Fermented cereals

A global perspective

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FOREWORD

This is the second document in the Agricultural Service Bulletin series on food fermentations in developing countries. This bulletin not only documents information on fermentation technologies which is rapidly being lost, but potential areas for the development and improvement of cereal fermentations are highlighted by each author.

I thank all of the authors who have contributed to the preparation of this document.

It is hoped that this document will generate wider interest in and contribute to the development and improvement of small-scale food fermentations in the developing world.

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CHAPTER 1

CEREALS: RATIONALE FOR FERMENTATION

INTRODUCTION

The global importance of cereal crops to the human diet and moreover to the written history of man and agriculture cannot be overstated. Cereal grains are the fruit of plants belonging to the grass family (*Gramineae*). The sustenance provided by cereals is frequently mentioned in The Bible, and they are by many other criteria the most important group of food crops produced in the world. Cereal crops are energy dense, containing 10 000-15 000 kJ/Kg, about 10-20 times more energy than most succulent fruits and vegetables. Nutritionally, they are important sources of dietary protein, carbohydrates, the B complex of vitamins, vitamin E, iron, trace minerals, and fibre. It has been estimated that global cereal consumption directly provides about 50 percent protein and energy necessary for the human diet, with, cereals providing an additional 25 percent protein and energy via livestock intermediaries. Some cereals, notably wheat, contain proteins that form gluten, which is essential for making leavened bread. Although dried cereal grains constitute living cells that respire, when kept in an appropriate environment, whole grains can be stored for many years. In 1996, world cereal production amounted to more than two billion tonnes (Figure 1). Major cereal crops produced worldwide include wheat, rice, maize and barley (Figure 2). Other major cereal crops produced include sorghum, oats, millet and rye. Asia, America, and Europe produce more than 80 percent of the world's cereal grains. Wheat, rice, sorghum and millet are produced in large quantities in Asia; corn and sorghum are principal crops in America, and barley, oats and rye are major crops in the former USSR. and Europe (Chaven and Kadam, 1989).

Cereals have a variety of uses as food. Only two cereals, wheat and rye, are suited to the preparation of leavened bread. The most general usage of cereals is in cooking, either directly in the form of grain, flour, starch, or as semolina, etc. Another common usage of cereals is in the preparation of alcoholic drinks such as whiskey and beer (barley, sorghum), vodka (wheat), American bourbon (rye), Japanese sake (rice), etc. A variety of unique, indigenous fermented foods, other than leavened breads and alcoholic beverages, are also produced in regions of the world that rely mainly on plant sources of protein and calories. In developed countries, that obtain most of their protein from animal products, cereals are increasingly used as animal feed. More than 70 percent of the cereal crop produced in developed countries is fed to livestock whereas, in developing countries, 68-98 percent of the cereal crop is used for human consumption (Betschart 1982; Chaven and Kadam, 1989).

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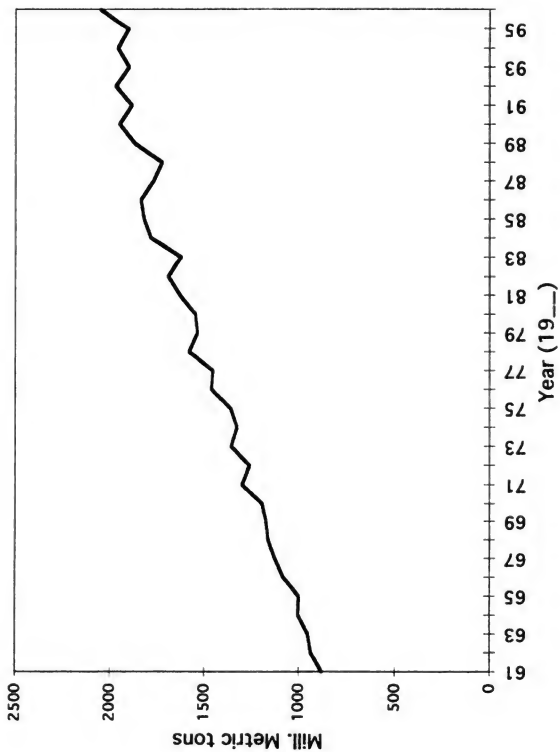
Figure 1 – Global cereal production from 1961-1996. Data from FAO, Rome
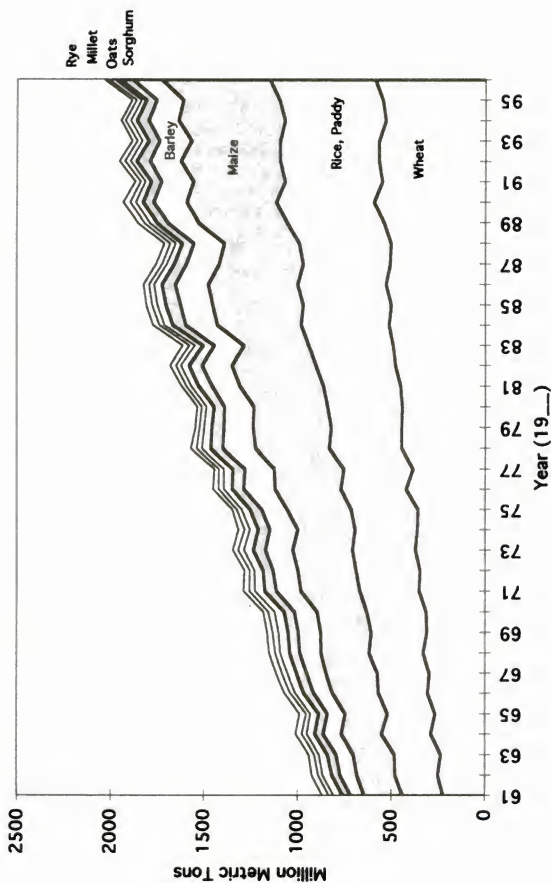
Figure 2 – Global production of major cereal crops from 1961-1996. Data from FAO, Rome

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HISTORY

The origin of farming practice appears to be located in the "Fertile Crescent", a wide belt of Southeast Asia, which includes Southern Turkey, Palestine, Lebanon and North Iraq. Abundant rainfall occurred in the highlands of this area where there still exist a wide variety of wild cereals. *Triticum dicoccoides* (wheat) and *Hordeum spontaneum* (barley) were collected by local dwellers. There is evidence that the people of Uadi el-Natuf Tell of Southeast Asia were the first grain cultivators at about 7 800 BC (Table 1). By 5 000 BC, wild animals became rare forming only 5 percent of the diet, while cereals and farmed animals provided a sizeable part of man's food (Furon, 1958). Early wild wheat (*Triticum*) and barley (*Hordeum*) species were diploid carried few seeds and abscissed from the plant at maturation, thus making harvest difficult. Polyploid plants can originate in nature but have little chance for self-propagation without cultivation (Raven *et al.* 1986). The cultivation and irrigation of cereals allowed the expansion of polyploid grains. The polyploid grains exhibit less genetic variation,

Table 1. Estimates of origin and early cultivation of cereals[1]

CEREAL	TIME	LOCATION
Wheat	7 000 BC	Near East
Barley	7 000 BC	Near East
Rice	4 500 BC	Asia
Maize	4 500 BC	Central America
Millet	4 000 BC	Africa
Sorghum	4 000 BC	Africa
Rye	400 BC	Europe
Oats	100 AD	Europe
Triticale	1 930 AD	USSR., Europe

[1] Source-McGee, 1984

since each gene is represented in several copies, causing more genetic uniformity and considerable increase in stability and yield. The first stable lines of polyploid cereals were identified as early as 6 000 BC. On the other hand, genetic variability in diploid wild types was essential in order to develop plants adapted to different environmental conditions and geographic areas (Feldman and Sears, 1981). *Triticum turgide dicoccoides* was crossed with *Triticum fanschii* to give *Triticum aestvum*, the progenitor of actual wheat. *T. aestvum* has 42 chromosomes compared to the 14 chromosomes *of T. monococcum*. Today there are more than 20 000 cultivars of *T. aestvum* over the world. In Roman times, *T. dicoccoides* (spelt) was used for bread making and *T. vulgaris* (siligo) was used for soups because of it's low gluten content (Simoons, 1982). Wheat and barley were initially cultivated in Southeast Asia, whereas rice was cultivated in Asia, maize in America, and sorghum and millet in Africa (Table 1). Over the last 200 years active programmes in genetic selection and manipulation have changed the character of the original *Triticacee* from few grains and low gluten to abundant grains rich in gluten forming proteins. Triticale (genus *Triticosecale*) is a relatively new cereal that was developed by crossing wheat and rye in order to combine the tolerance of rye for poor soil and climatic conditions with the superior technological characteristics of wheat (Bushuk and Larter, 1980).

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CEREALS

Wheat

Wheat is one of the oldest of all cultivated plants. Today, there are more than 50 000 cultivars of wheat in existence and as a result wheat can be grown in a relatively wide range of climatic conditions. Growing best in temperate climates, it is susceptible to disease in warm, humid regions and cannot be grown as far from the equator as can rye and oats. Wheat was brought to America early in the seventeenth century where it came to prominence in the Great Plains by 1855 (McGee, 1984). Different types of wheat are classified based on planting season and endosperm composition. Wheat holds a special place amongst the cereals because upon mixing wheat flour with water, an elastic matrix called "gluten" required for the production of leavened breads is formed. "Hard wheats" tend to contain relatively high levels of starch and relatively low levels of protein, while the reverse is true for "soft wheats". High protein flours are best suited for pastas and breads, while flour from soft wheats is excellent for cakes and pastries, etc.

Rice

The second most abundant cereal crop originated in the Indian subcontinent and Africa. Today, 90 percent of the world rice crop is grown in Asia (FAO, 1996). Alexander the Great is credited with introducing rice to Europe around 300 BC. Growing rice requires more water than other cereal crops, although rice is a highly productive crop. There are several thousand-rice cultivars, which may differ in colour, aroma, and grain size. The main commercial distinction between rice types is the grain size, i.e. long, medium and short. Long grain rice, also called "Indian", tends to separate relatively easily on cooking and is dry and flaky. Short grain rice, also called "Japanese" is sticky, moist and firm when cooked. Unlike wheat, rice is most often consumed as grain rather than as flour. Different grades of milling include brown rice (hull removed), unpolished rice (hull, bran and most of germ removed), and polished rice (aleurone layer removed from unpolished rice). Since polishing removes most of the lipid, the latter product is relatively stable during storage. The discovery that rice bran can alleviate beriberi led to the discovery of the vitamin thiamine. The traditional technique of parboiling rice in India and Pakistan (also called "converted rice") prior to milling improves the nutritional quality of the grain by allowing the B vitamins in the bran and germ to diffuse into the endosperm.

Wild rice is native to the Great Lakes region of North America where native Indians originally harvested it from the wild. Although wild rice is now cultivated, it is expensive and accounts for less than 1 percent of the American rice market. The rice is first fermented to develop a nutty flavour and to ease hulling.

Maize

Corn was originally cultivated in Central America and became the staple of the Incas of Peru, the Mayas and Aztecs of Mexico and early cliff dwellers of the American Southwest. Columbus brought corn back to Europe where it became a popular crop in the south. Different types of maize are classified on the basis of their protein content and the hardness of the kernel.

These include pop, flint, flour, Indian and sweet corns. Much of the niacin in corn is in a bound form and this led to pellagra in areas where corn became the food staple. It was not until the late 1920s that pellagra was identified as a vitamin deficiency. The traditional practice by early American natives, of boiling corn in 5 percent lime or ashes, releases bound niacin making it available as a nutrient.

Millet and Sorghum

Millet and sorghum are often grouped together because their growing conditions, processing and uses are similar. Millets are native to Africa or Asia and have been cultivated for more than 6 000 years. Millets grow well in arid regions with poor soils and are valued for their relatively high protein content among the cereals. Sorghum originated in East Africa and today is an important food crop in Africa, Asia, India and China where it is made into porridge, unleavened bread ("roti" in India) and beer. Whole grain sorghum flour has a relatively short shelf-life and production of low-fat sorghum flour requires removal of about 20 percent of the grain weight by abrasion (Perten, 1983). In North America, millet and sorghum are used primarily as livestock feed.

Barley

In the United States, barley is mostly used for feed, brewing and alcohol production with only about 2 percent used for human food. Barley flour is produced by abrasion dehulling, followed by milling of the "pearled" barley. Shellenberger (1980) reviewed the uses of barley flour and grits in products such as soups, dressings and baby foods.

Oats

About 95 percent of the world oat crop is used for livestock feed (McGee 1984). However, oat consumption as human food has recently increased to 19 percent in the United States (Bowers, 1992), perhaps due to the reported health benefits of the soluble fibre of oats. Oats thrive in a moist, cool climate and became an important crop in Northern Europe at the beginning of the seventeenth century. Oats have a relatively minor status among cereals because they are more difficult to process and are unstable due to their high lipid content and lipase activity. Reports of the possible blood cholesterol lowering effect of oat bran have increased the popularity of its use for human food in developed countries.

Rye

Rye appears to have originated in central Asia around 4 000 BC as a weed contaminating barley and wheat and became domesticated around the Baltic Sea in 400 BC where it grows well in a cool, moist climate with poor soil (McGee, 1984). It was traditionally a popular grain for bread making in Northern and Eastern Europe. Rye flour has a relatively low gluten content compared to wheat flour, but contains a unique class of carbohydrates (pentosans) that facilitate bread making. The milling of rye yields a flour that is classified based on colour or ash content (Rozsa, 1976).

BOTANICAL STRUCTURE OF CEREALS

All cereals belong to the taxonomic family known as the *Gramineae*. Other globally important crops in the *Gramineae* family include sugar cane and bamboo. Botanically speaking, cereal grains called caryopse, are types of "dry" fruit (Figure 3). A botanical fruit is defined as the ripened ovary or the ovary and adjoining parts. The flesh of the fruit may originate from the floral receptacle, from carpillary tissue, or from extrafloral structures, such as bracts. The caryopse fruit structure differs from that of other fruit (e.g. fleshy fruits) in that a thin, dry fruit wall is used together with the seed coat. Cereals are sometimes thought of as seeds by the layman since the bulk of their tissue is the true seed; i.e. the part resulting from sexual reproduction of the plant. Seeds are produced within the fruit, which serves to protect and aid their dispersal for propagation in a variety of ways for different plants. The seed is the result of sexual reproduction of the plant when the flower is fertilized and is the organ of propagation. Kernel structure is important with respect to minimizing damage during grain harvest, drying, handling, storage, milling, germination and in enhancing nutritional value (Pomeranz and Bechtel, 1978). Selection of cultivars with a large embryo or without hulls for example, will improve the protein content if the entire grain is consumed.

The seed portion of cereals consists of numerous components (Figure 3) which basically include three parts: a seed coat or testa (bran), storage organ or nutritive reserve for the seed (endosperm), and a miniature plant or germ. The fruit tissue consists of a layer of epidermis and several thin inner layers a few cells thick. The aleurone layer which is just below the seed coat, is only a few cells thick, but is rich in oil, minerals, protein and vitamins. Starch and protein are located in the endosperm, which represents the bulk of the grain and is sometimes the only part of the cereal consumed. Starch is housed in the form of subcellular structures called granules that are embedded in a matrix of protein. The developing endosperm contains protein bodies, which become a continuous phase as the grain matures. There is generally a gradient of more protein and less starch per cell from the outer to the inner region of the endosperm. The diameter, shape, size distribution and other characteristics of starch granules vary with different cereals. Starch granules range in size from 3-8 μm in rice, 2-30 μm in corn and 2-55 μm in wheat. Reserve proteins in the endosperm are in the form of smaller "protein bodies" that range in size from 2-6 μm, become disordered, and adhere to the starch granules in the mature grain of species like wheat.

MAJOR CHEMICAL COMPONENTS OF CEREAL GRAINS

Compositionally, cereals consist of 12-14 percent water, 65-75 percent carbohydrate, 2-6 percent lipid and 7-12 percent protein. Cereals are quite similar in gross composition being low in protein and high in carbohydrate (Table 2). Oats and maize however contain relatively large amounts of lipid. Oats contain at least 10 percent lipids, one-third of which are polar (phospho- and galacto-lipids). The lipid content of maize ranges between 0.4 percent and 17 percent, most of which are triacylglycerides (Eliasson and Larsson, 1993). Different cultivars of a given type of cereal exhibit compositional variability.
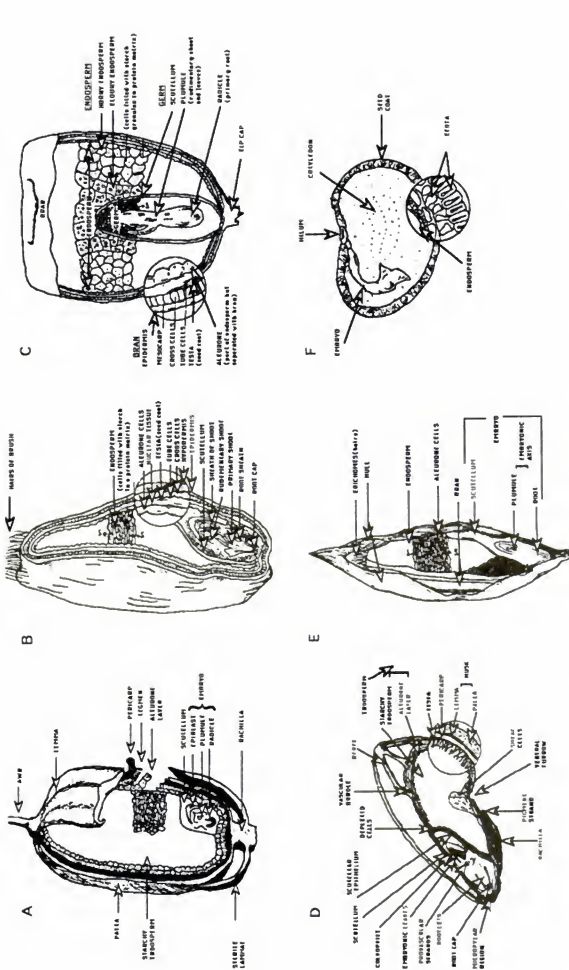
Figure 3 –Diagrammatic illustrations of cereal grain (caryopis fruit): A. Rice; B. Wheat, C. Maize, D. Barley, and E. Oat. An example of a true seed crop (soybean) is shown for comparison. From Haard and Chism (1996)

The chemical components of cereals are not uniformly distributed in the grain (Table 3). Hulls and bran are high in cellulose, pentosans and ash. The aleurone layer of wheat contains 25 times more minerals than the endosperm, whereas the lipids are generally concentrated in the aleurone and germ. The endosperm, which contains mostly starch, has a lower protein content than the germ and the bran, and is low in fat and ash.

Proteins

Early workers divided the proteins of wheat into four solubility classes called Osborne fractions: albumins, which are water soluble; globulins, which are soluble in salt solutions, but insoluble in water; gliadins, which are soluble in 70-90 percent alcohol; and glutenins, which are insoluble in neutral aqueous solutions, saline, solutions, or alcohol. The respective protein fractions from wheat are also applicable to other cereals and are generally known as albumins, globulins, prolamines, and glutelins. The distribution of these protein fractions varies among different cereals (Table 4). There is considerable variation in the solubility classes among the cereals and also to some extent within each species of cereal. Albumins range from 4 percent in maize to 44 percent in rye, globulins from 3 percent in maize to 55 percent in oats, prolamins from 2 percent in rice to 55 percent in maize, and the glutelins from 23 percent in oats to 78 percent in rice.

Table 2. Proximate composition of cereal grains[1]

CEREAL	CRUDE PROTEIN	CRUDE FAT	ASH	CRUDE FIBRE	AVAILABLE CARBOHYDRATE
Brown Rice	7.3	2.2	1.4	0.8	64.3
Sorghum	8.3	3.9	2.6	4.1	62.9
Rye	8.7	1.5	1.8	2.2	71.8
Oats	9.3	5.9	2.3	2.3	62.9
Maize	9.8	4.9	1.4	2.0	63.6
Wheat	10.6	1.9	1.4	1.0	69.7
Barley	11.0	3.4	1.9	3.7	55.8
Pearl Millet	11.5	4.7	1.5	1.5	63.4

Table 3. Distribution of major components of wheat grain [1]

FRACTION	PROPORTION	PROTEIN	LIPID	MINERALS
Whole grain	100	12	2.0	2.0
Endosperm	80	10	1.2	0.6
Aleurone	8	18	8.5	15.0
Seed coat	8.5	6	1.0	3.5

[1] Percent dry weight; from Alais and Linden (1991)

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Among the Osborne fractions in cereals, the prolamin fraction has been the most studied (Eliasson and Larsson, 1993). This fraction is called gliadin in wheat, secalin in rye, hordein in barley, avenin in oats, and zein in maize. The fraction includes several protein bands when analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis under both reducing and non-reducing conditions. The high molecular weight subunits of prolamins constitute a higher percentage of the total in wheat than in other cereals (Shewry and Mifflin, 1985). The baking quality of wheat flour from different varieties is influenced by the glutelin content (Eliasson and Larsson, 1993); however, rice flour, with it's high glutelin fraction, does not form gluten. The albumin and globulin fractions of cereals are also a complex mixture of proteins; however, they are of relatively low molecular masses and remain unchanged in size following reduction of their disulfide bonds. It is now recognized that cereal proteins exhibit biochemical polymorphism and can be distinguished through electrophoresis of the gliadin fraction (Alais and Linden, 1991).

Table 4. Distribution of proteins in Osborne solubility classes [1]

CEREAL	ALBUMIN	GLOBULIN	PROLAMIN	GLUTELIN
Wheat	9-15	6-7	33-45	40-46
Rye	10-44	10-19	21-42	25-40
Barley	12	8-12	25-52	52-55
Oats	10-20	12-55	12-14	23-54
Rice	5-11	10	2-7	77-78
Sorghum	4	9	48	37
Maize	4-8	3-4	47-55	38-45

[1] Data from Eliasson and Larsson (1993); Alais and Linden (1991)

Osborne fractions from different cereals exhibit similarities in the proportions of the amino acids glutamine (Glx), proline (Pro), glycine (Gly), and cysteine (Cys) (Wiesner *et al.* 1980) (Table 5). Glutamine, proline and glycine are principal amino acids in all cereal protein fractions. Differences in amino acid composition from wheat cannot explain the poorer baking performance of cereals such as rye and barely.

