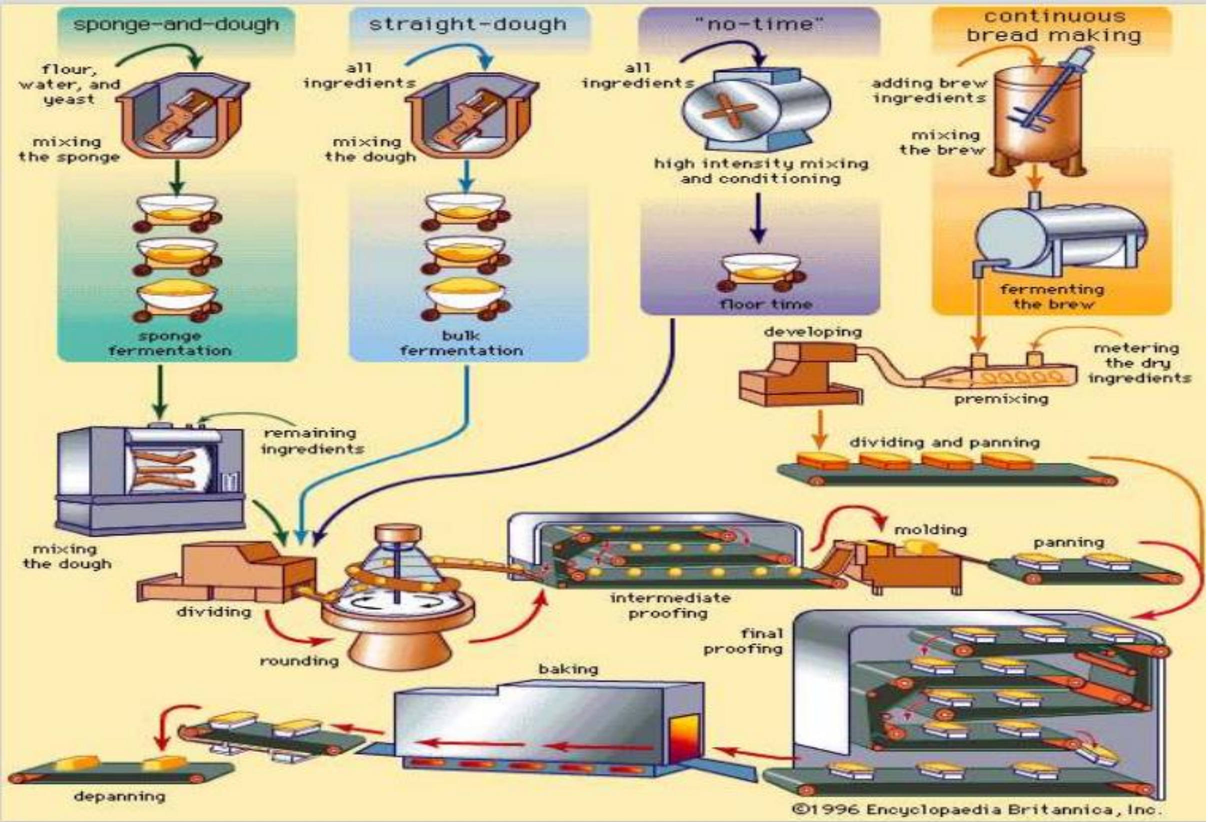
Bread Making Process

1. **Mixing** has two functions: to evenly distribute the various ingredients and allow the development of a protein (gluten) network to give the best bread possible.
2. Once the bread is mixed it is then left to rise **(ferment).**
3. Any large gas holes that may have formed during rising are released by **kneading.**
4. **Moulding** the dough into desired loaf shape.
5. During the **final rising** the loaf fills with more bubbles of gas, and once this has proceeded far enough they are transferred to the oven for baking.
6. The loaf is then placed in a preheated oven to **bake**. Such a high heat will kill the yeast, thus stopping its process of rising and growth.
7. The whole loaf is **cooled** to about 35°C before slicing and wrapping can occur without damaging the loaf

Steps in Yeast Bread Production



1. Mise en place (scale and prepare ingredients)
2. Mixing
3. Fermentation
4. Punching
5. Scaling
6. Rounding


sponge-and-dough
straight-dough
"no-time"
continuous bread making
flour, water, and yeast
mixing the sponge
sponge fermentation
all ingredients
mixing the dough
bulk fermentation
all ingredients
high intensity mixing and conditioning
floor time
adding brew ingredients
mixing the brew
fermenting the brew
developing
metering the dry ingredients
premixing
dividing and panning
remaining ingredients
mixing the dough
dividing
rounding
intermediate proofing
molding
panning
final proofing
baking
depanning
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Prevents
• Diabetes
• Cardiovascular disease
• Breast cancer
• Gallstones
Promotes
• Weight loss
• Dental and bone health
• Hormonal balance
• Gastrointestinal health

Idli Production

- In idli made with a 1:1 ratio of black gram to rice, batter volume increased about 47 percent 12 to 15 hours after incubation at 30°C.
- The pH fell to 4.5 and total cidity rose to 2.8 percent (as lactic acid).
- Using a 1:2 ratio of black gram to rice, batter volume increased 113 percent and acidity rose to 2.2 percent in 20 hours at 29°C.
- Reducing sugars (as glucose) showed a steady decrease from 3.3 milligrams per gram of dry ingredients to 0.8 milligrams per gram in 20 hours, reflecting their utilization for acid and gas production.
- Soluble solids increased,whereas soluble nitrogen decreased.
- Flatulence-causing oligosaccharides, such as stachyose and raffinose, are completely hydrolyzed.

Organisms and their role in Idly

- Idli and dosa are both products of natural lactic acid fermentation. L. mesenteroides and S. faecalis develop during soaking.
- Then continue to multiply following grinding. Each eventually reaches more than 1 × 109 cells per gram, 11 to 13 hours after formation of the batter.
- These two species predominate until 23 hours following batter formation. Practically all batters would be steamed by then. If a batter is further incubated, the lactobacilli and streptococci decrease in numbers and P. cerevisiae develops. L. mesenteroides is the microorganism essential for leavening of the batter and, along with S. faecalis,is also responsible for acid production. Both functions are essential for producing a satisfactory idli.

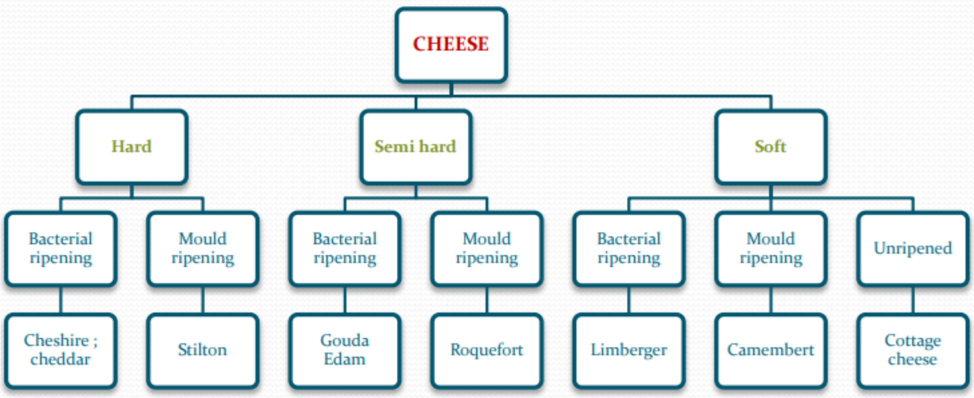
Fermented Milk Products..

- Cheese
- Srikhand
- Kefir
- Yoghurt
- Acidophilus Milk.

Cheese is made up of casein. Varieties of cheese are differentiated according to their

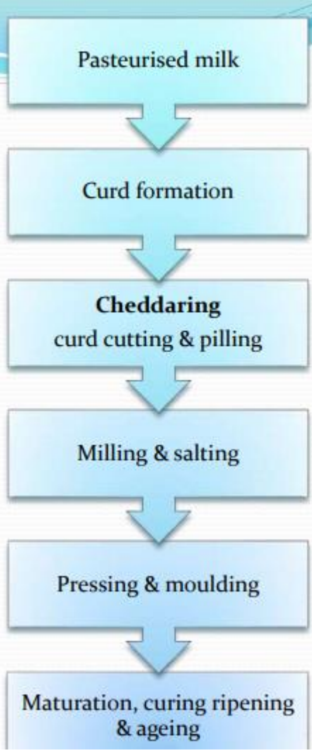
- Flavour
- Texture
- Type of milk

* Salts & seasoning added
* Type of bacteria & mould species used in ripening
* Manufacturing & processing method



Production of cheese

- **Curd formation:** pasteurised whole milk is brought to a temperature of 31'C, starter & required colouring matter is added. After 30 min rennin is added, stirred & allowed to set curd.
- **Curd cutting:** into small cubes
- **Curd cooking:** heated to 38°C & held for 45 min. curd is stirred to prevent matting.
- **Curd drainage:** whey is drained off & curd is allowed to mat.
- **Cheddaring:** cutting matted curd into blocks turning them at 15 min interval & then piling. It is then passed to curd mill which cuts the slab into strips.
- **Salting the curd:** to draw out the whey from curd & as preservative.
- **Pressing**: overnight



Examples of cheese, the microbes involved and the category they can be placed

CHEESE		MICROORGANISMS
SOFT, UNRIPENED	Cottage	*Lactococcus lactis* *Leuconostoc citrovorum*
	Cream	*Streptococcus cremoris*
	Neufchatel	*Streptococcus diacetilactis*
SOFT, RIPENED 1 – 5 MONTHS	Brie	*Lactococcus lactis* *Penicillium candidium* *Streptococcus cremoris* *Penicillium camemberti* *Brevibacterium linens*
	Camembert	*Lactococcus lactis* *Streptococcus cremoris* *Penicillium candidium* *Penicillium camembert*
	Limburger 	*Lactococcus lactis* *Brevibacterium linens* *Streptococcus cremoris*

CHEESE	MICROORGANISMS	CHEESE
SEMISOFT, RIPENED 1 – 12 MONTHS	Blue 	*Lactococcus lactis* *Penicillium roqueforti* *Streptococcus cremoris* *Penicillium glaucum*
	Brick 	*Lactococcus lactis* *Brevibacterium linens* *Streptococcus cremoris*
	Gorgonzola 	*Lactococcus lactis* *Penicillium roqueforti* *Streptococcus cremoris* *Penicillium glaucum*
	Monterey	*Lactococcus lactis* *Streptococcus cremoris*
	Meunster	*Lactococcus lactis* *Brevibacterium linens* *Streptococcus cremoris*
	Roquefort 	*Lactococcus lactis* *Penicillium roqueforti* *Streptococcus cremoris* *Penicillium glaucum*

CHEESE		MICROORGANISMS
HARD, RIPENED 3 – 12 MONTHS	Edam 	*Lactococcus lactis,* *Streptococcus cremoris*
	Gruyere 	*Lactococcus lactis* *Lactobacillus helveticus* *Streptococcus thermophilus* *Propionibacterium sheranii or* *Lactobacillus bulgaricus and* *Propionibacterium* *freudenreichii*
	Swiss 	*Lactococcus lactis* *Lactobacillus helveticus* *Propionibacterium shermanii or* *Lactobacillus bulgaricus and* *Streptococcus thermophilus*
VERY HARD, RIPENED 12 – 16 MONTHS	Parmesan 	*Lactococcus lactis* *Lactobacillus bulgaricus* *Streptococcus cremoris* *Streptococcus thermophilus*

Secondary Microbes

Large holes: *Propioni bacterium freudenreichii subsp. Shermaniee*

White moulds: *Penicillium camembertii, P. caseiocolum* and *P. candidum*

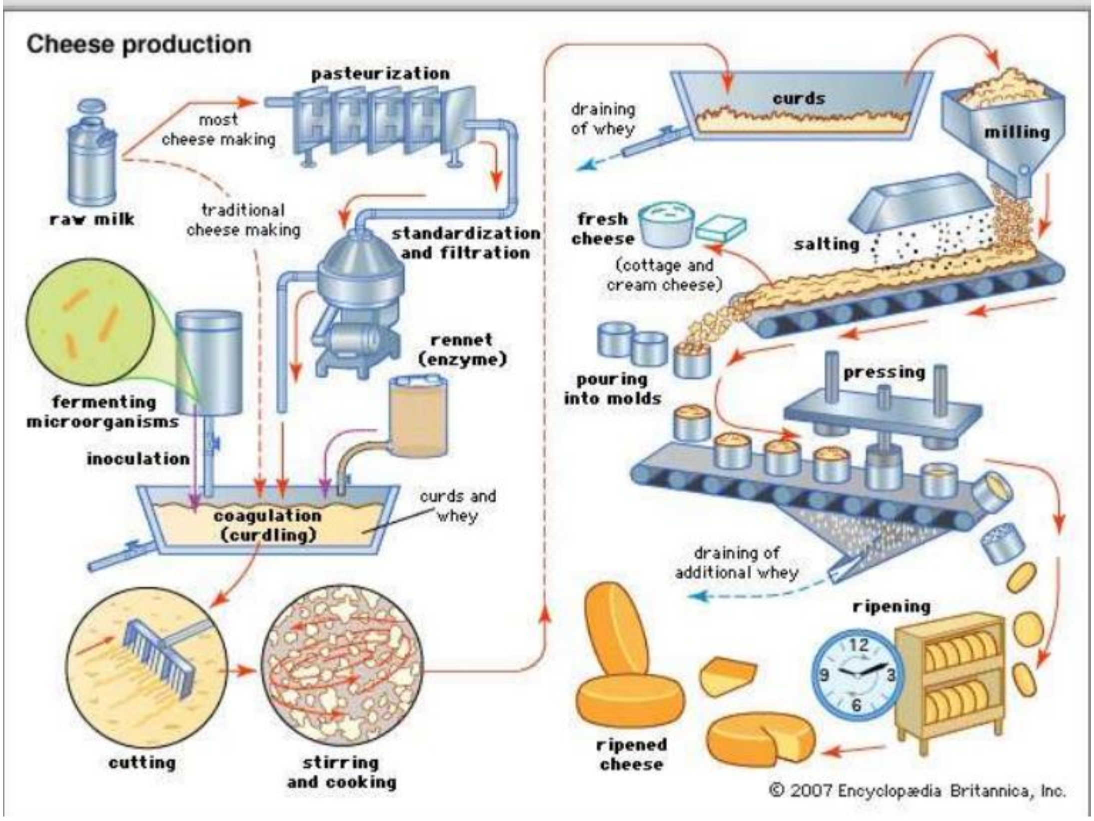
Blue/green moulds: *Penicillium roqueforti, P. glaucum*

Ripening adjuncts: Bacterial or yeast cultures added in addition to the regular LAB cultures

Attenuated cultures which are not intended to grow but only to contribute their enzymes.

Some microbes involved in cheesemaking

Species	Major Known Function	Product
Propionibacterium shermanii	Flavour and Eye formation	Swiss cheese family
Lactobacillus bugaricus *Lactobacillus lactis* *Lactobacillus helveticus*	Acid and flavour	Yoghurt, Swiss, Emmental, and Italian cheeses
Lactobacillus acidophilus	Acid	Acidophilus buttermilk
Streptococcus thermophilus	Acid	Emmental, Cheddar, and Italian cheeses, and yogurt
Streptococcus durans *Streptococcus faecalis*	Acid and flavour	Soft Italian, cheddar, and some Swiss cheeses.
Leuconostoc citrovorum *Leuconostoc dextranicum*	Flavour	Cultured buttermilk,, cottage cheese, and starter cultures.



Contd..

- **Ripening:** 60 days to 12 months depending on the flavour required under controlled conditions of temperature & humidity.
- Changes from a bland tough rubbery mass to a full flavoured soft product.
- Rennin splits protein into peptones & peptides.
- Increases the B-vitamins & improves cooking quality.

Cheese has limited keeping quality & requires refrigeration, should be kept cold & dry i.e., wrapped in wax paper or metal foil.

Health Benefits of Cheese

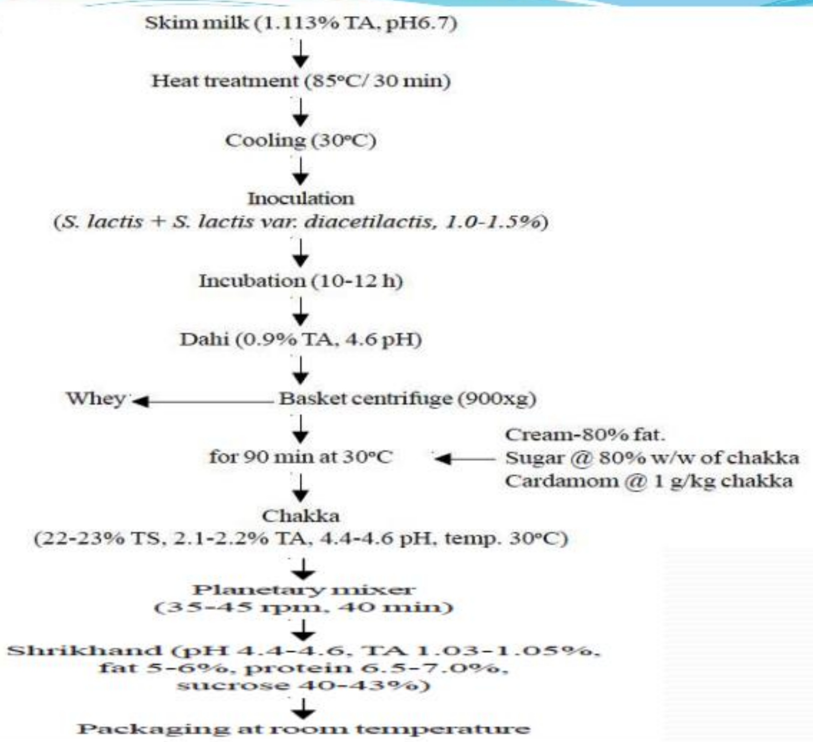
- Cheese contains a host of nutrients like calcium, protein, phosphorus, zinc, vitamin A and vitamin B12. Calcium is one of the nutrients most likely to be lacking in the American diet.
- The high-quality protein in cheese provides the body with essential building blocks for strong muscles.
- If you are lactose intolerant, many cheeses, particularly aged cheeses such as Cheddar and Swiss, contain little or no lactose and are often well tolerated.

Srikhand

Shrikhand: fermented product made by concentrating curd by removing whey & to which sugar, flavor & condiments are added.

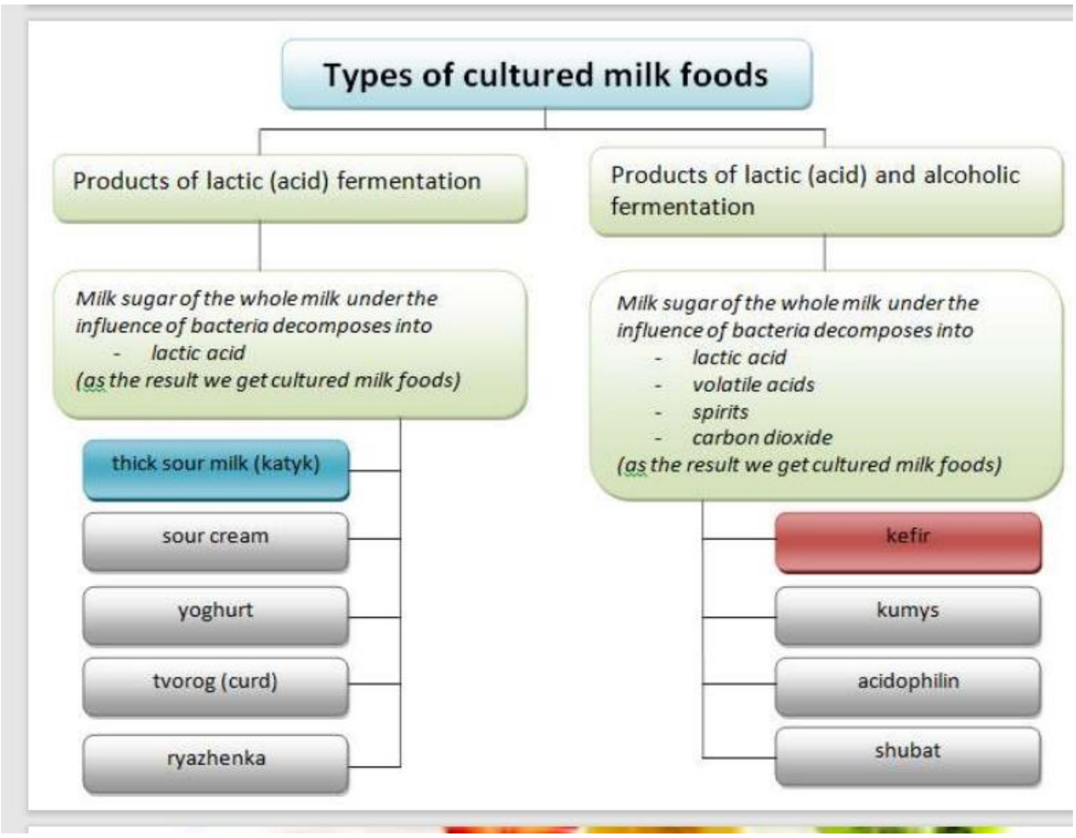
Manufacture of Shrikhand

- The method of manufacture of shrikhand involves the preparation of curd or dahi by fermentation of milk with starter culture, preparation of chakka by draining whey from the curd and blending additives like sugar, color, flavour, species and fruits to obtain a desired composition and consistency.



Nutritional Benefits

- Presence of calcium aids in bone health
- Presence of calcium makes teeth strong & healthy
- Aids in better sleep
- Helps to gain weight
- It helps in absorption of Calcium & Vitamin B
- Keeps you feel full
- Exfoliates dead skin from body
- Gives a smoother skin Nourishes with a soft, supple and a glowing skin
- Reduces dandruff



Kefir

- ✓ Kefir is a beverage produced by the action of lactic acid bacteria, yeast and acetic acid bacteria on milk, which produces a distinctive fermented milk product with unique properties
- ✓ It is produced by adding a starter culture called "kefir grains" directly to milk. The kefir grains are a mass of several different bacteria and yeasts embedded in a complex matrix of protein & carbohydrate.
- ✓ Traditionally prepared kefir contained 1-2% alcohol, due to the complex fermentative process of kefir grains. But new and improved production methods have resulted in much lower levels.
- ✓ The mouth feel of kefir is described to be 'prickly' & 'sparkly'

Method

1. Preparations soy milk kefir grains

- Milk kefir grains
- Water kefir grains

2. Study difference soy milk kefir

- **Characteristics**
  - Physical
  - pH
  - Titratable acidity
  - Reducing sugar
  - Alcohol
- **Microorganism**
  - Lactic acid bacteria
  - Yeast

Kefir

- Kefir is a fermented milk product which is believed to have originated from the Caucasus Mountains in Eastern Europe.
- Commercial Kefir is produced from a milk product that is heated to remove possibly pathogenic bacteria. Then, a mixture of bacteria and yeasts is added for fermentation. This starter culture is called kefir grains which has a cauliflower like appearance. This is what gives kefir a unique taste and texture. Home grown Kefir only requires grains.
- The bacteria used in kefir production is **Lactobacillus caucasius** which turns the lactose to lactic acid. This results to its tangy taste.
- Meanwhile, the yeasts, **Saccharomyces kefir and Torula kefir**, ferment lactose into a small amount of carbon dioxide and alcohol. This plays a role

CAMEL KEFIR

- Kefir is the ancient beverage of the people from the Caucasus Mountain, who attributed their long healthy lives to traditional (raw) Kefir.
- Kefir derived from Turkish word Keif "good feeling".
- Kefir is obtained from the fermentation of the fresh camel by Kafir cultures
- No preservative, no sugar added.

Kefir Benefits

- Nutrient source: Kefir contains essential nutrients and minerals that are important for proper functioning of the body such as protein, vitamin D, calcium, and calories
- Probiotics: It aids in the maintenance of digestive health and prevents growth of harmful bacteria in the intestines. Also, helps in prevention of digestive disorders.
- Eases off lactose intolerance
- Weight loss due to its low calorie contents
- Possible cancer prevention

Yoghurt

Yogurt is a variety of curd. Whole, low fat, skim milks & even cream can be used to make yogurt.

- In production of yogurt, a mixed culture of streptococcus thermophilus, lactobacillus acidophilus is usually added to to the pasteurised milk & incubated at 42-46°C.

- Increase in folic acid concentration during fermentation.
- Fermented milk is useful for a wide variety of disorders like colitis, constipation, diarrhoea, gastroenteritis, diabetes & hyper cholesteremia.

Making yogurt

Milk containing lactose

↓

Lactic acid bacteria added to the milk

↓

Bacteria feed off lactose in milk

↓

Lactic acid produced increases the milk acidity

↓

Milk protein caesin thickens and coagulates to produce yogurt

Yoghurt production

- Fermenting milks with different micro-organisms has also provided an opportunity to develop a wide range of products with different flavours, textures, consistencies and, more recently, health attributes. These include:
- **Live yogurts**, which contain harmless bacteria that are added to the milk and are still present and alive.
- **Probiotic yogurts**, which contain live probiotic micro-organisms that are suggested to be beneficial to health.
- **Bio yogurts**, which are very popular and are made using bifidobacteria. Bio yogurt has a milder, creamier flavour which is less acidic than some other varieties and has shown to aid digestion and promote good health.

YouTube - How It's Made Yogurt 1min 25secs

HOW TO MAKE YOGHURT

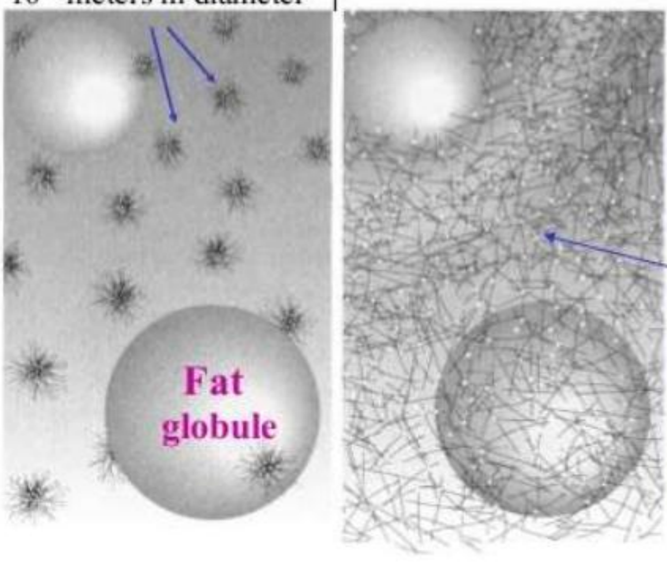
Making Yogurt in 4 Simple Steps

1. Start with Cow, Sheep, or Goat milk.
2. Heat milk to 80 °C. Two purposes:
   - destroy existing bacteria
   - "condition" the proteins = begins the denaturing process
   (a whey protein molecule binds to a casein molecule which disrupts the casein bundles allowing them to make short branched micelle chains)
3. Cool milk to 40 °C and innoculate with bacteria
4. Incubate at 30 °C to 45 °C

BACTERIA IN YOGHURT

Milk
Casein protein micelles (bundles)
10^{-7} meters in diameter

Yogurt
Bacteria produce acid



Acid causes Casein bundles to fall apart into separate casein molecules.

These rebind to each other in a network that traps water.

=> makes a gel

Yogurt as probiotic food

Yogurt is basically a probiotic food with live and active cultures. It contains different kinds of bacteria that are believed to be beneficial to your overall health.

Four major strains of bacteria to look for:

* Lactobacillus acidophilus,

* Lactobacillus bulgaricus,

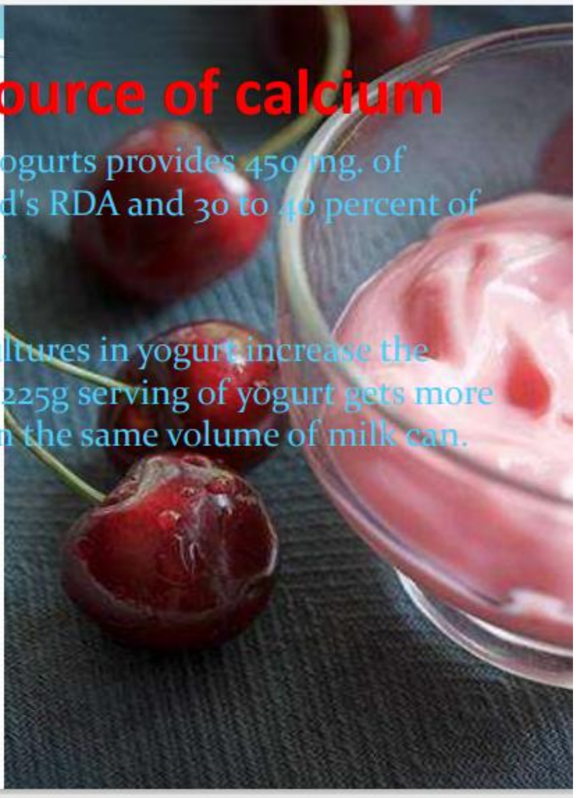
* Streptococcus thermophilus, and

* Bifidobacteria

YOGURT Nutrifacts-

- Yogurt is rich in potassium, calcium, protein and B vitamins, including B-12
- Yogurt is easier to digest than milk
- Yogurt contributes to colon health
- Yoghurt strengthens and stabilizes the immune system
- Research shows women who eat 4 cups of yoghurt/week have less vaginal and bladder infections
- The lactic acid of yoghurt is a perfect medium to maximize calcium absorption
- Yogurt can be used as an effective douche

Yogurt - A rich source of calcium



An 225g serving of most yogurts provides 450 mg. of calcium, one-half of a child's RDA and 30 to 40 percent of the adult RDA for calcium.

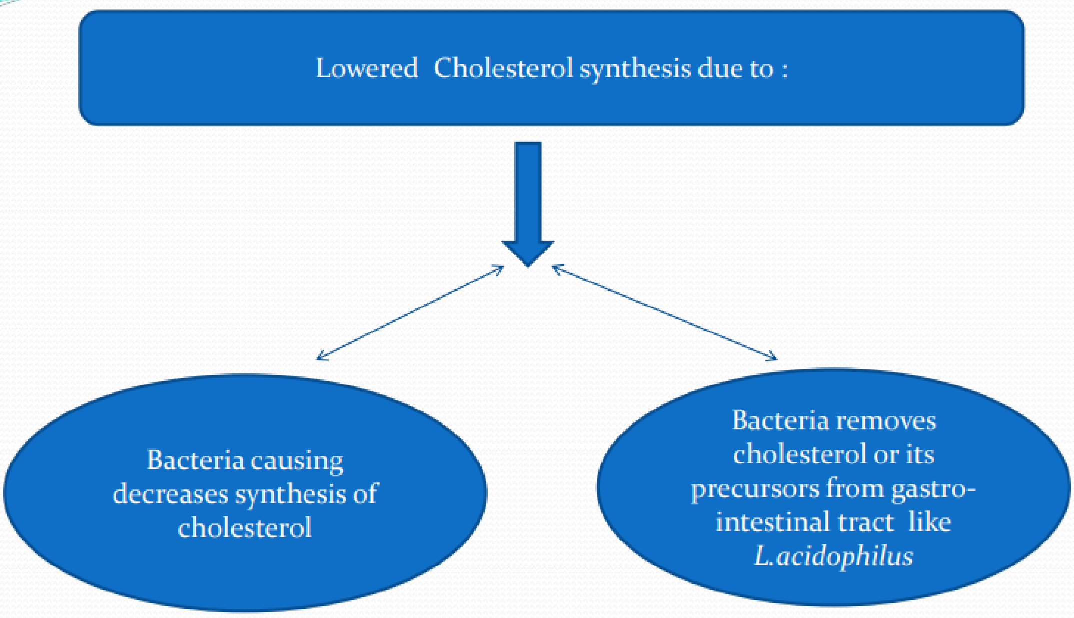
Because the live-active cultures in yogurt increase the absorption of calcium, an 225g serving of yogurt gets more calcium into the body than the same volume of milk can.

CHOLESTEROL CONTROL

- Although cholesterol is important and necessary for human health, high levels of cholesterol in the blood have been linked to damage to arteries and cardiovascular disease.
- Major dietary sources of cholesterol include cheese, egg yolks, beef, pork, poultry, fish, and shrimp.
- Cholesterol contributes to atherosclerosis - a condition that greatly increases the risk of heart attack and stroke - by suppressing the activity of a key protein that protects the heart and blood vessels

Yoghurt as Cholesterol Reducer

- Yogurt contains a factor that inhibits the synthesis of cholesterol from acetate.
- This factor may be either 3-hydroxy-3-methylglutaric acid
- orotic acid plus thermophilus milk and methanol solubles pf thermophilus milk on liver cholesterol.



Acidophilus Milk

- Acidophilus milk, also known as sweet acidophilus milk or probiotic milk.
- Milk that has been fortified with Lactobacillus acidophilus or other friendly bacteria cultures such as
    i. *Lactobacillus bulgaricus,*
    ii. *Bifidobacterium bifidum,* or
    iii. *Streptococcus theromphilus.*

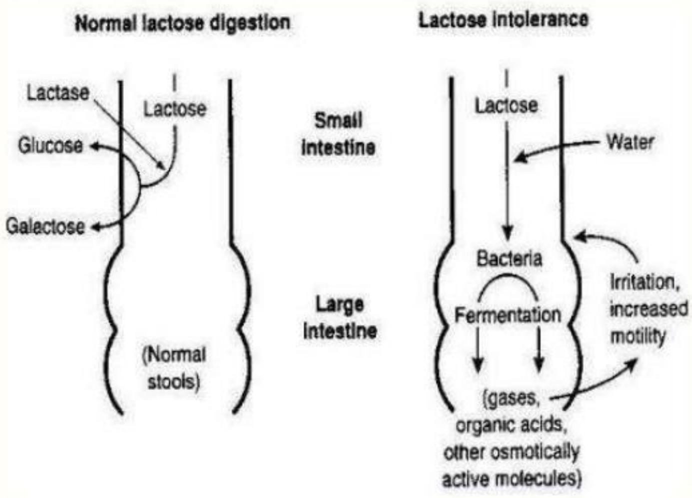
LACTOSE INTOLERANCE

- **Lactose intolerance**, also called **lactase deficiency** and **hypolactasia**, is the inability to digest lactose,a sugar found in milk and some dairy products. Lactose intolerant individuals have insufficient levels of lactase—the enzyme that metabolizes lactose—in their digestive system .

SYMPTOMS:

- Abdominal bloating and cramps, flatulence, diarrhea, nausea, borborygmi (rumbling stomach) and/or vomiting after consuming significant amounts of lactose.

Lactose Intolerance



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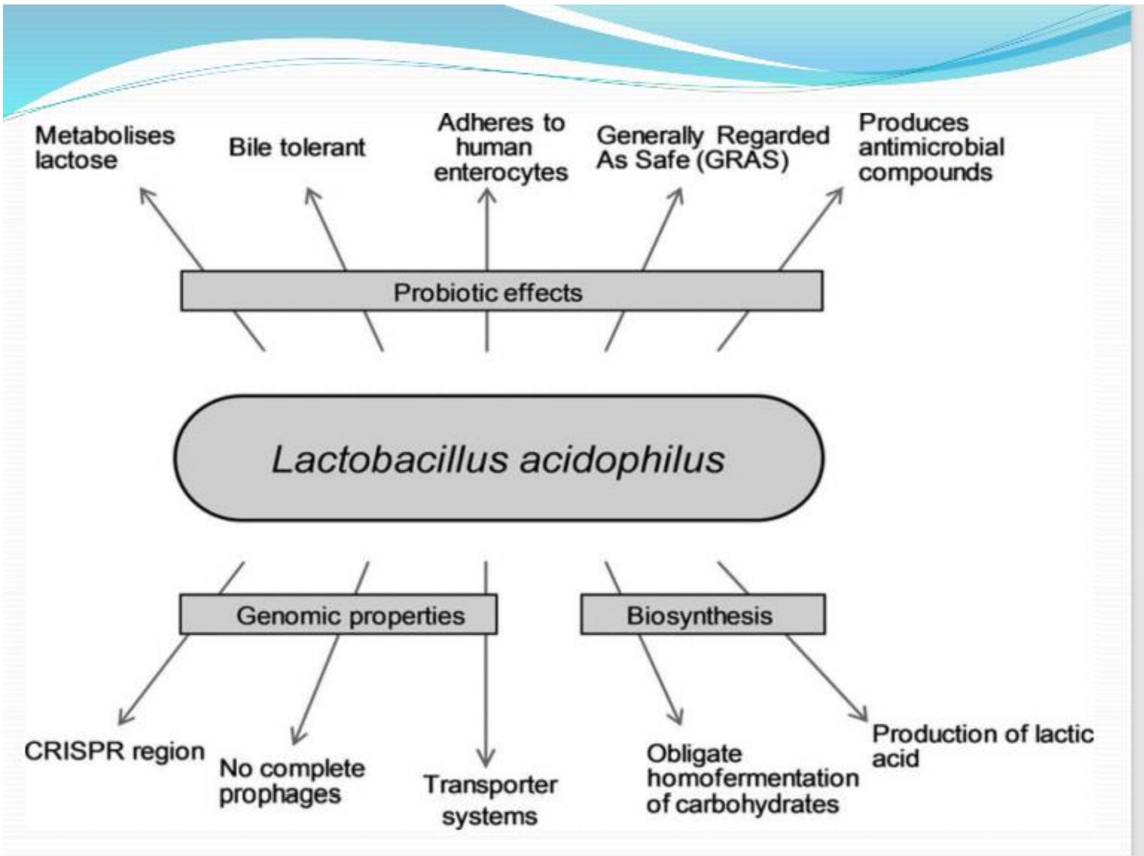
- **"Sweet" acidophilus** milk has been reported by some to prevent symptoms of lactose intolerance, whereas others have found this product to be ineffective.
- Developed by M.L. Speck and co-workers, it consists of normal pasteurized milk to which is added large numbers of viable *L. acidophilus* cells as frozen concentrates.
- As long as the milk remains under refrigeration, the organisms do not grow, but when it is drunk, the consumer gets the benefit of viable *L. acidophilus* cells.
- It is "sweet" because it lacks the tartness of traditional acidophilus milk.


Metabolises lactose
Bile tolerant
Adheres to human enterocytes
Generally Regarded As Safe (GRAS)
Produces antimicrobial compounds
Probiotic effects
Lactobacillus acidophilus
Genomic properties
Biosynthesis
CRISPR region
No complete prophages
Transporter systems
Obligate homofermentation of carbohydrates
Production of lactic acid

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

B.SC – MICROBIOLOGY

UNIT – III - FOOD FERMENTATION AND TECHNIQUES

PREPARATION OF CULTURED BUTTER MILK, COMPOSITION AND QUALITY CHARACTERISTICS

Introduction

Drinking of butter after churning dahi in to country butter is a very common habit in India. This product has most of the fermented milk solids except fat which goes in butter.It also has mixed lactic acid bacteria, especially Lactococci and Leucostocs,which gives it a typical diacetyl flavour.

Manufacturing cultured butter milk on industrial scale involve selection of good quality raw material,standard cultures and optimized process of fermentation, packaging and storage.

True buttermilk is the fluid remaining after cream is churned into butter. If butter is made from sweet cream, its buttermilk has approximately the same composition as skim milk. Cultured butter milk is prepared by souring true butter milk or more commonly, skim milk with a butter starter culture that produces a desirable flavor and aroma.

Starter Culture

Cultured butter milk is prepared with the help of normal mesophilic lactic acid bacteria.

There should be satisfactory balance between acid and flavor producers in the starter.

Making of Cultured Butter Milk

Selection of Milk

The quality of raw material decides the quality of final product. The raw milk selected for CBM manufacture should normal composition, be free from off flavor and odours and free from inhibitory substances. It should have lower microbial count.

Standardization of Milk

It is usual practice to standardize milk for fat and solids-not-fat content looking to legal requirements and also as per consumer demands. Generally skim milk is the starting material and it may be added with approximately 1.7% fat. In certain commercial processes, fat is added as granules in cold fermented butter milk.Sodium chloride at the rate of 0.1 – 0.2% and sodium citrate at the rate of 0.1 – 0.2% may be added for enhancement offlavor.

Heat Treatment

Proper heat treatment of at least pasteurization equivalence is mandatory for CBM production. This is to ensure destruction of pathogens and make the product safe for human consumption. It also helps in proving suitable environment for the cultures. Based on earlier experiences and scientific evidences, it found that heating at 85° for 30 min is the optimum heat treatment for CMP production which gives best quality product in terms of flavour and shelf-life. Heating below 82°C or above 88°C causes a weak body, that allows whey separation in cultured butter milk.

Cooling

Milk must be cooled down to inoculation temperature as soon as holding period is over. Inoculation in hot milk will destroy the culture. It should be 22-25°C for cultured buttermilk.

Inoculation

The milk should be inoculated with appropriate mesophilic starter culture just after cooling. Inoculation rate varies from 0.5 to 2.0%, depending upon the starter activity and time of incubation At the time of inoculation,starter should have 0.80-0.85% acidity. Over ripe culture are not suitable as the lactic streptococci have passed their peak of acidity. If it is under ripenedi.e. less than 0.8% lactic acid, it will not have sufficient number of aroma bacteria.

Incubation

The typical incubation temperature for CBM is 21.6°C. There are several reasons for selecting 21-22°C as incubation temperature.

- Any departure of more than a degree or two above or below will cause imbalance in the bacterial mixture and will adversely affect the product.
- At lower temperature, the starter organisms grow too slowly, and curdling is delayed excessively
- At much higher temperature, the streptococci overgrow aroma bacteria and product lack flavor and aroma.
- At higher incubation temperature the curd forms at a lower acidity and it tends to shrink, causing whey separation.
- Use of lowest practicable temperature inhibits the growth of undesirable bacteria that survive the heat treatment given to the milk.

Cooling, Agitation and Dilution

After desired stage of ripening, the curd must be cooled rapidly to avoid over ripening. Mixing may be necessary to hasten cooling, but it should not be done in a manner that incorporate air. Excessive agitation decreases the stability of butter milk and increases whey separation. Gentle agitation is required to break curd and to have efficient and quick cooling. At the stage dilution of the product may also may be done to adjust solids level to desired level. Some manufacturers also add some spices and condiments to falvour it . The final cooling of the product should be to less than 5°C.

Packaging & Storage

Bottle or cartons packaging material are commonly used. Packaging material should not excessively increase the microbial load in the product. Product will have better shelf-life if stored below 5°C.

Characteristics of Good Quality Cultured Butter Milk

A good quality butter milk, after packaging has a pH 4.5 and a possess smooth viscous body giving a slow even flow when poured. The flavor should be clean acid with an integrated aromatic diacetyl and free volatile acid background. It exhibits no free whey or whey separation. The keeping quality of good buttermilk at 5°C is approximately 2 weeks

The standards for quality and hygiene apply to butter milk too. In India the standards for contamination prescribe that coli form count should be less than 10 per ml and yeast & mold count should be less than 100 per ml.

YOGURT PROCESSING:

This month's Processing column on the theme of "How Is It Processed?" focuses on yogurt. Yogurt is known for its health-promoting properties. This column will provide a brief overview of the history of yogurt and the current market. It will also unveil both traditional and modern yogurt processing techniques.

Yogurt's History and the Market

Yogurt is a fermented milk product with a custard-like consistency that differentiates it from other fermented milk products. Yogurt is thought to have originated in Mesopotamia thousands of years ago. Evidence has shown that around 5000 B.C. domesticated goat and sheep milk was stored in gourds in warm climates and formed a curd, which was an early form of yogurt produced by naturally present bacterium. It is only in the last 30–40 years that yogurt has become popular in the United States.

The FDA Code of Federal Regulations defines yogurt as the food produced by culturing cream, milk, partially skimmed milk, or skim milk alone or in combination with a bacterial culture that contains the lactic acid–producing bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. There are many types of yogurt, including low fat, no fat, creamy, Greek, drinking, bio-yogurt, organic, frozen, and even yogurt for babies.

Yogurt Processing

Modification of Milk Composition.

Yogurt processing begins with modification of the incoming milk composition. This process typically involves reducing the fat content and increasing the total solids in the milk. A standardizing clarifier and/or a separator (centrifuge) is used to separate fat from milk and reduce the fat content. Alternatively, milk fat can be added to skim milk to obtain the desired fat level. Depending on the type of yogurt, the final fat content ranges from 1% to 10%. The final fat content affects yogurt consistency and viscosity. The milk fat content also affects the fermentation rate. From the separator, the milk is placed in a storage tank. It is then tested for fat and solids content. The solids content of the milk is then increased to around 16%, with 11%–14% of that being solids-nonfat (SNF). SNF components include lactose, protein, and minerals. SNF in yogurt typically ranges from 9% to 16%. Traditionally, either some water is evaporated off of the milk to increase the solids content, or concentrated milk, milk powder, whey protein concentrate, or casein powder is added to accomplish that objective. More recently, ultrafiltration and reverse osmosis technologies have been used to increase the SNF. Increasing the solids content increases the firmness of the yogurt and improves its nutritional value and stability. Increases in SNF also increase the duration of the fermentation process. After the solids are adjusted, stabilizers such as pectin and gelatin are frequently added to increase yogurt body and texture, as well as to prevent syneresis in the final product. Sweeteners can be added to improve flavor and consumer appeal.

Pasteurization and Homogenization.

Pasteurization and homogenization are the next steps in the process. Pasteurization is very important because it destroys microorganisms in the milk that may interfere with the fermentation process. It also denatures the whey proteins, resulting in improved body and texture. Pasteurization also helps release compounds in the milk and removes dissolved oxygen, both of which stimulate growth of the starter cultures. Pasteurization can be a continuous or batch process whereby the milk is heated to a high temperature for a set amount of time. Heating to 85°–90.5°C for 30–60 minutes is common.

Homogenization subjects milk fat globules to severe conditions that disrupt the membrane surrounding globules, breaking them up into smaller, more consistently dispersed emulsion particles. This produces a smoother and creamier end product with greater stability. Homogenization is typically accomplished using a homogenizer at high pressures. Homogenization pressures commonly applied range from 10–20 MPa. The milk is forced through small openings at high pressures and the fat globules are broken due to the shear. Alternative homogenization methods include ultra high pressure processing, ultrasound, and microfluidization. Ultra high pressure homogenization has been introduced commercially and produces firmer yogurts with higher water-holding capacities. High intensity ultrasound generates high pressures, temperatures, and shear gradients during homogenization. Microfluidization causes homogenization via shear, turbulence, and cavitation.

The images featured in this month’s column show the Cornell University dairy processing plant, which houses new tanks for raw product storage, a new separator, a high temperature/short time pasteurizer, homogenizer, and other processing equipment for yogurt manufacturing.

Fermentation. After the milk is pasteurized and homogenized, it is cooled to 40°–44°C and inoculated with a lactic acid–producing culture. During this stage, the curd is formed and the yogurt's textural characteristics and taste are developed. For a fermented product to be labeled as yogurt it needs to contain two live bacterial strains of *Lactobacillus bulgaricus* and *Streptococcus thermophiles*. Ratios of 1:1 are commonly employed. In addition, yogurt starter cultures may contain other microorganisms like *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus helveticus, Bifidobacterium bifidus, Bifidobacterium infantis,* and others. Cultures are typically added at concentrations of about 2%. The yogurt is then held at this temperature for three to four hours while the incubation process takes place. During this time, the lactose is reduced, proteins aggregate, and flavor compounds develop.

Depending on the type of yogurt, the inoculated milk is either incubated in a hygienically sealed stainless steel, stirred vessel or consumer-sized sterile packages to incubate in a temperature-controlled environment. Stirred yogurt is fermented in bulk and then poured into final consumer packages. During the process, the final coagulum is broken by the stirring prior to cooling and packaging. As a result, the texture of stirred yogurt is less firm than set yogurt. Drinking yogurt is processed similarly to stirred yogurt. Greek yogurt or concentrated yogurt is processed in the same manner as stirred yogurt. Following the breaking of the coagulum, Greek yogurt is strained through a filter. The straining process separates out some of the whey, resulting in a thicker, higher-protein yogurt. Alternatively, the yogurt can be concentrated by boiling off some of the water under vacuum, or the yogurt can be manufactured from evaporated milk. Set yogurt, known as French yogurt, is allowed to ferment in the container it is sold in and has a firm gel texture.

Lactic acid level (or pH) is used to determine when the yogurt is ready for cooling, which is often accomplished quickly by blast chilling to stop the fermentation process. A value of at least 0.9% acidity and a pH of about 4.4 are the current minimum standards for yogurt manufacture in the United States. To attain a yogurt with a pH of 4.0, the cooling process should begin when the fermented milk reaches a pH of 4.3–4.4. To extend the shelf life of the product, yogurt can be heat treated after culturing is complete to destroy viable microorganisms. Lastly, flavors are frequently added to chilled fermented yogurt. This is referred to as Swiss-style yogurt.

Alternatively, sundae-style yogurt can be obtained when the fruit or fruit flavoring is added to the bottom of the consumer package.

Recent Innovations in Yogurt

Recent research has focused on development of new flavors, increased nutrition, and longer-lasting yogurt products. Icelandic skyr strained yogurt and goat milk yogurts are a few novel yogurt products. Development of savory yogurts through the addition of spices, savory additives, espresso, and vegetables is another new trend, as is inclusion of grains such as oats in yogurts. High-protein Greek yogurts and probiotic yogurts with low or no lactose are also gaining in popularity. In addition, research on new processing innovations to obtain longer-lasting yogurts is underway, as is research on characterizing and improving the nutritional benefits of yogurt. Ancient people called yogurt the "food of the gods," and today yogurt continues to have an outstanding reputation and a promising future as a healthy processed food product.

ACIDOPHILUS AND BIFIDUS PRODUCTS

Introduction

Several products containing live lactobacilli or bifidobacteria have been developed in various forms using milk as the base material or by supplementation of cereals, oats, fruits, vegetables and other juices. Certain products are strictly hygienically made and prescribed by medical professionals for specific ailments

Acidophilus Products

Sour acidophilus milk

Acidophilus milk is a sour product that has been allowed to ferment under conditions that favour the growth and development of a large number of *Lactobacillus acidophilus* organisms. This acidophilus milk is considered as a probiotic since it aids in the well being of the consumer. Acidophilus milk differs from Indian dahi or curd in body, texture, consistency, flavour, chemical composition and in antibacterial activities

Sweet acidophilus milk

As natural fermented acidophilus milk was sour and having medicinal type of flavour, it was thought appropriate to sell as non-fermented milk. This gave birth to sweet acidophilus milk. It is probiotic dairy product based on unfermented milk. It is produced by adding concentrated probiotic bacteria to intensively heat treated and chilled milk. Heat treatment is necessary to achieve sufficient microbiological stability during storage of the final product.
In some cases it is also prepared by adding concentrated cells of *Lb. acidiophilus* in chilled pasteurized milk.

Acidophilus yoghurts

Yoghurt is a popular product in many parts of the world. Many probiotic products have been developed taking yoghurt as a base. In some products, acidophilus or other probiotic bacteria are added as a supplement or in other cases one of the yoghurt culture is replaced by *Lb. acidophilus*.
The acidophilus yoghurt produced from cow milk is popular in Germany, USA, Scandinavia, Australia, and many other countries,. It is believed that human intestinal strains of acidophilus culture increase the beneficial value of the yoghurt made with them. The starter culture consists of yoghurt culture (*Streptococcus thermophilus and Lactobacillus delbruckii subsp. bulgaricus*) and *Latobacillus acidophilus*.

A product known as ACO-Yoghurt is also reported from Switzerland and U.K with improved dietetic and therapeutic values of the yoghurt. The starter cultures consist of yoghurt culture and culture of an intestinal strain of *Lb. acidophilus*,

Acidophilus bifidus yoghurt

This product is very popular in Germany, United States, Japan and several other countries, obtained from cow milk. The product was first manufactured in Germany in order to improve the nutritive as well as the therapeutic value of yoghurt. The products involve three groups of bacteria, a. Yoghurt culture (*Streptococcus thermophilus and Lactobacillus delbruckii subsp. bulgaricus*), b. Strains of *Lb. acidophilus* and c. *Bifidobacterium bifidum or B. longum.*
The final product is expected to contain 10^7 per ml each of *Lb. acidophilus* and *B. bifidum* and large numbers of Yoghurt organisms.

Acidophilin

It is the product originated from the USSR, obtained from cow's milk which is set or stirred, flavor may vary from mild to acid depending on the quality of starter culture. The principle of processing is mixed acid and alcoholic fermentation involving *Lb. acidophilus, Lb. lactis* subsp. *lactis or Lb. delbruckii* subs*p bulgaricus* and Kefir culture) at the rate of 6 – 9 %.

Acidophilus cream

The product has originated from Czechoslovakia, obtained from cream of cow milk. The cultures involved is *Lb. acidophilus*. The process involves cream with 40 – 45 % fat, which is homogenized, pasteurized, added with sugar syrup and then inoculated with the culture of *Lb. acidophilus.*

Acidophilus ice cream

Ice cream is one of the most popular dairy products and probiotics can be easily dispensed through this medium. The acidophilus ice cream finds its origin from USA. It is prepared with a mix added with concentrated *Lb. acidophilus* culture (10^9 per ml) and frozen.

Now several reports are available wherein the study of viability of *Lb. acidophilus* in ice cream has been studied. Recently, Amul launched 'Prolife', a probiotic icecream containing Lactobacilli and Bifidobacteria.

Acidophilus yeast milk

The manufacture of the product using the culture *Lb. acidophilus* and lactose fermenting yeast was mainly for the therapy of the gastrointestinal disorders and tuberculosis. The viability of the acidophilus bacteria is expected to improve when they are grown together with yeasts. The product has a final acidity of 0.8-1.0% and contains about 0.5% ethanol in addition to carbon dioxide.

Bifidus Products

Bifidus milk

The product finds its origin from Germany. The product is named accordingly to the bacteria used in fermentation. *Bifidobacteria sp* are the predominant intestinal flora and the major components of large intestine of human adults. Bifidus milk is produced in small quantities in some of the European countries. The consumption is linked to the dietetic and therapeutic values rather than the sensory properties. However, as Bifidobacteria ferment milk producing more of acetic acid than lactic acid, it has a vinegar like flavour and people do not enjoy it as a food. It remained a product to be used for therapeutic purposes. Further, Bifidobacteria are anaerobic in nature and hence it is very difficult to grow them in milk.

Bifidus baby foods

Several formulations containing Bifidobacteria are in the market intended for use as baby foods or infant food formulae. The product aims to increase the population of bifidobacteria in intestinal tract.