Sulfhydryl-disulfide interchanges are the major reactions responsible for the formation of wheat dough. The gliadin and glutenin fractions of wheat represent 80-85 percent of the wheat endosperm protein and these fractions together make up the gluten. It appears that a specific pattern of interaction between low molecular weight glutenin (<90 KDa) and high molecular weight glutenin (> 90 Kda) is important for the development of a visco-elastic gluten (MacRitchie, 1992).

Carbohydrates

In general, carbohydrates constitute about 75 percent of the solid content of cereals. In cereals, as in other plant tissues, carbohydrates are localized in (1) the cell wall, (there are

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especially thickened walls in supporting tissues of husk and seed coat) (2) plastids, where starch constitutes the largest proportion of carbohydrates in all cereals, and (3) in vacuoles or the cytoplasm.

The principal constituents of cell walls are cellulose, hemicelluloses, pectins, and lignin. The hemicelluloses are a heterogeneous group of polysaccharides that contain numerous kinds of hexose and pentose sugars and in some cases residues of uronic acids. These polymers are classified according to the predominant sugar residue and are individually referred to as xylans, arabinogalactans, etc. Cell walls are the main components of "dietary fibre". Fibre constituents may reduce the biological availability of protein, minerals and other nutrients, such as vitamin B1 in rice. On the other hand, there is now considerable evidence for the beneficial role played by fibre in health and disease (Anderson *et al*. 1990). Dietary fibre absorbs water and provides roughage for the bowels, assisting intestinal transit. The crude fibre content of cereals varies a great deal, ranging from as low as 0.5 percent for brown rice to as high as 10.9 percent for oats (Chaven and Kadam, 1989; Eliasson and Larsson, 1993).

The principal carbohydrate of all cereals is starch, representing 56 percent (oats) to 80 percent (maize) of the grain dry matter (Eliasson and Larsson, 1993). Cereal starches are similar in composition, having 74-79 percent amylopectin, 25-30 percent amylose, and 1 percent lipid. High-amylose and high amylopectin ("waxy") cereal cultivars have also been developed. The baking performance of cereal starches of similar amylose and amylopectin contents (1:4) are however different, with maize starch exhibiting particularly poor qualities (Hoseney *et al*. 1971) (Table 6). The presence of lipid in cereal starches is a distinguishing feature of these starches (Morrison *et al*. 1984). Gelatinization temperatures of different cereal starches also show considerable variation. For example, maize and rice starches gelatinize at temperatures 10-20^{0}C higher than wheat, rye or oat starches (Eliasson and Larsson, 1993). There is also considerable variation in the transition temperatures of starches within species. Interactions of cereal starches with protein and lipids are known to influence physicochemical characteristics such as gelatinization and retrogradation.

Table 5. Partial amino acid composition (mole percent) of Osborne fractions from different cereals[1]

	WHEAT	RYE	BARLEY	OATS	RICE	MAIZE
ALBUMINS						
AMINO ACID Glx	21	23	14	13	15	13
Pro	10	12	8	6	5	9
Gly	7	7	10	13	10	10
Cys	3	2	4	7	2	2
Lys	3	3	4	5	5	4
GLOBULINS						
AMINO ACID						
Glx	16	17	13	16	15	11
Pro	7	8	7	5	6	6
Gly	9	9	10	10	10	11
Cys	4	2	3	2	4	3
Lys	4	4	5	5	3	5
PROLAMINS						
AMINO ACID						
Glx	38	36	36	35	20	20
Pro	17	19	23	10	5	10
Gly	3	5	2	3	6	3
Cys	2	2	2	3	1	1
Lys	1	1	1	1	1	trace
GLUTELINS						
AMINO ACID						
Glx	31	20	25	19	16	16
Pro	12	10	15	6	5	12
Gly	8	9	7	8	8	7
Cys	1	1	1	1	1	2
Lys	2	4	3	3	3	2

[1] Data from Wiesner *et al.* (1980)

Table 6. Bread loaf volume with various cereal starches [1]

STARCH SOURCE	LOAF VOLUME (ml)
Wheat	80
Barley	78
Rye	77
Rice	68
Oats	58
Maize	48

[1] Data from Eliasson and Larsson, (1993).
Wheat flour was reconstituted with indicated cereal starch

Flours prepared from oats, barley, and rye contain a relatively high percentage (5-25 percent of total carbohydrates) of non-starch polysaccharides. The pentosan fraction of cereals is a complex mixture of branched polysaccharides with an arabinoxylan backbone containing small amounts of glucose and ferulic acid. Rye flour contains a relatively high content of water-soluble pentosans, which are capable of absorbing large amounts of water to form gels. Wheat contains fewer pentosans than rye, and has a higher xylose/arabinose ratio than does rye (Eliasson and Larsson, 1993). The ß-glucans of barley play an important role in beer production and those of oats are of interest because of their health benefits as dietary fibre. Chemically, these molecules contain both $(1 \rightarrow 3)$ and $(1 \rightarrow 4)$ linkages of D-glucopyranose. Their high viscosity and slimy consistency can cause wort filtration problems in brewing. The molecular weights of ß-glucans from rye, oats, and barley are known to differ (Woods *et al*. 1991).

Lipids

Oats and maize are unique amongst the cereals in that cultivars may contain a relatively high lipid content, e.g. >10 percent for oats and as high as 17 percent for some maize cultivars compared to about 2-3 percent for wheat and most other cereals. The polar lipid content of oats is greater than that of other cereals since much of the lipid fraction is contained within the endosperm. In most cereals the lipid fraction is concentrated in the germ and in the bran milling fractions (Table 7). About one-third of oat lipids are polar (8-17 percent glycolipids and 10-20 percent phospholipid). On the other hand, maize lipids are predominately acyltriglycerides in cultivars having a high total lipid content. The distribution of lipid classes is similar in wheat, barely and rye which contain about 65-78 percent non-polar lipid, 7-13 percent galactolipid and 15-26 percent phospholipids (Morrison, 1978). In wheat, the glycolipids play an important role in gluten development during bread making (Pomeranz and Chung, 1978).

Table 7. Crude lipid contents of rice and wheat milling fractions [1]

GRAIN/FRACTION	% CRUDE FAT DWB
Rice	
Brown rice	2-4
Milled endosperm	<1
Bran	15-22
Embryo	15-24
Polished	9-15
Wheat	
Whole grain	2
Pericarp	1
Aleurone	9
Starch endosperm	1
Germ	10

[1] Adapted from Haard (1996)

The major fatty acids in cereal grain lipids are linoleic, oleic and palmitic (Haard and Chism, 1996) (Table 8).

Table 8. Principal fatty acids (percentage) of some cereal oils [1]

FATTY ACID	CORN	WHEAT	RYE	RICE
C:14:0	-	3	6	1
C:16:0	6	18	11	28
C:18:0	2	7	4	2
C:18:1	44	31	18	35
C:18:2	48	57	35	39
C:18:3	-	4	7	3

[1] Adapted from Haard (1996)

NUTRITIONAL QUALITY OF CEREALS

Cereals, together with oil seeds and legumes, supply a majority of the dietary protein, calories, vitamins, and minerals to the bulk of populations in developing nations (Chaven and Kadam, 1989). Some components of cereal nutritive value are summarized in Table 9. The following synopsis of cereal nutrition has been adopted from a review by Chavan and Kadam (1989). Cereal grains are low in total protein compared to legumes and oilseeds. Lysine is the first limiting essential amino acid for man, although rice, oats and barley contain more lysine than other cereals. Corn protein is also limiting in the essential amino acid tryptophan, while other cereals are often limiting in threonine. The annual global yield of essential amino acids from major cereals has been compared to a hypothetical population of 3 billion adults and 2 billion children (Phillips, 1997) (Table 10). Accordingly, if all cereals were effectively and

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fully utilized for human consumption they would more than meet humanity's needs for essential amino acids

Table 9. Comparative nutritive value of cereal grains [1]

FACTOR	Wheat	Maize	Brown rice	Barley	Sorghum	Oat	Pearl millet	Rye
Available CHO (%)	69.7	63.6	64.3	55.8	62.9	62.9	63.4	71.8
Energy (kJ/100 g)	1570	1660	1610	1630	1610	1640	1650	1570
Digestible energy (%)	86.4	87.2	96.3	81.0	79.9	70.6	87.2	85.0
Vitamins (mg/100 g)								
Thiamin	0.45	0.32	0.29	0.10	0.33	0.60	0.63	0.66
Riboflavin	0.10	0.10	0.04	0.04	0.13	0.14	0.33	0.25
Niacin	3.7	1.9	4.0	2.7	3.4	1.3	2.0	1.3
Amino acids (g/16 g N)								
Lysine	2.3	2.5	3.8	3.2	2.7	4.0	2.7	3.7
Threonine	2.8	3.2	3.6	2.9	3.3	3.6	3.2	3.3
Met. and Cys.	3.6	3.9	3.9	3.9	2.8	4.8	3.6	3.7
Tryptophan	1.0	0.6	1.1	1.7	1.0	0.9	1.3	1.0
Protein quality (%)								
True digestibility	96.0	95.0	99.7	88.0	84.8	84.1	93.0	77.0
Biological value	55.0	61.0	74.0	70.0	59.2	70.4	60.0	77.7
Net protein utilil.	53.0	58.0	73.8	62.0	50.0	59.1	56.0	59.0
Utilization protein	5.6	5.7	5.4	6.8	4.2	5.5	6.4	5.1

[1] Adapted from Chavan and Kadam (1989)

Table 10. Annual global yield of essential amino acids from major cereals and global human requirements[1]

AMINO ACID	Wheat	Rice	Maize	Sorghum	Total	Human Requirement	% Provided
	kg x 1000	kg x 1000	kg x 1000	kg x 1000	kg x 1000	kg x 1000	
Lysine	130	85	104	8	327	223	147
Met. and Cystine	162	78	135	11	386	158	244
Threonine	132	90	140	13	376	138	272
Isoleucine	219	85	143	14	461	158	292
Tryptophan	60	26	27	4	118	34	347
Valine	209	127	189	16	540	148	365
Leucine	313	189	484	47	1033	230	449
Phen. and Tyr.	404	199	339	30	972	164	593

[1] Adapted from Phillips (1997). Yield of amino acid from cereals is global production x amino acid profile x digestibility; human requirement is based on hypothetical population of 3 billion adults and 2 billion children; percentage provided is the calculated global production from wheat, rice, maize and sorghum divided by estimated global requirement by the hypothetical human population.

Barley, sorghum, rye and oat proteins have lower digestibilities (77-88 percent) than those of rice, maize and wheat (95-100 percent). The biological value and net protein utilization of cereal proteins is relatively low due to deficiencies in essential amino acids and low protein availability (Chaven and Kadam, 1989). The digestible energy of rice is significantly better than that of other cereals (Table 9).

Cereals also provide B-group vitamins and minerals, although refining results in losses of these nutrients (Miller, 1996) (Table 11). The endosperm of wheat contains only about 0.3 percent ash. Phosphorous, potassium, magnesium, calcium and traces of iron and other minerals are found in cereals (Bowers, 1992). Barley and wheat provide 50 and 36 mg Ca/100 g respectively. Barley provides 6 mg of iron per 100 g; millet provides 6.8; oats, 4.6 and wheat, 3.1. In contrast, soybeans provide more of these nutrients, i.e. Ca (210 mg/100 g) and Fe (7 mg/100 g) (Haard and Chism, 1996). Some grains, notably barley, sorghum, and oats, contain appreciable amounts of crude fibre (Table 2) and are referred to as coarse grains. The nutritive and sensory value of cereal grains and their products are, for the most part, inferior to animal food products. Methods that can be employed to improve the nutritive value of cereals include traditional genetic selection, genetic engineering, amino acid and other nutrient fortification, complementation with other proteins (notably legumes), milling, heating, germination and fermentation.

Table 11. Influence of milling on the trace mineral content of wheat [1]

MINERAL	Whole wheat mg/100 g	White Flour mg/100 g	Wheat Germ mg/100 g	Wheat bran mg/100 g	Loss %
Iron	4.3	1.1	6.7	4.7-7.8	76
Zinc	3.5	0.8	10.1	5.4-13.0	78
Manganese	4.6	0.7	13.7	6.4-11.9	86
Copper	0.5	0.2	0.7	0.7-1.7	68
Selenium	0.06	0.05	0.11	0.05-0.08	16

[1] Adapted from Miller (1996)

ANTINUTRIENTS AND TOXIC COMPONENTS IN CEREALS

Cereals and other plant foods may contain significant amounts of toxic or antinutritional substances. In this regard, legumes are a particularly rich source of natural toxicants including protease inhibitors, amylase inhibitors, metal chelates, flatus factors, hemagglutinins, saponins, cyanogens, lathyrogens, tannins, allergens, acetylenic furan and isoflavonoid phytoalexins (Pariza, 1996). Most cereals contain appreciable amounts of phytates, enzyme inhibitors, and some cereals like sorghum and millet contain large amounts of polyphenols and tannins (Salunkhe *et al.* 1990). Some of these substances reduce the nutritional value of foods by interfering with mineral bioavailability, and digestibility of proteins and carbohydrates. Since legumes are often consumed together with cereals, proper processing of cereal-legume mixtures should eliminate these antinutrients before consumption (Chaven and Kadam, 1989; Reddy and Pierson, 1994). Relatively little is known about the fate of antinutrients and toxicants in traditional fermented foods.

Phytates

Phytic acid is the 1,2,3,4,5,6-hexaphosphate of myoinositol that occurs in discrete regions of cereal grains and accounts for as much as 85 percent of the total phosphorous content of these grains. Phytate reduces the bioavailability of minerals, and the solubility, functionality and digestibility of proteins and carbohydrates (Reddy *et al.* 1989). Fermentation of cereals reduces phytate content via the action of phytases that catalyze conversion of phytate to inorganic orthophosphate and a series of myoinositols, lower phosphoric esters of phytate. A 3-phytase appears to be characteristic of micro-organisms, while a 6-phytase is found in cereal grains and other plant seeds (Reddy and Pierson, 1994).

Tannins

Oligomers of flavan-3-ols and flavan-3, 4-diols, called condensed tannins, occur widely in cereals and legumes (Haard and Chism, 1996). These compounds are concentrated in the bran fraction of cereals (Salunkhe *et al.* 1990). Tannin-protein complexes can cause inactivation of digestive enzymes and reduce protein digestibility by interaction of protein substrate with ionizable iron (Salunkhe *et al.* 1990). The presence of tannins in food can

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therefore lower feed efficiency, depress growth, decrease iron absorption, damage the mucosal lining of the gastrointestinal tract, alter excretion of cations, and increase excretion of proteins and essential amino acids (Reddy and Pierson, 1994). Dehulling, cooking and fermentation reduce the tannin content of cereals and other foods.

Saponins

These sterol or triterpene glycosides occur widely in cereals and legumes (Shiraiwa *et al.* 1991). Their hemolytic activity and surface-active properties detect saponins. Although the notion that they are detrimental to human health has been questioned (Reddy and Pierson, 1994), they have been reported to cause growth inhibition (Cheeke, 1976).

Enzyme Inhibitors

Protease and amylase inhibitors are often found in seed tissues including cereal grains. Trypsin-, chymotrypsin-, subtilisin-inhibitor, and cysteine-protease inhibitors are present in all major rice cultivars grown in California, although the individual inhibitor amounts are quite variable and are concentrated in the bran fraction (Izquerdo-Pulido *et al.* 1994). They are believed to cause growth inhibition by interfering with digestion, causing pancreatic hypertrophy and metabolic disturbance of sulphur amino acid utilization (Reddy and Pierson, 1994). Although these inhibitors tend to be heat stable, there are numerous reports that trypsin inhibitor, chymotrypsin inhibitor, and amylase inhibitor levels are reduced during fermentation (Chaven and Kadam, 1989; Reddy and Pierson, 1994).

FERMENTED CEREALS

Animal or plant tissues subjected to the action of micro-organisms and/or enzymes to give desirable biochemical changes and significant modification of food quality are referred to as fermented foods (Campbell-Platt, 1994). Fermentation is the oldest known form of food biotechnology; records of barley conversion to beer date back more than 5 000 years (Borgstrom, 1968). According to Steinkraus (1995), the traditional fermentation of foods serves several functions:

"1.   Enrichment of the diet through development of a diversity of flavours, aromas, and textures in food substrates.
2.   Preservation of substantial amounts of food through lactic acid, alcoholic, acetic acid, and alkaline fermentation.
3.   Enrichment of food substrates biologically with protein, essential amino acids, essential fatty acids, and vitamins.
4.   Detoxification during food fermentation processing.
5.   A decrease in cooking times and fuel requirements."

Aside from alcoholic fermentation and the production of yoghurt and leavened bread, food fermentation continues to be important primarily in developing countries where the lack of resources limits the use of techniques such as vitamin enrichment of foods, and the use of energy and capital intensive processes for food preservation. The technology of producing many indigenous fermented foods from cereals remains a household art in these countries (Chaven and Kadam, 1989). Prospects for applying advanced technologies to indigenous

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fermented foods (Wood, 1994) and for the production of value-added additive products, such as colours, flavours, enzymes, antimicrobials, and health products (Cook, 1994) during food fermentation have been reviewed.

Special mention should be made of the microbiological risk factors associated with fermented foods. The safety of fermented foods has been recently reviewed (Nout, 1994). Cases of food-born infection, and intoxication due to microbial metabolites such as mycotoxins, ethyl carbamate, and biogenic amines have been reported in fermented foods. Major risk factors include the use of contaminated raw materials, lack of pasteurization, and use of poorly controlled fermentation conditions. On the other hand, non-toxigenic micro-organisms can serve to antagonize pathogenic micro-organisms and even degrade toxic substances such as mycotoxins (Nakazato *et al*. 1990) in fermented foods.

Indigenous Fermented Cereal Foods

Most bacterial fermentations produce lactic acids; while yeast fermentations result in alcohol production. Many of the indigenous fermentation products of cereals are valued for the taste and aroma active components produced and are used as seasonings and condiments. Chaven and Kadam (1989) compiled a summary of flavour compounds formed in such products. A number of fermented products utilize cereals in combination with legumes, thus improving the overall protein quality of the fermented product. Cereals are deficient in lysine, but are rich in cystine and methionine. Legumes on the other hand are rich in lysine but deficient in sulphur containing amino acids. Thus, by combining cereals with legumes, the overall protein quality is improved. The Chinese concept of "fan" (rice) and "tsai" (other vegetables) for a balanced and interesting diet is seen throughout the world (Campbell-Platt, 1994).

Importance and Benefits of Fermented Cereals

Fermented foods contribute to about one-third of the diet worldwide (Campbell-Platt, 1994). Cereals are particularly important substrates for fermented foods in all parts of the world and are staples in the Indian subcontinent, in Asia and in Africa. Fermentation causes changes in food quality indices including texture, flavour, appearance, nutrition and safety. The benefits of fermentation may include improvement in palatability and acceptability by developing improved flavours and textures; preservation through formation of acidulants, alcohol, and antibacterial compounds; enrichment of nutritive content by microbial synthesis of essential nutrients and improving digestibility of protein and carbohydrates; removal of antinutrients, natural toxicants and mycotoxins; and decreased cooking times.

The content and quality of cereal proteins may be improved by fermentation (Wang and Fields, 1978; Cahvan *et al*. 1988). Natural fermentation of cereals increases their relative nutritive value and available lysine (Hamad and Fields, 1979) (Figure 4). Bacterial fermentations involving proteolytic activity are expected to increase the biological availability of essential amino acids more so than yeast fermentations, which mainly degrade carbohydrates (Chaven and Kadam, 1989). Starch and fibre tend to decrease during fermentation of cereals (El-Tinay *et al*. 1979). Although it would not be expected that fermentation would alter the mineral content of the product, the hydrolysis of chelating agents such as phytic acid during fermentation, improves the bioavailability of minerals. Changes in the vitamin content of

cereals with fermentation vary according to the fermentation process, and the raw material used in the fermentation. B group vitamins generally show an increase on fermentation (Chavan *et al*. 1989) (Figure 5). During the fermentation of maize or kaffircorn in the preparation of kaffir beer, thiamine levels are virtually unchanged, but riboflavin and niacin contents almost double (Steinkraus, 1994).

Reddy and Pierson (1994) reviewed the effect of fermentation on antinutritional and toxic components in plant foods. Fermentation of corn meal and soybean-corn meal blends lowers flatus producing carbohydrates, trypsin inhibitor and phytates (Compreeda and Fields, 1981; Chompreeda and Fields, 1984). However, fermentation of cereals with fungi, such as *Rhizopus oligosporus*, has been reported to release bound trypsin inhibitor, thus increasing its activity (Wang *et al*. 1972). Fungal and lactic acid fermentation have also been reported to reduce aflatoxin B_1, sometimes by opening of the lactone ring, which results in complete detoxification (Nout, 1994).