Some of the commercial baby foods are

- The dried formulae product called **Lactana – B**, containing lactulose and viable *B. bifidum,* produced from modified milk.
- The liquid formulae product called **Bifiline**, containing viable *Bifidobacteria*, first developed by the Russians in 1982. It is made by using milk formulae called Malutka (Acidophilus baby foods) and selected strain of *Bifidobacteria*.

· The dried formulae product called **Femilect**, containing viable *Bifidobacteria*, which was developed in Czechoslovakia in 1984, made by fermenting heat treated cream (12% fat) with mixed culture consisting of *B. bifidum, Lb. acidophilus and P. acidilactici.* The final product when reconstituted has 28°Th (0.25% T.A) and contains 10^8-10^9 / ml viable culture bacteria.

Bifidus yoghurt

The product is made with yoghurt cultures supplemented selected cultures of *Bifidobacterium bifidum or B. longum.* The product is made either by simultaneous fermentation or by mixing into cultured yoghurt separately cultured bifidus milk at a desirable ratio.

Bifighurt

This is a commercial product from Germany. The starter culture used in this product is *Bifidobacterium longum C KL 1969 of B. longum and S. thermophilus.* The final product contains 95% L (+) lactic acid and about 10^7 *bifidobacteria* per ml.

Biogarde

This product also is from Germany, known for its mild acidic taste and flavor. The manufacturing procedure of the product was developed by **Bioghurt**^{R} Company in Germany. The Biogarde^{R}*Lactobacillus acidophilus, Bifidobacterium longum and Streptococcus thermophilus*. **Biogarde**^{R} produces fermented milk as well as icecream. culture contains

Biokys

The technology of biokys as a health product was developed in Czechoslovakia, where the product is commercially produced. It is a cultured sour cream like beverage obtained by fermenting milk with a mixed culture of *bifidobacteria, acidophilus bacteria, and Pediococcus acidilactici*.

KEFIR PRODUCTION

The word kefir is derived from the Turkish word *keyif*, which means "feeling good" after its ingestion.

The kefir beverage is originally from the Caucasus Mountains, a traditional product highly consumed in Eastern Europe, Russia and Southwest Asia.

Currently, an increase in kefir consumption in many countries has been reported, due to its unique sensory properties and long history associated with beneficial effects on human health.

Kefir is characterized by its distinct flavour, typical of yeast, and an effervescent effect felt in the mouth.

The main products of kefir fermentation are lactic acid, ethanol and CO_2, which confer this beverage viscosity, acidity and low alcohol content.

Minor components can also be found, including diacetyl, acetaldehyde, ethyl and amino acids contributing to the flavour composition.

This drink differs from other fermented dairy products because it is not the result of the metabolic activity of a single or a few microbial species.

Kefir grains play a natural starter culture role during the production of kefir and are recovered after the fermentation process by milk straining.

These grains are composed of microorganisms immobilized on a polysaccharide and protein matrix, where several species of bacteria and yeast coexist in symbiotic association.

In this ecosystem there is a relatively stable microorganism population, which interacts with and influences other members of the community.

This population provides the synthesis of bioactive metabolites, which are essential for grain growth and microorganism inhibition, such as food pathogens and contaminants.

Kefir grains vary in size, from 0.3 to 3.0 cm in diameter, are characterized by an irregular, multilobular surface, united by a single central section, and their color varies from white to yellowish white. The grains are elastic and have a viscous and firm texture.

Although the kefir drink can be found in many countries, in Brazil the grains are not available commercially, and are culturally donated from person to person.

In kefir, lactic acid bacteria (LAB) are primarily responsible for the conversion of the lactose present in milk into lactic acid, which results in a pH decrease and milk preservation.

Other kefir microbial constituents include lactose-fermenting yeasts that produce ethanol and CO_2. Non-lactose fermenting yeast and acetic acid bacteria (AAB) also participate in the process.

After fermentation the grains increase in about 5-7% of their biomass.

During their growth in milk, the microorganism proportions in the grains differ from those present in the final product.

This difference is associated with the fermentation process conditions such as fermentation time, temperature, degree of agitation, type of milk, grain/milk inoculum ratio and microorganism distribution, among others.

Traditionally, classical microbiological methods are used to study kefir microbiota.

While these methods are useful, in some cases they are not discriminating enough to identify closely related or new species.

Because of the microbial symbiotic association present in the grains, the growth and survival of individual strains are dependent on the presence of each other.

Often, when microorganisms are isolated from the grains, they do not grow well in milk and/or show reduced biochemical activity.

Therefore, independent cultivation methods have been used as a complement to conventional methods in the study of kefir grain microbiota.

The polymerase chain reaction technique, coupled to electrophoresis in denaturing gradient gel (PCR-DGGE) has proved appropriate for analyzing complex microbial consortia, while the partial sequencing of the gene coding for 16S rRNA has been used for species.

However, some studies show that the PCR-DGGE technique does not allow the detection of significant changes during kefir fermentation, probably due to the relative stability of the dominant population in this community.

Homofermentative LAB, including;

Lactobacillus species, such as *L. delbrueckii* subsp. *bulgaricus, L. helveticus, L. kefiranofaciens* subsp. *kefiranofaciens, L. kefiranofaciens* subsp. *kefirgranum* and *L. acidophilus*;

Lactococcus spp. Such as *L. lactis* subsp. *lactis* and *L. lacti*s subsp. *cremoris* and *Streptococcus thermophilus* have been identified in kefir grains and in the fermented beverage, as well as heterofermentative LAB, including *L. kefiri*, *L. parakefiri*, *L. fermentum* and *L. brevis*, and citrate-positive strains of *L. lactis* (*L. lactis* subsp. *lactis biovar diacetylactis*), *Leuconostoc mesenteroides* subsp. *cremoris*, and *Leuconostoc mesenteroides* subsp. *mesenteroides*.

The use of citrate by citrate-positive strains results in the production of key compounds that contribute to typical kefir flavour.

Kefiran produced by L. kefiranofaciens is a branched, water-soluble polysaccharide, containing equal amounts of D-glucose and D-galactose. The production of this polysaccharide is stimulated when L. kefiranofaciens grows in co-culture with S. cerevisiae.

AAB species have been isolated and identified in both kefir grain and the kefir beverage.

However, in some countries, the presence of these species is considered undesirable and has received less attention, even though they play an essential role in both the microbial consortium and the sensory characteristics of the final product.

Although they produce metabolites that contribute to the desirable and typical kefir sensory properties, kefir yeast are less studied than kefir bacteria.

The main yeast capable of fermenting lactose found in kefir and kefir grains are *Kluyveromyces marxianus/Candida kefyr, Kluyveromyces lactis* var. *lactis, Debaryomyces hansenii* e *Dekkera anomala*, while the non-lactose fermenters include *Saccharomyces cerevisiae, Torulaspora delbrueckii, Pichia fermentans, Kazachstania unispora, Saccharomyces turicensis, Issatchenkia orientalis* and *Debaryomyces occidentalis*.

The complex interactions between yeast and bacteria and their interdependence in kefir grains are not completely understood. However, when the bacteria are separated from the grain, yeast will not grow as efficiently (Cheirsilp et al., 2003; Farnworth and Mainville, 2008; Rattray and O'Connel, 2011).

Due to its high capacity to metabolize lactose (Rea et al., 1996), the genus Lactococcus tends to grow faster than yeast in milk (Rea et al., 1996; Tamime, 2006). This genus hydrolyzes lactose, producing lactic acid and a suitable environment for yeast growth (Tamime, 2006).

Yeasts synthesize complex B vitamins and hydrolyze milk proteins, using oxygen to produce CO2 and ethanol.

The interaction between yeast and lactic acid bacteria can be stimulated or inhibited by the growth of one or both, in co-cultures.

These microorganisms can compete for nutrients for growth, or may produce metabolites that inhibit or stimulate one another. Some yeast species are proteolytic or lipolytic, providing amino acids and fatty acids.

Species such as Debaryomyces hansenii and Yarrowia lipolytica assimilate the lactic acid formed by LAB, raising the pH and stimulating bacteria growth.

The production of vitamin B by Acetobacter spp. also favors the growth of other microorganisms present in kefir grains.

During fermentation, the grains increase in size and number, and are usually recovered from the fermented milk and reutilized.

If carefully preserved, they may retain their activity for years.

The main marker to assess the symbiotic relationship between the different microorganisms is increased biomass during fermentation.

Kefir grains can be preserved lyophilized, dry or wet, but constant washing reduces their viability.

However, grain stored in these conditions present different microbiological profiles than fresh grain.

Dried grains maintain their activity for 12-18 months while wet grains maintain activity for 8-10 days.

Different preservation methods have been tested, with freezing being considered the best method.

Grain lyophilization has also been tested, but resulted in reduced lactose metabolism, as well as modifications in the bacterial profile, which was different from the original grain profile.

There are three main ways of producing kefir (I) the artisanal process, (II) the commercial process by the Russian method and (III) the commercial process using pure cultures.

Other substrates may also be used, such as milk from other animal species, coconut milk, soybean milk, fruit juices and/or sugar and molasses solutions.

The traditional artisanal production involves milk inoculation with a variable amount of grains and fermentation for a period between 18-24 h at 20-25 ºC.

At the end of the fermentation process the grains are sieved and can be used for a new fermentation or kept (1-7 days) in fresh milk, while the kefir beverage is stored at 4 ºC, ready for consumption.

The initial inoculum concentration of the grains (grain/milk proportion) affects the pH, viscosity, final lactose concentration and the microbiological profile of the final product.

Agitation during fermentation also influences kefir microbial composition, favoring the development of homofermentative lactococci and yeast. Incubation at temperatures above 30 ºC stimulates the growth of thermophilic LAB, while being a disadvantage for yeast growth and mesophilic LAB.

The second method, known as the "Russian method", allows for the production of kefir on a larger scale, and uses a process of fermentation in series, from the percolate resulting from the first fermentation of the grains (fermented without the grains or mother culture).

Different methods can be used in the industrial process of kefir production, but all based on the same principle.

The milk is inoculated with pure cultures isolated from kefir grains and commercial cultures.

The maturation phase can be performed or not, consisting of maintaining the kefir at 8-10 ºC for up to 24 h, to allow microorganism, primarily yeast, growth, contributing to the specific flavour of the product.

Omission of this step is associated with development of atypical flavour in kefir.

During storage, the CO2 production by yeast or heterofermentative LAB can cause bloating in the product package, a fact that should be considered in the choice of packaging.

PROBIOTIC PRODUCTS, PRODUCTION

Introduction

Milk and dairy products have been used as the base material for manufacture of majority of probiotic products as milk is has an excellent nutritional value. Among the probiotic cultures, Lactobacilli and Bifidobacteria are enjoying GRAS status and are considered as the most promising microbes for manufacture of probiotic and synbiotic products. This is basically because the selected species of these groups are autocathonous to the intestinal tract and hence can give the maximum advantages in the host. Hence, most of the probiotic products in the market are milk based and are prepared using Lactobacilli and/or Bifidobacteria.

Types of Products

The traditional acidophilus milk is sour,non-pleasant and having rather medicinal type flavour. Bifidobacteria produce more of acetic acid than lactic acid and hence they also give a vinegar type flavour in the basic product. Further, as both these cultures are slow growers in milk, the initial milk used in their preparation is severely heated. That makes it almost sterile so that the chance of survivors overgrowing during incubation can be avoided. This milk also give a cooked type of flavour to natural Acidophilus or Bifidus milk. These limitations did not make these products popular. However, increasing number of reports on their heath benefits, tempted the scientists to develop alternate technologies to make these products more palatable or acceptable. This was overcome by 2-3 different approaches.

1. Not to ferment- The growth and flavour problems were coming when the milk was allotted to fermented. This can be avoided by not fermenting milk. That lead to

development of non-fermented products, that contained only viable cells of these organisms in chilled milk.

2. Blending with other bacteria- To enhance the flavour it was thought to supplement additional microflora as starter culture that give good flavour.
3. Concentration and Drying – This changed the form of consumption and improved the shelf-life.
4. Carry acidophilus and bifidus in other popular products.

Based on these concepts number of products has been developed, which can be grouped as under:

1. Traditional fermented products

- Acidohpilus Milk (sour)
- Bifidus Milk

2. Non-fermented milks

- Acidohpilus sweet milk

3. Blending with yoghurt cultures

- Acidophilus yoghurt
- ACO yoghurt
- Acidohpilsbifidus yoghurt
- Bioghurt
- Bifighurt

4. Blending with other cultures

- Acidophilin
- Acidophilus yeast milk

5. Concentrated products

- Acidophilus cream
- Acidophilus paste

6. Dried Products

- Acidophilus powders
- Infant/Baby foods
- Tablets
- Capsules

7. Blending in other products

- Acidophilusice cream
- Probiotic cheese
- Probiotic fruit juices

Following is the brief description of production methods and effect of different processing steps on quality and microbiology of the product taking example of acidophilus milk.

Acidophilus Milk

Acidophilus milk is a sour product that has been allowed to ferment under conditions that favour the growth and development of a large number of *Lactobacillus acidophilus* organisms. This acidophilus milk is considered as a probiotic since it aids in the well being of the consumer. Acidophilus milk differs from Indian dahi or curd in body, texture, consistency,flavour, chemical composition and in antibacterial activities.

Manufacture of Probiotic Acidophilus Milk

Acidophilus sour milk, as the name indicates, is a sour milk beverage made out of standardized milk by acidifying it with pure culture of acidophilusrods. The steps involved in its manufacture are given in Figure 1.

The acidophilus milk thus obtained is sour with 1 to1.25% lactic acid and pH 3.7 - 4.0. The product should contain more than200-300 million viable *L. acidophilus* organisms per ml, which possess satisfactory antibiotic effect against *E.coli* as well as other pathogenic and non-pathogenic undesirable bacteria of the intestinal tract. The viability of the organism is of primary importance in the use of the acidophilus milk.

Effect of processing steps on quality of the product

Each of the processing steps shown in Figure 1 affects the quality of the product and probiotic microflora in it. The manufacturing technology is centered at the'care of culture'. The selection of raw material, processing conditions, environment, storage, etc. should be decided in such a way that the culture exhibits predictable behaviour and probiotics are available in maximum viable numbers. .

Selection of Raw Milk

The basic principle to be kept in mind is that ‘good quality raw material can give good quality end products’. The raw milk selected here is meant for allowing the growth of live microorganisms. It must support good growth of the culture. It should be fresh, have normal composition,free from mastitis, free from antibiotics and other inhibitors, free from off-flavours and should have low bacterial count. Buffalo milk should be preferred for set types of product as it gives firm curd while cow milk may be good for stirred product, which will be smoother and uniform.

Pretreatments to milk

Pre warming of milk to about 45 C is required to facilitate filtration/ clarification to remove extraneous matter from milk. The manufacturer has to standardize the milk to meet legal requirements for fat and SNF (Solids-not-fat). Technologically, good quality set product is obtained from the milk having 13-15% total milk solids. Fat do not have significant role to play infermentation, but contributes to integrated pleasant flavour and richness to the product. About 3% fat is sufficient to have good quality product, while SNF can be increased to 10-12%, preferably by concentration or by supplementation with skim milk powder.

Heat Processing

The milk intended for fermented product manufacture is generally heated to 80-85°C for30 min. or 90-95°C for 5-10 min. However,the milk for acidophilus milk manufacture is nearly boiled. This higher heat treatment is useful from many angles to the product. It supports good growth of the culture as it destroys other competing microflora, inactivates many other natural inhibitory substances in milk, drives out oxygen and also produce some growth stimulating agents in milk. Higher heat treatment also improves gel stability, thereby reducing the problems of whey separation in the curd.

Cooling

Just after heating, the milk is cooled to incubation temperature, which is around 37-40°C for acidophilus cultures. Adding culture in hot milk destroys the culture.

Inoculation

The tempered milk is inoculated with 1-5% of active culture of *Lactobacillus acidophilus*. The exact rate of inoculation depends upon the type of culture.

Incubation

After adding culture in the milk, it is uniformly mixed without aeration. It is then incubated in bulk or in the same tank, if stirred product is to be made. If set-product is required, the milk is filled in retail containers before incubation. Incubation is purely a biological process during which the culture grows and brings necessary transformations in milk to get a desirable fermented product. In general, incubation temperature should be kept around 37°C. However, precise temperature of incubation can be determined based on the strains of the culture and their combination used in the product. The period of incubation varies between 4-16 h, depending upon the rate of acid production by the culture in the milk. However, the best end point to stop fermentation is just after the milk sets. Setting takes place at about 0.6% acidity and the remaining acidity required in the product can develop while cooling. During incubation, the milk is very sensitive to mechanical disturbances and other changes. Hence, it should not be disturbed.

Cooling

As soon as the curd sets or desired acidity in the product is achieved, it must be cooled. The rate of cooling affects the quality characteristics of the product and should be decided according to the per cent lactic acid expected in the final product. Rapid cooling may lead to more contraction of gel and separate more whey, while too slow cooling may sour the product. In thermophilic cultured products, two stage cooling is preferred, i.e. in first stage cooling from 40°C to 20°C and in the second stage from 20°C to 5°C in cold store.

In the stirred products, cooling and stirring are simultaneously done. It is advisable to stir the product at lower temperature to reduce the problems of wheying-off. In most cases, the product is stirred at about 20 C and also blended with colour, flavour, fruits, nuts and other additives and then packed in retail containers. The product is to be stored at less than 5°C,until its consumption.

The stirred product can be packed in consumer convenient sizes in attractive cups,polyethylene pouches or cartoons. However, the packaging material used, should be thoroughly sanitized so that it does not add spoilage microorganism to the product. The distribution should always be through cold-chain.

At all times care must be taken to see that sufficient level of live probiotic cells are present in the product upto the end of shelf-life.

SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

B.SC – MICROBIOLOGY

UNIT – IV - FOOD FERMENTATION AND TECHNIQUES

PROCESSING OF MEAT AND MEAT PRODUCTS

Introduction

The meat processing involves the slaughter of animals and fowl, processing of the carcasses into cured, canned, and other meat products, and the rendering of inedible and discarded remains into useful by-products such as lards and oils. Meat is exposed to a series of wide range of processes viz. curing or preserving processes such as salting, wet pickling, drying, cooking and canning, sausage manufacture, ham curing. All these processing techniques are aimed at inhibiting the microbial spoilage and increasing the shelf life of the meat. Major principles involved in meat processing are use of heat, low temperature, smoking, modified atmosphere packaging and ionizing radiations. The methods of preservation are mainly grouped in three categories i.e. control by temperature, by moisture and by lethal agents (bactericidal, fungicidal etc.)

Preservation of Meat

Use of low temperatures

Chilling and freezing are most commonly used preservation system for meat and meat products.

Chilling

Chilling is most widely used technique to preserve raw and processed meat. Chilling preserves muscle tissue by retarding the growth of microorganisms and by slowing many chemical and enzymatic reactions. Storage temperature may vary from - 1.4 to 2.2�C for storage of beef for 30 days depending upon the number of microorganisms. Carcass should go to the cooler as soon as possible and its inner most part should be able attain below 10�C within 12 hrs of slaughter in order prevent undesirable off-flavours and bone taints due bacterial growth. An ideal temperature of storage for meat should 1�C above its freezing point.

During post mortem cooling and subsequent refrigerated storage, control of relative humidity (around 90%) is very important. The undesirable moisture is lost from the surface, the weight reduction becomes of economic important and meat pigments myoglobin might get oxidized to brown metamyoglobin. However, a small amount of moisture loss from the surface is desirable since this tends to retard growth of microorganisms.

Freezing

Freezing is an excellent process for preserving the quality of meat for long periods. Freezing is often used to preserve meats during shipment over long distances or for holding until long times of storage. Its effectiveness depends on ice crystal formation and rate of lowering of temperature. When the temperature of storage is below - 18�C, changes occur at a very slow rate in the muscle of warm blooded animals. Quality of frozen meat depends on various factors such as rate of freezing, packaging etc. When muscle tissue is frozen rapidly, small both intra � and extra � cellular ice crystals are formed which cause little damage to the meat structure. While large ice crystals are formed in slow rate of freezing causing compactness of muscle fiber. The process of denaturation can be accelerated with a resulting decrease in water holding capacity of tissue. Loss of water holding capacity of the muscle along with mechanical damage to cells by ice crystals is responsible in large parts of thaw exudates. To protect quality loss due to changes in protein, anti-freezing compounds or cryoprotectants i.e. polydextrose, polyphosphate are added to meat formulations. Rapid freezing can be obtained by using air blast freezers either on batch or continuous basis which employs -20 to -40�C cold air. Large size meat cuts are vacuum packaged to prevent lipid oxidation and discoloration due to formation of metmyoglobin. Retail meat is packed in low permeability films with better mechanical strength e.g. Sarlyn **Use of heat**

The canning of meat is a very specialized technique in that the procedure varies considerably with the meat product to be preserved. Since meat products are low acid foods so the rate of heat penetration is fairly low. Commercially canned meats can be divided into two groups on the basis of heat processing used ;

(a) Meats that are heat processed in an attempt to make the can contents sterile.
(b) Meats that are heated enough to kill part of spoilage organisms but must be kept refrigerated to prevent spoilage.

Processing temperature for shelf stable canned cured meat is 98�C. Treatment of meat surfaces with hot water to prolong the storage time has been suggested. Although this may result in loss in nutrients and damage in colour. Actin is the most heat labile muscle protein becoming insoluble at 50�C. Denaturation of muscle proteins decreases their water holding capacity. This decrease in water holding capacity may produce desirable juiciness, Provided free water is not expelled from the tissue. During heating, fat is melted. Adipose tissue cells are ruptured and there is a significant redistribution of the fat. When meat is eaten warm, the melted fat serves to increase palatability of the product by giving desirable mouth feel, especially at the end of chewing period, when most of the aqueous juices are lost. Myoglobin also undergoes denaturation. The red pigment heme is oxidized to brown pigment hemin. Canned meat loaf can be manufactured substituting a part of the meat with high calcium coprecipitate. It is observed that 20% meat can be replaced with high calcium milk protein coprecipitate in chicken meat loaf without affecting the quality of the end product.

Dehydration

Deprivation of available moisture (reduction of water activity) for microbes not only prevent their growth but also kills them, thus results in increased shelf life and better quality product. Water may be made unavailable either by dehydration, freeze drying or by increasing exrtacellular osmotic pressure as is done in curing. Drying meats can be successfully employed for both raw and cooked meat. However, the quality of the final reconstituted product is superior when meat is cooked prior to dehydration. There is a loss in native structure of protein as measured by loss of water holding capacity during temperature from 0 to 20�C. This is caused by denaturation of sarcoplasmic proteins. The next major loss in water holding capacity begins in the temperature range of 40 � 50�C due to denaturation of contractile proteins. Collagen is rapidly converted to gelatin at around 100�C. Texture is most severely altered by dehydration. The tough texture of dehydrated meat can be overcome by preparing products of intermediate levels of water.

Smoking

Smoking is often used with salting and curing. It gives desired flavour, aroma and aids in preservation. It was noted that preservative substances added to the meat together with the action of heat during smoking have a germicidal effect and that drying of the meat together with chemicals from the smoke inhibit microbial growth during storage. Smoke consists of phenols, alcohols, organic acids, carbonyl compounds and hydrocarbons. The desirable effects of smoking of meat can be listed as below:

- Meat preservation through aldehydes, phenols and acids (anti-microbial effect)
- Antioxidant impact through phenols and aldehydes (retarding fat oxidation)
- Smoke flavour through phenols, carbonyls and others (smoking taste)
- Smoke colour formation through carbonyls and aldehydes (attractive colour)
- Surface hardening of sausages/casings through aldehydes (in particular for more rigid structure of the casing)

Production of smoke

Smoke is produced by burning of wood or its saw dust which consist of 40-60% cellulose, 20-30% hemicelluloses, 20-30% lignin. A temperature gradient exists during thermal decomposition of wood. Outer surface temperature is generally above 212^{o}F during dehydration process. Co, CO_2 and volatile medium chain organic acids e.g. acetic acid are released during dehydration and distillation process. When internal moisture level reaches to zero, the temperature rapidly rises to 570-750�F. Once the temperature falls within this range thermal decomposition occurs and smoke is given off.

Nature of smoke

Although the smoke at the point of generation exists in a gaseous state, it rapidly goes into a vapor & particle phase. The vapor phase contains the more volatile component & is largely responsible for the characteristic flavor & aroma of smoke. As soon as smoke is generated numerous reactions and condensation occurs. Aldehyde & phenol condense to form resins which represent about 50% of the smoke component & are believed to provide most of color is smoked meats. Polyphenols are also formed by condensation.

The amount and ratio of smoke deposition on the product is influenced by smoke density, smoke house air velocity and its RH, and surface of product being smoked

Cooking during smoking

Cooking is often done simultaneously with smoking of meat. In fact cooking is often more important than smoking in meat processing. Cooking equines careful control of the smoking and heating process to give best results.

Liquid smoke preparations

Liquid smoke is used by some processors. It is sprayed on the product before cooking. It has some positive effects over natural wood smoke.

(1) It doesn�t require the installation of a smoke generator and which usually requires a major financial outlay.

(2) Process is more repeatable, as the composition of liquid smoke is mane constant.

(3) Liquid smoke can be prepared so the particle phase is removed and thereby possible problems from the carcinogens can be alleviated.

Liquid smoke is generally prepared from hardwoods; The final product is composed primarily of the vapor phase & contains mainly phenols, organic acids, alcohols & corbonyl compounds. They don�t contain poly hydro carbons (PHC).

The there are two types of smoking cold at 15 to 18�C (up to 26�C) and hot temperatures of +60 to 80�C. Cold smoking is used for fermented meat products (raw-cured ham, raw-fermented sausage) and precooked-cooked sausage (liver and blood sausages). Hot smoking is used for a range of raw-cooked sausages, bacon and cooked ham products.

Modified atmospheric storage

Fresh meat held at refrigerated temperature has a limited shelf life because of microbial growth. Modified atmosphere refers to the adjustment in the composition of the atmosphere surrounding the product. At higher concentration of CO_2 surface browning of meat occurs due to the oxidation of myoglobin and hemoglobin pigments to ferric state. The most desirable concentration of CO_2 to use in a modified atmosphere is a compromise between bacterial inhibition and product discoloration.

Ionising radiation

Ionising radiation constitutes the potentially useful form of preservation. Besides from its desirable ability to inactivate micro � organisms, it also has the undesirable effect of altering meat pigments. Sterilizing doses of ionizing radiation results in the breakdown of various lipids and proteins to often undesirable odours. Tenderization of muscle may also occur during this treatment. Temperature of $\leq$ 80�C or below greatly reduces undesirable effect without affecting lethal effect on microorganisms. Generally enzymes are not inactivated by irradiation treatment, it is necessary to heat approximately 70�C prior to irradiation and storage.

Processing of Meat Products

Comminuted meat products

Comminution is the mechanical process of reducing raw materials to small particles called as minced meat. Depending upon the final use of the communited meat the degree of comminution is done which differs among various processed products and is often a unique characteristic of a particular product ranging from very coarsely comminuted (to produce non-emulsified sausages like salamis and summer sausages), to finely comminuted, (to produce emulsified sausages like frankfurters, bologna, etc). Sausages are usually defined as comminuted seasoned meats, stuffed into casings; they may be smoked, cured, fermented and heated. They are made from any edible part of the slaughtered, veterinary-inspected animal, and a series of nonmeat ingredients.