Another benefit of fermentation is that frequently the product does not require cooking, or the heating time required for preparation is greatly reduced (Steinkraus, 1994).
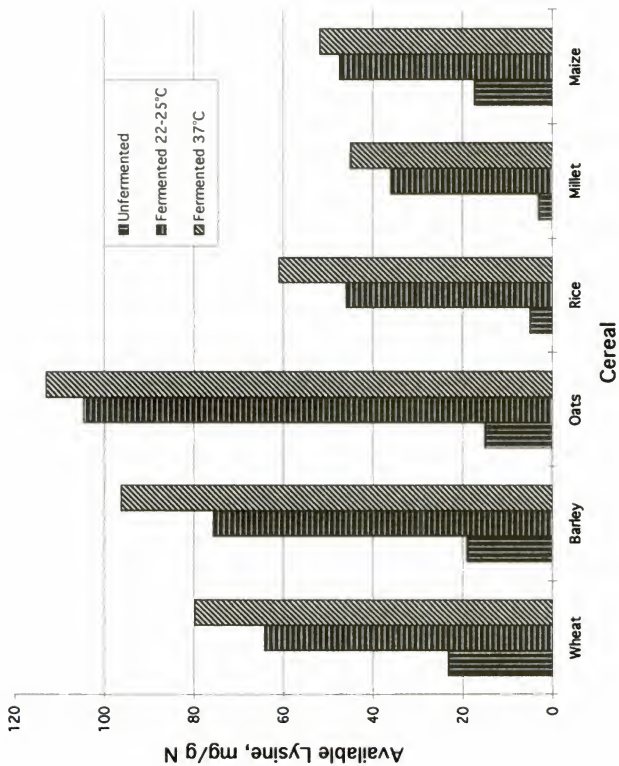
Figure 4 – Influence of natural fermentation of cereals on available lysine. Data from Hamad and Fields (1979)
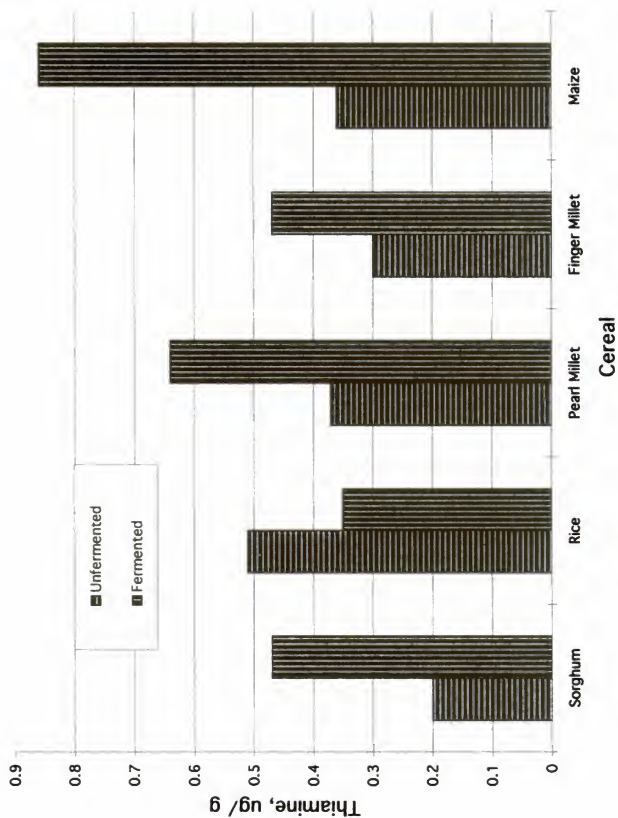
Figure 5 – Influence of natural fermentation of cereals on the thiamine content. Data from Chavan and Kadam (1989)

Need for Additional Research

Some advantages of traditional fermentation are that they are labour-intensive, integrated into village life, familiar, utilize locally produced raw materials, inexpensive, have barter potential and the subtle variations resulting, add interest and tradition to local consumers. From this perspective, research leading to new fermentation technologies should be sensitive to social and economic factors in developing countries. Rapid displacement of traditional foodstuffs in developing countries with technologies developed in more affluent countries may result in centralized production, distribution problems, less local involvement in food processing, less employment in some areas, less nutritionally adequate substitutions in raw materials, displacement of traditional arts, loss of unique local know-how, dependence on importation of equipment and materials, initially require the use of outside consultants, and may otherwise not meet local needs as fully as traditional fermented products. On the other hand, indigenous fermentation may have a number of problems, i.e. they are uncontrolled and often unhygienic, labour intensive, seen as primitive by some people, are normally not integrated into the economic mainstream, difficult to tax, have limited export potential (Wood, 1994) and in some cases, the impact on nutritive value and safety is questionable.

Specific microflora involved with indigenous fermentation is, in many cases, not known at this time. Specific information on microflora appears to be lacking for several indigenous fermented cereal products. The microbiology of many of these fermentations is undoubtedly quite complex. Much indigenous cereal fermentations involve the combined action of bacteria, yeast and fungi. Some microflora may participate in parallel while others may participate in a sequential manner with a changing dominant flora during the course of the fermentation. The specific microflora involved may vary somewhat from village to village and from family to family within the same village. The identification of specific microflora involved is needed to amplify and control such positive factors as the excretion of lysine by strains of *Lactobacillus plantarum* (Newman and Sands, 1984) and the metabolic detoxification of mycotoxins by *Rhizopus oryzae* (Nout, 1994); as well as to minimize or prevent negative factors such as growth and metabolism of pathogenic and toxinogenic bacteria, e.g. bongrek acid and toxoflavin formation by *Pseudomonas cocovenenans* (Ko, 1985). Identifying and providing a practical means of using appropriate starter cultures is advantageous due to the competitive role of micro-organisms and their metabolites in preventing growth and metabolism of unwanted micro-organisms. A strong starter may reduce fermentation times, minimize dry matter losses, avoid contamination with pathogenic and toxigenic bacteria and moulds, and minimize the risk of incidental microflora causing off-flavour, etc. According to Nout (1994) optimization of starter cultures may be achieved by either conventional selection or mutation, or by recombinant-DNA techniques to result in increased levels of safety. Relatively little is known of the contribution of microflora to the formation of desired flavour notes during such fermentation. Genes for flavour and other beneficial enzymes that come from incidental microflora may be incorporated into starter bacteria to facilitate more subtle and ancillary aspects of the fermentation along with primary events such as lactic acid production, thus preserving the distinctive nature of products made in different regions.

The contribution of specific enzymes to indigenous cereal fermentation is perhaps even less understood than that of micro-organisms. It is likely that there is considerable

synergy between complementary enzymes from the cereal itself and from the micro-organisms. One known example of this is the reduction of phytates resulting from 6-phytases of cereal origin and 3-phytases of microbial origin (Reddy and Pierson, 1994). Another is the synergy of cereal enzymes and yeast in bread making (Fox and Mulvihill, 1982). It is interesting that fermentation of cereals (e.g. bread making and brewing) in the Western world was adversely affected in some ways by the introduction of modern dehydration and storage techniques that minimized fungal contamination and incipient germination. Partial germination of cereal in the field and contamination with otherwise innocuous fungal contaminants contribute enzymes, notably α-amylase and proteases, which aid this fermentation. Today, essentially all beer production and continuous bread making in the West is achieved with the aid of added enzymes (Tucker and Woods 1995). A similar situation may occur in developing countries, i.e. as improvements in cereal handling are introduced to minimize post-harvest losses and mycotoxin formation, the otherwise improved crop may be less suitable in some ways for traditional fermentation. Hence, basic information is needed on the contribution of cereal enzymes and other constituents to indigenous fermentation. With this information in hand, consideration can then be given to use of enzyme supplements and other additives to improve the rate and quality of fermentation.

Another consideration for future research is the contribution of the aforementioned enzyme inhibitors in cereal fermentation. In addition to their already discussed significance as antinutrients in the finished product, protease, amylase and other enzyme inhibitors are expected to influence the rate and extent of important bioconversions that occur during indigenous fermentation. The concentration and spectrum of enzyme inhibitors varies considerably between cereal cultivars (Izquirdo-Pulido *et al.* 1994). Notwithstanding the benefits of the continual introduction of new cereal varieties (Meikle and Scarisbrick, 1994), given the genes for enzyme inhibitors are part of the defensive system of plants against insects and other pests, it is possible that introduction of "improved" cereal cultivars in developing countries may adversely affect the utility of cereals for indigenous fermentation. For this reason, basic research on the participation of cereal enzyme inhibitors in the process may provide useful insights on the need for including tests for inhibitors prior to introducing new varieties in areas that extensively utilise cereal fermentation to produce staple foodstuffs.

As pointed out by Wood (1994), there is a possible backlash if consumers in developing countries abandon traditional fermented foods for "smart", sophisticated products popularized in Europe and America. For example, the replacement of indigenous fermented cereal drinks with cola beverages could have a significant negative impact on daily nutrition of many consumers in developing countries. Study of traditional fermentation will undoubtedly yield new information that will expand our global knowledge of science and impact technology throughout the world. Thus, basic research of indigenous cereal fermentation will lead to "inward" as well as "outward" technology transfer.

CONCLUSIONS

Traditional fermentations are likely to remain an important part of global food supply; many may evolve into fermentations involving the use of starter cultures, enzyme additives and controlled environmental conditions, and others may benefit from genetic modification of the cereal or starter bacteria.

Further research should be directed towards identifying the benefits and risks associated with specific indigenous fermented cereals, elucidating the contributions of micro-organisms, enzymes and other cereal constituents in the fermentation process; and developing starter cultures, unique microbial strains for nutritive improvement and detoxification, and testing of new cereal varieties for their suitability as fermentation substrates.

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CHAPTER 2

CEREAL FERMENTATIONS IN AFRICAN COUNTRIES

INTRODUCTION

Africa is one of the lowest producers of cereals globally (Table 1). Major cereals grown in Africa include maize, rice, sorghum and millet (Table 1). Cereals are more widely utilized as food in African countries than in the developed world. In fact, cereals account for as much as 77 percent of total caloric consumption in African countries (Mitchell and Ingro, 1993), and contribute substantially to dietary protein intake in a number of these countries. A majority of traditional cereal-based foods consumed in Africa are processed by natural fermentation. Fermented cereals are particularly important as weaning foods for infants and as dietary staples for adults.

This Chapter reviews the production of a number of traditionally fermented cereals in African countries.

Table 1. Production of cereals (tonnes) in Sub-Saharan Africa

	1997	Percentage of world production
Maize	24 798	4.2
Millet	10 950	38.9
Rice	11 321	2.0
Sorghum	17 400	28.2
Wheat	3 140	0.5

Source: FAO, 1997

CLASSIFICATION OF FERMENTED CEREALS

Fermented cereal-based food products produced in African countries can be classified on the basis of either the raw cereal ingredients used in their preparation, or the texture of the fermented product.

Classification on the basis of raw cereal ingredients:

a) wheat based foods, e.g. bouza, kishk
b) rice based foods, e.g. busa
c) maize based foods, e.g. ogi, bread, kenkey
d) millet based foods, e.g. kunuzaki
e) sorghum based foods, e.g. pito, ogi, bogobe, kisra, burukutu, kisra, injera
f) barley based foods, e.g. beer

Classification on the basis of texture:

a) liquid (gruel). e.g. ogi, mahewu, burukutu, pito, uji
b) solid (dough) and dumplings, e.g. kenkey, agidi
c) dry (bread), e.g. kisra, injera

PRE-FERMENTATION PROCESSING OF CEREALS

Pre-fermentation processing of cereals is largely dependent on the end product desired. In most cases, grains are sun-dried prior to fermentation. Treatments such as washing, steeping, milling and sieving are pre-fermentation processing steps applied in the preparation of fermented gruels, while milling and sieving are required as pre-fermentation processing steps in the production of dry fermented foods such as bread.

FERMENTED CEREAL-BASED FOODS

Indigenous fermented foods prepared from major cereals are common in many parts of Africa. Some are used as beverages and breakfasts or snack foods while a few are consumed as staples and weaning foods (Tables 2 and 3).

Fermented Gruels and Non-Alcoholic Beverages

Ogi

Ogi is a porridge prepared from fermented maize, sorghum or millet in West Africa. It is a staple of that region, and serves as a weaning food for infants. The traditional preparation of ogi (Figure 1) involves soaking of corn kernels in water for one to three days followed by wet milling and sieving to remove bran, hulls and germ (Odunfa, 1985; Akinrele 1970). The pomace is retained on the sieve and later discarded as animal feed while the filtrate is fermented (for two to three days) to yield ogi, which is a sour, white starchy sediment. Ogi is often marketed as a wet cake wrapped in leaves or transparent polythene bags. It is diluted to a solids content of 8 to 10 percent and boiled into a pap, or cooked and turned into a stiff gel called "agidi" of "eko" prior to consumption.

Microbiological and nutritional studies by Akimrele (1970) showed that the lactic acid bacterium *Lactobacillus plantarum*, the aerobic bacteria *Corynebacterium* and

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Aerobacter, the yeasts *Candida mycoderma*, *Saccharomyces cerevisiae* and *Rhodotorula* and moulds *Cephalosporium*, *Fusarium*, *Aspergillus* and *Penicillium* are the major organisms responsible for the fermentation and nutritional improvement of ogi. Odunfa (1985) determined that *L. plantarum* was the predominant organism in the fermentation responsible for lactic acid production. *Corynebacterium* hydrolysed corn starch to organic acids while *S. cereviseae* and *Candida mycoderma* contributed to flavour development.

Substantial nutrient losses occur during the various steps of ogi processing. According to Lagunna and Carpenter (1951) steeping, milling and sieving are the processing steps during which considerable nutrient losses take place. Much of the protein in cereal grains is located in the testa and germ, which are usually sifted off during processing. These losses have been evaluated and reported by several workers (Hamad and Fields, 1979; Oke, 1967).

Table 2. Fermented non-alcoholic cereal based foods in Africa

Product name	Area of production	Substrate	Microorganisms involved	Textural characteristics of product
Ogi	Nigeria, Benin	Maize, sorghum or millet	*Lactobacillus* sp. and yeasts	Soft or stiff gel
Bogobe	Botswana	Sorghum	Unknown	Porridge
Koko and kenkey	Ghana	Maize, sorghum or millet	*Lactobacillus* sp. and yeasts	Dough
Mawe	Dahomey	Maize	*L. fermentum*, *L. cellobiosis*, *L. brevis*, yeasts – *Candida Krusei* and *S. cerevitae*	Dough
Mahewu (magou)	South Africa	Maize sorghum or millet	*L. delbruечkii*, and *L. bulgaricus*	Liquid
Uji	East Africa	Maize, sorghum or millet	*Lactobacillus* sp.	Liquid
Kisra	Sudan	Sorghum	Unknown	Dough
Injera	Ethiopia	Sorghum	*Candida guilliermondii*	Dough

Table 3: Alcoholic beverages produced from cereals in Africa

Product name	Area of Production	Substrate	Starter	Menstrum
Burukutu	Ethiopia Nigeria (north) Northern Ghana	Guinea corn and cassava	Yeasts and lactic acid bacteria	Liquid
Pito	Nigeria (Bendel) Ghana	Guinea corn and maize	Moulds, yeast and *Lactobacillus* spp.	Liquid
Kaffir beer	South Africa	Kaffir corn (or maize)	*Lactobacillus* spp. and yeasts	Liquid
Busaa (maize beer)	East Africa	Maize	Yeasts and *Lactobacillus* spp.	Liquid
Malawa beer	Uganda	Maize	*Candida krusei*	Liquid
Zambian opaque maize beer	Zambia	Maize Sorghum	Yeasts	Liquid
Merissa	Sudan	Sorghum	Lactic acid bacteria, acetic acid bacteria	Liquid
Seketeh	Nigeria (south)	Maize	Unknown	Liquid
Bouza	Egypt	Wheat or maize	Unknown	Liquid
Talla	Ethiopia	Sorghum	Unknown	Liquid
Kishk	Egypt	Wheat and milk	*Lactobacillus* spp., yeasts and *Bacillus* spp.	Liquid

Corn

⇩

Clean

⇩

Steep for 2 to 3 days

⇩

Wet mill

⇩

Sieve and discard pomace

⇩

Ferment filtrate and allow to sediment
for 1-3 days

⇩

OGI
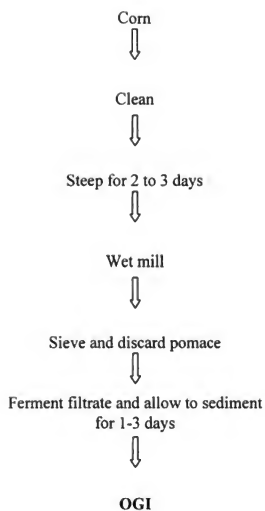
Figure 1: Flow diagram for the preparation of ogi.

Efforts are currently under way in Africa to modify the processing of ogi with a view to enhancing its nutritive value, shelf-life and possible therapeutic qualities. A protein-enriched ogi containing 10 percent soya flour was developed by the Federal Institute of Industrial Research (FIIRO), Oshodi, Lagos, Nigeria (Akinrele, 1970; Akinrele *et al.* 1970). Olukoya *et al.* (1994) reported the development of an ogi product (dogik) having therapeutic properties on the basis of its ability to control diarrhoea among infants. This finding is of great relevance since ogi is used as a popular weaning food for children in African countries.

Odunfa *et al.* (1994) investigated the possibility of improving the limiting lysine level in ogi. Fifty mutants from *L. plantarum* and seven mutants from a yeast strain were selected from thialysine-resistant cultures capable of overproducing lysine, and analysed for lysine production. Up to a 12-fold increase in lysine production was observed for *L. plantarum* and a three to four fold increase for yeasts was observed. Utilization of the mutants as starter cultures resulted in a three-fold increase in the lysine content of ogi. Banigo *et al.* (1974) and Adeniji and Potter (1978) reported the use of high lysine corn for improving the nutritional value of ogi.

Dehydration of ogi by drum or tray drying has been shown to prolong its shelf-life (Plahar and Leung, 1983). Drum drying was however reported to destroy heat-sensitive nutrients in ogi (Labuza, 1972). Adeniji and Potter (1978) reported an appreciable loss in the available lysine content of ogi as a result of drum drying.

Recent studies have sought to optimize the role of *Lactobacillus* species in the safety of fermented foods. Olasupo *et al.* (1995) determined bacteriocin-producing *Lactobacillus* isolates to be active against common food-borne pathogens including *Salmonella*. This bacteriocin also improved the shelf-life of "jellied" ogi, extending it by ten days (Olasupo *et al.* 1997).

Banku

Banku is a popular staple consumed in Ghana. It is prepared from maize and/or from a mixture of maize and cassava (Owusu-Ansah *et al.* 1980). Preparation procedures for banku are summarized in Figure 2. The preparation of banku involves steeping the raw material (maize or a mixture of maize and cassava) in water for 24 hours followed by wet milling and fermentation for three days. The dough is then mixed with water at a ratio of four parts dough to two parts water or four parts dough to one part cassava and two parts water. Continuous stirring and kneading of the fermented dough is required to attain an appropriate consistency during subsequent cooking. Microbiological studies of the fermentation process revealed that the predominant micro-organisms involved were lactic acid bacteria and moulds (Beuchat, 1983). Owusu-Ansah *et al.* 1988 reported the development of a quick-cooking fermented "banku" using a drum-drying process.

Kenkey

This is a fermented maize dough, which is popularly consumed in Ghana. During the production of kenkey, the dough is divided into two parts: one part, the "aftata" is cooked into a thick porridge, while the other uncooked part is later mixed with the "aflata". The resulting mixture is moulded into balls and wrapped in dried maize husk or plantain

leaves, after which it is steamed.   It is interesting to note that kenkey varieties vary widely throughout Ghana.   In Northern Ghana, sorghum is sometimes used instead of maize for preparation of the dough.

Microbiological studies of kenkey production by Jespersen *et al*. (1974) highlighted the significance of yeasts and moulds in the production of the fermented maize dough.   A mixed flora consisting of *Candida, Saccharomyces, Penicillium, Aspergillus* and *Furasium species* were found to be the dominant organisms during the preparation of this food product.   Halm *et al*. (1993) concluded that a homogenous group of obligatively heterofermentative *lactobacilli* related to *L. fermentum and L. reuteri* plays a dominating role during kenkey production.

<div align="center">

Corn

⇓

Clean

⇓

Steep for 24 hr

⇓

Wet mill

⇓

Ferment for 3 days

⇓

Dough formation

⇓

Stir and knead

⇓

Cook

⇓

BANKU

</div>

Figure 2. Flow diagram for the preparation of banku

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```
                    Maize
                     ⇩
                    Clean
                     ⇩
             Steep (24-48 hrs)
                     ⇩
                    Mill
                     ⇩
             Dough formation
                     ⇩
               Ferment for 72 hr