Sausages

Sausages are meat products that are salted & usually seasoned or spiced and are an example of comminuted meet products that are generally recognized as emulsified, stuffed, linked, smoked, and cooked meat products. Based on the product characteristics and processing methods, they are broadly divided into three categories: **fresh sausages, cured sausages and fermented sausages**. In all cases meat is communited to reduce meat and fat particle size (grinding, mincing, chopping, or flaking), mixing with ingredients, stuffing into specific casing, linking to obtain specific lengths and finally, packaging. Sausages might be of ground and emulsion type. In the ground variety of sausages discrete particles of meat are seen on the other hand, in emulsion type sausages fat is emulsified & stabilized by lean component. Sausages were developed to utilize low- quality meats such as trimmings head, shoulder & by- products of the meat. The processing of sausages is a continuous sequence of steps (Fig 23.1), which are all equally important.

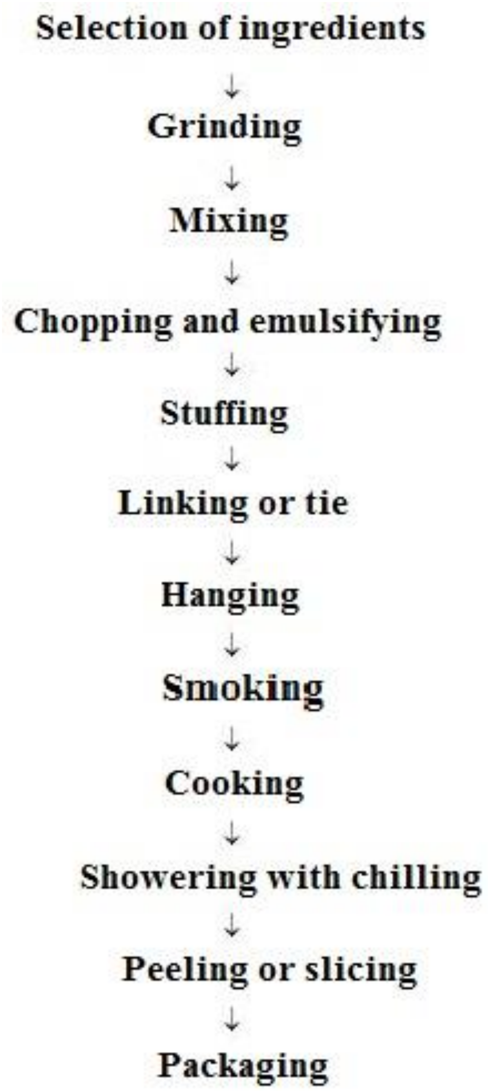


Fig. 23.1 Process flow diagram of sausages manufacture

i. Selection of Ingredients

Sausage ingredients include:

- Meat - based on consideration of fat/protein; moisture/protein and myoglobin concentration
- Moisture - added as ice at time of chopping in a number of fresh and smoked sausages
- Curing ingredients - salt, sodiuim nitrite and/or nitrate and sugar
- Seasonings - may include spices, such as black peper, paprika, mace and cinamon; herbs that may include thyme and savory; vegetables such as garlic and onion and other substances, such as flavor enhancers
- Fillers and binders - occasionally used to improve color, binding properties, slicing characteristics, altering flavor or reducing costs
- Ascorbic acid - used to improve color in smoked sausages
- Other additives - may include liquid smoke

Milk protein have been utilized as fillers, binders and extenders in cooked, comminuted meat products to reduce cook shrink and formulation cost, as well as to improve emulsifying capacity, emulsion stability, water binding, potential nutritive value and slicing characteristics. These proteins significantly increase the gel strength of meat proteins and it has been shown that there has a synergistic effect between milk proteins and salt soluble meat proteins, through covalent cross linkages.

Addition of caseinate stabilizes the meat emulsion as required in the sausage mix. It thickens the gravy during frying and prevents it running out, but excess incorporation of caseinate may result in drying up of the sausages. Further addition of water absorbent materials becomes essential when sodium caseinate concentration in sausages exceeds 5%. The greater water holding capacity, lower viscosity and lower cooking losses of sausage batters containing 2% sodium caseinate in comparison to all meat control were observed.

The coprecipitates have good potential in various meat products such as frankfurters, sausage batter and luncheon meats as meat replacers or extenders. Sausage acts as a good medium for the use of coprecipitates. The finely, dispersed dairy protein matrix in sausages also can act as a moisture binding agent, thus, developing the desirable chewy texture besides controlling shrinkage during storage and deformation while slicing. Addition of milk coprecipitate in combined boiled sausages resulted in increased pH, reduced nitroso pigments and increased residual nitrites content in the end product. It is found that both high and low calcium coprecipititates improved the emulsifying capacity, emulsion stability and water holding capacity of meat emulsion in fresh sausages at the 20% replacement level. Supplementation with dairy coprecipitates into boiled beef pork sausage batters up to 30% of meat protein yields emulsion with increased pH, enhanced water binding ability and improved adhesion properties.

Grinding

Meat chunks of variable size and shape with variable fat contents are ground to form uniform cylinders of fat and lean. The screw feed in the barrel of the grinder conveys the meat & presses it in to holes of the grinder plate. The rotating blade cut the compressed meat and aids in filling the grinder plate holes.

Mixing

Cylinders of fat and lean obtained by grinding are tumbled in a mixer to give a uniform distribution of fat and lean particles. This can be used for coarse ground sausages or for emulsion type sausages by utilizing a chopper or emulsifier and with suitable additions of required ingredient to obtain the desired texture & uniformity of composition.

Chopping

It is often used as a means of batching the sausage mix, the mixed batch being transferred to an emulsifier or acquiring the desired texture.

Emulsifying

This machine combines the principle of grinding and chopping. Emulsifier machine handles large volumes of meat rapidly to produce a desired texture. Speed of handling material and high degree of disintegration of meat tissue help in obtaining desired textures. In the preparation of sausage, the protein and water of the meat mixture form a matrix than encapsulates the fat portion. In a meat emulsion the protein myosin acts as the primary emulsifying agent. The addition of salt to the product is to release the myosin from the muscle fiber. The emulsion is generally formed by mixing the meat with salt and other ingredients in a chopper, which aids in disrupting the fibers and facilitates the release of myosin.

Stuffing

Sausage emulsion also known in the trade as mix sausage dough or batter is transferred to stuffers for extending the mix or emulsion into **casings**. At this point, the size and shape of the product is determined. Generally three type of stuffing devices are used.

- Piston
- Pump
- Combination of piston & pump

In the past, the casing of the sausages were made from animal casings, however this was a limiting factor for the production of sausages. Today, the casings are made of cellulosic and regenerated collagen. The limiting factor now, is the supply of meat and the cost of it. Fermented sausages are further subjected for the fermentation and maturation. Fermentation of meat constituents results in flavor development, improvement of shelf life and improved quality and food safety. Sausage batter is inoculated with the started bacteria composed of **selected lactic acid bacteria (LAB)** i.e. homofermentative lactobacilli *(Lb pentosus, Lb plantarum, Lb sake, Lb curvatus)*, pediococci (*Pediococcus acidilactici*, *Pediococcus cerevisiae*) and gram positive catalase positive cocci (GCC) i.e. non-pathogenic, coagulase-negative staphylococci (*Staphylococcus carnosus, Staphylococcus xylosus, Staphylococcus piscifermentans)* . Small manufacturers use spontaneous fermentation without adding starter culture.

Linking and tying

After the emulsion is stuffed in to casings, the encased mass is tied with thread of fastened with metal clips. In the case of small sausages such as Frankfurters stuffed casing are twisted or drawn together to produces links either by hand or with mechanical devices.

Large sausages items are tied or slipped on one end with a hanging tie and suspended from a smoke stick or hook so the entire surface is free from contact with the equipment. This permits a good flow of air around the sausage in the smoke house and prevents touch marks and spotting due to contact with adjacently hanging product.

Smoking & cooking

The draped smoker picks are placed on smoke trees or trolleys with 12-18 specs per tree. The smoke house operation is essentially a specialized drying and cooking operation in which sausage emulsion is coagulated. Encased sausage at the time of introduction in to the smoke house usually has an internal temp of 60-70�F. During cooking this rises to 155 to 160�F.

Chilling

After smoking and cooking the product is showered with cold water and than chilled by refrigeration chilling is frequently done with a brine solution by dipping or spraying the products. (a 6% salt brine) balanced within leaching of salt from the sausage and imbibing of water by the sausage.

Peeling & packaging

After properly chilling the product usually to an ultimate temp of 35 to 40�F, the cellulosic casings on frankfurter and slicing bologna are removed. This is known as the peeling operation.

Semi dry sausages

Semi dry sausages are usually made from pork or beef or a mixture of the two and are characterized by a moisture content ranging from 40- 45%, e.g. summer sausage, G�teborg Sausage, Cerevelat, Thuringian, Holsteiner. They have excellent keeping quality with need of little refrigeration because

(1) Some reduction in microbiogical contamination is achieved in the cooking process
(2) A high salt to moisture ratio contributes to retarding bacterial growth
(3) A low pH (5.3 or less) provides the tangy flavor and serves a protective food and good keeping quality is achieved with a pH of 4.8 to 5.0 and with a total acidity of 0.75 to 1% lactic acid.

Grind pork trimmings

↓

Place the trimmings in a mixer; add the salt sugar spices, cure and starter culture and mix for 2 to 3 minute.

↓

Regrind the mix

↓

Hold the mix for 12- 72 hr at 40°F

↓ stuff

Warm water shower for 2 min to wash the sausage surface free of any adhering particles

↓

Smoke the encased sausage for 16 hr at 110°F

↓ Shower with cold water /15 min

Allows to stand at room temp so the surface dies and then chill to 40°F

Fig. Manufacturing method of semi-dry sausages

Dry sausages

Semi-dried sausages are smoked and cooked to varying degrees, whereas dry sausages are not cooked and only with some products smoke is applied. The manufacture of dry sausages is more difficult to control than that of semidried sausages. Overall processing time may require up to 90 days. As a result of this prolonged holding the sausages are vulnerable to chemical, microbiological degradation. However, when prepared properly the finished sausages are usually stable and can be held with little or no refrigeration. Examples of dry sausages are Geneva salami, Pepperoni, mortadella etc.

Cured meat products

Curing of meat involves the essentially addition of **sodium chloride, sodium nitrite or sodium nitrate** and adjuncts to meat for increasing shelf-life and to obtain desirable colour and flavour. Sugar may or may not be added along with other ingredient to improve flavour. Curing can be done for both raw/cooked meats cut products as well for comminuted meat products e.g. sausages and similar preparations. Most popular raw cured meat includes ham and bacon which are pork products. However, the technique can be applied to any meat group.

Ingredients used in curing

(i) Salt
(ii) Sugar
(iii) Nitrate or nitrite

} Essential curing ingredients

(iv) Phosphates
(v) Spices
(vi) Baking Soda
(ii) Sodium erythroborate
(iii) Ascorbic acid
(iv) Mono sodium glutamate
(v) Hydrolysed vegetable proteins,
(vi) vinegar

} Optional ingredients/curing adjuncts

Commonly used salt sodium chloride (occasionally KCl) is most essential ingredients and it significantly inhibits growth of microorganism including *Clostridium botulinum* due to increase in the osmotic pressure of the medium and dehydration of the muscle. Salt if used alone results in dark coloured, unpalatable dry harsh and salty product. Therefore, it is recommended to be used in combination with sugar and nitrite and nitrate. Salt should be of good quality. Generally dry salting utilizes higher levels of salts; however, acceptable level of salt is about 3% for most of the meats and about 2% for bacon. Nitrite/nitrate has as well a small inhibitory effect on *C. botulinum*. However, it plays very important role in colour fixation of the cured meat. On the other hand sugar contributes to flavour and colour development due to mailard browning and also helps in increasing shelf life by controlling of bacterial growth. Endogenous low molecular weight components in the sarcoplasma of the meat promote the formation of nitric oxide, myoglobin and nitrite decomposition.

Chemistry of curing process and meat colour development

During the dry curing process salt in dry form is rubbed on the surface of the meat whereas in meat wet curing meat portion is immersed in the curing solution. The latest techniques of curing includes use of artery pumping, multiple needle injection, thermal or hot cures, tumbling, massaging, are employed to accelerate the curing the processes. In all cases, salt diffuses into the meat, causing some of the expelled protein to diffuse back in and the meat to swell. The salt � protein complex binds the water well thus the water holding capacity of proteins generally increases during curing. The final meat contains increased ash due to the absorbed salts. Generally salting results in darkening of the colour. To counteract the effect of salt nitrite/nitrates are added to salt which fix the desirable pink colour of the meat. In the curing, nitrite reacts with muscle pigment myoglobin to give purple-red coloured nitroso-myoglobin. On the cooking this is further converted into nitrosomyochrome which gives typical pink clour to the meat. It is further claimed that nitrite has a significant beneficial effect on the flavour of cured meats by preventing oxidation through the antioxidative activity of nitric oxide-myoglobin and s � nitrocysteine, a component found during the curing process.

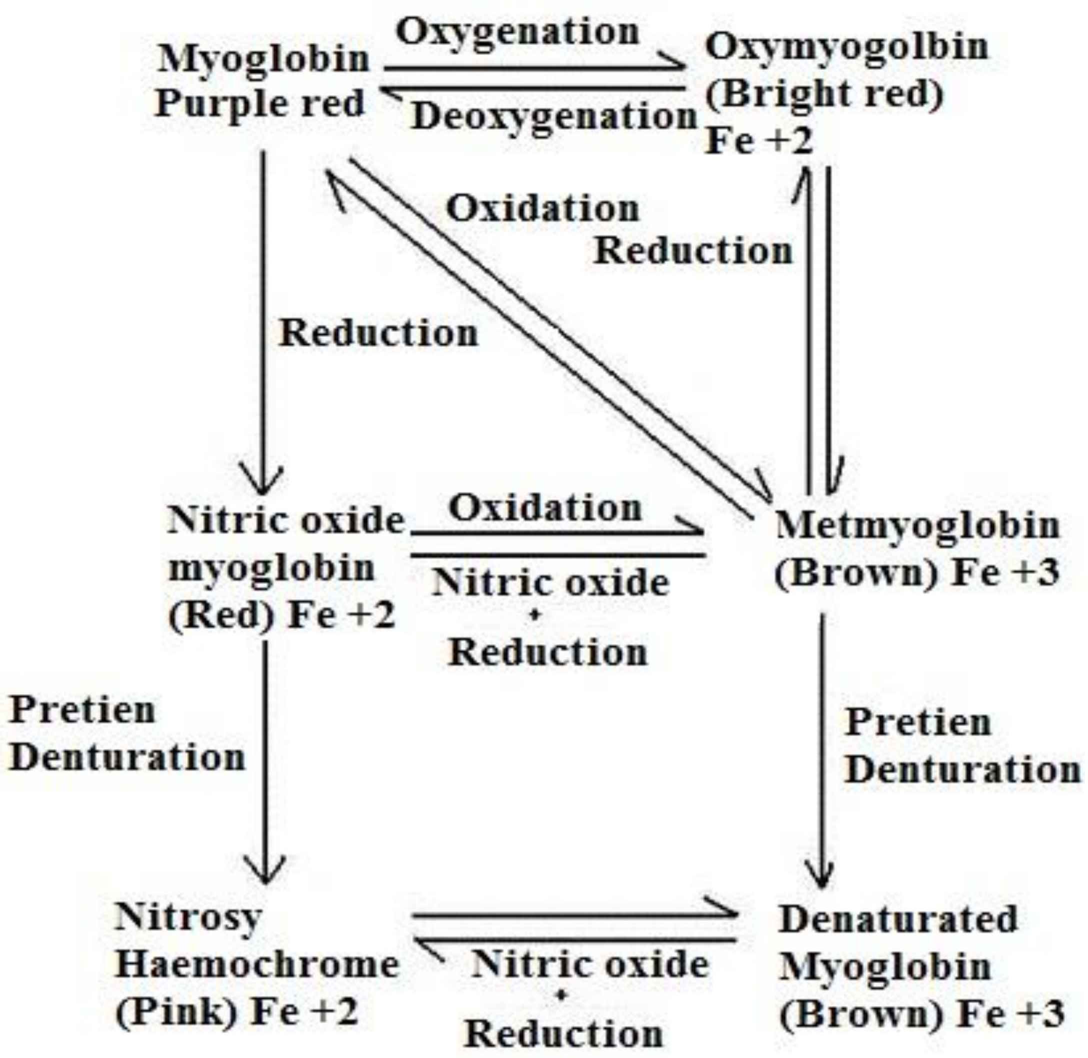


Fig. Colour fixation in meat

A major detrimental change that can occur in cured meat during storage is the oxidation of nitric oxide hemochromagen (pink) or nitric oxide myoglobin (red) to brown metamyoglobin (Fig.). The rate of oxidation increases with increasing oxygen content, therefore cured meat should be preferably packaged in a container from which oxygen is excluded.

Acceptable levels of nitrite used in meat and meat products are 100-200 ppm. The use of nitrite in cured meat may be hazardous if it is used at higher concentration with improper mixing, as it reacts with amines, especially secondary amines, to form N � nitrosamines, which may be carcinogenic. High temperature may also induce nitrosamine formation.

Fermentation

• Originally the word ‘fermentation’ was proposed and used by the botanists to mean the conversion of glucose molecule to alcohol by bacteria in anaerobic condition. • Later this word was used in organic chemistry.

• Now-a-days this term ‘fermentation’ is widely used in food science, biotechnology, microbiology, agriculture and fisheries.

• The simplest definition of the term in this modern age is the conversion or transformation of a complex biomolecule into simpler compounds either by bacteria or by enzymes.

• The final product may vary depending on the condition, storage, and nature of the process etc.

• The final product may be liquid or paste. Fermentation may also be defined as follows-

• Fermentation is the transformation of organic substances into simpler compounds by the action of enzymes and microorganisms. (Mackei et.al.1971)

• Fermentation is an old technology of preservation of highly perishable fresh water or marine animals. It is described as a process on which the complex protein molecules in the fish are broken down by the action of organic catalyst; enzymes into simple protein molecule. How this is introduced in Fish Processing

• Fermentation is practiced to have a food of particular flavour and taste.

• Sometimes the process is practiced or used to store the huge catch.

• The fish fermentation process defers from one country to another.

• In most cases, the process is accelerated by the use of salt as well as by added carbohydrates. The final product in such cases is liquid.

• However, in the fermentation of fish, where salt or carbohydrates are not used, the end product is semisolid paste.

• A series of exceedingly complex biochemical reactions take place during fish fermentation process. The advantages of traditionally fermented fish products

• High acceptability • Low cost • Ease of preparation • Safety • Improved digestibility and absorbability.

Types of fermented fishery products:

1.Products primarily involving enzymatic hydrolysis.

ii. Products preserved by microbial fermentation.

Different fermentation processes result in 3 distinct types of products:

a. fish largely retains original form: cured texture and aroma, as in shidhal of Bangladesh

b. original fish reduced to the form of paste: red/brown, salty, as in Nga-pi of Bangladesh

c. flesh is reduced to a liquid: salty taste, cheese like aroma, as in fish sauce.

Amano (1962) divided fermented fish products into 3 categories according to the processing technologies applied as:

1.traditional products mainly fermented by the action of enzymes present in flesh and entrails to which salt is added;

ii. traditional products fermented by the combined effects of flesh and gut enzymes supplemented with microbial enzymes supplied in the form of starter culture on flesh and entrails added with salt;

iii. non-traditional products manufactured by accelerated fermentation, acid ensilage and chemical hydrolysis.

Adams et al. (1985) divided the traditional fermented fish products according to the substrate used in the fermentation process as:

i. Products made from fish/shrimp and salt; ii. Products made from fish/shrimp, salt and carbohydrate. Saisithi (1987) proposed a complete classification based on both the type of substrate and the source of enzymes used in the fish fermentation process as:

i. traditional fermented fish in which the fish is fermented by the combined action of fish enzymes and bacterial enzymes normally present in the fish/ salt mixture;

ii. products in which the fish and a carbohydrate mixture are fermented mostly by bacterial enzymes normally present in the fish/salt/carbohydrate mixture;

iii. products in which the fish is fermented mostly by fish tissue enzymes and the carbohydrate is fermented by yeast and molds added in the form of starter culture.

List of fermented fish products of South-East Asian countries

1. Fish sauces (where the flesh is reduced to a liquid)

Product name	Country	Species used
Nuoc-mam / Nuoc-nhut	Thailand, Malaysia, Vietnam, Cambodia	Different kinds of small sea fish mainly clupeids and carangids
Nuoc-mam ruoc	Thailand	Prepared from shrimps
Patis	Philippines	Made from shrimps
Nam-pla	Thailand	Made preferably from *Stolephorus*
Tuk-trey	Cambodia	
Petis	Indonesia	
Ketjap-ikan	Indonesia	Shrimps
Nga ngapi	Myanmer	Shrimp and prawn
Budu	Malaysia	Shrimps

2. Fish pastes (a product in which the fish is reduced to the form of paste)

Product name	Country	Species used
Bagoong	Philippines	Sea fish –anchovies, ambassids and small shrimp *Atya* sp.
Prahoc	Cambodia	Cyprinids and others are used
Padec	Laos	
Phaak / Mam-chao	Cambodia	
Trassi	Indonesia	It can be made from fish as well as shrimp
Nga ngapi	Myanmer	Fish previously sun dried
Trassi udang`	Indonesia	Plankton containing very small shrimps
Nga seinsa	Myanmer	Small shrimp and prawn
Ngapi	Myanmer	Big fish "ngapi gaung"

3. Salted fish not dried or partially dried

Name of the product	Country	Species used
Pedah	Thailand, Indonesia	Pla thu and pla lang

4. Miscellaneous products

Name of the product	Country	Species used
Sheedal shutki	Bangladesh	*Puntius* spp. mainly along with other species

Preparation of fish sauce

• Fish sauce is the most important fermented fishery product as it is produced and consumed in large quantities in all most all Southeast Asian countries.

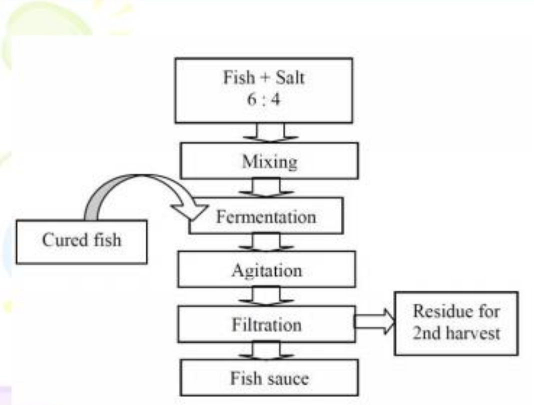
• Salt is the main constituent, comprising up to 30%

. • Protein comes next, varying from 6–12%.

• The processing of fish into sauce involves hydrolysis of fish protein to peptides and amino acids that are water soluble.

• The time of sauce fermentation varies from 6 months to 1 year.

• Aging is an important step and helps to develop the aroma characteristic to sauce.



Preparation of fish sauce

Fermented fish in Bangladesh

• Sheedal shutki also called semi-fermented fish products is an ancillary type of fishery product but it has some scientific basis.

• For the production of sheedal shutki Puntius stigma known as minnow or Indian minor carps are mainly used.

• In some cases Puntius ticto is also used due to difficulties in sorting and species identification of Puntius stigma and Puntius ticto.

• Besides Puntius sp. snake head in the North-West part of Rangpur and foli in Kishoregang is also used for fermentation.

Production of sheedal shutki by traditional method •

The fish after collection are gutted as quickly as possible without any pre washing and then directly exposed to sun light.

• Mean while clay vats are soaked with fish oil in such a manner that absorption of oil from the body of fish doesn't occur during ripening.

• Before placing into the vats the dried fishes are sprinkled with water and kept overnight.

• On the next day clay vats are packed up to their rims with the fish so treated.

• The mouth of the vats is closed successively with banana leaf and a heavy layer of clay in order to create an anaerobic condition.

• The vat is kept for 3 months in this condition.

• The product so prepared is locally known as 'sheedal shutki'.

Fig. Process flow chart for Sheedal shutki

Improvement suggestions for the betterment of the product

• In some cases sprinkling with water is done by river water. In such cases tube well water or distilled water can be used.

• Instead of earthen vats cemented tank or mosaic tank can be used.

• If we can use fermentative bacteria, then fermentation will be completed with in 2/3 week s instead of 3-6 months.

• Usually banana leaf and thick layer of clay are used as covering materials to ensure anaerobic condition.

• But if we replace these covering materials with a modern technique, the product will be more hygienic and acceptable to the consumers.

Nutritional composition

Sl. No	Parameters	Semi fermented fish
1	Crude protein	30%
2	Lipid	17%
3	Moisture	38%
4	Minerals	
	(i) Sodium	247mg/100g
	(ii)potassium	163mg/100g
	(iii)calcium	621mg/100g
	(iv)phosphorus	440mg/100g
	(v)magnesium	50mg/100g
	(vi)zinc	3.4mg/100g
	(vii)iron	3mg/100g

NGA-PI •

Nga-pi is a fermented fish product in which the original fish is reduced to the form of a paste.

• The fermentation involves the breakdown of wet protein into simpler substances which are themselves stable at normal temperatures.

• This breakdown is partial and is controlled by the addition of salt.

• Typically, the fish or shrimp are ground to a paste with a little amount of salt.

• The paste is subjected to alternate sun-drying and grinding before being packaged to mature in an air-tight container.

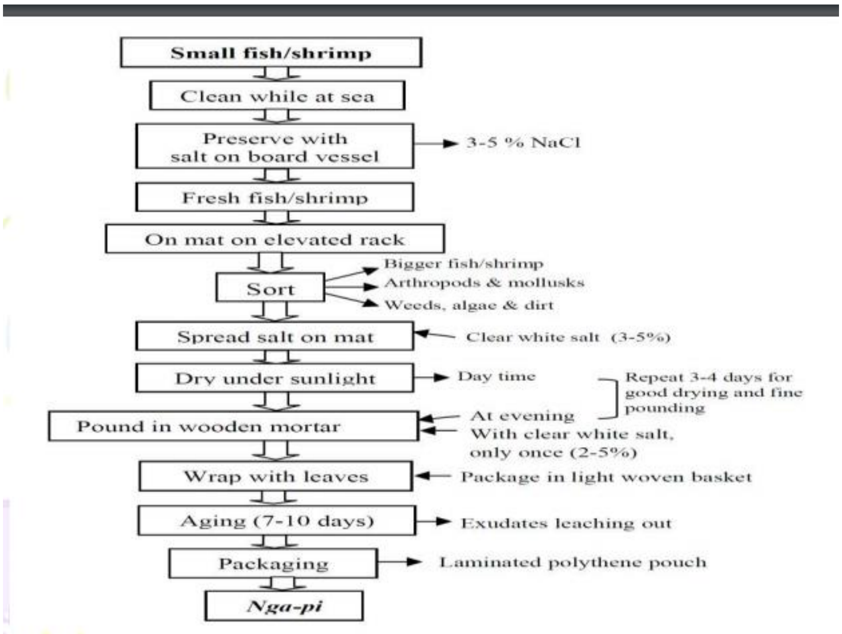
• The moisture content of a typical paste varies from 35 to 50%.

• Therefore, almost half of the water present in the fresh raw material will have been lost during processing.

• A good quality Nga-pi is produced from Acetes shrimp with small proportion of Mysid shrimp.

• The yield varies from 40 to 50% of raw shrimp.

• A typical analysis of good quality Nga-pi is : pH 7.6-7.8, moisture 27-40%, ash (including salt) 20 – 24 %, salt 13 – 18 % and protein 30 - 40%.



Tea Fermentation:

Tea plants can be categorized on the **type of plant**, the appearance of the dry leaves, or the color of the tea's liquor. But what's used is a more scientific way of categorizing all teas, which is based on the processing that the tea leaves have been put through. These methods were first developed in China and continue to be practiced today across most tea producing regions of the world.

Classification of teas

From one tea plant, it is possible to derive six types of tea: **green tea**, yellow tea, **white tea**, **oolong tea**, **black tea** and post-fermented tea. Each tea type can be further broken down by their styles–which can result from modifications in the processing method– type of tea plant, the cultivar, or even the intention of the tea maker.

Tea processing techniques

Between two and seven procedures are involved in the processing of fresh tea leaves; the addition or exclusion of any of these stages results in a different type of tea. Each of these procedures is carried out in a climate-controlled facility to avoid spoilage due to excess moisture and fluctuating temperatures.

Withering refers to the wilting of fresh green tea leaves. The purpose of withering is to reduce the moisture content in the leaves and to allow the flavor compounds to develop. While it can be done outdoors, controlled withering usually takes places indoors. Freshly plucked leaves are laid out in a series of troughs and subjected to hot air forced from underneath the troughs. During the course of withering, the moisture content in the leaf goes down by about 30%, making the leaf look limp and soft enough for rolling. Additionally, the volatile compounds in the leaf, including the level of caffeine and the flavors, begin to intensify. A short wither allows the leaves to retain a greenish appearance and grassy flavors while a longer wither darkens the leaf and intensifies the aromatic compounds.

Fixing or "kill-green" refers to the process by which enzymatic browning of the wilted leaves is controlled through the application of heat. It is held that the longer it takes to fix the leaves, the more aromatic will be the tea. Fixing is carried out via steaming, pan firing, baking or with the use of heated tumblers. Application of steam heats the leaves more quickly that pan firing, as a result of which steamed teas taste 'green' and vegetal while the pan-fired ones taste toasty. This procedure is carried out for green teas and yellow teas.

Oxidation results in the browning of the leaves and intensification of their flavor compounds. From the moment they are plucked, the cells within the tea leaves are exposed to oxygen and the volatile compounds within them begin to undergo chemical reactions. It is at this stage that polyphenolic oxidase, including theaflavin and thearubigin, begin to develop within the leaves. Theaflavins lend briskness and brightness to the tea while thearubigins offer depth and fullness to the liquor that's produced.

In order to bring out specific intensities in flavors, tea makers control the amount of oxidation the leaves undergo. Controlled-oxidation is typically carried out in a large room where the temperature is maintained at 25-30º C and humidity stands steady at 60-70%. Here, withered and rolled leaves are spread out on long shelves and left to ferment for a fixed period of time, depending on the type of tea being made. To halt or slow down oxidation, fermented leaves are moved to a panning trough where they are heated and then dried.

Due to oxidation, the leaves undergo a complete transformation and exhibit an aroma and taste profile that's completely different from the profile of the leaves that do not undergo this process. Less oxidized teas tend to retain most of their green color and vegetal characteristics due to a lower production of polyphenols. A semi-oxidized leaf has a brown appearance and produces yellow-amber liquor.

In a fully oxidized tea, amino acids and lipids break down completely, turning the leaves blackish-brown. The flavors in such a tea are more brisk and imposing.

Rolling

Rolling involves shaping the processed leaves into a tight form. As a part of this procedure, wilted/fixed leaves are gently rolled, and depending on the style, they are shaped to look wiry, kneaded, or as tightly rolled pellets. During the rolling action, essential oils and sap tend to ooze out of the leaves, intensifying the taste further. The more tightly rolled the leaves, the longer they will retain their freshness.

In order to keep the tea moisture-free, they are dried at various stages of production. Drying enhances a tea's flavors and ensures its long shelf-life. Also, drying brings down the tea's moisture content to less than 1%. To dry the leaves they are fired or roasted at a low temperature for a controlled period of time, typically inside an industrial scale oven. If the leaves are dried too quickly, the tea can turn abrasive and taste harsh.

Aging

Some teas are subjected to aging and fermentation to make them more palatable. Some types of Chinese Pu-erh, for example, are aged and fermented for years, much like wine.

COFFEE FERMENTATION

Some of our very favorite foods and beverages are fermented: chocolate, wine, beer, bread, sauerkraut, kimchi, and… coffee? Well, yes and no: While fermentation is involved in the production of a majority of the world's coffee, coffee itself isn't a fermented beverage like spirits or kombucha are. Instead, when we talk about coffee fermentation, we're referring to what the coffee fruit undergoes between the time that the cherries are harvested and the seeds are fully dried and ready for export.

The word "fermentation" describes the process of metabolization of sugars and other compounds by microorganisms like yeasts and bacteria, which will consume those compounds and convert them into fuel for their own use, leaving behind useful by-products like ethanol and different acids. Those converted compounds are absorbed into the cellular structure of the seed and, when heat is applied to those seeds in the roaster, the compounds will be what transform into the flavors we love in coffee: Complex tastes, fruity acids, and other good, or at least interesting, stuff.

Understanding Coffee Fermentation, Processing, and Flavor

While it's an integral part of coffee's post-harvest processing and plays a large role in the development of coffee flavor, fermentation is not a very widely understood aspect of coffee's production cycle, though fermentation does occur in almost every style of coffee preparation. We often use the words “fermentation” and “processing” interchangeably and incorrectly: While they are related, they’re not identical. Fermentation is a natural occurrence that is undertaken by living microorganisms that are in search of energy sources; processing is a purely agricultural set of mechanical and technical steps designed to prepare a crop product for export. While fermentation can be used as part of processing, that distinction is important.

When we think about fermentation, we try to consider many variables, such as: the ripeness of the fruit; the ambient temperature in the environment as well as the temperature in the coffee as it is; say, piled or soaking in fermentation tanks or spread on patios drying, the local population of microorganisms; the water activity and moisture content throughout the drying process; and even more. It is a very difficult process for producers to track, and so most of the ways we discuss fermentation are colloquial, anecdotal, or “layman’s terms.”

The microorganisms that perform fermentation processes are found almost everywhere, and they will begin to consume fuel as soon as they have access to it, which means that fermentation can actually begin before the coffee cherry is even picked. The more fruit material that is exposed to the environment and to the population of microorganisms (when the skin is removed from the cherry, for instance), the faster this process can happen.

Different types of bacteria and yeast populations—which will vary based on location, climate, and health of the local ecosystem—consume different compounds and, so, convert them into different by-products, but generally speaking they will continue to ferment whatever useful material is available to them until they are no longer able to survive. For instance, in a Natural process coffee, they will ferment the fruit material around the seed until either all of the fuel is metabolized or until the environment is too dry for them to live. In a Washed process, the fermentation might happen all the way through the drying process, depending on how much mucilage was left on the parchment after the washing was completed.

Anaerobic Fermentation

As producers consider the effect of fermentation more and more on the quality and profile of their coffee, they are adopting different and interesting techniques to their repertoires in order to diversify their offerings. One method that’s becoming more popular is fermenting coffee in a controlled anaerobic environment, meaning that the coffee is held in a vessel without any presence of oxygen during some of all of the duration of fermentation.

The fermentation process itself is already anaerobic, meaning that the yeast and bacteria that do the work of fermenting a coffee cherry’s sugar content do not need oxygen to be present in order to successful complete their mission or transform the organic material. (This is why we specify that it’s the *environment* that’s anaerobic in these cases.) One of the main benefits of holding the coffee in an oxygen-depleted environment, then, is to slow the fermentation process, which allows a totally different spectrum of flavors to express. Where in a Washed process the controlled fermentation might last 12–24 hours on average, anaerobic environment fermentation can take 96 hours or longer, depending on the thermal retention inside the tank.

Different fermentation-tank materials will have different thermal retention, and producers choose wisely based on the desired effect. Stainless steel is a common material, as is thick plastic. The tanks will contain a one-way valve that allows the producer to remove oxygen from the vessel and release CO_2 created during the fermentation process; careful monitoring of the coffee fruit’s pH as well as the temperature inside the tank is pivotal to ensuring the success of the process.

After fermentation, the coffee’s processing can be completed in any number of ways: The fruit can be fully removed as in a Washed process, the pulp can be removed but the mucilage left on as in a Honey process, or the cherries can be dried whole as in a Natural. The length of

drying time will vary based on this last step as well as the environment around the drying area.
Fermentation and Processing
On our Processes page, we attempt to outline when and how fermentation occurs during the most commonly found post-harvest processing methods, while also recognizing that there are no hard-and-fast rules in coffee, and your mileage may vary. In our coffee traceability profiles, we attempt to share as much information as can reasonably be gathered and provided by producers, knowing that there is not an efficient and concise way to share all the relevant details.

CACAO PROCESSING

Fermentation is an essential part of turning raw cacao into delicious chocolate. This processing step contributes to the development of flavor and the acidity of the final product. But what actually happens during the process?

Read on to learn more about the microbial activity, chemical reactions, and other elements involved in cacao fermentation.

Lee este artículo en español ¿Qué Sucede Durante la Fermentación Del Cacao?

Fresh cacao beans that have been bisected to reveal a violet inner seed, in Ecuador. Credit: Jeana Cadby

FERMENTATION IN A BEANSHELL

Fermentation is essential to flavor development and the final acidity of cacao beans. In fact, it's been stated that without fermentation, cacao would have no flavor at all. But one thing should be clarified: cacao beans are not fermented.

Rather, yeasts, bacteria, and enzymes ferment the juicy white pulp, or *baba*, that surrounds the cacao beans. The beans endure the heat, acid, and enzyme effects from the fermentation of the pulp and are transformed, both internally and externally, as a result.

The pulp itself is sterile before it's removed from the pod, but naturally occurring yeast and bacteria soon find their way to the pulp when the pod is opened. These microbial inoculants come from a variety of sources in the local environment. This could be the outside of the pod, workers' hands, visiting insects, or simply from the air. Sometimes the cacao is introduced to selected inoculants with a planned microbial cocktail.

An open cacao pod is visited by fruit flies in Vietnam. Credit: Jeana Cadby

DIFFERENT FERMENTATION METHODS

Fermentation methods vary depending on regional preferences and availability of resources. Individual producers will make different choices in the type and size of the fermentation vessel they use, the number of days they ferment cacao for, how often they turn the cacao, pulp preconditioning (pod storage prior to fermentation), and other factors.

But generally speaking, all cacao fermentation follows a similar process including an anaerobic phase and an aerobic phase. The specific details of each stage are influenced by a multitude of factors, not limited to pod ripeness, climactic and ambient weather conditions, pod quality, and batch size.

Fermenting cacao beans in the Philippines. Credit: Jeana Cadby

THE ANAEROBIC PHASE

Anaerobic conditions are simply environments that lack oxygen. In cacao, the pulp surrounding snugly packed beans creates a juicy barrier that blocks air from entering the system. The pulp is composed of water, high levels of sugars (sucrose, glucose, fructose), and various acids.

These sugars and the high acidity in the pulp create ideal conditions for microorganisms. The main players during this phase are yeasts, lactic-acid-producing bacteria, and pulp enzymes.

Using anaerobic respiration, yeasts quickly consume simple sugars and produce carbon dioxide, ethanol, and low amounts of energy. Lactic acid producing bacteria convert citric acid, glucose, and other carbohydrates in the pulp into lactic acid. Microbes are working around the clock to produce ethanol and lactic acid. You can see tiny bubbles of carbon dioxide gurgle up to the surface of the cacao in this stage.

In the early stages of fermentation, active yeast breaks down sugar found in the cacao pulp and produce bubbles of carbon dioxide. Credit: Jeana Cadby

Enzymes also help to break down the pulp, transforming it into a liquid that runs off and is known as sweatings. As the mass is broken down, there is more space for air to enter the process. Citric acid is also broken down and runs off with the sweatings, helping to increase the overall pH of the fermentation. The combination of rising pH and increased airflow marks the beginning of the aerobic phase of fermentation.

Workers in the Philippines turn fermentation boxes to introduce more air. Credit: Jeana Cadby

THE AEROBIC PHASE

Aerobic conditions are environments that contain oxygen. In cacao processing, producers mix and move the beans, a process known as "turning", to incorporate oxygen. The influence of this oxygen varies based on fermentation style, vessel, insulation, and the size of the ferment. But turning the beans will always make the fermentation process more uniform across the batch.

The aerobic stage is bursting with heat. During this step, acetic-acid-producing bacteria dominates and oxidizes ethanol and acids (citric, malic, lactic) to produce acetic acid. This acetic acid is further broken down into carbon dioxide and water by the oxygen.

Breaking down ethanol generates energy, which is expressed as heat. These **exothermic reactions** increase the overall temperature of the cacao. As the beans are turned, heat escapes and the overall temperature drops, but it then builds again through the introduction of more oxygen.

The combination of intense heat and the diffusion of ethanol and acetic acid into the cacao beans breaks down the cell walls. They will no longer germinate and the damaged internal structure becomes a venue for chemical activities that develop the flavor precursors associated with chocolate.

In Costa Rica, the outer pulp of fermenting beans has been shed and they are bisected to reveal oxidized juices. Credit: Jeana Cadby

Raw cacao beans have a bitter and astringent taste, due to their high phenolic content. Anthocyanins are one group of these polyphenols, and it both contributes to astringency and provide the reddish-purple color.

Fermentation allows the enzymatic breakdown of proteins and carbohydrates inside the bean, creating flavor development. This is aided by microbes, which create the perfect environment through the fermentation of the cacao pulp surrounding the beans.

In Vietnam, a producer checks baskets of fermenting cacao covered in banana leaves. Credit: Jeana Cadby

A fermented cacao bean won't taste like cacao nibs – for that you need to dry and roast the beans. But fermentation is an essential process to create the chemical compounds we associate with that desirable chocolate flavor

The Benefits of Fermented Foods Our digestive system is composed of a network of beneficial bacteria that are responsible for assisting our digestive system to digest food, absorb nutrients, battle harmful bacteria, and eliminate toxins. When these bacteria are killed off due to food additives, antibiotic drugs, processed foods, our gut health is affected. Eating the right kind of fermented foods and avoiding foods that feed unhealthy bacteria can help nourish our healthy gut bacteria and balance the ratio of beneficial-to-bad bacteria, which will eventually reflect on our overall health and well being. To achieve the proper ratio of beneficial-to-bad bacteria you need to increase the raw fruits and vegetables, cultured and properly fermented foods, limit sugary foods and anything that your body converts to sugar quickly like refined grains and processed foods. So eat more organic foods, especially greens and all vegetables, fiber-rich foods which cleanse the body like flax, chia and psyllium, and eat a variety of anti-fungal foods like coconut, turmeric, ginger, garlic, onion, oregano, cruciferous vegetables, cloves, cinnamon, coriander and olive oil.

WHAT ARE FERMENTED FOODS

Fermented foods contain healthy live bacteria known as probiotics and are foods that have gone through a process during which this bacteria converts the starches and sugars in that food into lactic acid and acetic acid. Fermented foods have high nutritional values (vitaminK2, trace minerals, B-vitamins and probiotics), are easy to prepare and are economical. Fermentation is an old food preservation method and was used by the Romans who consumed sauerkraut, Ancient Indians who enjoyed “ Lassi” a pre-dinner yogurt drink, Bulgarians who are known for their high consumption of fermented milk and Kefir, Turks who are famous for their Ariana drink alongside their meals, Asians who pickle cabbage, turnips, eggplant, cucumbers, onions, squash, and carrots and Middle Easterners who use yoghurt to accompany almost every meal.

MOST POPULAR FERMENTED FOODS

1. Sauerkraut Made from fermented cabbage rich in B vitamins and probiotics.

2. Kimchi Similar to sauerkraut but spicier and known as Korean kraut. It may contain peppers and other vegetables. Rich in antioxidants.

3. Pickles Made with cucumbers and spices. The best brands will just include organic cucumbers, salt (preferably sea salt) and water. Several brands also include herbs and spices like dill or even garlic and onion Rich in probiotics and minerals like silica.

4. Milk Yogurt, Ariana, Kefir These cultured foods that are made with milk can regulate your digestive tract. Unlike fermented foods milk is mixed with certain types of live cultures like acidophilus and are kept in a stabilized environment to ensure the right cultures develop. Different types of milk can be used with the best being goat or sheep milk.

5. Coconut Yoghurt, Coconut Kefir Is a great option if you’re a dairy-free eater. Choose homemade raw coconut yogurt that contains antiviral nutrients like lauric acid and caprylic acid known to fight and kill yeasts and other forms of bad bacteria in the body.

6. And: green bananas, fermented dark chocolate, tempeh, kombucha, seed cheese, tofu, sour cream, wine, beer, brewed ginger ale, cottage cheese, whey, soy sauce, yeasted breads (sourdough),Tabasco Sauce, Worcestershire Sauce, vinegar, “aged” cheeses like parmesan, blue cheese, and feta cheese.

Health aspects of fermented foods.

Kombucha is a type of sweetened black tea that uses fermentation to promote the growth of good bacteria.

The bacteria turn the sugar in the tea to alcohol. As a result, kombucha contains a low level of alcohol but not enough to cause intoxication.

The authors of a review article on kombucha conclude that it may promote immune system health and could also counter some metabolic disorders.

The chemicals that kombucha bacteria produce include antioxidants. Antioxidants counter the effects of free radicals, which experts believe play a role in a wide variety of illnesses, including cancer and chronic inflammation.

Kefir

Kefir is a fermented dairy product that is similar to yogurt but has a thinner consistency. Some people drink it, while others prefer to top cereal with it or mix it into other foods.

Kefir is high in protein, making it a good option for vegetarians. Protein can also help people feel fuller for longer, which can help support weight loss efforts.

According to a 2017 analysis, kefir offers probiotic benefits, such as improved digestive health. It may also help lower blood pressure and act as an anti-inflammatory agent, but more research is necessary to confirm these effects.

Miso and tempeh

Miso and tempeh are soybean-based fermented foods that are popular in Japanese cooking.

Miso is best known as the primary ingredient of miso soup, while tempeh is a popular meat substitute similar to tofu. Soybeans are rich in protein, so tempeh and miso are excellent choices for people who do not eat meat.

Research from 2016 suggests that the process of fermenting soybeans may release beneficial peptides, which are amino acids that help regulate the body’s functions. These bioactive peptides may:

- reduce the risk of diabetes and cancer

- fight infections
- lower blood pressure

Apple cider vinegar

Apple cider vinegar is a popular folk remedy that can also add flavor to salads, recipes, and some teas.

In addition to its fermentation benefits, the authors of a 2014 review noted that research in animal models and test tubes suggests that apple cider vinegar may have the following properties:

- antioxidative
- anti-diabetic
- antimicrobial
- anti-tumor
- anti-obesity
- anti-hypertensive
- cholesterol-lowering

Fermented vegetables

Pickles and sauerkraut are among the most popular fermented foods. These foods are easy to add to salads, sandwiches, and other dishes.

Many vegetables are high in fiber and contain important vitamins and minerals. Vegetables that people commonly ferment include:

- okra
- broccoli
- beets
- ginger

- mustard greens
- eggplant

Benefits of fermented foods

Share on PinterestFermented vegetables, such as kimchee, contain probiotics.

All fermented foods contain potentially beneficial bacteria, and some contain other organisms, such as yeast. These microbes act as probiotics, supporting gut health.

The benefits of fermented foods may include treating or reducing the symptoms of:

- *Clostridium difficile*, a bacterial infection
- diarrhea due to antibiotics
- infectious diarrhea
- ulcerative colitis
- irritable bowel syndrome
- Crohn's disease

As an imbalance in the gut microbiome can allow yeast to multiply, probiotics may reduce the risk of yeast infections and thrush, especially following treatment with antibiotics.

Weaker evidence suggests that beneficial gut bacteria may play a broader role in overall health. Probiotic-rich foods, such as fermented products, might reduce the symptoms of numerous conditions, including:

- depression
- urinary tract infections
- osteoporosis
- respiratory health issues
- hormonal disorders

- kidney and liver dysfunction
- diabetes
- cavities
- gingivitis

Any food made with beneficial bacteria potentially offers these benefits, so people who want to try probiotics can choose from among a wide variety of options.

How to read the label

Not all pickled foods are fermented. It is best to check the label to look for the mention of "live bacteria," "fermented," or "probiotics."

Fermented foods that contain a wide range of bacteria are more likely to offer extensive health benefits. If possible, choose fermented foods that list several different bacterial strains.

Some fermented foods, such as pickles, tend to be high in sodium. People concerned about their sodium intake, especially those with diabetes and cardiovascular disease, should check the sodium content that the label lists.

Homemade fermented foods are unregulated and may not have labels. People seeking specific health benefits from fermented foods should choose store-bought options with clear nutritional information.

How to ferment foods at home

Share on PinterestSome people choose to ferment foods at home.

There are several different methods of fermenting foods. The taste of the food can vary depending on the chosen method, so it is important to research which process is best for each specific food.

In general, people can ferment foods by following these steps:

1. Prepare the vegetables by chopping or shredding them. Some vegetables may taste better when a person ferments them whole.
2. Make a brine. The easiest method is to use a starter brine that already contains a culture. Alternatively, a mixture of either sea salt and water or sea salt, water, and whey can also work. Use between one-half of a tablespoon (tbsp) and 1 tbsp of salt per cup of water depending on taste preferences.
3. Put the vegetables in a sealable jar, such as a mason jar. Completely cover them in the brine. It is important to submerge the vegetables fully to prevent them from molding.

4. Leave the jar in a location with a stable room temperature for several days. When the mixture begins bubbling or smells like pickles, it is ready. Move it to the refrigerator. The food is now ready to eat.

Summary

As with many health foods, fermented foods are not suitable for everyone. People with weakened immune systems due to health conditions, such as HIV, or certain medications should avoid homemade fermented foods and check with a doctor whether it is safe to eat store-bought fermented foods.

Even good bacteria can affect some medications, so it is best to talk to a healthcare professional about specific health concerns before making any significant dietary changes.

For most people, however, fermented foods are a safe and healthful addition to a balanced diet. They may be the perfect antidote to gut bacteria problems.

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UNIT – V - FOOD FERMENTATION AND TECHNIQUES

Benefits & Drawbacks & Trends for Food Processing

Food processing is a transforming course from raw ingredients into foods, or from one form of food into another. It involves many different steps such as raw materials (harvested crops or butchered animal products) treatment, ingredients mixing, transmission, forming and packing, etc., during which are applied a wide variety of sanitary machines and equipment, for example, tanks, ***sanitary valves***, ***fittings*** and ***pumps*** during ingredients mixing and transmission, grinders in the raw materials treatment step and different molds doe forming, etc..

Benefits

Food processing can yield many benefits including:

- Elimination of toxin, bacteria and other unhealthy compositions. Some process can even give foods better mouthfeel and easier absorption.
- Longer shelf-life and facilitation for transportation, storage and preservation, which can enable people enjoy non-seasonal foods.
- More attractive appearance of foods
- A wider variety of food forms that are targeted for different demands. For example, nowadays in the market foods for weight loss, organic foods, certain-ingredient-reinforced foods are all available.

Drawbacks

Every processing of food can have some effect on the food's original nutrition structure, slight or huge. Vitamin C, for example, is destroyed during heating and therefore canned fruits have a lower content of vitamin C than fresh ones. According to a nutrient retention table for several foods created by the USDA in 2004, in the majority of foods, processing reduces nutrients by a minimal amount. On average any given nutrient may be reduced by 5%-20%.Another drawback of food processing is the possibility of food additives. The same addictive may cause varied health effects on different persons. In the USA, only food additives that have been approved as safe for human consumption by authorized organism are allowed, at specified levels, for use in food products.Last, because of the complex process of food processing (mixing, grinding, chopping and emulsification, etc.), processed foods will be exposed to a number of contamination risks.

PROCESSING OF VEGETABLES:

Vegetables consist of a large group of plants consumed as food. Perishable when fresh but able to be preserved by a number of processing methods, they are excellent sources of certain minerals and vitamins and are often the main source of dietary fibre. The consumption of vegetables has increased significantly as consumers have become more health-conscious. Owing to the perishable nature of the fresh produce, international trade in vegetables is mostly confined to the processed forms.

Structure And Composition

Vegetables can be classified by edible parts into root (*e.g.,* potatoes and carrots), stem (asparagus and celery), leaf (lettuce and spinach), immature flower bud (broccoli and brussels sprouts), and fruit (tomatoes and cucumbers).

Aging and spoilage

Depending on the class of vegetable, there are differences in the structure, size, shape, and rigidity of the individual cells. The fresh market shelf life and processing requirements are also very different. Vegetable cells, as plant cells, have rigid cell walls and are glued together by various polysaccharides such as cellulose, hemicellulose, and pectin. Once vegetables are harvested from the fields, the cells, now deprived of nutrient supplies normally obtained from soils and the air, go into senescence, or aging. The most noticeable structural change in senescent vegetables is softening, or loss of texture. Softening is caused by natural enzymatic reactions that degrade the plant cell walls. A large group of enzymes is involved in the senescence stage, including cellulase, pectinase, hemicellulase, proteinase, and others. After these enzymes break open the cells, chemical oxidation reactions take place and the vegetables develop off-flavours and loss of nutritional value. Broken cells are also much more easily subject to microbial attacks, which quickly lead to spoilage. In addition, even though the vegetables may be packaged or bagged, the plant cells continue to respire, or break down carbohydrates for energy needs. Respiration leads to loss of quality, so that eventually the products are unsuitable for human consumption.

Nutritional value

The four quality factors of vegetables are colour, texture, flavour, and nutritive values. Fresh vegetables are purchased on the basis of colour and texture, but repeated purchases are made on the basis of flavour and nutritional content. The major nutrients contributed by vegetables to the human diet are dietary fibre (both soluble and insoluble), minerals (calcium, phosphorus, iron, sodium, potassium), and vitamins (vitamin C, vitamin A, thiamine, niacin, folic acid).

Certain vegetables contribute lipids to the diet, mostly in the form of unsaturated oils. Roots and legumes can be important contributors of dietary proteins—especially in developing countries, where animal proteins are scarce. One potential nutritional problem of obtaining proteins from a single vegetable source is the low concentration of essential amino acids in vegetables. Twenty common amino acids are considered to be building blocks of proteins for the body. Of these 20, the body cannot synthesize eight; these eight must be obtained from foods. Most vegetable proteins are low in one of the eight essential amino acids; for example, corn is low in lysine, and soybeans are low in methionine. However, if proteins are obtained from a proper mixture of vegetables, there will not be a nutritional problem.

It is a common misconception that fresh vegetables are always superior in nutritional value to processed vegetables. Several investigations have shown frozen or canned vegetables can actually have higher nutritional value than fresh products. Fresh vegetables are subject to quality and vitamin losses during transportation and storage, whereas processing before these losses occur can yield a nutritionally superior product. Research has shown that a major cause of nutrient loss in vegetables is in the draining of cooking or processing liquids.