Cooked dough <                      > Raw dough

                     ⇩
              Mix (Aflatasation)
                     ⇩
              Mould into balls
                     ⇩
           Wrap with maize husks
                     ⇩
            Boil for about 3 hrs
                     ⇩
                   KENKEY
```
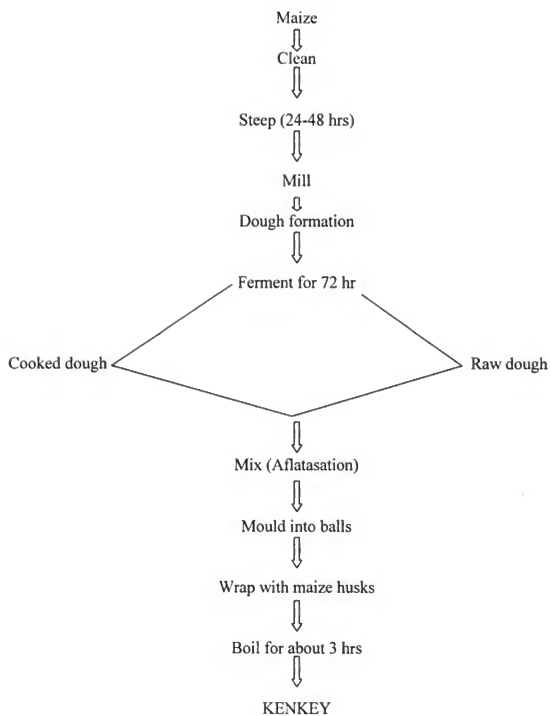
Figure 3. Flow chart for the traditional preparation of kenkey

Mahewu

This is a fermented maize meal commonly consumed as a staple among black South Africans. It is traditionally prepared by adding one part of maize meal to 9 parts of boiling water. The suspension is cooked for ten minutes, allowed to cool and then transferred to a fermentation container. At this stage, wheat flour (about 5 percent of the maize meal used) is added to serve as a source of inoculum. Fermentation occurs in a warm sunny place within 24 hours. *Streptococcus lactis* is the main fermenting organism in traditionally prepared mahewu (Hesseltine, 1979).

Mahewu is known to offer some advantages over ogi in that boiling both the maize meal and water for steeping eliminates the initial wild fermentation by fungi, etc. Furthermore, it is pre-cooked and requires only mixing prior to consumption. Mahewu consists of coarse maize particles while ogi contains very fine pasty maize particles.

Mahewu is currently produced on an industrial scale (Figure 4) as a dry food product which is marketed as a pre-cooked ready-mix powder. The industrial production of mahewu therefore spurs the need for the development of starter cultures. Schwigart and Fellingham (1963) evaluated the use of various lactic acid bacteria as starters in mahewu fermentation and determined that *Lactobacillus delbruckii* and *Lactobacillus bulgaricus* produced the most acceptable mahewu at a temperature of $50^{0}C$, which was determined to disallow the growth of unwanted micro-organisms. Van Noort and Spence (1976) of Jabula Foods Limited, South Africa, produced a more acceptable mahewu product at room temperature using a combination of starters including an acid-producing bacterium, a yeast and a non-acid producing bacterium. The identity of the various organisms used was not however disclosed by these workers.

Mawe

Mawe is a sour dough prepared from partially dehulled maize meal, which has undergone natural fermentation for a one to three-day period. Houhonigan (1994) conducted studies on mawe production. An estimated 14-16 percent of total maize production in Cotonou, Benin, is used for mawe production. Quantitatively mawe is less important than ogi, but is suitable as a basis for the preparation of many dishes, including those prepared from ogi (Figure 5). Mawe is produced using both a traditional (home) process (Figure 6) and a commercial (Figure 7) process. The commercial process for mawe production was developed to meet quality requirements of urban mawe consumers (Hounhouigan, 1994).

Traditional mawe production involves cleaning maize by winnowing, washing in water and crushing in a plate disc mill. The crushed maize is screened by sieving whereby grits and hulls are separated by gravity and the fine endosperm fraction collected in a bowl.

Maize meal

⇓

Mix in warm water to give 8% solids content

⇓

Cook at 121°C for 15 minutes

⇓

Cool

⇓

Inoculate
(5% wheat flour or an adapted pure culture of
Lactobacillus delbrueckii)

⇓

Incubate at 30-50°C for wheat innoculum, or at 45°C for *L. delbrueckii* innoculum

⇓

Ferment for 36 hrs with mixing only at the beginning of fermentation

⇓

Heat for 10-15 mins under pressure (7 psi)

⇓

Spray or drum dry

⇓

MAHEWU

Figure 4: Industrial preparation of mahewu
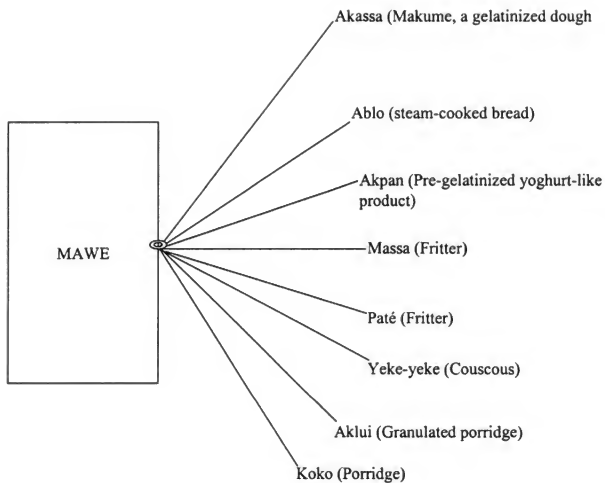
Figure 5. Main dishes prepared from mawe

MAWE

Akassa (Makume, a gelatinized dough

Ablo (steam-cooked bread)

Akpan (Pre-gelatinized yoghurt-like product)

Massa (Fritter)

Paté (Fritter)

Yeke-yeke (Couscous)

Aklui (Granulated porridge)

Koko (Porridge)

42

Maize grains

⇩

Clean and wash

⇩

Crush

⇩

Screen and dehull; discard hull

⇩

Soak in water for 2-4 h and drain

⇩

Grind

⇩

Knead to form a dough

⇩

Ferment for 1-3 days

⇩
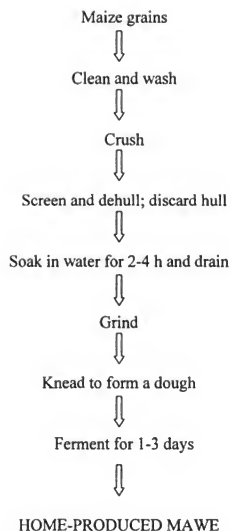
HOME-PRODUCED MAWE

Figure 6. Flow diagram of the home process of mawe production

Maize grains

⇓

Clean and wash

⇓

Crush

⇓

Screen and dehull

⇓

Soak and wash; discard hull, and germ

⇓

Drain

⇓

Add water and allow to stand for 2-4 hours

⇓

Grind

⇓

Add water and knead to form a dough

⇓

Ferment for 1-3 days

⇓
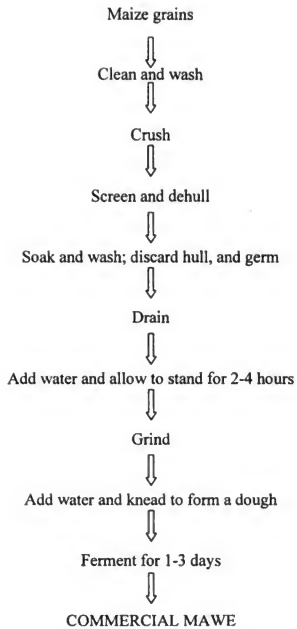
COMMERCIAL MAWE

Figure 7. Flow diagram for the commercial production of mawe

The grits are not washed but home dehulled, following which they are mixed with the fine fraction moistened over a 2-4 hour period and milled to a dough. The kneaded dough is then covered with a polyethylene sheet and allowed to ferment naturally to a sour dough in a fermentation bowl, or wrapped in paper or polyethylene. In the commercial process, which takes place entirely in a milling shop, the grits are washed by rubbing in water, following which, the germ and remaining hulls are floated off and discarded along with the water. The sedimented endosperm grits are subsequently blended with the fine endosperm fraction.

The main difference between the traditional and the commercial process of mawe production is that hulls and germs are removed during the commercial processing of mawe. Commercial mawe is whiter in appearance than home-produced mawe and has better swelling and thickening characteristics, but is of lower nutritional value. A compositional study of mawe resulting from both the traditional and commercial processes showed that average moisture contents varied between 45 and 47 percent and did not differ significantly. The titratable acidity of home-made and commercial mawe samples was similar (1.2 – 1.4 percent w/w as lactic acid), but home-made mawe was of a slightly higher pH (Table 4). The crude protein, crude fat, crude fibre and ash contents of home-made mawe were higher than those of commercial mawe since more hulls and germ were retained during home production (Hounhouigan et al. 1993).

Dominant micro-organisms in mawe preparation include lactic acid bacteria (mainly *Lactobacillus fermentum* and its biotype *L. cellobiosis, L. brevis)* and yeasts (*Candida krusei* and *Saccharomyces cerevisiae* (Table 3).

Table 4: Chemical Characteristics of Mawe

	Home Produced Mawe	Commercially Produced Mawe	
	(collected from homes) n = 20	(fresh from mill) n = 15	(sold at the market) n = 15
pH	4.2	3.9	3.8
Titratable acidity (% w/w, as lactic acid)	1.2	1.1	1.4
Moisture content (%)	46.8	45.9	45.1
Crude protein (% dwb)	9.2	8.3	8.2
Crude fat (% dwb)	2.3	0.9	1.0
Crude fibre (% dwb)	0.7	0.4	0.4
Ash (% dwb)	1.1	0.6	0.6
Soluble carbohydrate (% dwb)	86.7	89.8	89.8

* Source: Hounhnigan *et al.* (1993).

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Bread and Pancakes

Injera

Injera is the most popular baked product in Ethiopia. It is a fermented sorghum bread with a very sour taste (Stewart and Getachew, 1962) and is the undisputed national bread of Ethiopia. The baked product is referred to by different names depending on the locality of production in Ethiopia. It is referred to as "bidena" in Oromigua, "taeta" in Giragigua, and "solo" in Walaytigna. According to a report by Gebrekidan and Gebrettiwat (1982) over 8 percent of total sorghum production in Ethiopia is used for "injera" production. The sorghum grains are dehulled manually or mechanically and milled to flour which is subsequently used in the preparation of injera (Figure 8).

<div align="center">

Sorghum flour

⇓

Mix with water, 4:1 *w/v*

⇓

Knead to form a dough

⇓

Mix with starter
(Fermented yellowish liquid saved from
previously fermented dough)

⇓

Knead

⇓

Add water

⇓

Ferment for 48 h

⇓

Add water and allow to stand for 1 hour

⇓

Bake on hot
greased clay griddle metal till holes begin to form on top

⇓

INJERA

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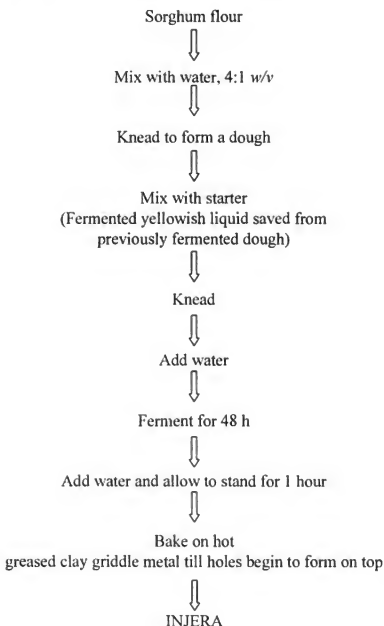
Figure 8. Flow diagram for the preparation of injera

On the basis of production procedures three types of injera are distinguishable: (i) thin injera which results from mixing a portion of fermented sorghum paste with three parts of water and boiling to yield a product known as "absit" which is, in turn, mixed with a portion of the original fermented flour (ii) thick injera, which is reddish in colour with a sweet taste, is a "tef" paste that has undergone only minimal fermentation for 12-24 hours; (iii) komtata-type injera, which is produced from over-fermented paste, and has a sour taste. The paste is baked or grilled to give a bread-like product. Yeasts are the major micro-organisms involved in the fermentation of the sweet type of injera (Beuchat, 1983).

The comparative chemical composition of injera prepared from different cereals (Gebrekidan and Babrettwat, 1982) is shown in Table 5. There is little variation in the nutrient composition of injera prepared from different cereals, which indicates the potential for the use of cereals other than sorghum in the production of injera.

Kisra

This is a thin pancake-like leavened bread prepared from whole sorghum flour. It is a dietary staple in the Sudan. This fermented sorghum bread has a very sour taste (Ejeta, 1982). It is prepared by mixing sorghum flour with water to give a thick paste which is allowed to ferment for 12-24 hours, following which the paste is thinned to a desirable consistency with water just prior to baking (Figure 9).

Ejeta (1982) conducted an evaluation of the effect of sorghum variety on kisra quality. Cultivars with a white chalky pericarp and without a subcoat were judged to have the best sensory properties. El-Tinay *et al.* (1979) reported that there was a slight increase in protein and fibre and an appreciable decrease in carbohydrate (starch and sugars) during the fermentation of kisra. An amino acid analysis of kisra prepared from three different cultivars of sorghum indicated slight differences in the levels of the various amino acids (Table 6).

Table 5: Nutritional composition of Injera (per 100g) prepared from different cereals

Nutrient	Sorghum	Tef	Corn	Finger Millet	Barley	Wheat
Energy (cal)	193	162	185	172	167	172
Moisture (%)	52.0	59.8	54.0	56.1	58.0	57.4
Protein (g)	7.1	4.2	5.0	3.8	3.5	5.4
Fat (g)	0.6	0.6	0.7	0.3	0.3	0.9
Carbohydrates (g)	39.8	33.9	39.6	38.4	37.5	35.6
Fibre (g)	0.9	1.7	0.7	4.0	0.9	0.9
Ash (g)	0.5	1.5	0.7	1.4	0.7	0.7
Calcium (mg)	10	64	27	169	16	28
Phosphorus (mg)	111	129	120	103	128	155
Iron (mg)	3.5	30.5	2.1	17.3	4.2	3.3
ß-Carotene equiv. (ug)	0	0	Trace	Trace	0	0
Thiamin (mg)	0.17	0.21	0.14	0.14	0.12	0.14
Riboflavin (mg)	0.08	0.07	0.06	0.01	0.05	0.09
Niacin (mg)	1.7	0.8	0.7	0.2	3.0	2.4
Ascorbic acid (mg)	0	1	2	1	0	1

* Source: Gebrekidam and Gebrelfiwat (1982)

Sorghum flour
⇓
Mix with water (60:40 *w/v*)
⇓
Ferment for 12 – 24hrs
⇓
Add water to form a thin slurry
⇓
Bake on hot oiled pan
⇓
KISRA
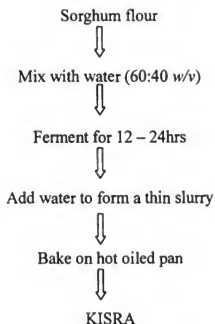
Figure 9. Flow diagram for the preparation of kisra

Kishk

Kishk is a fermented product prepared from parboiled wheat and milk (Figure 10). It is consumed in Egypt and in most Arabian countries (Morcos *et al.* 1973a). During the preparation of kishk, wheat grains are boiled until soft, dried, milled and sieved in order to remove the bran. Milk is separately soured in earthenware containers, concentrated and mixed with the moistened wheat flour thus prepared, resulting in the preparation of a paste called a hamma. The hamma is allowed to ferment for about 24 hours, following, which it is kneaded and two volumes of soured salted milk are added prior to dilution with water. Alternatively, milk is added to the hamma and fermentation is allowed to proceed for a further 24 hours. The mass is thoroughly mixed, formed into balls and dried.

Kishk is a highly nutritious food, having a protein content of about 23.5 percent. It is of a high digestibility, and high biological value. Micro-organisms responsible for fermentation include *Lactobacillus plantarum*, *L. brevis*, *L. casei*, *Bacillus subtilis* and yeasts (Beuchat, 1983; Odunfa, 1985). Kishk is usually overheated to improve its keeping quality.

Bogobe

Bogobe is a sorghum porridge prepared in Botswana from fermented and non-fermented sorghum (Figure 11). Fermented bogobe is a soft porridge, known as *ting* while non-fermented bogobe is a thick porridge called *monokwane* (Boising and Nancy, 1982). Information relevant to micro-organisms involved in the fermentation of bogobe, and the nutritional changes, which occur during fermentation, is still scant.

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Table 6: Essential amino acid profiles for flour, fermented dough, and kisra produced from three sorghum cultivars*

Amino acids (mg/g)	Dabar			Sorghum Cultivars Ferita			Mayo		
	Flour	Fermented Dough	Kisra	Flour	Fermented Dough	Kisra	Flour	Fermented Dough	Kisra
Threonine	4.2	4.6	4.7	3.3	5.1	4.3	4.6	4.1	3.5
Valine	6.9	6.7	8.0	5.5	7.9	6.1	8.1	6.2	5.4
Methionine	1.2	2.4	1.1	1.3	2.7	0.9	1.3	2.0	1.2
Isoleucine	6.0	4.9	4.7	4.7	5.9	4.8	6.7	4.8	3.8
Leucine	19.1	15.1	14.0	14.0	20.6	14.3	20.8	15.5	11.1
Phenylalanine	7.9	6.8	7.4	6.0	8.4	5.6	9.8	6.5	5.0
Lysine	3.4	3.3	3.3	3.5	3.1	3.7	3.4	3.3	2.6

Note: Dough fermented at 28°C for 18 hours at pH 3.7.

* Source: El-Tinay et al. (1979)

Wheat grains covered with water

⇩

Heat slowly to boiling and simmer until soft

⇩

Wash with cold water   Sour milk by churning in
                              skin bags
⇩   ⇩

Dry on mats   Concentrate

⇩

Grind

⇩

Remove seed coats by sieving

⇩

Place in pots and moisten with
lightly salted boiling water ⟹   Mix to form a paste

                                  ⇩

                              Ferment for 24 hrs

                                  ⇩

                              Mix and add whey

                                  ⇩

                              Dilute with milk or water to
                              give a syrupy consistency

                                  ⇩

                              Ferment for 24 hrs

                                  ⇩

                              Mix and form into small
                              balls

                                  ⇩

                              Place on mats and sun dry

                                  ⇩

                              KISHK

Figure 10: Flow diagram for the preparation of kishk

Sorghum grains

⇩

Wash with water

⇩

Dehull (mechanical or manual)

⇩

Discard bran and grind to a coarse meal

⇩

Add lukewarm water (1:1 w/v) to form a slurry

⇩

Allow to ferment in a closed environment for 24 hours

⇩

Cook in boiling water for 12 – 15 min.

⇩
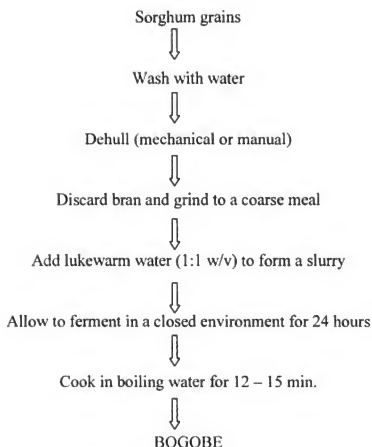
BOGOBE

Figure 11. Flow diagram for the preparation of bogobe from sorghum

Alcoholic Beverages

Kunu-Zaki

This is a millet-based non-alcoholic fermented beverage widely consumed in the northern parts of Nigeria. This beverage is however becoming more widely consumed in southern Nigeria, owing to its refreshing qualities. Adeyemi and Umar (1994) described the traditional process for the manufacture of kunu-zaki. This process involves the steeping of millet grains, wet milling with spices (ginger, cloves, and pepper), wet sieving and partial gelatinization of the slurry, followed by the addition of sugar, and bo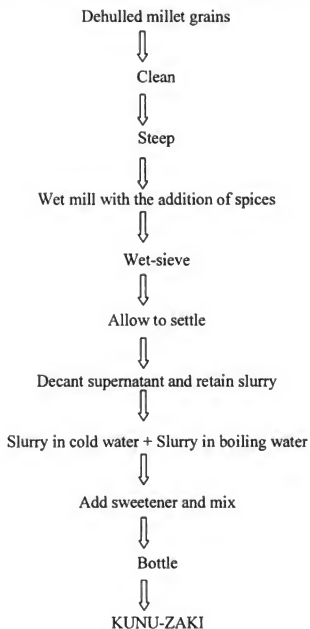ttling (Figure 12). The fermentation which occurs briefly during steeping of the grains in water over a 8-48 hour period is known to involve mainly lactic acid bacteria and yeasts.

Sopade and Kassum (1992) highlighted the significance of rheological characteristics in processing, quality control, sensory evaluation and structural analysis of kunu-zaki. Increasing temperatures reduced viscosity but did not alter the rheological characteristics of the product. The time of shear (up to 1 hr) did not appreciably alter the viscosity.

Storage studies conducted by Adeyemi and Umar (1994) revealed that the product had a shelf-life of about 24 hours at ambient temperature, which was extended to eight days by pasteurization at $60^{\circ}C$ for 1 hour and storage under refrigeration conditions. Studies are currently under way at the Federal Institute of Industrial Research Oshodi (FIIRO), Lagos,

Nigeria, to produce kunun-zaki of improved shelf-life. FIIRO has been able to preserve kunun-zaki effectively for 90 days, with the use of chemical preservatives.

Dehulled millet grains

⇩

Clean

⇩

Steep

⇩

Wet mill with the addition of spices

⇩

Wet-sieve

⇩

Allow to settle

⇩

Decant supernatant and retain slurry

⇩

Slurry in cold water + Slurry in boiling water

⇩

Add sweetener and mix

⇩

Bottle

⇩

KUNU-ZAKI

Figure 12. Flow chart for the traditional preparation of kunu-zaki

Burukutu

This is a popular alcoholic beverage of a vinegar-like flavour, consumed in the Northern Guinea savannah region of Nigeria, in the Republic of Benin and in Ghana. The preparation of burukutu involves steeping sorghum grains in water overnight, following which excess water is drained. The grains are then spread out on to a mat or tray covered with banana leaves and allowed to germinate. During the germination process, the grains are watered on alternate days and turned over at intervals. Germination continues for four to five days until the plumule attains a certain length. The malted grains are spread out in the

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sun to dry for one to two days, following which the dried malt is ground into a powder. Gari (a farinaceous fermented cassava product) is added to a mixture of the ground malt and water in a ratio of one part gari to two parts malt and six parts water. The resulting mixture is allowed to ferment for two days, following which it is boiled for approximately 4 hours and allowed to mature for a further two days. The resulting product is a cloudy alcoholic beverage.

Sorghum malt contains primarily yeasts and moulds as the indigenous microflora. Micro-organisms associated with the fermentation include yeasts mainly *Saccharomyces cerevisiae* and *S. chavelieri* and the bacteria, *Leuconostoc meseteroides*.

The pH of the fermenting mixture decreases from about 6.4 to 4.2 within 24 hours of fermentation and decreases further to 3.7 after 48 hours. At the termination of the two-day maturing period *Acetobacter sp.* and *Candida sp.* (Faparusi *et al.* 1973) are the dominant micro-organisms. Boiling prior to maturation eliminates lactics and other yeasts. Fully matured burukutu beer has an acetic acid content, which varies between 0.4 and 0.6 percent.

Pito

Pito is the traditional beverage of the Binis in the mid-western part of Nigeria. It is now very popularly consumed throughout Nigeria owing to its refreshing nature and low price. Pito is also widely consumed in Ghana. The preparation of pito involves soaking cereal grains (maize, sorghum or a combination of both) in water for two days, followed by malting, and allowing them to sit for five days in baskets lined with moistened banana leaves. The malted grains are ground, mixed with water and boiled. The resulting mash is allowed to cool and later filtered through a fine mesh basket. The filtrate thus obtained is allowed to stand overnight, or until it assumes a slightly sour flavour, following which it is boiled to a concentrate. A starter from the previous brew is added to the cooled concentrate, which is again allowed to ferment overnight. Pito, the product thus obtained, is a dark brown liquid which varies in taste from sweet to bitter. It contains lactic acid, sugars, amino acids and has an alcohol content of 3 percent (Ekundayo, 1969). Organisms responsible for souring include *Geotrichum candidum* and *Lactobacillus* sp. while *Candida* sp. are responsible for the alcoholic fermentation.

Merissa

This is an alcoholic drink, which is widely consumed in the Sudan. It is prepared from sorghum and millet by a relatively complex process. Brewing takes place in three distinct phases (i) "ajeen" fermentation, a lactic souring of sorghum, (ii) "debosa" fermentation, a starter activating phase and (iii) "merissa" fermentation, an alcoholic fermentation.

The fermentation of merissa is similar to that applied in the preparation of other African alcoholic beverages. Lactic acid and acetic acid bacteria and yeasts at a pH of about 4.0 accomplish Ajeen fermentation, an alcoholic content of 1 percent and lactic acid content of 2.5 percent. At the final stage of merissa fermentation, however, the alcoholic content increases to about 6 percent.

Bouza

Bouza, a fermented alcoholic beverage produced from wheat in Egypt, has been known by the Egyptians since the days of the Pharaohs (Morcos *et al.* 1973). It is a thick, pasty yellow beverage with an agreeable taste and produces a sensation of heat when consumed. It is prepared by coarsely grinding wheat grains, placing a portion of them (three-quarters) in a wooden basin and kneading them with water into a dough. The dough is cut into thick loaves, which are very lightly baked. Meanwhile, the remainder of the grains (approximately one-quarter of the total amount of wheat grains) is moistened with water, germinated for three to five days, sun-dried, ground and mixed with the loaves of bread which are soaked in water in a wooden barrel. Bouza from a previous brew is added to serve as an inoculum. The mixture is allowed to ferment at room temperature for a 24-hour period, following which the product is sieved to remove large particles and diluted with water to a desired consistency.

Like other opaque beers, bouza has a very short shelf-life and is expected to be consumed within a day. Its pH increases to between 3.9 and 4.0 and its alcoholic content to between 3.8 and 4.2 percent within a 24-hour period.

SCOPE FOR IMPROVEMENT OF FERMENTED FOODS

Indigenous fermented foods are produced at the household level in a majority of African countries. Increasing industrialization and urbanization trends in these countries will however dictate the need for larger scale production of fermented foods of consistent quality. Additionally, variation of the quality attributes of these foods to meet the demands of the sophisticated and varied palates of industrialized communities will eventually be required.

Upgrading the production of fermented foods from the household to the industrial level will necessitate several critical steps:

1. **Isolation and identification of the micro-organisms associated with the fermentations**

Micro-organisms associated with indigenous fermentations need to be isolated, properly identified and preserved preferably in a recognised culture collection for future use.

2. **Determination of the role(s) of the micro-organism(s)**

The biochemical role(s) of micro-organisms associated with food fermentations needs to be determined through chemical analysis of products released by the micro-organisms under controlled laboratory conditions.

3. **Selection and genetic improvement of micro-organisms**

Micro-organisms responsible for effecting important changes in the food during fermentation should be selected and subjected to genetic improvement geared toward maximising desirable quality attributes in the food and the limiting any undesirable attributes.

4.   Improvement in process controls for the manufacture of fermented foods

Improvements in the quality and quantity of fermented foods may be achieved by manipulating environmental factors such as temperature, moisture content, aeration, pH, acidity, etc. which influence the activity of micro-organisms during the fermentation process.

5.   Improvement in the quality of raw materials used in the production of fermented foods

Both the quality and the quantity of fermented foods may be improved by choosing raw materials other than those traditionally used for their production.

6.   Laboratory simulation of the fermented foods

Prior to pilot scale production, and (ideally) after all the five stages above have been well studied, fermented products may be produced under laboratory conditions. Laboratory simulation of fermented foods will involve the production of fermented foods by inoculating microbial isolates having desirable properties, into raw materials.

7.   Pilot stage production

The pilot stage is the first clear departure from small-scale production and should be based on the result of laboratory experiments.

8.   Production or industrial plant stage

The production stage is the culmination of all the previous efforts and should lead to the availability of food of predictable and consistent quality on a large scale.

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CHAPTER 3

CEREAL FERMENTATIONS IN COUNTRIES OF THE ASIA-PACIFIC REGION

INTRODUCTION

The Asia-Pacific region is characterized by its tropical and subtropical humid climate, which is suitable for rice paddy cultivation and mold growth. The consumption of rice as a staple food, and the high population density which limits animal husbandry practices in that Region, has resulted in a typical food processing technology - cereal fermentation with molds. Molds and other microorganisms convert unpalatable carbohydrates of low digestibility and proteins into palatable sugars and amino acids respectively, with a high conversion efficiency. The soybean protein conversion ratio into amino acids in a traditional Korean soysauce fermentation for example, is over 75 percent, which is approximately 15 times higher than the feed protein conversion ratio in beef production, and 6 times higher than that in pork production (Lee and Jul, 1982).

Cereal consumption in the Asia-Pacific region varies in accordance with geographic and climatic conditions. Inhabitants of the tropical Southeastern regions consume primarily rice, while those in subtropical and temperate zones of the Northeastern region including Northern China, Republic of Korea and Japan consume wheat, buckwheat, barley, corn, millet and soybeans in addition to rice. Table 1 shows the contribution of cereals to the diet in the Asia-Pacific region in 1995. Countries of the Mecong delta basin, known as the origin of fish fermentation technology derive up to 80 percent of their total caloric intake from rice. Ishige (1993) discussed the correlation between rice eating habits and fish sauce consumption. On the other hand, countries of the Far East, China, Republic of Korea and Japan, known as the soybean sauce culture zone, consume less rice than countries of Southeastern Asia. Inhabitants of that region typically consume short grain rice (Japonica type). Rice consumption in this region has decreased owing to recent economic growth.

According to statistical data, 47 percent of the total caloric intake in Korea and 43 percent in Japan were supplied by rice in 1965. These percentages decreased to 35 percent and 23 percent respectively in 1995, due to incorporation of other foods in the diet. In China on the other hand, wheat and sorghum are mostly consumed. India has cereal consumption patterns, which are similar to those of China, but with the consumption of much less wheat. These differences in cereal consumption patterns have resulted in variations in cereal fermentation practices in countries of the Asia-Pacific region.

Table 1. Contribution of cereals to the energy supply of people in Asia-Pacific Region (1995)

Country	Total energy intake (Kcal)	(% of total energy supply)			
		Rice	Other cereals	Meat	Other foods
China	2 734	35	`31	10	24
Korea	3 285	35	15	6	44
Japan	2 887	24	16	6	54
Philippines	2 255	40	15	6	39
Vietnam	2 250	70	3	5	22
Cambodia	2 021	80	3	3	14
Thailand	2 434	56	3	5	36
Malaysia	2 889	33	10	8	49
Indonesia	2 752	56	10	2	32
India	2 394	31	3	1	35

Source: Samsung Economics Research Institute, (1997)

THE HISTORY OF CEREAL FERMENTATION TECHNOLOGY IN ASIA

It is often mentioned that today's modern biotechnology originated from the alcohol fermentation of primitive peoples. Since indigenous fermented foods were produced by natural fermentation, the origin of cereal fermentation technology is obscure. Unlike fruit and milk fermentations, cereal fermentation requires a saccharification process, which is accomplished with some difficulty. One primitive method of cereal saccharification would be chewing raw cereals and spitting them into a vessel in order to allow saccharification to occur through the action of salivary amylase, followed by alcoholic fermentation by natural yeasts. Another method of cereal saccharification is through the malting process. Malting occurs naturally through wet damage of cereals during storage, and is used for beer manufacture in Europe. However, in Asia the malting process is rarely used in traditional fermentation processes. Instead, fermentation starters prepared from the growth of moulds on raw or cooked cereals are more commonly used. The use of fermentation starters might very well have its origins in the process of Euchok, the daughter of the legendary king of Woo of 4 000 BC, known as the Goddess of rice-wine in Chinese culture (Lee, 1984). Fermentation starters are referred to as *chu* in Chinese, *nuruk* in Korean, *koji* in Japanese, *ragi* in Southeast Asian countries and *bakhar ranu* or *marchaar* (*murcha*) in India (Batra and Millner, 1974).

The first documentation of *chu* was found in Shu-Ching written in the Chou dynasty (1 121-256 BC), in which it is stated that *chu* is essential for making alcoholic beverages. It is speculated that man must have discovered *chu* much earlier than was documented in the literature (Yokotsuka, 1985). According to Chi-Min-Yao-Shu written by Jia-Si-Xie of Late-Wei kingdom in the 6th century, dozens of preparation methods for *chu*, the cereal fermentation starter, were described (Yoon, 1993). Methodology for *chu* preparation is very

similar to that for *shi*, or Korean *meju* preparation, which is a mouldy starter prepared from soybeans, for soysauce fermentation.

The use of *chu* for rice-wine production was commonly practised in the Spring and Fall and Warrior Periods of China (from seventh to third centuries BC) and the beginning of the Three Nations' Periods in Korea ('from the first century BC to the second century AD). This process must have been transferred from Korea to Japan in the third century by Inborn, according to Kojiki, or Chin, whose memorial tablet is kept in a shrine, Matsuo Taisha, in Kyoto, Japan (Lee, 1995).

In the indigenous process of rice-wine production, saccharification, souring and alcoholic fermentation proceed almost simultaneously. Lactic acid fermentation of cereals is, therefore, an old process in East Asia, which occurs naturally in rice-wine fermentation, but has limited application in other food processing when compared to lactic fermentations practised in Europe and Africa (Lee, 1994). Sour bread prepared from rice can be found in some areas of the Asia-Pacific region, but is most commonly produced in the Philippines. Lactic acid fermentation of cereals has been used more significantly as a preservative in fish fermentations. Historically, lactic fermentation of fish was associated with salt production, irrigated rice cultivation and the seasonal behaviour of fish stock. The Mekong basin was most probably the place of origin of these products, and Han Chinese (200 BC to 200 AC) learned of it when they expanded south of the Yangtze River. Fish products prepared by lactic acid fermentation remain common in Laos, Kampuchea, and in the north and the Northeast of Thailand (Ishige, 1993).
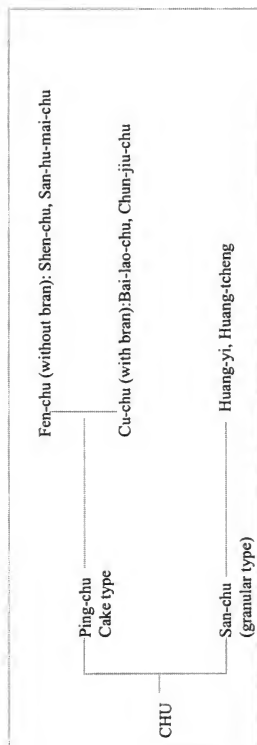
When considering the historical background and the technical aspects of cereal fermentations in the Asia-Pacific region, fermentation starters prepared from cereals should be discussed prior to the products of fermentation.

Fermentation starters

As mentioned above, *chu* is commonly used in the Asia-Pacific region as an enzyme source for the degradation of complex plant tissue to produce cereal-wines, soysauce, fish and meat sauce, sour bread, and fermented porridges and snacks. Table 2 summarizes the names of *chu* in different countries, and their ingredients.

According to Chi-Min-Yao-Shu (AD 530-550), *chu* was prepared from barley, rice and wheat, and can be classified as described in Figure 1 (Yoon, 1993). Ten different types of *chu* were described in Chi-Min-Yao-Su, all of which were used for the fermentation of alcoholic beverages. Granular types were used for soysauce and soybean paste fermentation.

Cake type *ping-chu* is identical to *nuruk* in Korea, and granular type *san-chu* is similar to Japanese *koji*. Since cake type *ping-chu* was described in Chi-Min-Yao-Su written in 530-550 AD, it should be corrected that cake type *ping-chu* was developed later in the Han dynasty (947-979 AD) as reported by Yokotsuka (1985). Figure 2 outlines the processing methodology for the production of *shen-chu* from barley and *chun-jiu-chu* from wheat as described in Chi-Min-Yao-Shu. According to the book, the appropriate season for the preparation of *chu* is July in which the ambient temperature varies between 20°C and 30°C in

Figure 1. Classification of fermentation starters described in Chi-Min-Yao-Su

CHU
- Ping-chu
  Cake type
  - Fen-chu (without bran): Shen-chu, San-hu-mai-chu
  - Cu-chu (with bran):Bai-lao-chu, Chun-jiu-chu
- San-chu
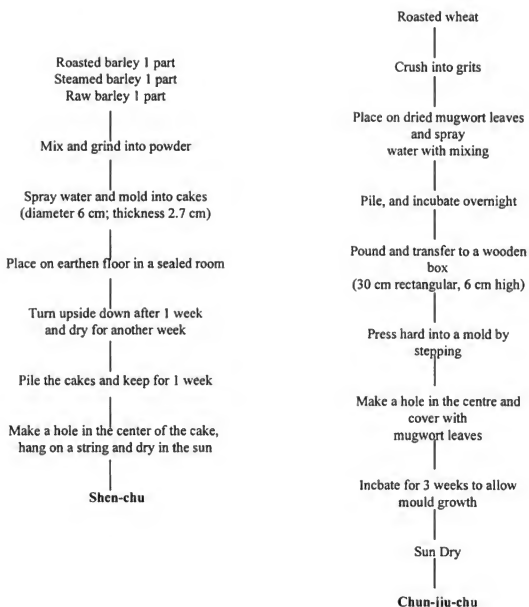 (granular type)
  - Huang-yi, Huang-tcheng

Northern China and the Korean peninsular. The procedures for preparing these starters are very similar to those for the preparation of *nuruk* in Korea.

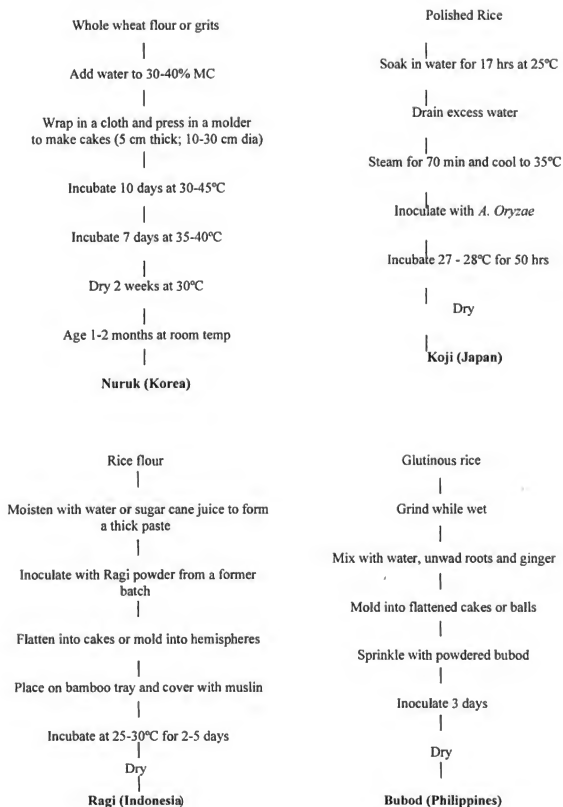**Table 2. Fermentation starters used in different countries**

Country	Name	Ingredients	Shape	Microorganism
China	Chu	Wheat, barley, millet, rice (whole grain, grits or flour)	granular or cake	*Rhizopus* *Amylomyces*
Korea	Nuruk	Wheat, rice, barley (whole grain, grits or flour)	large cake	*Aspergillus* *Rhizopus* yeasts
	Meju	soybean (whole seed)	large ball	*Aspergillus* *Bacillus*
Japan	Koji	wheat, rice (whole grain, grits or flour)	granular	*Aspergillus*
Indonesia	Ragi	rice (flour)	small cake	*Amylomyces* *Endomycopsis*
Malaysia	Ragi	rice (flour)	small cake	
Philippines	Bubod	rice, glutinous rice (flour)	small cake	*Mucor, Rhizopus* *Saccharomyces*
Thailand	Loopang	bran	powder	*Amylomyces* *Aspergillus*
India	Marchaa	rice	flat cake	*Hansenula anomala* *Mucor fragilis* *Rhizopus arrhizus*

According to Yokotsuka (1985), *chu* may either be yellow *(huang)* possibly due to *Aspergillus oryzae,* or white probably due to *Rhizopus* and *Mucor*. *Huang-chu* was widely used for alcoholic fermentation as well as for the fermentation of soybean foods. Three types of *huang-chu* have been described, *huang-yi, huang-tcheng* and *nu-chu*. *Huang-yi* is prepared from crushed wheat, which is washed, soaked in water until sour and then drained and steamed. After cooling, the steamed wheat is piled to a thickness of 6 cm and covered with leaves for seven days following which it is covered with yellow mycelia and spores. *Chu* thus prepared is then sun dried.

During the preparation of *huang-tcheng*, wheat flour mixed with water is shaped into a ball or cake, which is steamed, cooled and then covered with leaves until it develops cultures of moulds. *Nu-chu* is prepared from cooked rice, which is shaped into a cake and then cultured with moulds (Yokotsuka, 1985). Wheat *chu* originated in the Northern part of China and the Korean Peninsular, while rice *chu* originated in the South. This is reflected by the main ingredients of the fermentation starters prepared today in the countries of the South-Pacific region as shown in Table 2.