Harvesting and storing

Most leafy vegetables that do not require harvesting by mechanical device are cooled immediately after harvest to remove field heat, sorted to remove debris, washed to remove dirt, and bundled or packed for shipping and retail. In most cases vegetables are bundled as whole plants, since cutting will injure the cells and liberate ethylene, which promotes senescence and shortens shelf life. Low-temperature storage is essential in the handling of quality leafy vegetables. On the other hand, storing below refrigerated temperature may lead to chilling injury of certain vegetables and to rapid loss of quality. In developing countries where refrigeration is not available, postharvest losses of fresh vegetables can be as much as half the total harvest.

For roots and legumes, the harvesting of which is normally done by machines, some sorting and grading are performed either in the field or at collection stations. Bulk handling of these vegetables is common, and few additional steps of preparation are performed before distribution. For vegetables that need to be stored for long periods of time, treatments to avoid microbial spoilage, insects, and small-animal invasion may be necessary. For some vegetables such as cucumbers, a washing and waxing step may be taken to improve the shelf life and the attractiveness of the produce.

Packaging

Provided in response to demands for convenient foods, minimally processed fresh produce has gained popularity in the marketplace. These vegetables go through additional preparation steps of washing, sorting, grading, cutting, and packaging into retail-size containers. In order to extend the shelf life of these products, vacuum packing and modified-atmosphere (MA) packaging are practiced. In most cases air is replaced by an atmosphere high in carbon dioxide and low in oxygen. This modified atmosphere can slow the respiration rate and therefore the senescence of cut vegetables. The most common products in American and European markets are various types of cut lettuces with shredded carrots, cabbages, and other vegetables. Modern packaging techniques employing “clean room” concepts make it possible for such vegetable products as salad mix and stir-fry mix to have shelf lives approaching those of the whole plants. The products can be shipped by refrigerated containers to overseas locations and still have a shelf life long enough to reach consumers.

Minimally processed vegetables normally do not contain any preservatives and have not gone through any heat or chemical treatment. The disadvantage of these products is that refrigeration storage is essential, limiting its practice to developed countries.

Processing Of Vegetables

Because of the varied growing and harvesting seasons of different vegetables at different locations, the availability of fresh vegetables differs greatly in different parts of the world. Processing can transform vegetables from perishable produce into stable foods with long shelf lives and thereby aid in the global transportation and distribution of many varieties of vegetables. The goal of processing is to deter microbial spoilage and natural physiological deterioration of the plant cells. Generally, the techniques include blanching, dehydrating, canning, freezing, fermenting and pickling, and irradiating.

Blanching

After vegetables have been washed clean, they must undergo blanching (heating) in hot water at 88° C (190° F) for two to five minutes or with steam in a conveyor at 100° C (212° F) for one-half to one minute. Blanching inactivates natural enzymes that would cause discoloration and off-flavours and aromas. It also serves to reduce the number of microorganisms and to render vegetables limp for easy packing into containers. For some vegetables, such as spinach, snap beans, and collards, the blanching step also serves to remove harsh flavours.

After blanching the vegetables must go through rapid cooling in either cold water or cold air for better quality retention. The vegetables are then ready for the various food-processing methods described below.

Dehydration

Drying is probably the oldest method of preserving foods. The removal of water from vegetables is accomplished primarily by applying heat, whether it be through the radiant energy of the sun or through air heated by electrical energy. A major advantage of removing water is a reduction in volume and weight, which aids in storage and transportation of the dried products. Modern drying techniques are very sophisticated. Many machines are available to perform tunnel drying, vacuum drying, drum drying, spray drying, and freeze-drying. Although freeze-drying produces a food of outstanding quality, the cost is high, and it has not been used widely in vegetable products.

One of the most familiar dehydrated products is instant potatoes. Almost all the mashed potato dishes served in restaurants and institutions are rehydrated instant potatoes. In restaurants and institutions dehydrated potato granules are used, while dehydrated flakes are preferred for home cooking. Potato granules have high bulk density and are easy to handle in large quantity. However, they produce mashed potatoes with a pasty texture—an effect caused by the rupture of cells during processing, so that starch is released from the cells. Mashed potatoes made from flakes, on the other hand, have a mealy texture comparable to that of freshly prepared mashed potatoes. The major difference in the processing of these two dehydrated products is in the drying steps. For granules, air-lift drying is used to bring the product to 10–13 percent moisture. After screening to proper granule size, the product is dried to 6 percent moisture in a fluidized-bed drier. In the making of flakes, a steam-heated drum drier is used to bring a flattened sheet of potato solids to final moisture content before it is broken into a suitable size for packaging. Although a considerable quantity of the potato cells are ruptured during the breaking of the dried sheet, the reconstituted product has an acceptably mealy texture because the potatoes are subjected to a precooking and cooling treatment as well as the addition of a monoglyceride emulsifier.

A small amount of sulfite may be used in producing certain dried vegetables. The sulfite serves as an antimicrobial agent, aids in heat transfer, and (in the case of potatoes) acts as a blanching agent. A small percentage of the consumer population is allergic to sulfite. Although the rehydrated product contains little or no sulfite, consumer concerns are forcing the industry to search for economically feasible sulfite replacements.

Canning, method of preserving food from spoilage by storing it in containers that are hermetically sealed and then sterilized by heat. The process was invented after prolonged research by Nicolas Appert of France in 1809, in response to a call by his government for a means of preserving food for army and navy use. Appert's method consisted of tightly sealing food inside a bottle or jar, heating it to a certain temperature, and maintaining the heat for a certain period, after which the container was kept sealed until use. It was 50 years before Louis Pasteur was able to explain why the food so treated did not spoil: the heat killed the microorganisms in the food, and the sealing kept other microorganisms from entering the jar. In 1810 Peter Durand of England patented the use of tin-coated iron cans instead of bottles, and by 1820 he was supplying canned food to the Royal Navy in large quantities. European canning methods reached the United States soon thereafter, and that country eventually became the world leader in both automated canning processes and total can production. In the late 19th century, Samuel C. Prescott and William Underwood of the United States set canning on a scientific basis by describing specific time-temperature heating requirements for sterilizing canned food

PROCESSING OF MILK

Milk is the most valuable protein food that widely consumed by people all over the world. The milk as a raw food is easily available on various dairy farms that are processed to the increases the variety of nutrients.

Milk is collected from the farmers and trasnported to milk plants for its processing into mass market milk and other dairy products such as: cream, butter, cheese, casein, yogurt, etc.

Milk Reception units

The milk is transported on tank trucks and delivered to the different dairy and milk plants. Milk is then pumped from the milk tanks into the milk reception units, where the milk is de-aerated and tested and then pumped over again to the storing units or the processing line. In the milk reception units, milk is measured and tested, air is eliminated and the milk is cooled before further processing or storing.

Milk Storage units

Milk can be then stored into tanks or pumps, or can go directly into the processing line from the milk reception units. Milk will be stored in tanks or silos along different phases of the processing line, these inter processing tanks will be automated into the processing line. Aseptic milk storage is required if the milk has followed an aseptic process.. Storage systems can vary from a very basic system that is manually handled , combined alongside CIP systems and sterilization units.

CIP or SIP

CIP**(cleaning in place**) systems are a requirement throughout the whole manufacturing process so as to guarantee hygienic maintenance of the installations and milk hygiene.

Milk sterilization

The process of reducing the microorganism is called sterilization. Depending on the amount and type of microorganism that need to be killed, and the shelf life of the product it is achieved using different processes like pasteurization, UHT, HTST or filtration.

For the production of **fresh milk**, the chosen process is **pasteurization**

for the production of **ESL milk, UHT** or several filtration systems can be used.

The most marketable **fluid milk** nowadays is either **UHT or HTST**.

Pasteurization is the process of treating the milk at a high temperature, and then chilling it so as to extend its shelf-life and reduce microbial growth, whilst retaining the maximum natural qualities possible.

HTST (High Temperature/Short Time).

Milk is put through a continuous process of very high temperatures for a very short time. This process conserves milk qualities better than more aggressive processes such as UHT or aseptic processing, although it leaves it with a shorter shelf life.

UHT (Ultra High Temperature).

Although pasteurization deactivates most of the microbial growth, to extend milk shelf-life further, milk needs to be further sterilized. The most commonly used method in the industry nowadays is UHT (ultra-high temperature) which consists of a continuous sterilization process where milk is heated at very high temperatures and then chilled numerous times in a continuous process. The aim is to kill all micro-organisms and prolong milk shelf-life. Most of the milk you can buy today is put through a UHT process.

ESL (Extended Shelf Life) Milk is fresh milk with an extended shelf life. Other microbiological reduction methods are used versus heat methods such as pasteurization, HTST or UHT.

Milk Standardization - Cream separation

The fat content of raw milk varies depending on the type of cow, cow feeding, age, timing, etc. This is why milk follows a standardization process where either raw milk or even sterilized or pasteurized milk is separated into cream and skimmed milk by the cream separation machines. Part of the cream is added back into the skimmed milk in exact proportions in order to precisely define the fat content of the milk and standardize it. The rest of the cream is processed to produce products such as cream, condensed milk, butter, etc, using technologies such as evaporation, mixing, drying, etc.

Mixing

Milk can be enriched with vitamins, calcium or other types of ingredients. These should be mixed and blended in batch or continuous processes in mixing units.

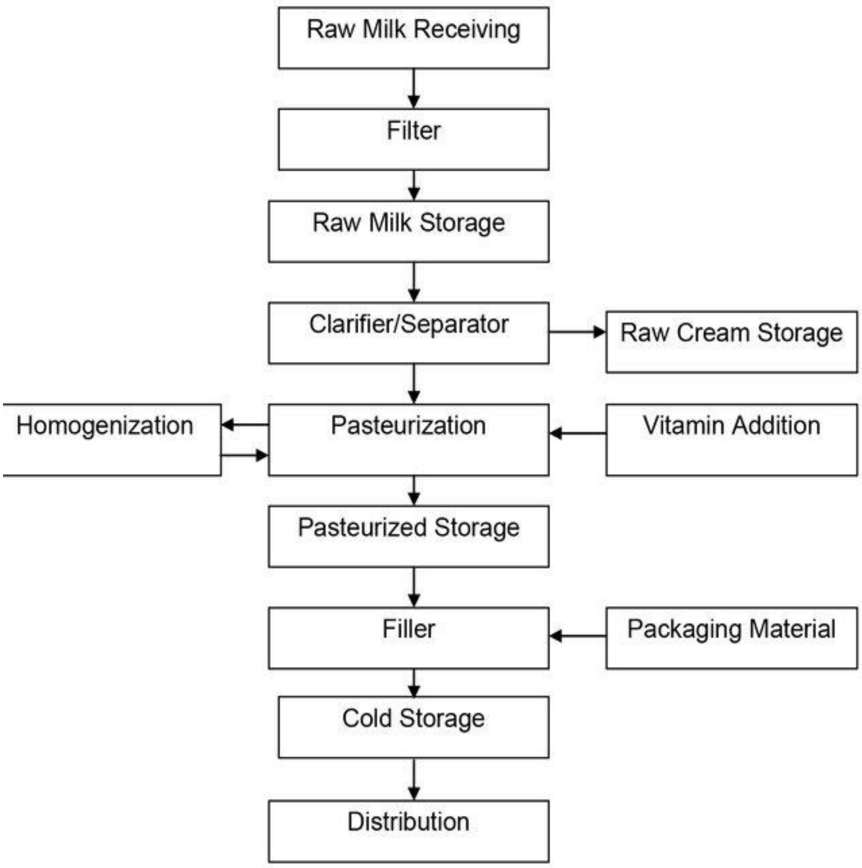
Homogenization

This process is done by machines called homogenizers, whose objective is to prevent the cream from seperating from the rest of the liquid once the milk is stored.

Milk Filling and Packaging technologies

Once the product manufacturing process is finished, the milk passes to the filling and packaging process. Milk can be packed into different types of packages: carton, glass, pouches, PET bottles, etc. Sterilized milk that needs to have a long shelf life should be filled and packed using aseptic technologies. In this case, previous sterilization of the package should also be done.

Flowchart on processing of milk:



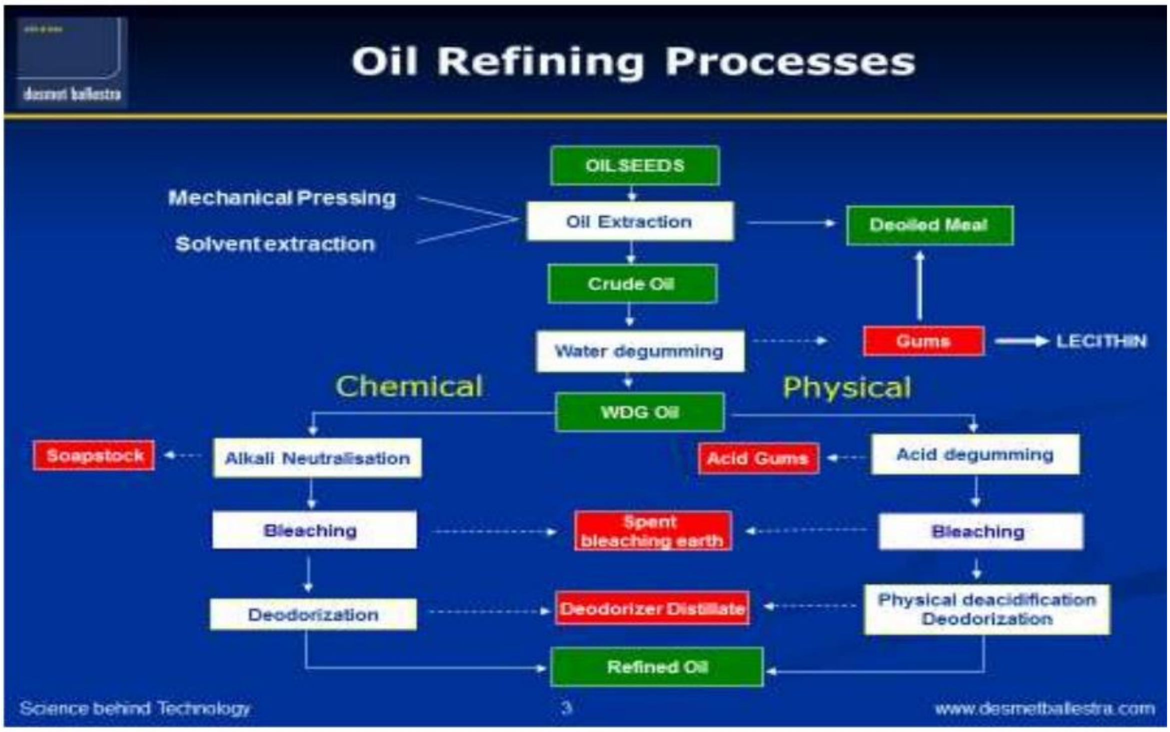
Edible Oil Processing

INTRODUCTION:

- Crude vegetable oil obtained from various oil milling units is further refined before use for edible purposes.
- Refined edible oil is a process where free fatty acids are volatized, condensed and recovered simultaneously with vacuum de-colouring operation.
- Sometimes, refining process is limited to simple physical treatment such as heating and filtering in regard to refining of superior quality of crude oil.

MANUFACTURING PROCESS:

- Special pre-treatment steps which are a combination of
- De-gumming and Blending under special operating conditions eliminate all impurities and render oil fit to be processed at elevated temperature under vacuum.
- Various steps involved in refining are:
    1. Super cleaning
    2. Contobleaching
    3. De-acidification
- All these processes are very well standardized and practiced in the country since long.



Specifications of Edible Oil Refinery:

- Capacity 5 tons per day i.e. 4 batches of 1250 kg each
- All the main vessels i.e. two neutralizers, one bleacher and one deodorizer are properly arranged on the first floor of the steel structure. So all these vessels are hanging on the steel structure. Just below the two neutralizers, two soap pans are resting on the ground floor in which soap stocks is collected. There is a steam is a steam pipe arrangement in the soap pans also.
- Steel structure has size of 14ft.x 14ft. First floor is 9ft. above the ground level. There are 8 columns of double channel which supports the entire steel structure. It has proper staircase and raining on all the slides of steel structure at the first floor and also on staircase. Two filter presses are also accommodated on the first of the steel structure.
- Two oil tanks i.e. raw oil tank and bleached oil tank are accommodated under the structure on the ground floor. Then cooler, thermic fluid boiler, two stream generators, vacuum pumps, water pumps, oil pumps and refined oil tank are arranged on the ground floor around the steel structure outside the square of 14ft. x14ft. of the steel structure. So total space occupied is about 30ft. * 30ft.
- 40ft, tower is erected just near the deodorizer and its complete structure is supported from the ground floor and also it is attached with the refinery structure. Barometric condenser is arranged at 40ft. hight to create proper vacuum.
- All types of pipelines are interconnected as per the requirement of the refinery oil pipelines, vacuum pipelines, steam pipelines water pipelines, thermic fluid pipelines. At all appropriate places, proper valves are provided in the pipelines.
- Neutralizer is provided with thermic fluid coil for heating the oil.
- Bleacher is provided with double pipe coil. One is for thermic fluid and another is for cooling water.
- Cooler is provided with double pipe coil. Both for cooling water.
- Neutralizer is open on the top having conical bottom.
- Bleacher has dished ends on the both the sides. Similarly, deodorizer has dished ends on both the sides. Cooler has also dished ends on both sides.

Process Description of Edible Oil Refinery:

For refining the edible oil, there are these basic processes in the refinery.

- First process is neutralizing the oil in the neutralizer to remove the free fatty acids (FFA) by adding caustic soda.
- Oil is heated up to about 60 degree C by thermic fluid coils and oil is stirred by stirrer.
- Then soap stock formed due to chemical reaction is allowed to settle at the bottom of the neutralized from where it is taken out into soap pan.
- Neutralized oil is drawn into the second vessel called bleacher where colour of oil is removed by bleaching process with aid of chemicals such as carbon black and bleaching earth. Oil is generally heated up to 110degree C by thermic fluid coils. Stirring is also continued. Bleaching process is done under vacuum.

Process Description of Edible Oil Refinery:

- Bleached oil then goes to the filter press where bleaching earth and chemicals are separated and clean bleached oil is then drawn to deodorizer where oil is heated above 110degree C through thermic fluid coils and then live steam is given to the oil from the bottom steam nozzles and temperature of oil is raised up to 200 to 220 degree C through thermic fluid coils. Entire process is done under high vacuum. Thus, smell is removed from the oil in the deodorizer. Then it goes to cooler where water circulating coils take away heat and oil is cooled. Again, it goes to second filter press where completely refined and transparent colour less oil is obtained.
- Thermic fluid boiler, vacuum pump, barometric condenser, catchalls, steam generator etc. play their role in the refining process. So, these equipment’s are part of the refinery and connected with the vessels through pipelines.

REFINING:

- Refining produces an edible oil with characteristics that consumers desire such as:
- Bland flavour
- Odor
- Clear appearance
- Light color
- Stability to oxidation
- Suitability for frying.

REFINING:

- The following precautionary measures are taken during refining in order to avoid undesirable autoxidation and polymerization reactions:
    1. Absence of oxygen
    2. Avoidance of heavy metal contaminations
    3. Maintaining the processing temperature as low and duration as short as possible.

REFINING:

- Refining process comprising the following steps:
    1. Lecithin removal
    2. Degumming
    3. Free fatty acid removal
    4. Bleaching
    5. Deodorization

Removal of lecithin:

- This processing step is special importance for soybean oils.
- Water (2-3%) is added to crude oil, there by enriching the phospholipids in the oil/water interface.
- The emulsion thus formed is heated up to 80 degree C and then separated or clarified by centrifugation.

Degumming:

- Finely dispersed protein and carbohydrate are coagulated in oil by addition of phosphoric acid (0.1 % of oil weight).
- A filtering aid is then added and the oil is clarified by filtration.

Removal of free fatty acids (deacidification):

- The removal of fatty acids with 15% sodium hydroxide (alkali refining) is most frequently used method.
- Technically, this is not very simple since fat hydrolysis has to be avoided and moreover the sodium soap which tends to forms / stable emulsions, has to be washed out by hot water.
- After vacuum drying, the fat or oil may contain only about 0.05% free fatty acids and 60 to 70 ppm of sodium soaps.

- When fat or oil is treated with diluted phosphoric acid, the content of sodium soaps decreases to 20 ppm and part of the trace heavy-metal ions is removed.

Bleaching:

- In order to remove the plant pigment (chlorophyll, carotenoids) and autoxidation products, the fats or oil is stirred for 30 min in the presence of Al-silicates in a vacuum at 90degree C.
- The bleached oil is removed from the adsorbent by filtration.
- The retained by the absorbent can be recovered by hexane extraction and recycled into refining process.

Deodorization:

- Deodorization is essentially vacuum steam distillation.
- The volatile compounds, together with undesirable odorants present in the fat or oil, are separated in refining steps.
- Deodorization takes place min 20 in to 6h depending on the type of fat or oil and content of volatile compunds.

Parameters of Food Processing

Food Processing

Food processing is the transformation of agricultural products into food, or of one form of food into other forms. Food processing includes many forms of processing foods, from grinding grain to make raw flour to home cooking to complex industrial methods used to make convenience foods.

Primary food processing is necessary to make most foods edible, and secondary food processing turns the ingredients into familiar foods, such as bread.

Tertiary food processing has been criticized for promoting overnutrition and obesity, containing too much sugar and salt, too little fiber, and otherwise being unhealthful in respect to dietary needs of humans and farm animals.

To ensure purity, freshness and safety of the food products at the time of consumption. BENEFITS OF FOOD QUALITY PARAMETERS. Benefits of food processing include toxin removal, preservation, easing marketing and distribution tasks, and increasing food consistency. We should take parameters in food processing.

When designing processes for the food industry the following performance parameters may be considered:

- Hygiene, e.g. measured by number of micro-organisms per mL of finished product
- Energy efficiency measured e.g. by "ton of steam per ton of sugar produced"
- Minimization of waste, measured e.g. by "percentage of peeling loss during the peeling of potatoes"
- Labor used, measured e.g. by "number of working hours per ton of finished product"
- Minimization of cleaning stops measured e.g. by "number of hours between cleaning stops".

Adding Sodium

One of the main sources for sodium in the diet is processed foods. Sodium is added to prevent spoilage, add flavor and improve the texture of these foods.

Food Quality

Quality is an important **food** manufacturing requirement, because **food** consumers are susceptible to any for **Food quality** is the **quality** characteristics of **food** that is acceptable to consumers. **Food** of contamination that may occur during the manufacturing process.

Quality Parameters

Several **quality parameters**, which differ in chemical composition, can define the makeup of each product. These **parameters** refer to the chemical, microbiological, nutritional and physical factors that make up the coconut liquid products its unique properties, which also influences the products shelf life.to any form of contamination that may occur during the manufacturing process.

Intrinsic and Extrinsic Factors of Food Spoilage

Intrinsic factors of food spoilage are those inherent factors that are associated with the food and which in several ways affect the overall physical and chemical composition of the food. Intrinsic factors are food related factors; and they are generally referred to as the physiochemical properties of food. These intrinsic factors of food spoilage include the nutrient makeup of the food, the acidity or alkalinity of the food, water activity, moisture content, buffering capacity and the pH of the food.

Intrinsic and Extrinsic Factors

Intrinsic factors act from within an individual, **extrinsic factors** wield their influence from the outside (i.e., they are environmental, cultural, or related to lifestyle). **Extrinsic factors** can have a sizeable impact on a person's health and can affect medical decision-making.

Intrinsic and Extrinsic Parameters of Food Processing

All foods possess a set of conditions called intrinsic parameters. These parameters can be influenced by another set of conditions called extrinsic parameters. Together, these two groups of parameters have great influence on the number and kinds of microorganisms occurring in and on a food and their physiologic activities. Intrinsic parameters All foods conditions called of food include pH, *moisture, oxidation-reduction potential (presence or* possess a set of *absence of oxygen), nutrient content, occurrence* of antimicrobial constituents, and biologic structures.

All microorganisms have a minimum, maximum, and optimal pH tolerance a moisture requirement an oxygen-tension requirement and a nutrient requirement. By knowing these parameters, one can predict the presence and growth potential of specific microorganisms in certain types of foods. A pH of 4.5 is considered the demarcation line between acidic foods (<pH 4.5) and basic foods (>pH 4.5). Yeast and molds can grow down to pH 1 whereas bacteria cannot grow below pH 3. Thus, acidic foods such as citrus fruits and carbonated soft drinks will be spoiled more by yeasts and molds than by bacteria. On the other hand, in a more basic food (>pH 4.5) bacteria will outgrow yeasts and molds owing to their higher metabolic rates in a favorable growth environment. Moisture content is another important parameter. This is usually expressed as water activity (Aw). Most moist foods are in the range of 0.95 to 1.00 Aw). When the Aw drops to 0.9, most spoilage bacteria reach their minimum level. Most spoilage yeasts have their minimum at 0.88, and molds have theirs at 0.80.

pH	Optimum	Min	Max
Bacteria	6-8	4.5-5	9
Yeast	4.5-6	2-3	11
Mold	3.5-4	2-3	11

Thus, in dry food products yeasts and molds grow much better than bacteria, and in moist food bacteria will outgrow yeasts and molds. The role of oxygen tension in and around food also has a great impact on the type of organisms growing there. Bacteria can be aerobic, anaerobic, or facultative anaerobic, so they can grow in a variety of oxygen levels (although different types will grow in different oxygen-tension environments). Yeast can grow both aerobically and anaerobically. Most molds, however, cannot grow anaerobically. In a properly vacuum-packaged food, for example, one should not find mold growing. The amount of oxygen measured in terms of oxidation-reduction potential also dictates the types of bacteria that can grow in the food. Disrupting the oxygen tension of a food (e.g., grinding a piece of meat to make ground beef from a steak) makes it easier for aerobic organisms to spoil the food.

Nutrient content (water; source of energy for metabolism; source of nitrogen, vitamins, and growth factors; and minerals) of different foods will support different types of microbes. In general, a food nutritious for human consumption is also a good source of nutrients for microbes. Some foods have natural antimicrobial compounds, such as eugenol in cloves, allicin in garlic, and lysozyme in egg, that can suppress the growth of some microbes. Biologic structures of some foods are also important for the prevention of microbial invasion. An example is the skin of an apple. When the apple is bruised, microbes can easily enter the fruit and spoil it.

Extrinsic parameters of food also play an important role in the activities of microbes. Temperature of storage greatly influences the growth of different classes of microbes. The amount of moisture in the environment (relative humidity) also influences the absorption of moisture or the dehydration of the food during storage and thus also influences the growth of different organisms. Varying the gaseous environment in storage will also change the types and growth rates of different organisms during storage of the food items. And last, the length of time of food storage also influences the spoilage potential by microbes in the food.

Aerobic and anaerobic bacteria can be identified by growing them in test tubes of thioglycolate broth:
1: **Obligate aerobes** need oxygen because they cannot ferment or respire anaerobically. They gather at the top of the tube where the oxygen concentration is highest.
2: **Obligate anaerobes** are poisoned by oxygen, so they gather at the bottom of the tube where the oxygen concentration is lowest.
3: **Facultative anaerobes** can grow with or without oxygen because they can metabolize energy aerobically or anaerobically. They gather mostly at the top because aerobic respiration generates more ATP than either fermentation or anaerobic respiration.
4: **Microaerophiles** need oxygen because they cannot ferment or respire anaerobically. However, they are poisoned by high concentrations of oxygen. They gather in the upper part of the test tube but not the very top.
5: **Aerotolerant organisms** do not require oxygen as they metabolize energy anaerobically. Unlike obligate anaerobes however, they are not poisoned by oxygen. They can be found evenly spread throughout the test tube.

Conclusion

Thus, intrinsic and extrinsic parameters of food are of great concern to food microbiologists. Skillful manipulation of these parameters by food microbiologists will result in more stable, more nutritious, fresher, and safer foods for the consumer.

MEAT PROCESSING

Introduction

The meat processing involves the slaughter of animals and fowl, processing of the carcasses into cured, canned, and other meat products, and the rendering of inedible and discarded remains into useful by-products such as lards and oils. Meat is exposed to a series of wide range of processes viz. curing or preserving processes such as salting, wet pickling, drying, cooking and canning, sausage manufacture, ham curing. All these processing techniques are aimed at inhibiting the microbial spoilage and increasing the shelf life of the meat. Major principles involved in meat processing are use of heat, low temperature, smoking, modified atmosphere packaging and ionizing radiations. The methods of preservation are mainly grouped in three categories i.e. control by temperature, by moisture and by lethal agents (bactericidal, fungicidal etc.)

Preservation of Meat

Use of low temperatures

Chilling and freezing are most commonly used preservation system for meat and meat products.

a. Chilling

Chilling is most widely used technique to preserve raw and processed meat. Chilling preserves muscle tissue by retarding the growth of microorganisms and by slowing many chemical and enzymatic reactions. Storage temperature may vary from - 1.4 to 2.2�C for storage of beef for 30 days depending upon the number of microorganisms. Carcass should go to the cooler as soon as possible and its inner most part should be able attain below 10�C within 12 hrs of slaughter in order prevent undesirable off-flavours and bone taints due bacterial growth. An ideal temperature of storage for meat should 1�C above its freezing point.

During post mortem cooling and subsequent refrigerated storage, control of relative humidity (around 90%) is very important. The undesirable moisture is lost from the surface, the weight reduction becomes of economic important and meat pigments myoglobin might get oxidized to brown metamyoglobin. However, a small amount of moisture loss from the surface is desirable since this tends to retard growth of microorganisms.

Freezing

Freezing is an excellent process for preserving the quality of meat for long periods. Freezing is often used to preserve meats during shipment over long distances or for holding until long times of storage. Its effectiveness depends on ice crystal formation and rate of lowering of temperature. When the temperature of storage is below - 18C, changes occur at a very slow rate in the muscle of warm blooded animals. Quality of frozen meat depends on various factors such as rate of freezing, packaging etc. When muscle tissue is frozen rapidly, small both intra and extra cellular ice crystals are formed which cause little damage to the meat structure. While large ice crystals are formed in slow rate of freezing causing compactness of muscle fiber. The process of denaturation can be accelerated with a resulting decrease in water holding capacity of tissue. Loss of water holding capacity of the muscle along with mechanical damage to cells by ice crystals is responsible in large parts of thaw exudates. To protect quality loss due to changes in protein, anti-freezing compounds or cryoprotectants i.e. polydextrose, polyphosphate are added to meat formulations. Rapid freezing can be obtained by using air blast freezers either on batch or continuous basis which employs -20 to -40C cold air. Large size meat cuts are vacuum packaged to prevent lipid oxidation and discoloration due to formation of metmyoglobin. Retail meat is packed in low permeability films with better mechanical strength e.g. Sarlyn.

Use of heat

The canning of meat is a very specialized technique in that the procedure varies considerably with the meat product to be preserved. Since meat products are low acid foods so the rate of heat penetration is fairly low. Commercially canned meats can be divided into two groups on the basis of heat processing used ;

(a) Meats that are heat processed in an attempt to make the can contents sterile.
(b) Meats that are heated enough to kill part of spoilage organisms but must be kept refrigerated to prevent spoilage.

Processing temperature for shelf stable canned cured meat is 98C. Treatment of meat surfaces with hot water to prolong the storage time has been suggested. Although this may result in loss in nutrients and damage in colour. Actin is the most heat labile muscle protein becoming insoluble at 50C. Denaturation of muscle proteins decreases their water holding capacity. This decrease in water holding capacity may produce desirable juiciness, Provided free water is not expelled from the tissue. During heating, fat is melted. Adipose tissue cells are ruptured and there is a significant redistribution of the fat. When meat is eaten warm, the melted fat serves to increase palatability of the product by giving desirable mouth feel, especially at the end of chewing period, when most of the aqueous juices are lost. Myoglobin also undergoes denaturation. The red pigment heme is oxidized to brown pigment hemin. Canned meat loaf can be manufactured substituting a part of the meat with high calcium coprecipitate. It is observed that 20% meat can be replaced with high calcium milk protein coprecipitate in chicken meat loaf without affecting the quality of the end product.

Dehydration

Deprivation of available moisture (reduction of water activity) for microbes not only prevent their growth but also kills them, thus results in increased shelf life and better quality product. Water may be made unavailable either by dehydration, freeze drying or by increasing exrtacellular osmotic pressure as is done in curing. Drying meats can be successfully employed for both raw and cooked meat. However, the quality of the final reconstituted product is superior when meat is cooked prior to dehydration. There is a loss in native structure of protein as measured by loss of water holding capacity during temperature from 0 to 20 C. This is caused by denaturation of sarcoplasmic proteins. The next major loss in water holding capacity begins in the temperature range of 40 to 50 C due to denaturation of contractile proteins. Collagen is rapidly converted to gelatin at around 100 C. Texture is most severely altered by dehydration. The tough texture of dehydrated meat can be overcome by preparing products of intermediate levels of water.

Smoking

Smoking is often used with salting and curing. It gives desired flavour, aroma and aids in preservation. It was noted that preservative substances added to the meat together with the action of heat during smoking have a germicidal effect and that drying of the meat together with chemicals from the smoke inhibit microbial growth during storage. Smoke consists of phenols, alcohols, organic acids, carbonyl compounds and hydrocarbons. The desirable effects of smoking of meat can be listed as below:

- Meat preservation through aldehydes, phenols and acids (anti-microbial effect)
- Antioxidant impact through phenols and aldehydes (retarding fat oxidation)
- Smoke flavour through phenols, carbonyls and others (smoking taste)
- Smoke colour formation through carbonyls and aldehydes (attractive colour)
- Surface hardening of sausages/casings through aldehydes (in particular for more rigid structure of the casing)

Production of smoke

Smoke is produced by burning of wood or its saw dust which consist of 40-60% cellulose, 20-30% hemicelluloses, 20-30% lignin. A temperature gradient exists during thermal decomposition of wood. Outer surface temperature is generally above 212^{o}F during dehydration process. Co, CO_2 and volatile medium chain organic acids e.g. acetic acid are released during dehydration and distillation process. When internal moisture level reaches to zero, the temperature rapidly rises to 570-750�F. Once the temperature falls within this range thermal decomposition occurs and smoke is given off.

Nature of smoke

Although the smoke at the point of generation exists in a gaseous state, it rapidly goes into a vapor & particle phase. The vapor phase contains the more volatile component & is largely responsible for the characteristic flavor & aroma of smoke. As soon as smoke is generated numerous reactions and condensation occurs. Aldehyde & phenol condense to form resins which represent about 50% of the smoke component & are believed to provide most of color is smoked meats. Polyphenols are also formed by condensation.
The amount and ratio of smoke deposition on the product is influenced by smoke density, smoke house air velocity and its RH, and surface of product being smoked

Cooking during smoking

Cooking is often done simultaneously with smoking of meat. In fact cooking is often more important than smoking in meat processing. Cooking equines careful control of the smoking and heating process to give best results.

Liquid smoke preparations

Liquid smoke is used by some processors. It is sprayed on the product before cooking. It has some positive effects over natural wood smoke.

(1) It doesnt require the installation of a smoke generator and which usually requires a major financial outlay.

(2) Process is more repeatable, as the composition of liquid smoke is mane constant.

(3) Liquid smoke can be prepared so the particle phase is removed and thereby possible problems from the carcinogens can be alleviated.

Liquid smoke is generally prepared from hardwoods; The final product is composed primarily of the vapor phase & contains mainly phenols, organic acids, alcohols & corbonyl compounds. They dont contain poly hydro carbons (PHC).

The there are two types of smoking cold at 15 to 18 C (up to 26 C) and hot temperatures of +60 to 80 C. Cold smoking is used for fermented meat products (raw-cured ham, raw-fermented sausage) and precooked-cooked sausage (liver and blood sausages). Hot smoking is used for a range of raw-cooked sausages, bacon and cooked ham products.

Modified atmospheric storage

Fresh meat held at refrigerated temperature has a limited shelf life because of microbial growth. Modified atmosphere refers to the adjustment in the composition of the atmosphere surrounding the product. At higher concentration of CO_2 surface browning of meat occurs due to the oxidation of myoglobin and hemoglobin pigments to ferric state. The most desirable concentration of CO_2 to use in a modified atmosphere is a compromise between bacterial inhibition and product discoloration

Ionising radiation

Ionising radiation constitutes the potentially useful form of preservation. Besides from its desirable ability to inactivate micro � organisms, it also has the undesirable effect of altering meat pigments. Sterilizing doses of ionizing radiation results in the breakdown of various lipids and proteins to often undesirable odours. Tenderization of muscle may also occur during this treatment. Temperature of $\leq$ 80�C or below greatly reduces undesirable effect without affecting lethal effect on microorganisms. Generally enzymes are not inactivated by irradiation treatment, it is necessary to heat approximately 70�C prior to irradiation and storage.

Processing of Meat Products

Comminuted meat products

Comminution is the mechanical process of reducing raw materials to small particles called as minced meat. Depending upon the final use of the communited meat the degree of comminution is done which differs among various processed products and is often a unique characteristic of a particular product ranging from very coarsely comminuted (to produce non-emulsified sausages like salamis and summer sausages), to finely comminuted, (to produce emulsified sausages like frankfurters, bologna, etc). Sausages are usually defined as comminuted seasoned meats, stuffed into casings; they may be smoked, cured, fermented and heated. They are made from any edible part of the slaughtered, veterinary-inspected animal, and a series of nonmeat ingredients.

Sausages

Sausages are meat products that are salted & usually seasoned or spiced and are an example of comminuted meet products that are generally recognized as emulsified, stuffed, linked, smoked, and cooked meat products. Based on the product characteristics and processing methods, they are broadly divided into three categories: **fresh sausages, cured sausages and fermented sausages**. In all cases meat is communited to reduce meat and fat particle size (grinding, mincing, chopping, or flaking), mixing with ingredients, stuffing into specific casing, linking to obtain specific lengths and finally, packaging. Sausages might be of ground and emulsion type. In the ground variety of sausages discrete particles of meat are seen on the other hand, in emulsion type sausages fat is emulsified & stabilized by lean component. Sausages were developed to utilize low- quality meats such as trimmings head, shoulder & by- products of the meat. The processing of sausages is a continuous sequence of steps (Fig 23.1), which are all equally important.

Selection of Ingredients

Sausage ingredients include:

- Meat - based on consideration of fat/protein; moisture/protein and myoglobin concentration
- Moisture - added as ice at time of chopping in a number of fresh and smoked sausages
- Curing ingredients - salt, sodiuim nitrite and/or nitrate and sugar
- Seasonings - may include spices, such as black peper, paprika, mace and cinamon; herbs that may include thyme and savory; vegetables such as�� garlic and onion and other substances, such as flavor enhancers
- Fillers and binders - occasionally used to improve color, binding properties, slicing characteristics, altering flavor or reducing costs
- Ascorbic acid - used to improve color in smoked sausages
- Other additives - may include liquid smoke

Milk protein have been utilized as fillers, binders and extenders in cooked, comminuted meat products to reduce cook shrink and formulation cost, as well as to improve emulsifying capacity, emulsion stability, water binding, potential nutritive value and slicing characteristics. These proteins significantly increase the gel strength of meat proteins and it has been shown that there has a synergistic effect between milk proteins and salt soluble meat proteins, through covalent cross linkages.

Addition of caseinate stabilizes the meat emulsion as required in the sausage mix. It thickens the gravy during frying and prevents it running out, but excess incorporation of caseinate may result in drying up of the sausages. Further addition of water absorbent materials becomes essential when sodium caseinate concentration in sausages exceeds 5%. The greater water holding capacity, lower viscosity and lower cooking losses of sausage batters containing 2% sodium caseinate in comparison to all meat control were observed.

The coprecipitates have good potential in various meat products such as frankfurters, sausage batter and luncheon meats as meat replacers or extenders. Sausage acts as a good medium for the use of coprecipitates. The finely, dispersed dairy protein matrix in sausages also can act as a moisture binding agent, thus, developing the desirable chewy texture besides controlling shrinkage during storage and deformation while slicing. Addition of milk coprecipitate in combined boiled sausages resulted in increased pH, reduced nitroso pigments and increased residual nitrites content in the end product. It is found that both high and low calcium coprecipititates improved the emulsifying capacity, emulsion stability and water holding capacity of meat emulsion in fresh sausages at the 20% replacement level. Supplementation with dairy coprecipitates into boiled beef pork sausage batters up to 30% of meat protein yields emulsion with increased pH, enhanced water binding ability and improved adhesion properties.

ii. Grinding

Meat chunks of variable size and shape with variable fat contents are ground to form uniform cylinders of fat and lean. The screw feed in the barrel of the grinder conveys the meat & presses it in to holes of the grinder plate. The rotating blade cut the compressed meat and aids in filling the grinder plate holes.

iii. Mixing

Cylinders of fat and lean obtained by grinding are tumbled in a mixer to give a uniform distribution of fat and lean particles. This can be used for coarse ground sausages or for emulsion type sausages by utilizing a chopper or emulsifier and with suitable additions of required ingredient to obtain the desired texture & uniformity of composition.

iv. Chopping

It is often used as a means of batching the sausage mix, the mixed batch being transferred to an emulsifier or acquiring the desired texture.

v. Emulsifying

This machine combines the principle of grinding and chopping. Emulsifier machine handles large volumes of meat rapidly to produce a desired texture. Speed of handling material and high degree of disintegration of meat tissue help in obtaining desired textures. In the preparation of sausage, the protein and water of the meat mixture form a matrix than encapsulates the fat portion. In a meat emulsion the protein myosin acts as the primary emulsifying agent. The addition of salt to the product is to release the myosin from the muscle fiber. The emulsion is generally formed by mixing the meat with salt and other ingredients in a chopper, which aids in disrupting the fibers and facilitates the release of myosin.

vi. Stuffing

Sausage emulsion also known in the trade as mix sausage dough or batter is transferred to stuffers for extending the mix or emulsion into **casings**. At this point, the size and shape of the product is determined. Generally three type of stuffing devices are used.

- Piston
- Pump
- Combination of piston & pump

In the past, the casing of the sausages were made from animal casings, however this was a limiting factor for the production of sausages. Today, the casings are made of cellulosic and regenerated collagen. The limiting factor now, is the supply of meat and the cost of it. Fermented sausages are further subjected for the fermentation and maturation. Fermentation of meat constituents results in flavor development, improvement of shelf life and improved quality and food safety. Sausage batter is inoculated with the started bacteria composed of **selected lactic acid bacteria (LAB)** i.e. homofermentative lactobacilli *(Lb pentosus, Lb plantarum, Lb sake, Lb curvatus)*, pediococci (*Pediococcus acidilactici*, *Pediococcus cerevisiae*) and gram positive catalase positive cocci (GCC) i.e. non-pathogenic, coagulase-negative staphylococci (*Staphylococcus carnosus, Staphylococcus xylosus, Staphylococcus piscifermentans)* . Small manufacturers use spontaneous fermentation without adding starter culture.

vii. Linking and tying

After the emulsion is stuffed in to casings, the encased mass is tied with thread of fastened with metal clips. In the case of small sausages such as Frankfurters stuffed casing are twisted or drawn together to produces links either by hand or with mechanical devices.

Large sausages items are tied or slipped on one end with a hanging tie and suspended from a smoke stick or hook so the entire surface is free from contact with the equipment. This permits a good flow of air around the sausage in the smoke house and prevents touch marks and spotting due to contact with adjacently hanging product.

viii. Smoking & cooking

The draped smoker picks are placed on smoke trees or trolleys with 12-18 specs per tree. The smoke house operation is essentially a specialized drying and cooking operation in which sausage emulsion is coagulated. Encased sausage at the time of introduction in to the smoke house usually has an internal temp of 60-70F. During cooking this rises to 155 to 160F.

ix. Chilling

After smoking and cooking the product is showered with cold water and than chilled by refrigeration chilling is frequently done with a brine solution by dipping or spraying the products. (a 6% salt brine) balanced within leaching of salt from the sausage and imbibing of water by the sausage.

x. Peeling & packaging

After properly chilling the product usually to an ultimate temp of 35 to 40F, the cellulosic casings on frankfurter and slicing bologna are removed. This is known as the peeling operation.

b. Semi dry sausages

Semi dry sausages are usually made from pork or beef or a mixture of the two and are characterized by a moisture content ranging from 40- 45%, e.g. summer sausage, G�teborg Sausage, Cerevelat, Thuringian, Holsteiner. They have excellent keeping quality with need of little refrigeration because

(1) Some reduction in microbiogical contamination is achieved in the cooking process
(2) A high salt to moisture ratio contributes to retarding bacterial growth
(3) A low pH (5.3 or less) provides the tangy flavor and serves a protective food and good keeping quality is achieved with a pH of 4.8 to 5.0 and with a total acidity of 0.75 to 1% lactic acid.

Grind pork trimmings

↓

Place the trimmings in a mixer; add the salt sugar spices, cure and starter culture and mix for 2 to 3 minute.

↓

Regrind the mix

↓

Hold the mix for 12- 72 hr at 40°F

↓ stuff

Warm water shower for 2 min to wash the sausage surface free of any adhering particles

↓

Smoke the encased sausage for 16 hr at 110°F

↓ Shower with cold water /15 min

Allows to stand at room temp so the surface dies and then chill to 40°F

Fig. Manufacturing method of semi-dry sausages

Dry sausages

Semi-dried sausages are smoked and cooked to varying degrees, whereas dry sausages are not cooked and only with some products smoke is applied. The manufacture of dry sausages is more difficult to control than that of semidried sausages. Overall processing time may require up to 90 days. As a result of this prolonged holding the sausages are vulnerable to chemical, microbiological degradation. However, when prepared properly the finished sausages are usually stable and can be held with little or no refrigeration. Examples of dry sausages are Geneva salami, Pepperoni, mortadella etc.

Cured meat products

Curing of meat involves the essentially addition of **sodium chloride, sodium nitrite or sodium nitrate** and adjuncts to meat for increasing shelf-life and to obtain desirable colour and flavour. Sugar may or may not be added along with other ingredient to improve flavour. Curing can be done for both raw/cooked meats cut products as well for comminuted meat products e.g. sausages and similar preparations. Most popular raw cured meat includes ham and bacon which are pork products. However, the technique can be applied to any meat group.

Ingredients used in curing

(i) Salt
(ii) Sugar
(iii) Nitrate or nitrite

} Essential curing ingredients

(iv) Phosphates
(v) Spices
(vi) Baking Soda
(ii) Sodium erythroborate
(iii) Ascorbic acid
(iv) Mono sodium glutamate

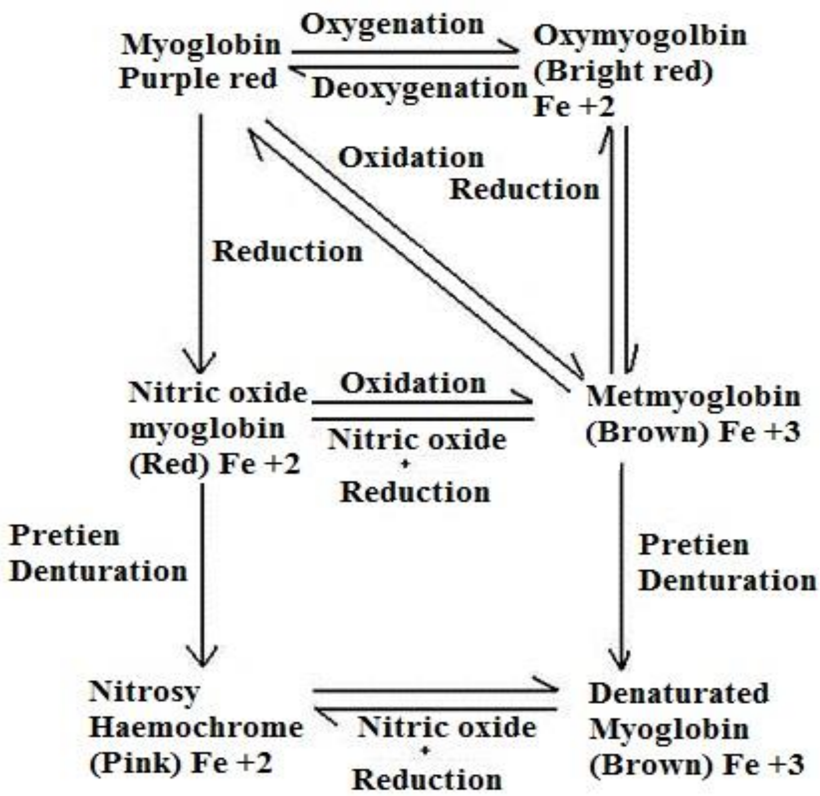
} Optional ingredients/curing adjuncts

(v) Hydrolysed vegetable proteins,
(vi) vinegar

Commonly used salt sodium chloride (occasionally KCl) is most essential ingredients and it significantly inhibits growth of microorganism including *Clostridium botulinum* due to increase in the osmotic pressure of the medium and dehydration of the muscle. Salt if used alone results in dark coloured, unpalatable dry harsh and salty product. Therefore, it is recommended to be used in combination with sugar and nitrite and nitrate. Salt should be of good quality. Generally dry salting utilizes higher levels of salts; however, acceptable level of salt is about 3% for most of the meats and about 2% for bacon. Nitrite/nitrate has as well a small inhibitory effect on *C. botulinum*. However, it plays very important role in colour fixation of the cured meat. On the other hand sugar contributes to flavour and colour development due to mailard browning and also helps in increasing shelf life by controlling of bacterial growth. Endogenous low molecular weight components in the sarcoplasma of the meat promote the formation of nitric oxide, myoglobin and nitrite decomposition.

Chemistry of curing process and meat colour development

During the dry curing process salt in dry form is rubbed on the surface of the meat whereas in meat wet curing meat portion is immersed in the curing solution. The latest techniques of curing includes use of artery pumping, multiple needle injection, thermal or hot cures, tumbling, massaging, are employed to accelerate the curing the processes. In all cases, salt diffuses into the meat, causing some of the expelled protein to diffuse back in and the meat to swell. The salt � protein complex binds the water well thus the water holding capacity of proteins generally increases during curing. The final meat contains increased ash due to the absorbed salts. Generally salting results in darkening of the colour. To counteract the effect of salt nitrite/nitrates are added to salt which fix the desirable pink colour of the meat. In the curing, nitrite reacts with muscle pigment myoglobin to give purple-red coloured nitroso-myoglobin. On the cooking this is further converted into nitrosomyochrome which gives typical pink clour to the meat. It is further claimed that nitrite has a significant beneficial effect on the flavour of cured meats by preventing oxidation through the antioxidative activity of nitric oxide-myoglobin and s nitrocysteine, a component found during the curing process.



Colour fixation in meat

A major detrimental change that can occur in cured meat during storage is the oxidation of nitric oxide hemochromagen (pink) or nitric oxide myoglobin (red) to brown metamyoglobin. The rate of oxidation increases with increasing oxygen content, therefore cured meat should be preferably packaged in a container from which oxygen is excluded.

Acceptable levels of nitrite used in meat and meat products are 100-200 ppm. The use of nitrite in cured meat may be hazardous if it is used at higher concentration with improper mixing, as it reacts with amines, especially secondary amines, to form N � nitrosamines, which may be carcinogenic. High temperature may also induce nitrosamine formation.

Grind Milling

Grinding:

Grinding (milling) is used for the size reduction of solid dry material. It may also improve the eating quality and/or suitability of the material for further processing.

Milling:

Milling is also used to crush cane sugar, to facilitate the extraction of sugar in sugar and rum factories.

Crushing:

Crushing covers, for instance, breaking the skin of berries and then crushing the berries to liberate the must. This process is necessary to facilitate the yeasts' multiplication and also to conduct traditional macerations before pressing.

Field of application:

Grinding/milling is applied in sectors in the food industry where dry solid materials are processed, for example, the animal feed industry, food products in flour milling industry, breweries, sugar industry, dairy industry (milk powder, lactose), etc.

Description of processing techniques, methods and equipment:

A whole range of grinding/milling techniques and equipment are available for application with different types of food.

Grinding/milling can be carried out dry or wet. In wet grinding/milling smaller particle sizes can be attained. Often dry grinding (milling) is combined with sieving or air classification, this results in particle size fractions.

Generally, cyclones are used as an integral part of the process to recover the particulate matter (dust) in extracted air. The recovered material is then reprocessed.

Common types of mills used in the food industry are:

a) Hammer mills:

A hammer mill consists of a horizontal or vertical cylindrical chamber lined with a steel breaker plate and contains a high-speed rotor fitted with hammers along its length. The material is broken apart by impact forces as the hammers drive it against the breaker plate.

b) Ball mills:

The mill consists of a slowly rotating, horizontal steel cylinder, half filled with steel balls (2.5 – 15 cm in diameter). The final particle size depends on the speed of rotation and on the size of the balls.

c) Roller mills:

The mill consists of two or more steel rollers which revolve towards each other and pull particles of the food material through the space between the rollers, the space being known as “the nip”. The size of the nip can be adjusted for different food materials

d) Disc mills:

Disc mills consist of either a single rotating disc in a stationary casing or two discs rotating in opposite directions. The food material passes through the adjustable gap between the disc and the casing or between the discs. Pin and disc mills have intermeshing pins fixed on the discs and casing. This improves the effectiveness of the milling.

e) High pressure grinding rolls:

A high pressure grinding roll, often referred to as HPGRs or roller press, consists out of two rollers with the same dimensions, which are rotating against each other with the same circumferential speed. The special feeding of bulk material through a hopper leads to a material bed between the two rollers. The bearing units of one roller can move linearly and are pressed against the material bed by springs or hydraulic cylinders. The pressures in the material bed are greater than 50 MPa (7,000 PSI). In general, they achieve 100 to 300 MPa. By this the material bed is compacted to a solid volume portion of more than 80%.

The roller press has a certain similarity to roller crushers and roller presses for the compacting of powders, but purpose, construction and operation mode are different.

Extreme pressure causes the particles inside of the compacted material bed to fracture into finer particles and also causes micro fracturing at the grain size level. Compared to ball mills HPGRs achieve a 30 to 50% lower specific energy consumption, although they are not as common as ball mills since they are a newer technology.A similar type of intermediate crusher is the edge runner, which consists of a circular pan with two or more heavy wheels known as mullers rotating within it; material to be crushed is shoved underneath the wheels using attached plow blades.

FOOD ADDITIVES SAFETY AND EFFECTS

Introduction:

With the advent of food processing, food additives play an important role in providing a food supply as well as meeting the consumers need.

Food Additives:

Food additives means any substance either natural or synthetic intentionally added to food for a

technological purpose in the processing, packaging, transport or storage of such food. Food additive is not normally consumed as a food by itself and not normally used as a typical ingredient of food.

The term does not include contaminants or substances added to food for maintaining or improving nutritional qualities as well as seasonings such as salt, herbs and spices.

Functions of Food Additives:

 Extending the shelf-life by protection against any oxidative deterioration

 Enhancing the flavor and odor

 Improving the texture and consistency of a food

 Stabilizing or retaining the color

 Enhancing the safety and quality by the inhibition of microbial growth.