```
        Roasted barley 1 part                    Roasted wheat
        Steamed barley 1 part                          |
          Raw barley 1 part                    Crush into grits
                |                                       |
    Mix and grind into powder          Place on dried mugwort leaves
                |                                and spray
    Spray water and mold into cakes          water with mixing
    (diameter 6 cm; thickness 2.7 cm)               |
                |                          Pile, and incubate overnight
  Place on earthen floor in a sealed room          |
                |                        Pound and transfer to a wooden
      Turn upside down after 1 week                box
        and dry for another week        (30 cm rectangular, 6 cm high)
                |                                       |
    Pile the cakes and keep for 1 week     Press hard into a mold by
                |                                  stepping
  Make a hole in the center of the cake,            |
  hang on a string and dry in the sun     Make a hole in the centre and
                |                                 cover with
            Shen-chu                           mugwort leaves
                                                        |
                                        Incbate for 3 weeks to allow
                                               mould growth
                                                        |
                                               Sun Dry
                                                        |
                                            Chun-jiu-chu
```

Figure 2. Flow charts for the solid fermentation of *Chu* preparation in Chu-Min-Yao-Shu written in the sixth century

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Whole wheat flour or grits
|
Add water to 30-40% MC
|
Wrap in a cloth and press in a molder
to make cakes (5 cm thick; 10-30 cm dia)
|
Incubate 10 days at 30-45°C
|
Incubate 7 days at 35-40°C
|
Dry 2 weeks at 30°C
|
Age 1-2 months at room temp
|
Nuruk (Korea)

Polished Rice
|
Soak in water for 17 hrs at 25°C
|
Drain excess water
|
Steam for 70 min and cool to 35°C
|
Inoculate with *A. Oryzae*
|
Incubate 27 - 28°C for 50 hrs
|
Dry
|
Koji (Japan)

Rice flour
|
Moisten with water or sugar cane juice to form
a thick paste
|
Inoculate with Ragi powder from a former
batch
|
Flatten into cakes or mold into hemispheres
|
Place on bamboo tray and cover with muslin
|
Incubate at 25-30°C for 2-5 days
|
Dry
|
Ragi (Indonesia)

Glutinous rice
|
Grind while wet
|
Mix with water, unwad roots and ginger
|
Mold into flattened cakes or balls
|
Sprinkle with powdered bubod
|
Inoculate 3 days
|
Dry
|
Bubod (Philippines)

**Figure 3. Flow charts for the preparation of solid-fermented starters in different
countries of the Asia-Pacific region**

Figure 3 compares the preparation processes of Korean *nuruk*, Japanese *koji*,
Indonesian *ragi*, and Philippine *bubod* (Steinkraus, 1983). *Nuruk, ragi* and *bubod* are similar
in that they are prepared by the natural fermentation of raw cereal powders, which are moulded

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into the shape of a cake or ball. *Koji* on the other hand is prepared by controlled fermentation of cooked cereals in a granular form, which are commonly inoculated with the mould, *Aspergillus oryzae*. Numerous types of microorganisms, moulds, bacteria and yeasts, are found in these naturally fermented products. *Aspergillus oryzae* (1×10^7 cfu/g), *Aspergillus niger* (1×10^7 cfu/g), *Rhizopus* (1×10^6 cfu/g), bacteria (1×10^7 cfu/g) and yeasts (1×10^5 cfu/g) were identified in *nuruk* (Kim 1968). The number of moulds (1×10^3-10^7/g), yeasts (1×10^5-10^7/g) and lactic acid bacteria (1×10^5-10^7/g) were observed to vary with the source and district of collection of *bubod* (Tanimura *et al.*1978). Important micro-organisms in *ragi* fermentations were moulds of *Amylomyces rouxii* and yeasts of *Endomycopsis burtonii* (Ko, 1972), while those in *loog-pang* were *Amylomyces, Aspergillus, Rhizopus, Mucor,* and *Absidia* (Pichyangkura and Kulprecha, 1977).

Table 3 compares the enzyme activities of Japanese *koji* and Korean *nuruk* (Nunokawa and Ouchi 1973; Kim, 1968). Enzyme activities in *koji* are generally higher than those in *nuruk*. This may be due to the fact that the pure cultures of *Aspergillus oryzae* on loose cereal granules allow maximum growth during the preparation of *koji*. In *nuruk* manufacture however, mould growth is confined mainly to the surface of the cake or ball, thus allowing yeasts and lactic acid bacteria to grow simultaneously and contribute to the deeper flavour notes of Korean rice-wine in later alcoholic fermentation stages.

Of 41 yeast strains isolated from Indonesian *ragi* and *tape* by Saono and co-workers (1977) 19 were amylolytic, none were proteolytic, but 14 were lipolytic. All mould isolates were amylolytic and lipolytic, and 89 percent also exhibited proteolytic activity.

Table 3. Main enzyme activities in *koji* and *nuruk*

Enzyme	*Koji*	*Nuruk*
α-Glucosidase (Wohlgemuth value D $^{40}_{30}$/g)	1225	256
Glucoamylase (mg glucose produced/hr/g)	201	260
Acid Protease (mg tyrosine produced/hr/g)	3674	181

Figure 4 outlines the preparation of solid-state fermented starters used for the preparation of soysauce in Korea and Japan. Korean *meju* is prepared from cooked whole soybeans; while Japanese *koji* is prepared from a mixture of roasted wheat and defatted soybean cake. *Koji* is prepared by the inoculation of *Aspergillus oryzae* in a controlled fermentation, while *meju* is prepared by spontaneous fermentation. The outer layer of the *meju* ball is over grown with moulds, while on the inside, bacteria, mainly *Bacillus subtilis* grow.

```
Wheat          Defatted soybean cake                    Whole soybeam
  |                    |                                        |
Roaster              Steam                          Soak in water overnight
  |                    |                                        |
Cooler                                              Cook for 2-3 hrs.
  |                                                              |
Storage tank                                Mash and mold into 1 kg balls or blocks
  |                                                              |
Roller                                          Dry surface for 1 week
  |_____|                                            |
                  |                        Hang on the ceiling with rice straw for 2-3 months
                  |                                            |
            Mixer crusher                                   Meju
                  |
            Inoculation with
            Aspergillus oryzae
                  |
         Incubation at 30°C, 72 hr
                  |
                Koji
```
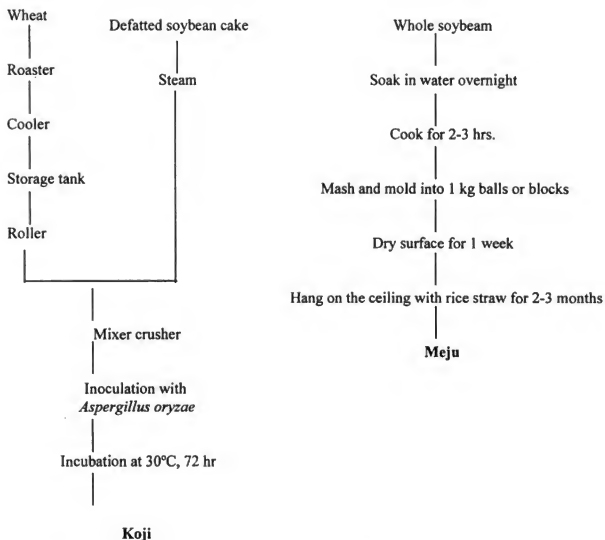
Figure 4. Flow chart for the preparation of solid-state fermented starters used in soysauce processing (Saone *et al.* 1986; Lee and Jul, 1982).

CLASSIFICATION OF FERMENTED CEREAL FOODS IN THE ASIA-PACIFIC REGION

Indigenous fermented foods may be classified according to a number of different criteria. They may be classified in accordance with the raw materials used, the major type of fermentation taking place, the usage of the product, and the district of production. In general, fermented products are classified according to usage of the products and the major fermentation process taking place; e.g. alcoholic foods and beverages, vinegars, breads, fermented porridges and snacks, and lactic acid fermented fish products. The type of cereals used in the fermentation process and the regional variation of the fermentation form the sub-classes of each category. Numerous types of fermented products prepared using various methodologies and having different physico-chemical and sensory characteristics within each category are described in the literature.

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More than 200 alcoholic beverages are described in Korean literature written between the seventeenth and nineteenth centuries. Some of these beverages are identical but differ in nomenclature; however, most of the products vary according to the methods of preparation, raw materials used and the season of production. Traditional Korean alcoholic beverages are classified in Table 4 (Lee and Kim, 1993). The term rice-wine (*chongju* or *yakju*) designates a filtered clear beverage containing an alcohol content of at least 15 percent, while rice-beer (*takju*) designates an unfiltered turbid beverage containing approximately 8 percent alcohol. These designations are applicable to most of the indigenous alcoholic beverages of the different countries described in this Chapter. The terminology describing alcoholic beverages in individual countries is generic, and represents numerous varieties of a single product in a country. Methods of producing alcoholic beverages, and their microbial and biochemical characteristics are also described here.

Table 4. Classification of traditional Korean alcoholic beverages (Lee and Kim, 1993)

Brewing Methods	Brewing Time	Separation Process	Raw Materials
Brewed Wine & Beer (*yakju, takju*)			rice, corn
Pure cereal Brew			glutinous rice
Single brew	short	no filtration	
Double brew	short	filtration or not	
Third brew	long	filtration	
Fourth brew	long	filtration	
Medicinal herb added brew			herbs
Fragrant plant added brew			fragrants
Distilled liquor (soju)			
Pure cereal		distillation	rice, corn
Medicinal herb added			sorghum, barley
Fragrant plant added			glutinous rice
Others			
Mixture of wine and spirits			rice, glutinous rice
Fruit wine (fruit-extracts in ethanol)			plum, grape, apricot etc
Oddly brewed			cereals

Alcoholic Food and Beverages

The most important fermentation products of cereals in the Asia-Pacific region are acids and alcohols, which are both flavour compounds and food preservatives. Alcohol fermentation is more important than acid fermentation in this region in terms of the relative amounts of cereals used for fermentation and the varieties of the products produced. Alcoholic fermentation of cereals also involves acid fermentation, which prevents the growth of spoilage and pathogenic microorganisms at the initial stage of the fermentation, but causes quality deterioration during storage.

Alcoholic beverages have played an important role in human spiritual and cultural life both in Eastern and Western societies. Unlike in Europe and the Middle East, where indigenous alcoholic beverages are produced primarily from fruit, alcoholic beverages are produced from cereals in the Asia-Pacific region, and serve as an important source of nutrients. European beer uses barley malt as the primary raw material, while Asian beer utilizes rice with moulded starters as the raw material. Beverages vary from crystal-clear products to turbid thick gruels and pastes. Clear products which are generally referred to as *shaosingjiu* in China, *chongju* in Korea and *sake* in Japan, contain at least 15 percent alcohol and are designated as rice-wine. Turbid beverages, such as *takju* in Korea and *tapuy* in the Philippines which contain less than 8 percent alcohol along with suspended insoluble solids and live yeasts, are referred to as rice-beer. Examples of alcoholic beverages prepared from cereals in Asia-Pacific region are listed in Table 5.

The process of cereal alcohol fermentation using mould starters was well established in the year of 1 000 BC, and 43 different types of cereal wines and beers were described with detailed processing procedures in Chi-Min-Yao-Su (530-550 AD). Millet appeared to be the main ingredient for alcohol fermentation. Among the 43 product types described, 16 were prepared from millet, 11 from rice and 12 from Indian millet. The dried and powdered starter was mixed with water and steamed grains, and fermented for two to three weeks or up to five to seven months depending on the brewing method. Multiple brews prepared by adding newly cooked grains to the fermenting mash for two, three, four and up to nine times were described (Yoon, 1993). Figure 5 compares the processing methodology for the preparation of *quing-chu-jiu* as appeared in Chi-Min-Yao-Su with that for traditional *chong-ju* prepared in Korea.

The incubation period for each step of the brewing process varies from two days to one month depending on the fermentation temperature. Low temperatures (ca. 10°C) are better for improving taste and keeping quality of the wine. Wines are traditionally prepared in late autumn or early spring, when ambient temperatures are below 10°C in the Far Eastern region. The volume of wine produced is approximately equivalent to that of raw grain used (Lee and Kim, 1993).

The traditional method of rice-wine brewing was industrialized by Japanese brewers in the late 19th century, who adopted pure starter culture manufacturing technology from Europe and transferred it to Korea and China. Figure 6 shows the Japanese process for preparing rice-wine (Steinkraus, 1983).

Table 5. Examples of cereal alcoholic beverages prepared in the Asia-Pacific Region

Product name	Country	Major Ingredients	Micro-organisms	Appearance and Usage
RICE WINE				
Shaosinghjiu	China	rice	*S. cerevisiae*	clear liquid
Chongju	Korea	rice	*S. cerevisiae*	clear liquid
Sale	Japan	rice	*S. sake*	clear liquid
RICE BEER				
Takju	Korea	rice, wheat	Lactic acid bacteria *S. cerevisiae*	turbid drink
Tapuy	Philippines	rice, glutinous rice	*Saccharomyces Mucor Rhizopus Aspergillus Leuconostoc L.Plantarum*	sour, sweet liquid, paste
Brembali	Indonesia	glutinous rice	*Mucor indicus Candida*	dark brown liquid sour, alcoholic
Jaanr	India Himalaya	finger millet	*Hansenula anomala Mucor rouxianus*	sweet-sour alcoholic paste; mix with water
ALCOHOLIC RICE PASTE				
Khaomak	Thailand	glutinous rice	*Rhizopus, Mucor Saccharomyces Hansenula*	semisolid, sweet alcoholic
Tapai pulut	Malaysia	glutinous rice	*Chlamydomucor Endomycopsis Hansenula*	semisolid, sweet alcoholic
Tape-ketan	Indonesia	glutinous rice rice	*A. rouxii E. burtonii E. fibulinger*	sweet-sour alcoholic paste
Lao-chao	China	rice	*Rhizopus A. rouxii*	paste
Bhattejaanr	India Sikkim	glutinous rice	*Hansenula anomala Mucor rouxianus*	sweet-sour alcoholic paste
ALCOHOLIC RICE SEASONING				
Mirin	Japan	rice, alcohol	*A. oryzae A. usamii*	clear liquid seasoning

```
Mix 1 part chu with 8 parts water              Polished rice powder (4 parts)
     in an earthen jar                              Wheat flour (1/2 parts)
            |                                            water (8 parts)
            |                                                  |
    Incubate for 10 days                         Boil to make gruel and cool
            |                                                  |
                     Millet (4 parts)             Add muruk powder (1 part)
            |               |                                  |
                     Steam Cooking           Ferment for 12 days (mother brew)
            |                                                  |
                  Spreading and cooling          Add 12 parts of cooked rice cake
            |               |                                  |
            |               |                     Ferment for 12 days (2nd brew)
            |               |                                  |
      Mix evenly in the jar (1st brew)           Add 16 parts of steamed rice
            |                                                  |
Add 6 parts of cooked millet after 2-3 days (2nd brew)   Ferment for 12 days (3rd brew)
            |                                                  |
Add 7-8 parts of cooked millet to the fermented mash up    Put into a sack and press
    to 6 times in every 2-3 days (6 th brew)                 |
            |                                    Remove clear liquid and discard filter
      Remove clear liquid                                  cake

  Quing-chu-jiu  (Chi-Min-Yao-Su)                  Sam-hai-ju (Korean)
```

Figure 5. Flow chart for the production of rice-wines in China and Korea. (newly cooked cereals are added at the end of each step of the fermentation process)

Polished Rice
|
Wash and steep
|
Steam
|
Cool
|
Add spores of *Aspergillus oryzae*
|
Ferment   Sake yeast
| |
Koji   Water
| |
                              Yeast seed mash

Main mash
|
Add water
|
Ferment for about 3 weeks
|
Filter and discard filter cake
|
Fresh sake
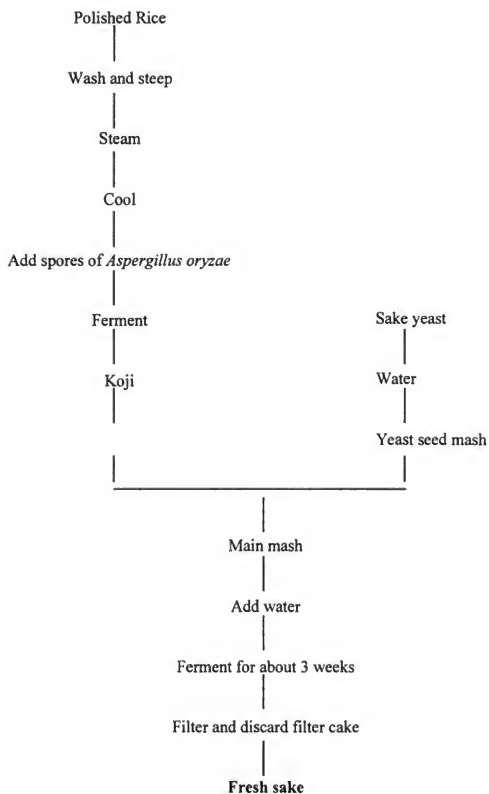
Figure 6. Flow chart for the Japanese sake brewing process.

Cereal beers are produced at a higher fermentation temperature, (ca. 20°C) than cereal wine, and are usually prepared by either single or double brewing. The fermentation starter powder is mixed with cooked cereals incubated at approximately 20°C for two to three days, following which it is filtered through a fine mesh, sieve or cloth. Figure 7 compares the preparation of Korean *takju* with that of Philippine *tapuy* (Steinkraus, 1983). Similar products such as *brem bali* in Indonesia (Saono *et al.* 1986) and *jaanr* and *bhatte jaanr* in Sikkim, India (Tamang *et al.* 1996; Batra and Millner, 1974) are also prepared in other Southeast Asian countries. Biochemical changes occurring during the fermentation of *takju* are summarised in Figure 8 (Kim, 1968).

Cereal-beers are abundant in micro-nutrients, such as the B vitamins, which are formed during the fermentation. Table 6 shows the approximate chemical composition of *takju*, which contains 7 percent alcohol (Korea Rural Nutrition Institute, 1991). Indonesian *brem bali* contains 16-23 percent reducing sugars and 6-14 percent ethanol (Saono *et al.* 1986).

Table 6. Approximate chemical composition of *takju* (Korea Rural Nutrition Institute, 1991)

Nutrient	Amount
Energy (kcal/100g)	55.0
Moisture (g)	90.7
Ethanol(%)	7.0
Protein (g/100g)	1.9
Fat (g/100g)	0.1
Carbohydrates (g/100g)	1.2
Ash (g/100g)	0.1
Calcium (mg/100g)	14.0
Phosphorus (mg/100g)	28.0
Iron (mg/100g)	0.8
Thiamine (mg/100g)	0.01
Riboflavin (mg/100g)	0.03

```
Polished Rice (4 parts)                    Glutinous Rice
         |                                       |
   Wash and steep                              Roast
         |                                       |
      Steam                            Cook without a cover
         |                                       |
Mix with 1 part powdered muruk           Cool on tray
and 10 parts water in an earthen jar            |
         |                          Innoculate with powdered bubod
 Ferment for 2-3 days                           |
         |                                 Pre-ferment
      Sieve                                      |
         |                                       |
   Takju (Korea)                         Tapuy (Phillipines)
```
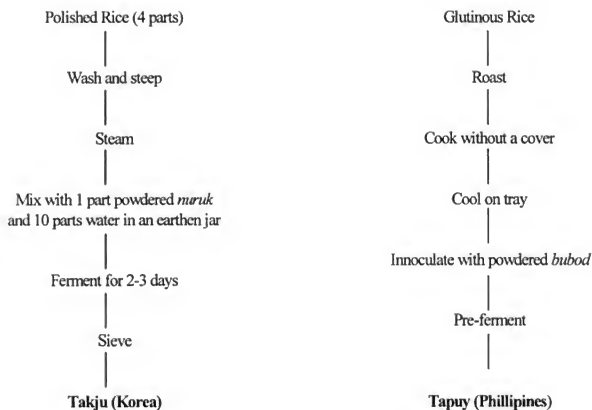
Figure 7. Flow chart for the processing of *takju* and *tapuy*.
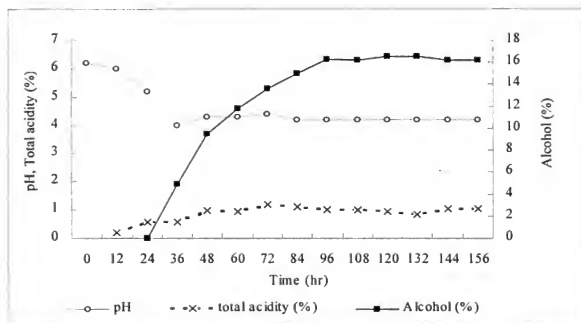
Figure 8. Changes in alcohol (percentage), pH and total acidity (percentage) during *takju* fermentation (Kim, 1968)

In the Southeast Asian countries, alcoholic fermentation has been used for the preparation of paste-type food products. Figure 9 outlines the processing procedure for the manufacture of *tape ketan* in Indonesia (Saono *et al.* 1986) and *bhattejaanr* in East Sikkim, India (Tamang *et al.* 1996). The major contributing microorganisms for *tape ketan* fermentation are *Amylomyces rouxii* and *Endomycopsis burtonii*. Figure 10 shows that during the course of fermentation, the pH decreases to 4.0 and ethanol content increases to 7 percent within 48-72 hours of fermentation, while starch and reducing sugars are decreased (Steinkraus, 1983).

Mirin is a traditional Japanese alcoholic seasoning prepared, from rice and *koji*. The digestion of rice by native enzymes in *koji* is carried out in ethanol in order to prevent microbial contamination. *Mirin*, the final product, is a clear liquid containing approximately 40 percent sugar, which is produced as a result of starch hydrolysis by *koji* enzymes. Approximately 80 percent of the sugar content of *mirin* is glucose (Takayama *et al.* 1997).

Glutinous rice	Glutinous rice
|	|
Wash	Wash
|	|
Soak	Cook
|	|
Steam	Cool in "mandro"
|	|
Cool to room temperature	Inoculate with marchaa and mix thoroughly
|	|
Mix with powdered *ragi;* spread (1 cm deep) on bamboo trays and cover with banana leaves	Place in a vessel/earthen pot and saccharify for 1-2 days
|	|
Incubate at room temperature for 48-72 hrs.	Ferment in an air-tight container for 2-8 days at ambient temperature
|	|
Tape ketan	**Bhattejaanr**

Figure 9. Flow chart for the manufacture of Indonesian *tape ketan* and *Indian Bhattejaanr* (Saono *et al.* 1986, Tamang *et al* 1996).

Figure 10. Biochemical changes occurring in *tape ketan* fermentation

Vinegar

Vinegar production is as ancient as is alcoholic fermentation, since acetic acid is produced in any natural alcoholic fermentation upon exposure to the air. In the Asia-Pacific region, vinegar prepared from cereal alcoholic fermentations is widely used in Northeastern regions, while vinegars from tropical fruits, such as coconut, sugar cane and pineapple, are prepared in Southeastern countries (Saono *et al.* 1986). Cereal vinegars may be divided into three classes: rice vinegar, rice-wine filtre cake vinegar and malt vinegar. Indigenous processes for the preparation of vinegars are natural or spontaneous fermentations brought about by the growth of *Acetobacter* on alcoholic substrates under aerobic conditions. Traditionally, degraded or poor quality rice-wines were used for the production of low-grade vinegars at the household level. Today, vinegars of high quality standards are produced by industry (Lim, 1984).

Rice vinegar is prepared from polished, unpolished or broken rice. The fermentation starter, *chu, nuruk* or *koji* prepared from rice is used for saccharification and alcohol fermentation as in the preparation of rice-wine. Slightly greater amounts of *nuruk* (ca. 30 percent of steamed rice) than are used in the preparation of rice wine are added to steamed rice, following which water is added (two to three parts water: one part raw rice). Fresh vinegar containing the appropriate organism is added to the fermented mash at a level of 8-20 percent of the weight of raw rice, and incubated at 30-35°C for one to three months. An additional storage period of two to three months is required for production of a high quality aged product (Ha, 1986).

Commercial vinegar is prepared from rice-wine filter cake in the Far-eastern countries. Filter cakes from rice-wine factories are collected and tightly packed into a storage tank for a one to two year period. The filter cake contains large amount of unused carbohydrates and proteins (Table 7), which are further hydrolyzed by inherent microorganisms and enzymes during storage, converting them into alcohol and other nutrients and flavour substances (Lim, 1984). The cake is slurried in two to three volumes of water prior to filtration. The filtrate is fermented with *Acetobacter* to produce filter cake vinegar. Figure 11 shows the industrial process for the preparation of rice-wine filter cake vinegar.

Rice-wine filtercake

|

Pack tightly in a storage tank for 1-2 years

|

Add 2-3 volumes of water

|

Stir 1-2 times/day for 7-10 days

|

Filter

|

Heat to 70°C

|

Mix Filter mash, filtrate and heated filtrate (2:1:1) to a temp of 36-38°C

|

Incubate at 36-38°C for 1-3 months

|

Age for 3-6 months

|

Clarify and filter

|

Heat, pasteurize and package

|

Rice-wine filtercake vinegar
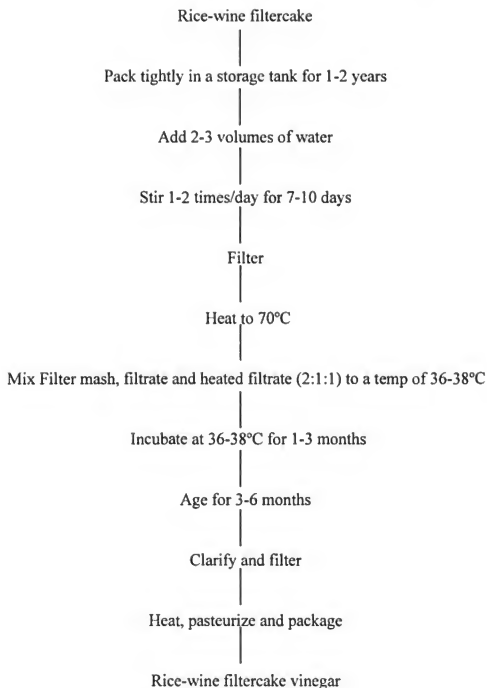
Figure 11. Flow chart for the processing of rice-wine filter cake vinegar.

Table 7. Proximate chemical composition of rice-wine filter cake

Total nitrogen	3.2%
Formol nitrogen	0.5%
Total sugar	22.0%
Reducing sugar	17.0%
Ethyl alcohol	12.4%

Fermented Breads, Noodles and Porridges

Large quantities of acid leavened bread and pancakes are consumed daily in India, Sri Lanka, Pakistan, Nepal, Sikkim, Tibet and neighbouring countries. *Idli*, *dosa* and *dhokla* are produced primarily in South India and Sri Lanka, and *jalebies* are consumed throughout India, Nepal and Pakistan.

Idli is a small, white acid-leavened and steamed cake prepared by bacterial fermentation of a thick batter prepared from carefully washed rice and dehulled black gram dhal. The rice is coarsely ground and the black gram is finely ground. *Dosa* batter is very similar to *idli* batter, except that the rice and black gram are finely ground. Following fermentation, the *dosa* is quickly fried as a thin, fairly crisp pancake and eaten directly. *Dhokla* is similar to *idli* except that dehulled Bengal gram dhal is used instead of black gram dhal in its preparation. The fermented batter is poured into a greased pie pan, and steamed in the open rather than in a covered *idli* steamer. Figure 12 shows the flow chart for idli production (Steinkraus, 1983).

During the production of these fermented breads, *Leuconostoc mesenteroides* and *Streptococcus faecalis* develop concomitantly at the soaking stage, and then continue to multiply following grinding (Mukherjee *et al.* 1965). *L. mesenteroides* is considered to be the microorganism essential for leavening of the batter and also responsible, along with *S. faecalis*, for acid production in *idli*, *dosa* and related products. These organisms appear to be associated with the ingredients and it is generally unnecessary to add an inoculum. Aerobic contaminants on the ingredients are eliminated partly by careful washing of the ingredients and partly by acidic conditions generated during the fermentation. Batra and Millner (1974) isolated *Torulopsis candida* and *Trichosporon pullulans* from *idli* batter and prepared authentic *idli* using the combined action of both yeasts. Both *T. pullulans* and *T. candida* imparted characteristic acidity. In addition, *T. candida* produced gas.

Jalebies are pretzel like, syrup-filled confections prepared from deep-fried, fermented wheat-flour dough. *Saccharomyces bayanus Sacc.* was isolated as a fermentor of wheat-flour paste during the preparation of *jalebies*. *Jalebies* prepared using that organism, could not be distinguished from a similar batch prepared using commercial baker's yeast as the fermentor. Acid-leavened bread and noodles prepared in the Asia-Pacific region are described in Table 7.

Although yeast fermented breads are widely consumed in the Asia-Pacific region, leavened bread type foods are not traditional staples of that region. The Chinese have traditionally consumed steamed bread or *mantou*, which is prepared by steaming yeast-leavened wheat dough, often filled with sweets, meats and vegetables.

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Black gram dahl

Wash and soak for 5-10 hrs

Gind finely in a mortar

White polished rice

Wash and soak for 5-10 hrs

Gind coarsely in a mortar

Combine slurries into a thick batter and mix well

Add salt for seasoning (approximately 1 % w/v)

Incubate overnight in a warm place (30-32°C)

Pour batter into small cups in idli cooker

Steam for 10 minutes

Ready for consumption
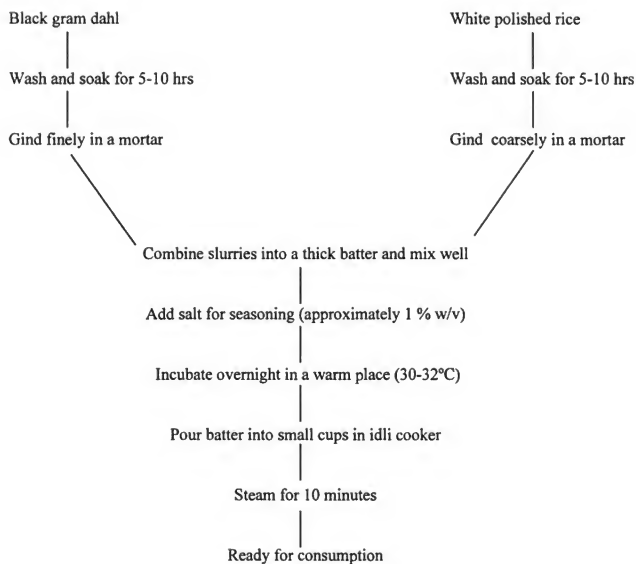
Figure 12. Chart for traditional Indian *idli* production (Steinkraus, 1983).

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Other types of breads are prepared primarily by acid fermentation of rice flour dough. These include Korean *kichudok* and Philippine *puto*, as shown in Table 8. These products are leavened, steamed rice cakes, which are similar to Indian *idli*, except for the fact that they do not contain any legumes. *Puto* is special in that it is prepared using year-old rice and the batter is neutralized at the mid point of the fermentation. Figure 13 outlines processing methodologies for the preparation of *kichudok* and *puto*.

Kichudok is prepared at the household level in Korea and is consumed on special occasions, while *puto* is normally consumed as a breakfast and snack in the Philippines. *Puto* is a common food of the lower-income group, but higher-income groups consume *puto* varieties containing added cheese, eggs etc., as delicacies. In a number of Philippine towns, the preparation of *puto* is an important cottage industry (Sanchez, 1977).

Brem is a special snack food prepared by the acid fermentation of rice in Indonesia. It is a solid cake, with a sweet and slightly sour taste, containing over 65 percent of glucose. Figure 14 shows essential steps in the processing of *brem*.

Table 8. Examples of acid-leavened bread and noodles used in Asia-Pacific region

Product Name	Country of Use	Major Ingredients	Microorganisms	Appearance & Usage
Idli	South India Sri Lanka	rice grits; black gram powder;	*L. mesenteroides* *S. fecalis* *T. candida* *T. pullulans*	steamed cake
Dosa	India	rice flour; black gram powder;	*L. mesenteroides* *S. faecalis* *T. candida* *T. pullulans*	griddled pancake
Dhokla	India	rice bengal gram	*L. mesenteroides* *S. faecalis* *T. candida* *T. Pullulans*	steamed cake
Jalebies	India Nepal Pakistan	wheat flour	*S. bayanus*	pretzel-like confection
Mantou	China	wheat flour	*Saccharomyces*	steamed cake
Kichudok	Korea	rice, takju	*Saccharomyces*	steamed cake
Puto	Philippines	rice, sugar	*L. mesenteroides* *S. faecalis* yeast	steamed cake
Brem	Indonesia	glutinous rice		cake
Mungbean starch	China Thailand Korea Japan		*L. mesenteroides* *L. casei* *L. cellobiosus* *L. fermenti*	noodle
Khanomjeen	Thailand	rice	*Lactobacillus sp.* *Streptococcus sp*	noodle
Me	Vietnam	rice	Lactic acid bacteria	sour food ingredient