Principles for using food additives:

 The food additives being used should present no risk to the health of the consumer at the levels of use.

 All food additives shall be used under conditions of good manufacturing practice(GMP)

 GMP includes the quantity of additive added to food shall be limited to the lowest possible level necessary to accomplish its desired effect

 The additive is prepared and handled in the same way as a food ingredient.

 The use of food additives is justified only when such use has an advantage, does not present a hazard to health of and does not deceive the consumer.

Types of Food Additives:

The different types of food additive and their uses are:

 Anti-caking agents – stop ingredients from becoming lumpy.

Examples: Table salt, milk powder, baking powder, cake mixes, grated cheese and instant soup mixes.

 Antioxidants – prevent foods from oxidizing or going rancid.

Examples: Vitamins C and E, selenium, and carotenoids such as beta-carotene, lycopene and lutein.

 Artificial sweeteners – increase the sweetness.

Examples: Aspartame, sucralose, saccharine.

 Emulsifiers – stop fats from clotting together.

Example: Lecithin, mono- and di-glycerols, ammonium phosphatide and xanthan gum.

 Food acids – maintain the right acid level.

Examples: Soft drinks, citrus fruits.

 Colors – enhance or add color.

Examples: Lycopene (E160d), Turmeric (E100)

 Humectants – keep foods moist.

Examples: Glycerin, sorbitol, propylene glycol.

 Flavors – add flavor.

Examples: Spices, herbs, edible yeast, wine.

 Mineral salts – enhance texture and flavor.

Examples: Calcium, sulphur, chlorine

 Preservatives – stop microbes from multiplying and spoiling the food.

Examples: Benzoates, sorbates, nitrates, vit E

 Thickeners and vegetable gums – enhance texture and consistency.

Examples: Agar, collagen, cornstarch

 Glazing agent – improves appearance and can protect food.

Example: Stearic acid, beeswax, shellac

 Gelling agents – alter the texture of foods through gel formation.

Example: Alginates, carrageenans

Effects of Food Additives:

It is often the additives that are used to give a food a marketable quality such as color that most commonly cause allergic reactions. Some of these hypersensitive reactions include:

- Digestive disorders – diarrhoea
- Nervous disorders – hyperactivity, insomnia and irritability
- Respiratory problems – asthma and sinusitis
- Skin problems – hives, itching, rashes and swelling.

It is important to realize that many of the symptoms experienced as a result of food sensitivities can be caused by other disorders. Medical diagnosis is important.

Applications of Food Additives:

The application of food additives has a rich history.

- Before the development of refrigeration and thermal processing, meat and fish were often salted to be preserved.
- The addition of sugar and vinegar was often used to retain the safety, flavor, and texture of fruits and vegetables.
- These and other practical food ingredients are readily used in the typical home kitchen and include baking soda, baking powder, yeast and food colorings.

Safety Assessment of Food Additives:

1. The toxicity of food additives is generally low.

2. The Joint Food Agriculture Organization / World Health Organization Expert Committee

on Food Additives (JECFA) is the international food safety authority responsible for collecting and evaluating scientific data on food additives and allocate a safety reference to the food additives evaluated.

3. The Acceptable Daily Intake (ADI) of a chemical is the estimate amount of a substance in food or water expressed on a body weight basis that can be ingested daily over a lifetime without any health risk.

4. A dietary intake above the ADI does not automatically mean that health is at risk. Transient excursion above the ADI would have no health consequences provided that the average intake over long period is not exceeded as the emphasis of ADI is a lifetime exposure.

5. A small proportion of the population may be intolerant to some food additives and may have acute effects

Example: Small amount of sulphur dioxide may cause bronchoconstriction and asthmatic reaction for certain people with allergic conditions.

Precautions:

JECFA recommended some precautions regarding food additives to the people. They are:

- The public were recommended to buy foods from reputable sources.
- The label of prepackaged food can be read carefully in particular the ingredient list for food additives added.
- In choosing foods those which have abnormal color, odor and texture should be avoided and the food which tastes abnormally cannot be consumed.
- Any abnormalities of foods can be reported to the authority for investigation and other

follow-up actions.

 Members of the public are advised to take a balanced diet so as to avoid excessive exposure to food additives from a small range of food items.

SEPERATION AND CONCENTRATION OF FOOD COMPONENTS

INTRODUCTION

Foods are complex mixtures of compounds and the extraction or separation of food components is fundamental for the preparation of ingredients to be used in other processes for e.g., cooking oils from oilseeds, sugar from cane or beet, or gelatine from connective tissue. For retrieval of high-value compounds, such as essential oils and enzymes e.g., papain from papaya for meat tenderisation or rennet from calf stomachs for cheese making. Each operation is used as an aid to processing and is not intended to preserve the food. Changes to both the organoleptic and nutritional qualities of products are caused by the intentional separation or concentration of food components, but generally the processing conditions cause little damage to these properties of foods.

There are other types of separation methods such as,

- Those used to clean foods by separating contaminating materials.
- Those used to sort foods by separating them into classes based on size, colour or shape.
- Those used to selectively remove water from foods using heat by evaporation.
- Those by dehydration.
- Those by crystallisation.
- By alcohol by distillation.
- Osmotic dehydration of fruits and vegetables, by soaking in concentrated solutions of sugar or salt respectively.

There are several types of separation. In physical separation of food components, by centrifugation, filtration, expression, solvent extraction and membrane separation are described.

There are two main categories:

1. Separation of liquids and solids where either one or both components may be valuable (e.g. fruit juices, pectin and coffee soluble), or liquid-liquid separation (e.g. cream and skimmed milk

2. Separation of small amounts of solids from liquids. The main purpose is purification of water or clarification of liquids such as wine, beer, juices, etc. and the solids are not a product.

CENTRIFUGATION

There are two main applications of centrifugation: separation of immiscible liquids and separation of solids from liquids. Separation of solid particles from air by centrifugal action in the `cyclone' separator is described in more detail in the section describing spay drying.

Centrifuges are classified into three groups for:

1.separation of immiscible liquids;

2 clarification of liquids by removal of small amounts of solids (centrifugal clarifiers);

3 removal of solids (desludging, decanting or dewatering centrifuges).

SOLID-LIQUID SEPERATION

The fruit fibres are suspended in juice. Sugar is crystallized from the solution and separated thereafter. The solid matter either floats or settles in the tank in due course of time because of the density difference between the two phrases. To achieve quick setting centrifugal force is used and the process is called centrifugal settling.

LIQUID-LIQUID SEPERATION

Because of density differences, the lighter liquid seperates from the heavier liquid, if allowed to stand for some time. Milk is a good example of emulsion where fat is in the finely dispersed state whereas the skim milk is in continuous phase.

SOLID-GAS SEPERATION

Solid particulates separation from agas stream is very common phenomenon in food processing operations. The seperaion of milk powder from the drying air stream coming from the drying chamber of a spray dryer after drying is a good example of solid-gas separation. The peripheral attachment required for this operation to accomplish is called cyclone separator.

FILTRATION

Filtration is the removal of insoluble solids from a suspension or feed slurry by passing it through a porous material a filter medium. The resulting liquor is termed the 'filtrate' and the separated solids are the 'filter cake'. Filtration is used to clarify liquids by the removal of small amounts of solid particles (e.g. from wine, beer, juices, oils and syrups).

SIEVING

Sieving is a method of using a sieve to distinguish small particles from bigger particles. It is used in flour mills or building sites. Impurities such as husks and stones are extracted from wheat at flour mill. They remove pebbles and stones from sand through sieving. It is a separation technique based on the difference in particle size. The sieve is responsible for retaining the larger particles. The sample is subjected to horizontal or vertical motion during sieving according to the method chosen. This causes a relative movement between the particles and the sieve; the individual particles either pass through the sieve mesh, or are retained on the sieve surface depending on their size.

MAGNETIC SEPERATION

Metal pieces in food products pose a safety risk to consumers and can damage processing equipment. Detection and removal of metal contaminants is becoming common practice in the food processing industry. One approach to reduce or eliminate metallic contamination is the use of magnetic separators. Sources of Metal Contamination Metal contamination may come from a variety of sources including:

- Incoming ingredients and raw materials.
- Processing equipment (grinders, crushers, etc.) general abrasion or vibration causing the loss of nuts and bolts.
- Inadequate personnel practices and environmental causes.

There are several types of materials used for magnetic separation such as,

Alnico magnets, Ceramic magnets, Rare earth magnets.

MEMBRANE CONCENTRATION

The separation or concentration of food components using membranes is well established, especially in the fruit processing, dairy processing and alcoholic beverage industries. It is also used to purify process water and treat wastewaters in a wide variety of food industries. There are seven types of membrane systems in use in food industries, grouped as follows according to the driving force for transport across the membranes.

Hydrostatic pressure systems: reverse osmosis, nanofiltration, ultrafiltration, microfiltration and pervaporation.

Systems where a concentration difference is the driving force: ion-exchange and electrodialysis.

NANO FILTERATION MICRO FILTERATION AND ULTRA FILTERATION:

The term nanofiltration is used when membranes remove materials having molecular weights in the order of 300±1000 Da. This compares to a molecular weight range of 2000±300 000 Da for ultrafiltration membranes, although there is overlap with microfiltration. Nanofiltration is capable of removing ions that contribute significantly to the osmotic pressure and thus allows operation at pressures that are lower than those needed for reverse osmosis. Ultrafiltration membranes have a higher porosity and retain only large molecules (e.g. proteins or colloids) that have a lower osmotic pressure. Smaller solutes are transported across the membrane with the water. Ultrafiltration therefore operates at lower pressures (50±1500 kPa). The most common commercial application of ultrafiltration is in the dairy industry to concentrate milk prior to the manufacture of dairy products or to selectively remove lactose and salts. In cheese manufacture, ultrafiltration has advantages in producing a higher product yield and nutritional value, simpler standardisation of the solids content, lower rennet consumption and easier processing.

PERVAPORATION

Pervaporation is a membrane separation technique in which a liquid feed mixture is separated by partial vaporisation through a non-porous, selectively permeable membrane. It produces a vapour permeate and a liquid retentate. Partial vaporisation is achieved by reducing the pressure on the permeate side of the membrane (vacuum pervaporation) or less commonly, sweeping an inert gas over the permeate side. There are two types of membrane, which are used in two distinct applications: hydrophilic polymers (e.g. polyvinyl alcohol or cellulose acetate) preferentially permit water permeation, whereas hydrophobic polymers.

EFFECTS ON FOODS AND MICRO-ORGANISM

Each of the unit operations described in this chapter is intended to remove components of the food and they are therefore used to intentionally alter or improve the sensory properties of the resulting products. The effects on nutritional value are difficult to assess in most operations and are usually incidental to the main purpose of altering eating qualities. However, with the exception of some types of solvent extraction, these operations take place at ambient temperatures and loss of heat-sensitive nutrients is not significant.

The main changes occur as a result of the physical removal of food components. In milk processing for example, the fat-soluble vitamins, retinol, carotene and vitamin D, are removed in the milkfat when it is separated from skimmed milk and concentrated in cream and butter. Conversely, water soluble vitamins and minerals are largely unchanged in skimmed milk, but substantially reduced in cream and butter. Both types of membrane retain proteins, fats and larger carbohydrates, but the larger pore size of ultrafiltration membranes allows sugars, vitamins and amino acids to be lost.

PHYTOCHEMICAL PROCESSING:

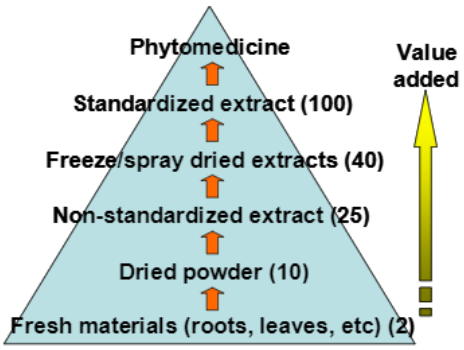
PHYTOCHEMICAL – comes from the Greek word “Phyto” for plant. It refers to every naturally occurring chemical presents in plants. Plant are also the source many modern pharmaceutical (drugs). It is estimated that approximately one quarter drugs contain plant extract or active ingredients obtained from plant substances. COSMECEUTICAL – is the term used to describe cosmetic containing ingredients that are bioactive, exerting effects on people. It is a blend of cosmetic and pharmaceutical which has appeared only in the nineties. Examples are anti-wrinkles creams, baldness treatments, moisturizers and sunscreens.

NUTRACEUTICAL – can be any substance that may be considered a food or part of a food that provide medical and health benefits, including the prevention and treatment of disease. Under this broad definition, nutraceutical might be isolated nutrients, dietary supplements or diets, processed foods, herbal products or genetically engineered “designer foods”.

OLEORESIN – are pure extractives of a spice or herb which contain concentrated natural liquid flavourings that contain both volatile and non-volatile flavour components. ESSENTIAL OILS – volatile part of the plant that are largely responsible for its characteristic aroma. It can be applied to enhance health through its holistic effects on the body.

There are several major steps in herbal product manufacturing starting from herbal crop planting to herbal product manufacturing and marketing. Chemical engineers are involved once the herb is harvested where quick preprocessing and correct storage is required. Preprocessing involves reducing the size of the herb through chopping and grinding to prepare for processing while good storage method ensures that the active phytochemicals are maintained before processing. Processing is a critical aspect of herbal production, especially due to the low yield of extracts. Processing methods are usually based on traditional methods such as high pressure water extraction for herbs which are traditionally boiled as decoctions. New innovative methods such as Supercritical Fluid

Extraction (SFE) where supercritical fluids such as carbon dioxide under high pressure are utilised to produce herbal extracts need to be developed to produce herbal products of higher yield, lower operating costs, and faster production times. Packaging and sale follow processing. Herbal products can be sold in a variety of forms such as capsules, tablets, tea bags, extracts and essential oils. Good Manufacturing Practice (GMP) is a code of practice used by the medical and health related industries including the pharmaceutical industry in an effort to maintain the highest standards of quality in the development, manufacture and control of medicinal products. In Malaysia, the GMP certification is issued by National Pharmaceutical Control Bureau (NPCB), which is issued as an annual Manufacturing License to which it can be revoked at any time if the facilities are found not to meet the standards of GMP. Herbal medicine products can only be sold by manufacturers who utilize GMP as it ensures that the herbal product safety and purity. In addition, manufacturers intending to export their products must ensure that their target markets accept their GMP practices.

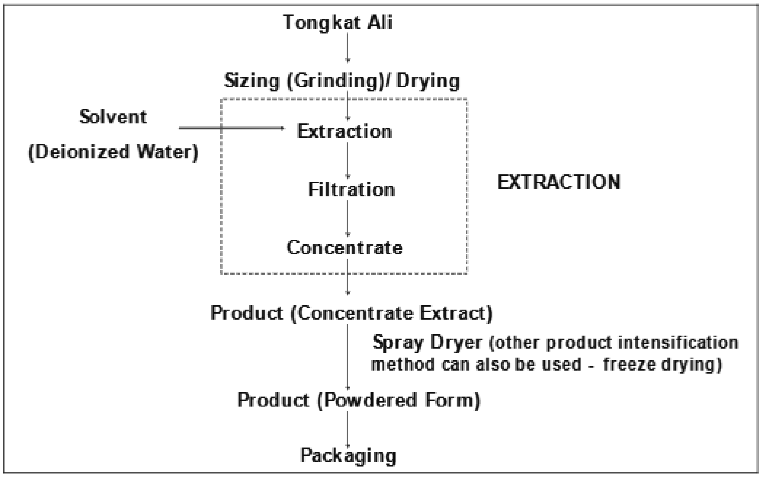


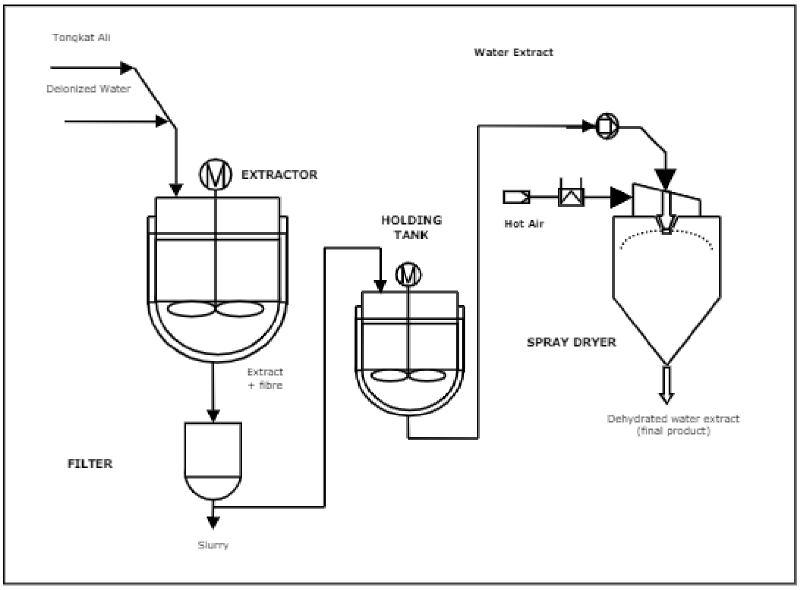
Unit Operation	Chemical Engineering	Phytochemical Processing
Mass Transfer	• Distillation • Solid Liquid Extraction • Supercritical Fluid Extraction	• Essential Oil extraction • Herbal leaching • Supercritical Fluid Extraction
Process Design	• Model based design • Process synthesis and design • Optimisation	• Model based design • Process synthesis and design • Optimisation
Bioprocess Engineering	• Downstream processing • Scale up	• Phytochemical approach • Scale up
Powder Technology	• Mixing	• Capsule preparation
Instrumentation and Measurement	• On line analysis • Process Control	• Phytochemical analysis • Process Control
Environmental Engineering	• Pollution prevention through substitution • Biomass conversion • Waste reduction	• Solvent design and substitution • Biomass conversion • Waste reduction

The Chemical Engineering Pilot Plant (CEPP) at Universiti Teknologi Malaysia (UTM) was set up in 1998 for the following objectives: • To bridge the funding gap between research findings and commercialised products • To assist Malaysia in building indigenous products and processes • To assist Malaysia in building up indigenous expertise Phytochemical processing was chosen as a focus area in the Fine Chemical section at the Pilot Plant. The primary emphasis in this area is on improving processing techniques for local herbal products as well as developing products for market testing as well as small scale production Several major equipment used for phytochemical processing include: • 300 litre Extraction vessel for producing extracts • Low Pressure Super Critical Extractor, a novel supercritical extraction process that uses Tetrafloroethane as a supercritical fluid which requires a lower pressure than carbon dioxide • Hydro distillation unit for essential oil production • Spray Dryer for herbal extract production • Freeze Dryer for herbal extract production • Centrifuge for concentrating and separating extracts • Homogenizer for mixing extracts • Turbo Extractor Distiller, an extractor that combines a grinder, extractor and distiller which can be used in producing essential oils more efficiently As the analysis of the raw material, herbal extract, pure phytochemical and final products are critical to quality assurance and process development, several analytical equipment are available at CEPP which comprise of: • High Performance Liquid Chromatograph (HPLC) • Liquid Chromatography – Mass Spectrometer • Spectrophotometer Significant CEPP Research Projects and Industrial Collaborations In the short time since it's formation in 1998, CEPP has been involved in several projects which include: 1. MHCP production from

Cinnamon 2. Phytochemical production from Zingiber Zerumbet and Curcuma Xanthorhizza 3. Production of oleoresins and essential oils from Zingiber officinale 4. Tongkat Ali production process development 5. Aromatherapy product development 6. Vitamin E product formulation A key concept in many of CEPP's projects is the biorefinery concept where raw plant material is totally fractionated and converted into a spectrum of valuable products. Cinnamon (kayu manis), a common spice used in Asian cooking, is one such plant where this concept can be applied. Among products

from Cinnamon include essential oils, oleoresins, extracts, and purified phytochemicals. One high value product from Cinnamon is Methyl Hydroxy Chalcone Polymer (MHCP), a phytochemical with scientifically proven anti diabetic properties. MHCP has been found to increase cellular glucose oxidation by a factor of up to 20 fold, improve the function of insulin receptors in cells, and has a strong anti-oxidant effect (Anderson and Schmidt 2002). An IRPA funded project entitled 'Production of Speciality Phytochemicals from Cinnamomum Zeylanicum' CEPP together with the UTM Science faculty as well as industry collaborators started in 2002. Its primary aim was to develop an environmentally safe and economically viable process to produce standardised Cinnamon extracts and purified MHCP. These extracts would be then used as functional food additives or phytomedicines. Apart from Cinnamon, CEPP has also been involved in the production of phytochemicals from members of the zingiberase (ginger) family, Zingiber Zerumbet (lempoyang) and Curcuma Xanthorrhiza (temu lawak). Another IRPA funded project together with the Science faculty and industry collaborators was started in 2001 to develop process design to extract these two species. The active ingredients in Lempoyang and Temu Lawak are zerumbone, which has anti viral and potentially anti HIV properties, and xanthorrizol, which has anti bacterial properties. The process design project involves process detailed design, scale up studies, total plant utilisation through biorefinery approach, and phytochemical purification. An example of the Turbo Extraction Distillation device and its applications is shown in Figure 6. Another product from the ginger family developed at CEPP are the essential oils and oleoresins from the household ginger, zingiber officinale. Ginger is freely available in Malaysia at a low price, therefore value added products can increase the value of this crop. In addition, many traditional cures in Malay, Indian, Chinese, and Indigenous groups are based on ginger (Ahmed and Sharma 1997). In collaboration with Universiti Malaysia Sabah, CEPP focused on the process design, process optimisation, and product commercialisation of essential oil and oleo resin production from ginger. Besides IRPA funded projects, CEPP also focuses strongly on industry based development projects. A key and visible success process development at CEPP is the development of the Tongkat Ali water extract production process, which is currently utilised by Phytes Biotek Sdn Bhd and the Forest Research Institute of Malaysia (FRIM). Phytes Biotek, the country's first Malaysian Venture Capital (MAVCAP) investment, is currently the largest commercial producer of Tongkat Ali extracts. ongkat Ali extraction by Phytes Biotek previously had an extraction time of over 4 hours and low yield. After process development at CEPP, it was found that high pressure and temperature extraction increased the yield and reduced the extraction time to part from herbal extracts such as Tongkat Ali, CEPP has also developed aromatherapy products, as shown in Figure 9. CEPP has helped created several formulations in different forms such as lotions, sprays, and candles. A successful set of formulations are currently being marketed by Fyto-Elegance, another MAVCAP funded company. CEPP has also done product formulations for spray dried Vitamin E from Palm Oil sources. The product formulation proved to be of high demand in countries such as Japan where in powder form it proved to be easily formulated and standardised.





Effects of food processing on food nutrition.

Nearly every food preparation process reduces the amount of nutrients in food. In particular, processes that expose foods to high levels of heat, light, and/or oxygen cause the greatest nutrient loss. Nutrients can also be "washed out" of foods by fluids that are introduced during a cooking process. For example, boiling a potato can cause much of the potato's B and C vitamins to migrate to the boiling water. You'll still benefit from those nutrients if you consume the liquid (i.e. if the potato and water are being turned into potato soup), but not if you throw away the liquid. Similar losses also occur when you broil, roast, or fry in oil, and then drain off the drippings.

Consuming Raw Foods

The amount of nutrient loss caused by cooking has encouraged some health-conscious consumers to eat more raw foods. In general, this is a positive step. However, cooking is also beneficial, because it kills potentially harmful microorganisms that are present in the food supply. In particular, poultry and ground meats (e.g. hamburger) should always be thoroughly cooked, and the surface of all fruits and vegetables should be carefully washed before eating. To learn more about preventing common food-borne diseases,

Grilling Meats

Outdoor grilling is a popular cooking method, primarily because of the wonderful taste it imparts on meats. It can also be a healthy alternative to other cooking methods, because some of the meat's saturated fat content is reduced by the grilling process. However, grilling also presents a health risk. Two separate types of carcinogenic compounds are produced by high-temperature grilling:

- **heterocyclic amines (HCAs)**
  HCAs form when a meat is directly exposed to a flame or very high-temperature surface. The creatine-rich meat juices react with the heat to form various HCAs, including amino-imidazo-quinolines, amino-imidazo-quinoxalines, amino-imidazo-pyridines, and aminocarbolines. HCAs have been shown to cause DNA mutation, and may be a factor in the development of certain cancers.
- **polycyclic aromatic hydrocarbons (PAHs)**
  PAHs form in smoke that's produced when fat from the meat ignites or drips on the hot coals of the grill. Various PAHs present in the resulting smoke, including benzo[a]pyrene and dibenzo[a,h]anthracene, adhere to the outside surface of the grilled meat. PAH exposure is also believed to be linked to certain cancers.

HCA and PAH content in meats can be dramatically reduced by slight alterations in your grilling method. In particular, the following practices will reduce the amount of HCAs and PAHs formed:

1. **Select leaner meats.**
   Leaner cuts of meat are less likely to drip fat on the grill and produce PAH-laden smoke.
2. **Marinate meats before grilling.**
   Researchers have determined that marinating meat prior to grilling, even for just a few minutes, can reduce HCA formation by 90% or more. It's believed that the marinade forms a protective barrier for the meat juices that prevents the HCA reaction from occurring.
3. **Grill at lower temperatures.**
   Lower temperature "roasting" also greatly reduces HCA formation.
4. **Prevent flare-ups.**
   Flames from grill flare-ups cause the formation of both HCAs and PAHs. Keep an eye on your grill and turn meats frequently to minimize the chance of flare-ups.
5. **Don't overcook meats.**
   While it's important to cook poultry and ground meats thoroughly, be careful not to overcook any meat. Well-done or burnt meats contain higher levels of HCAs than less

cooked meats. For thicker cuts of meat, use a meat thermometer to measure doneness rather than just guessing.

Typical Maximum Nutrient Losses (as compared to raw food)					
Vitamins	**Freeze**	**Dry**	**Cook**	**Cook+Drain**	**Reheat**
Vitamin A	5%	50%	25%	35%	10%
Retinol Activity Equivalent	5%	50%	25%	35%	10%
Alpha Carotene	5%	50%	25%	35%	10%
Beta Carotene	5%	50%	25%	35%	10%
Beta Cryptoxanthin	5%	50%	25%	35%	10%
Lycopene	5%	50%	25%	35%	10%
Lutein+Zeaxanthin	5%	50%	25%	35%	10%
Vitamin C	30%	80%	50%	75%	50%
Thiamin	5%	30%	55%	70%	40%
Riboflavin	0%	10%	25%	45%	5%
Niacin	0%	10%	40%	55%	5%
Vitamin B6	0%	10%	50%	65%	45%
Folate	5%	50%	70%	75%	30%
Food Folate	5%	50%	70%	75%	30%
Folic Acid	5%	50%	70%	75%	30%
Vitamin B12	0%	0%	45%	50%	45%
Minerals	**Freeze**	**Dry**	**Cook**	**Cook+Drain**	**Reheat**
Calcium	5%	0%	20%	25%	0%
Iron	0%	0%	35%	40%	0%
Magnesium	0%	0%	25%	40%	0%
Phosphorus	0%	0%	25%	35%	0%
Potassium	10%	0%	30%	70%	0%
Sodium	0%	0%	25%	55%	0%
Zinc	0%	0%	25%	25%	0%
Copper	10%	0%	40%	45%	0%