```
Polished rice                          Milled rice (500 g)
     |                                        |
Wash, soak in water              Wash and soak in water (3-4 hr)
     |                                        |
Drain and grind                    _____|_____
     |                            |                       |
Mix with Takju and water    Ferment 100 g portion    Hang remaining
     |                      18 hrs at room temp       portion (900 g) in
Liquid dough                      |                   muslin bag
     |                       Add sugar                for 24 hrs
Ferment overnight                 |                       |
     |                       Ferment 6 hrs                |
Steam in layers                   |_____|
     |                                    |
  Kichudok                       Mix and add 30-100 ml water
                                          |
                                   Ferment 9 hrs
                                          |
                             Add 300 g sugar, 12 g lye, 80 ml water
                                       and mix
                                          |
                                   Ferment 4-5 hr
                                          |
                                   Steam for 30 min
                                          |
                                        Puto
```

**Figure 13. Flow chart for preparation of Korean *kichudok* and Philippine *puto*
(Steinkraus, 1983)**

Glutinous rice

|

Wash and soak overnight

|

Drain

|

Cook by steaming for 30 min

|

Cool with air

|

Inoculate with Ragi and mix thoroughly

|

Place in a well-covered container

|

Incubate at room temp. for 5-6 days

Press ————————→ Press cake ←———————— Water

| |

Liquid I   Press ················→ Press cake II

| |

Heat   Liquid II

| |

Concentrated product ——→ Mix products

|

Spray into layers of 1 cm thickness on trays

|

Sun dry for 5-7 hrs

|
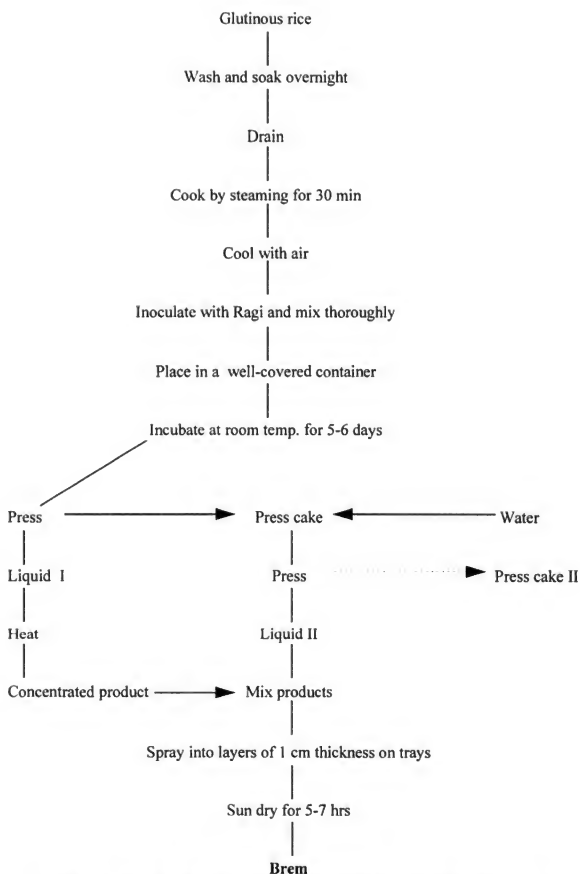
Brem

Figure 14. Flow chart for preparation of Indonesian *brem* (Steinkraus, 1983)

Mungbean starch is produced in most Asian countries, and mungbean starch noodles are dietary staples of the Chinese. The process for manufacturing mungbean starch involves an acidic bacterial fermentation (Wang, 1977). The mungbeans are hydrated by soaking in water and inoculated with 12-hour steep water from a previous fermentation to insure acidification of the beans. The principal microorganisms found in the steep water are *L. mesenteroides, L. casei, L. collobiosus* and *L. fermentum.* Lactic fermentation, which reduces the pH to about 4.0, protects the starch granules from spoilage and putrefaction that would occur in ground bean slurries.

Thai rice-noodle, *khanom-jeen*, is also prepared from acid-fermented rice (Rosanaphaiboon, 1987). Soaked rice is drained and fermented for at least three days prior to grinding. *Lactobacillus* species and *Streptococcus* species are involved in the acid fermentation.

Acid-fermented porridges, such as *ogi* and *uji* in African countries, are not common in the Asia-Pacific region.

Acid-fermented Fish and Meat Incorporated with Cereals

The storage life of perishable fish and meats can be extended by acid-fermentation with added carbohydrates and salts. Rice, millet, flour and even syrup or sugar are used as carbohydrate sources. A number of acid-fermented fish and meat products of different countries are listed in Table 9 (Lee, 1989). Acid fermentation and the keeping quality of the product are greatly influenced by the amount of added salt and carbohydrate (Jamias-Apilado and Mabesa, 1990; Mabesa and Babaan, 1993). Figure 14 outlines processing methodologies for the production of Korean *sikhae* and Philippine *balao-balao*. Both fresh water and seawater fish are preserved using this method. Millet is used as the carbohydrate source in the North-eastern countries, while in the Southeastern countries, rice is commonly used as the carbohydrate source.

Table 9. Examples of acid-fermented seafood, cereal, and meat mixtures.

Product Name	Country	Major Ingredients	Microorganisms	Usage
Sikhae	Korea	salt water fish cooked millet salt	*L. mesenteroides* *L. plantarum*	side dish
Nazerushi	Japan	salt water fish cooked millet salt	*L. mesenteroides* *L. plantarum*	side dish
Burong-isda	Philippines	fresh water fish rice salt	*L. brevis* *Streptococcus sp.*	side dish
Pla-ra	Thailand	fresh water fish salt roasted rice	*Pediococcus sp.*	side dish
Balao-balao	Philippines	shrimp rice salt	*L. mesenteroides* *P. cerevisiae*	condiment
Kungchao	Thailand	fresh water fish sweetened rice	*P. cerevisiae*	Side dish
Nham	Thailand	pork garlic salt rice	*P. cerevisiae* *L. plantarum* *L. brevis*	pork meat in banana leaves
Sai-krok-prieo	Thailand	pork rice garlic salt	*L. plantarum* *L. salivarius* *P. Pentosaccus*	sausage
Nem-chua	Vietnam	pork salt cooked rice	*Pediococcus sp.* *Lactobacillus sp.*	sausage

Figure 16 shows the microbial and biochemical changes of a typical lactic fermented fish product, *sikhae*, incubated at 20°C. The pH decreases rapidly during the first three to five days from 6.5 to below 5.0, while the texture softens within three to four days. The amino-N concentration increases steadily for 14 days, coincident with the attainment of optimum flavour. The number of lipolytic bacteria decreases rapidly during the initial stages of fermentation, while proteolytic bacteria increase until the twelfth day of fermentation and thereafter decrease rapidly. Acid forming bacteria increase rapidly, become the dominant microorganisms within one week of the fermentation and attain a maximum at 16 days. Flavour deterioration is associated with maximum growth of yeast and acid-forming bacteria (Lee *et al.* 1983).

```
        Flat fish                                    Live shrimp
           |                                            |
     Degut, wash                                   Wash and drain
    cut and drain                                       |
           |                                  Add 20 % (w/w) solar salt
Mix with salt and leave overnight                       |
           |                                   Allow to stand for 2 hrs
         Drain                                          |
           |                                    Drain and cut antennae
 Mix with cooked millet,                                |
    red pepper powder,                        Mix shrimp with cooked rice (1:4.8)
 minced garlic and ginger                               |
           |                                  Pack in glass jars and cover
Pack tightly in an earthen jar                          |
           |                                 Ferment at tropical room temperature
 Incubate at 20°C for 2 weeks                           |
           |                                        Balao-balao
     Gajami Sikhae
```

Figure 15. Flow chart for preparation of Korean *sikhae* and Philippine *balao-balao* (Lee, 1990)

Important bacteria for the lactic fermentation of Sikhae were identified as *Leuconostoc mesenteroides* and *Lactobacillus plantarum* (Souane, 1987). The role of these acid forming bacteria for the preservation of fish is apparent, but a more important factor is their ability to produce acceptable flavour during the fermentation process.

Fermented pork, *nham*, is a popular food in Thailand. It consists of fresh pork meat that is trimmed, minced, mixed thoroughly with salt, rice and seasoning and wrapped in small banana leaf packets. As is the case with Western fermented sausages, such as pepperoni and salami, *Pediococci sp. (P. cerevisiae)* are the main microorganisms associated with the fermentation. *Lactobacillus plantarum* and *L. brevis* have also been identified (Phithakpol, 1987).
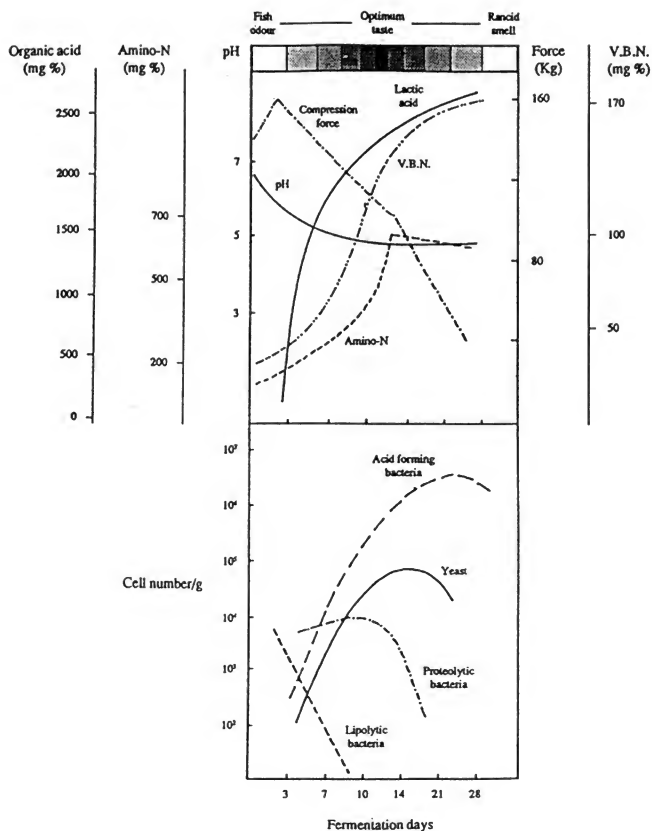
Figure 16. Microbial and biochemical changes during the fermentation of a lactic fermented fish product, *sikhae* (Lee, 1990).

RECENT DEVELOPMENTS

The production of rice-wine is a highly industrialized process in the Far Eastern countries. Numerous studies on *koji* moulds, *sake* yeasts and lactic acid bacteria, and their interactions during rice-wine brewing have been conducted in Japan. The application of modern biotechnology, particularly the application of immobilized microorganisms to brewing, has been studied widely and has substantially improved the traditional process of rice wine making. The use of recombinant DNA strategies for strain development is a hot issue in this area of research. Recent advances in Japanese brewing technology are well documented by Inoue and colleagues (1992).

Figure 17. Pasteurized and aseptically packaged Korean *takju* and *chongju*.

Extension of the shelf-life of rice-beer is perhaps the most important problem to be solved in Asia. Recently, optimum pasteurization conditions for *takju* were established and commercially applied (Lee *et al*, 1991; Lee and Kim, 1995). Under these conditions, the flavour of *takju* is stable for six months, and pasteurized and aseptically packaged Korean *takju* is now exported to Japan and to the United States (Figure 17).

Research geared toward the development of high protein lactic acid beverages from cereals has been conducted by several laboratories in East Asian countries (Lee, 1992). Pre-fermentation and extrusion cooking are desirable for improving the acceptability and yield of lactic fermented rice beverages (Lee *et al*. 1992; Souane, 1994). The organic acid composition, particularly the ratio of acetic acid and lactic acid produced by specific heterofermentative

lactic acid bacteria, is an important factor for the fresh fruity flavour of fermented rice beverages (Yi *et al.* 1993). Production of yoghurt-like beverages from cereals, (e.g. *risogurt*) has been investigated on a laboratory scale as well as a pilot plant scale (Mok, 1994; Collado *et al.* 1994). Although consumer surveys revealed that the rice yoghurts *risogurt* were highly acceptable, these products have not been commercialized.

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CHAPTER 4

CEREAL FERMENTATIONS IN LATIN AMERICAN COUNTRIES

INTRODUCTION

Cereal crops, in particular maize which has its origins in Mexico, are very important in Latin America and have been consumed in the fermented form for hundred of years. According to FAO statistical data, cereal production in Latin America and the Caribbean region during 1996 was as follows:

CEREAL	THOUSAND METRIC TONS
Wheat	25 429
Husked rice	20 505
Maize	69 348
Millet	47
Sorghum	8 813
Barley	2 022
Rye	47
Oats	852

The relative proportion of cereals fermented for human consumption in the Latin American region is unknown. The indigenous populations of Mexico and Peru in the Pre-Colombian era developed a number of traditional products based on maize. These fermented products are utilized as stimulants, in traditional medicine, as well as in religious ceremonies. Over the past twenty years consumption of many of these fermented cereals has declined due to urbanization.

This Chapter reviews fermented cereal products in Latin American countries.

FERMENTED BEVERAGES AND PORRIDGES

Among the cereals grown in Latin America, maize is most widely utilized, probably because Mexico is the centre of the origin of maize. Over hundreds of years indigenous populations developed and established processes for transforming maize into different types of products. Although the Latin American region is currently a net importer of maize (International Food Policy Research Institute, 1992), the situation differs from country to country: Brazil and Mexico are very important maize producers while most other countries are net importers of it. Fermented beverages and porridges consumed in the Latin American region are presented in Table 1. Of the products listed, chicha (maize beer) is the most important traditional fermented beverage in the region and also the most widely studied.

Chicha

Chicha is a clear, yellowish, effervescent, alcoholic beverage prepared from maize. It has a flavour similar to that of cider. The Andean Indians have consumed chicha for centuries. When prepared from pigmented maize varieties, its colour varies from red to purple. The alcoholic content of chicha varies between two and 12 percent (v/v).

The traditional production of chicha is a somewhat unique fermentation process in which saliva serves as the source of amylase for converting starch to fermentable sugars. Malting (germination) of maize kernels to produce the amylase required for starch conversion is an alternative procedure which is widely used in modern day processing. Frequently, salivation is combined with malting to yield chicha (Steinkraus, 1996).

Table 1.- Fermented Cereal Products Consumed in Latin America

Name	Description	Country
Abati	Alcoholic beverage based on maize	Paraguay, Argentina
Acupe	Beverage based on germinated maize, fermented and sweetened	Venezuela
Agua-agria	Non alcoholic beverage based on ground maize and water	Mexico
Arroz requemado	Fermented rice grains	Ecuador (Van Veen *et al.* 1968)
Atole	Non alcoholic porridge based on maize dough	Mexico
Atole agrio	Non alcoholic porridge based on black maize dough fermented four to five days	Mexico
Cachiri	Fermented beverage based on maize, manihot or fruits. It is produced in clay pots	Brazil
Champuz	Fermented beverage based on maize or rice	Colombia, Peru
Chicha	Alcoholic beverage based on pineapple, barley steep liquor and black maize dough. It is fermented for four days, following which brown sugar; cinnamon and clove are added.	Mexico
Charagua	Alcoholic beverage based on "pulque" syrup, chilli and toasted maize leaves, heated slowly and fermented	Mexico
Fubá	Germinated maize grains fermented in water	Brazil
Jamin-bang	Bread based on maize fermented for three to six days and cooked as a cake.	Brazil

Name	Description	Country
Napú	Beverage based on germinated, ground and fermented maize	Peru
Ostoche	Alcoholic beverage based on maize juice and "pulque" or brown sugar	Mexico
Pozol	Non-alcoholic acidic beverage based on maize liquor. Balls prepared from fermented dough are enveloped in banana leaves	Mexico
Quebranta huesos	Alcoholic beverage based on maize juice toasted maize and pirú fruits (Schinus molle)	Mexico
Sendechó	Alcoholic beverage (beer-like) based on germinated maize and red chilli. Dough is resuspended in water, boiled, bestowed, cooled and inoculated with Sendechó	Mexico
Sora	Alcoholic beverage based on germinated, ground, cooked and fermented maize	Peru
Tepache	Alcoholic beverage based on maize grains, brown sugar and water	Mexico
Tesgüino	Alcoholic beverage (beer-like) based on germinated maize, ground and cooked with fragments of plants that serve as enzyme sources	Mexico
Tocos	Dessert based on maize fermented for two to three months and cooked	Peru
Zarzaparrilla bark wine	Alcoholic beverage based on maize beer and zarzaparrilla bark	Mexico
Zambumbia	Alcoholic beverage based on toasted barley and water;	Mexico

Name	Description	Country
	fermented for three to four days, following which brown sugar is added	

Adapted from: Wacher-Rodarte, C. (1995). Alimentos y bebidas fermentados tradicionales. *Biotecnología Alimentaria*. pp. 313-349. García-Garibay, M., Quintero, R. and López-Munguía, A. (coord.). Limusa, México.

Production and consumption patterns - Chicha is produced in the Andean regions and sometimes in the lower altitude regions of countries such as Argentina, Bolivia, Brazil, Colombia, Ecuador and Peru.

In the Andean region, maize has always been of profound religious and magical significance, and chicha has played a role in fertility rites. Chicha was used to induce the "thunder god" to send rain, and was also used in sun and harvest festivals. Even today, chicha manufacture is a significant household or communal activity and mainly the Indians during religious and agricultural festivities and during important family and social events consume the beverage. A special annual festival designated the *Kayova* (*Cayua*) is dedicated to chicha production in Peru. This festival takes place in January at the beginning of the maize harvest (Cavero, 1986).

Substrates used in chicha production - The principal substrate used in chicha production is maize. Although any available maize may be used, certain types are considered to yield a high quality chicha. Alazan maize contains kernels which are light to dark red in colour, are early maturing, and highly drought resistant. Viru maize is yellow, requires a relatively long growing season, and is somewhat less drought resistant. Maize Negro is a purple-black variety used for preparing chicha in the Arequipa region of Peru. Other starchy materials such as manioc (yuca, cassava), sweet potatoes, or ripe plantains are used in chicha production. Quinoa, a grain (*Chenopodium quinoa*) is also used as a substrate in chicha production (Steinkraus, 1996).

Pre-fermentation processing - Starch hydrolysis is an essential step in the production of chicha. It is accomplished either through the salivation process, which utilizes salivary amylase (diastase), or through amylases generated during the malting (germination) of maize kernels.

In order to introduce salivary amylase into maize, it is first desirable to dry-grind the maize kernels. Dry grinding is performed by Indian chicha makers using either a primitive half-moon-shaped rocker stone mill or a mortar and pestle. The maize flour thus obtained is slightly moistened with water, rolled into a ball of appropriate size, popped into the mouth and thoroughly mixed with saliva using the tongue. The gob thus obtained is then flattened against the roof of the mouth with the tongue and placed in the sun to dry. The salivated gobs referred to as *muko* resemble the upper plate of a set of false teeth. They are sun-dried and ground in a stone mill. Muko production is generally carried out as a social event by groups of older women, sometimes with the help of young girls, who all sit in a circle.

During the malting/germination process, the kernels are soaked for 12 to 18 hours, generally overnight, then drained and kept in the dark usually about three days, under moist conditions until the plumules range between 0.25 and 0.5 cm in length. The kernels are then heaped and covered with burlap for one to two days, during which time the temperature rises until it is uncomfortable to place the hand in the mass of kernels. The kernels become white, parched, and covered with a thin layer of ash. Germinated grains are sun-dried, following which they are finely ground in a stone mill.

Chicha production from maize flour - Chicha is produced using a variety of methodologies. Methods of extracting the maize flour vary rather widely. In one method, an earthenware pot about 75 cm high and 85 cm in diameter is filled to one-third of its capacity with maize flour. When *muko* is used, additional nonsalivated flour may be added along with crude sugar or squash pulp. The pot is then filled with water and heated to approximately 75°C. Boiling water is not used since it produces an undesirable pasty consistency. The flour and hot water are thoroughly mixed for 1 hour and then allowed to settle and cool, following which three layers are formed: a top liquid layer called *upi*; a middle jelly-like layer, and a bottom layer which contains coarse particles (*hanchi*). The upi is scooped out with a gourd and is transferred to another earthenware pot. The middle layer is placed in a shallow pan, heated, and concentrated to a sugar-like product. The hanchi is pressed and filtered and the filtrate thus obtained is added to the upi. The upi is simmered for several hours until it becomes caramelized. This caramelized product, referred to as *misqui kheta*, is allowed to cool. Additional upi may be boiled for as long as 3 hours and then combined with the misqui kheta. After an incubation period of four days, the mixture of upi and misqui kheta begins to ferment and bubble violently. In the highlands, fermentation is complete and bubbling ceases in approximately six days, while in the hot lowlands, fermentation may be complete within two days. When bubbling ceases, the chicha is transferred to narrow-mouth pots and is ready for consumption. Froth is removed from the chicha with a cupped hand prior to consumption. This froth is used either as a furniture polish or as a starter for new batches.

An inoculum is not required, since the fermentation pots are never cleaned. After the chicha has been consumed, a layer of sediment remaining in the pot is filtered to obtain *sutu*, which resembles chicha in colour but lacks carbonation and the typical tangy flavour associated with cider. Sutu is reportedly of a higher alcohol content than chicha. Forty litres of chicha yield about 1 litre of sutu. Chicha production has not changed very much over the centuries (Steinkraus, 1996).

Chicha production from germinated grains - Germinated dried maize referred to as *jora* is ground in a stone mill, or by a village miller. The ground jora is referred to as *pachucho*. Pachucho is mixed with water and boiled in an earthenware pot for 3.5 hours. Water is added to replace moisture lost by evaporation. The mixture may then be slowly cooked for a further 24 hours. After cooling, handfuls of the mixture are rubbed in order to separate hulls and starch, and the pachucho is boiled for a further 4 hours. It is then cooled and filtered through a cloth or wire screen. The filtrate is collected in pots, which are used exclusively for fermentation and are therefore already inoculated with the necessary organisms. Fermentation proceeds for one day. The chicha is ready for consumption when the sweetness disappears and the flavour becomes semisharp. If not immediately consumed, it becomes increasingly sour and over time, turns to vinegar. Brown sugar or molasses may be added at the time of the

second boiling in order to increase the alcoholic content of the chicha. Forty pounds of pachucho yield approximately 7 gallons of chicha; and 100 pounds of shelled maize yields between 14 and 15 gallons of chicha (Steinkraus, 1996).

Microbiology of chicha - Yeasts, particularly *Saccharomyces cerevisiae*, and bacteria of the genus *Lactobacillus*, are the primary fermenting organisms in chicha produced in northern Argentina. Various yeasts (*S. cerevisiae*, *S. pastorianus* and *Mycoderna vivi*, *Oidium lactis* and *Monilia candida*), bacteria of the genera *Leuconostoc*, *Lactobacillus*, *Acetobacter*, and various moulds including *Aspergillus* and *Penicillium* are present and presumably active in Colombian chicha (Steinkraus, 1996).

Biochemical changes during chicha production - It has been reported that the natural mixture of organisms found in chicha has a greater ability to utilize starches, dextrins, and sugars than do pure cultures of *S. cerevisiae*. According to Steinkraus (1996) chicha organisms produced a total alcohol content of 13.4 percent (v/v) when fermenting a 30 percent solution consisting of 15 percent starch and dextrin and 15 percent fermentable sugars. Ninety percent of the total solids were fermented within ten days, and the total acid produced was 0.8 percent (Steinkraus, 1996).

Nutritive value of chicha - The nutritive value of chicha would be expected to be relatively high. The alcohol content of chicha supplies a caloric source. Chicha is also rich in the B vitamins. The riboflavine concentration is doubled, while thiamine and niacin remain fairly constant during the fermentation of chicha (Steinkraus, 1996).

Toxicoloy - There are no reports describing toxic reactions resulting from the consumption of chicha. If the starting maize were contaminated with mycotoxin-producing moulds such as *Aspergillu flavus* however, the chicha produced would likely contain aflatoxin.

Pozol

Pozol (from the Aztec *pozolli*, foamy) is a fermented maize dough formed into balls of various shapes and sizes ranging from 10 to 12 cm in length, 5 to 8 cm in width and 70 to 170 g in weight. Some unusually large pozol balls weigh 1 kg or more. Pozol is consumed by Indian and mestizo populations, mainly in the Southeastern states of Mexico, such as Chiapas, Tabasco, Campeche, Yucatan, and on a smaller scale in Veracruz, Oaxaca and Guatemala (Ulloa *et al*. 1987).

Consumption patterns -Balls of freshly prepared pozol, or pozol at various stages of the fermentation process are diluted with water to produce a whitish porridge which is consumed in the uncooked state as a basic food in the daily diet of large communities. The ratio of pozol dough to water varies between 1:2 and 1:3. Salt, toasted ground chilli pods, sugar, or honey may be added.

Particularly low-income individuals consume the beverage prepared from pozol during working hours, at meals, or as refreshment at any hour of the day. Some Indians, such as the Lacandones and Chamulas, carry balls of fermented and mouldy pozol as a provision for their journeys through the jungle. Pozol consumption varies between 80 and 1 000 g of undiluted pozol dough per person per day.

There are descriptions indicating the alimentary and ceremonial usage of this food by the Maya culture for several centuries prior to the Spanish conquest. Since then, pozol has been and continues to be consumed by ethnic groups such as the Chontales, Mayas, Lacandones, Tzeltales, Tzotziles, Tojolabales, Chamulas, Mames, Zoques and Zapotecos in Mexico. It is consumed by all social classes. Two basic types of pozol are distinguishable: a traditional-type prepared by the indigenous Indians and a mestizo-type, characterized by additional cooking of the dehulled grains (Cañas-Urbina *et al.* 1993).

The Lacandones utilize pozol mixed with water and honey to reduce, according to them, the fever of the sick. Present day Mayas offer pozol at ceremonies performed at immature, mature, and ready-to-harvest stages during the cultivation of maize.

Pozol is also consumed for the control of diarrhoea. Mouldy balls of pozol are said to have been used since ancient times as cataplasms in curing superficial infections and wounds. An *in vitro* antagonistic effect of pozol on several species of bacteria, yeasts and moulds, many of, which are pathogenic or potentially pathogenic to humans, has been reported.

Production - Pozol is prepared either domestically for consumption or on a small commercial scale according to traditional procedures handed down from generation to generation. In the production of pozol, 1 to 1 1/2 kg of kernels obtained by shelling cobs of maize (preferably white *Zea mays* L.), are boiled for 1 hour in a pot containing 1 to 2 litres of an approximately 10 percent (w/v) calcium hydroxide solution. During boiling, swelling of the kernels takes place, thus allowing the pericarp to be relatively easily peeled off the kernels. The kernels are cooled, rinsed with water, and drained resulting in what may be described as *nixtamal*. The nixtamal is ground in a manual metal mill to obtain coarse dough, which is manually shaped into balls. The balls are then wrapped in banana leaves to prevent desiccation, and fermented for one to 14 days or more depending on consumer preference and prevailing circumstances, Figure 1.

In the state of Tabasco, ground cacao beans are added to the dough prior to fermentation, to yield a fermented product called *chorote*. Ground coconut is also added to pozol (Cañas-Urbina *et al.* 1993) in the state of Yucatan.

Microbiology of pozol - During the initial 24 hours of pozol fermentation, bacteria outnumber yeasts and moulds and are probably responsible for the majority of acid produced. It has been reported that at the start of fermentation, traditional pozol contains lactic acid bacteria (10^4-10^6/g), aerobic mesophiles (10^4-10^5/g), *Enterobacteriacea* (10^2-10^3/g), yeast (10^2-10^4/g) and moulds (less than 10^3/g) at a pH of 7.3. After incubation for 30 hours at 28°C, bacterial counts increase to: 10^{10}/g lactic acid bacteria, 7×10^6/g aerobic mesophiles, 5×10^5/g *Enterobacteriaceae*, 10^6/g yeast and 10^4/g mould while the pH decreases to 4.6 (Wacher *et al.* 1993). Lactic acid bacteria, the predominant microbial flora of pozol includes strains of *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Lactobacillus confusus*, *Lactococcus lactis* and *Lactococcus raffinolactis* (Nuraida *et al.* 1995).

Corn
|

Boil in water containing calcium hydroxide
(this process is known as *nixtimalization*)
|

Grind to obtain a dough
|

Shape dough into balls and envelope with banana leaves
|

Allow to ferment for 1-14 days
|
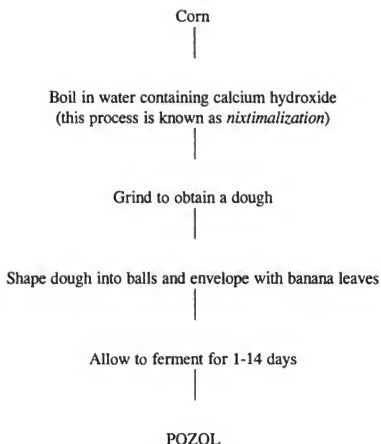
POZOL

Figure 1. Flow chart for the preparation of pozol

The majority of micro-organisms associated with maize kernels used in pozol preparation are destroyed by heat treatment during nixtamal production. Inoculation of the maize dough takes place during processing of the nixtamal, since individuals who prepare pozol do not take sanitary measures. Lactic acid bacteria, *Enterobacteriaceae*, and aerobic mesophiles are introduced at the grinding step (Wacher *et al*. 1993).

Geotrichum candidum, *Trichosporon cutaneum* and various species of *Candida* are always associated with pozol during the first few hours of fermentation. Yeasts associated with pozol include *Candida krusei*, *Trichosporon cutaneum*, *Hansenula fabiani*, *Kluveromyces fragilis*, *Candida guillermondii*, *C. parapsilosis*, *C. tropicalis* and *S. cerevisiae* (Ulloa *et al*. 1987). Moulds such as *Cladosporium clados-porioides* or *C. herbarum*, *Monilia sitophola*, and *Mucor rouxianus* or *M. recemosis* are also common in pozol balls as their surface progressively dries and their pH in lowered. Other species of moulds isolated from pozol include *Alternaria tenuis*, *Aspergillus flavus*, *Aureobasidium pullulans*, *Cladosporium herbarum*, *Epicoccum* sp., *Fusarium* sp., *Paecilomyces fumosoroseus*, *Rhizopus stolonifer*, *Trichoderma viride*, *Penicillium claviforme*, *P. cyclopium*, *P. expansum*, *P. italicum, P. lanoso-viridae* and *Phialophora richardsiae* (Ulloa *et al*. 1987).

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Bacteria isolated from pozol include *Bacillus cereus, Paracolobactrum aerogenoides, Agrobacterium azotophilum* and *Alkaligenes pozolis, Escherichia coli* var. *napolitana, Pseudomonas mexicana* and *Klebsiella pneumoniae* (Ulloa *et al.* 1987*).* Two newly identified bacterial species: *Agrobacterium azotophilum* and *Alkaligenes pozolis* have also been isolated from pozol.

A. azotophilum, originally isolated from pozol fixes atmospheric nitrogen both aerobically and anaerobically, in maize dough. It also exhibits nitrogen-fixing capability in several culture media, soil by-products, wastes of the sugar industry, and other substrates. *K. pneumoniae* has also been observed to act as a nitrogen fixer in some pozol samples. *In vitro* studies on *A. azotophilum* revealed that it exhibited different degrees of antagonism toward many micro-organisms. The chemical nature of the substance or substances, which are antagonistic towards the growth of these microorganisms, is not yet known. It is however believed that antifungal substances produced by *A. azotophilum* and/or other micro-organisms growing in pozol may prevent mould growth on pozol during the first few days of storage. Other factors such as the pH, moisture content, and temperature are presumably also important.

Flavour, biochemical and nutritional changes during pozol fermentation - Two essential changes that occur in maize dough during pozol fermentation are the development of an acidic flavour and a characteristic aroma which impart the refreshing properties of the product on ingestion. The acidic pH (pH 5.7) of the maize kernels is elevated to 7.5 by treatment with lime water. The maize dough, which has an initial pH of 6.8, attains a pH of 3.9 on the eighth day of fermentation. Its moisture content remains around 30 percent.

Improvement in the nutritive value of pozol over that of maize kernels is important to pozol consumers. Pozol is richer in protein, niacin, riboflavin, lysine, tryptophane, and some other nutrients than maize, however, maize contains more thiamine and phosphorous than pozol. In addition, on the basis of its essential amino acid composition and growth-promoting efficiency in albino rats, the protein quality of pozol was found to be better than that of maize. The nitrogen content of pozol was determined to be higher than that of unfermented maize dough.

Crude and soluble protein were significantly higher in nixtamal than in cooked maize and protein/ash ratios increased in nixtimal but not always in cooked maize. These findings suggest that fermentation is influenced by modifications in the dough caused by nixtamalization. Further investigations are required to ascertain the precise reasons for these changes (Loaeza-Chávez and Wacher-Rodarte, 1993).

Pathogenic or potentially pathogenic species of fungi, such as *Candida parapsilosis, C. tropicalis,* and *Phialophora richardsiae*, have been isolated from pozol. It has been shown that if the maize kernels used in the preparation of pozol are contaminated with the aflatoxins produced by *Aspergillus flavus,* most of these aflatoxins are destroyed by treatment with limewater and heat. Remaining toxins however persist throughout dough fermentation. One advantage of fermenting maize dough is that it can be preserved without refrigeration under the tropical conditions in which it is routinely eaten, owing to its low pH. A primary advantage however is the improvement of the nutritional qualities of this maize product due to the development of certain bacteria, yeasts and moulds.

Tesgüino

Tesgüino is a slurry-like, alcoholic beverage prepared by fermentation of germinated maize or maize stalk juice. The term tesgüino or *tejuino* comes from the Aztec *tecuin*, meaning heartbeat (Robelo, 1948). The Tarahumaras refer to tesgüino prepared from maize stalk juice as *paciki* and tesgüino prepared with the incorporation of the bark of certain species of *Rubiaceae*, as *batari*. The beverage is also referred to as sugiki. The Tepehuanos in Mexico refer to tesgüino prepared from germinated maize as *navaitai* and that prepared from the juice of maize stalks as *vougadi navaitai*. Navaitai is generally preferred over vougadi navaitai.

Tesgüino is consumed by several ethnic groups of northern and Northwestern Mexico. Yaquis and Pimas in Sonora, Tarahumaras in Chihuahua, Guarijíos in Chihuahua and Sonora, Tepehuanos in Durango, Huicholes in Jalisco and Nayarit and Zapotecos in Oaxaca all consume tesgüino. The mestizo populations of the states of Sonora, Chihuahua, Sinaloa, Durango, Nayarit, Jalisco and Oaxaca also consume it.

The beverage plays an important role in the everyday life of indigenous groups. It is the preferred beverage at any celebration, religious ritual, funerals, sporting games, or in the so-called *tesgüinadas*, which are some of the most important events in a Tarahumara's life. Tesgüino, when diluted with water, is commonly drunk by babies and infants, and is also drunk at meals during family reunions. The Tarahumaras, during their ball games, take a provision of tesgüino along with them and even drink it for strength prior to the games.

Mestizo populations consume tesgüino primarily as a refreshing beverage during hot weather. Tesgüino prepared by the Mestizos is of a relatively low alcoholic content and is not a basic dietary ingredient. The quantity of tesgüino consumed and frequency of its consumption varies with the age and type of consumer, season, and occasion of consumption. Usual consumption ranges between 250 ml and several litres per day.

Tesgüino is generally prepared by the fermentation of germinated maize, maize stalk juice or prepared juice obtained from mashed leaves of *Agave* sp.

Methodologies used in the preparation of *tesgüino* vary among ethnic groups. The most common method for preparing tesgüino is summarised in Figure 2. About 10 kg of dry maize kernels are soaked in water for several days, drained, and placed either in baskets in the dark or in a hole in the ground in order to allow them to germinate. The germinating kernels are protected from light, in order to prevent the formation of green and bitter sprouts. The germinated kernels are ground in a manual metal or stone mill and then boiled in water until the mixture turns yellow (about 8 hours). The liquid portion is then transferred to a clay pot, and catalysts are added. The most common catalysts in the vicinity of Cannon Urique in Chihuahua are bark (*batari*) or *kakwara* (*Randia echinocarpa, R. watsoni,* and *R. laevigata*) and *kaya* (*Coutarea pterosperma*), which are chopped, ground, and boiled for many hours prior to being added to the tesgüino.

At higher altitudes where pine trees grow, the catalysts used are leaves of *roninowa* (*Stevia serrata*), *rojisuwi* (*Chimaphila maculata*), and *ubitakuwari* (*Datura meteloides*); stems of *basiawi* (*Bromus arizonicus*), roots of *gotoko, otoko,* or *goto* (*Phaseolus metcalfei* and *Plumbago scandens*); *rawici kitakame* or "mouse's ear" (*Hieracium fendleri*); and two

unidentified plants, one of the *Graminea* species, and the other a legume, *gotoborisi*. The mixture is allowed to ferment for several days prior to consumption (Taboada *et al*. 1977 in Steinkraus, 1996).

Corn

|

Germinate in the dark

|

Grind to obtain a dough

|

Boil in water

|

Transfer liquid portions to a clay pot and add catalysts

|

Allow to ferment (2-3 days)

|
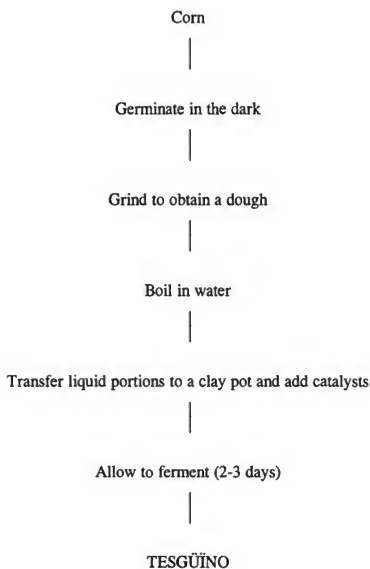
TESGÜÏNO

Figure 2. Flow chart for the preparation of tesgüino

In order to prepare tesgüino from maize stalks, the raw material either fresh or dry is macerated by pounding with a club, in the depression of a rock. The macerated material is then placed on a sieve made out of *awaka* (*Salix bonplandiana*) or *baka* (*Arundo donax* or *Phragmites communis*). Water is slowly poured over the macerated stems and juice is collected in a hollow pumpkin. The juice is mixed with water and boiled for several hours prior to the addition of catalysts. The mixture is allowed to ferment in a dark place for two to three days, until it develops a pleasant appearance and flavour before it is consumed.

The juice obtained from *Laphophora williamsii* (the hallucinogenic peyote), known as *jukuri*, or from *Ariocarpus fissuratus*, is sometimes added to the tesgüino in the Conchos river region of Mexico. The juice of peyote is not considered a catalyst but an additive that makes the ingestion of tesgüino more pleasant to consumers.

Several bacteria, yeasts and moulds have been isolated from tesgüino. These include *Lactobacillus, Streptococcus, Leuconostoc, Pediococcus, Saccharomyces, Candida, Cryptococcus, Hansenula, Brettanomyces, Pichia, Geotrichum* and *Penicillium* (Ulloa *et al.* 1987).

S. cerevisiae is an important micro-organism in the alcoholic fermentation of tesgüino. It has been isolated from samples of tesgüino obtained from different localities. The yeast inoculum is maintained on surfaces of the utensils and clay pots, which are used exclusively for the preparation of tesgüino. *Bacillus megaterium* has also been isolated from several samples of maize tesgüino and appears to be constant in the microflora of tesgüino. Since there are no standards for the preparation of tesgüino, the microflora of tesgüino varies according to the manufacturer, and the substrates and catalysts utilized in its preparation.

Micro-organisms initially detected in the gruel after cooking, filtering, and cooling are of homo- and hetero-lactic bacteria genera (*Lactobacillus, Leuconostoc, Pediococcus,* and *Streptococcus*) and increase throughout the fermentation. These organisms produce the lactic and acetic acids which give tesgüino some of its distinctively refreshing, acidic, slightly, acrid, flavour. Abundant yeast species consistently identified at various stages of the fermentation are alcohol producers, and include: *Candida guilliermondii, Hansenula anomala, S. cerevisiae,* and *S. kluyveri. S. cerevisiae,* and *S. kluyveri.* Other yeasts produce oxidative esters, which contribute to turbidity, aroma, and flavour. Thus tesgüino production is a lactic-alcoholic fermentation followed by an alcoholic-acetic acid fermentation. Of the yeasts isolated from tesgüino, *Brettanomyces intermedius, H. anomala,* and *S. cerevisiae* were also identified in the clay pots used for fermentation and serve as inocula.

Acetylene reduction detected in samples of tesgüino from the Tarahumara region, indicates the presence of nitrogen-fixing microorganisms. Such organisms have not however been isolated from tesgüino.

Fermentation catalysts may serve as sources of vitamins, enzymes or other growth factors. Basiawi (*Bromus arizonicus*), for example, supplied survival factors to a gram-negative strain isolated from tesgüino, significantly favoured the stationary phase of the organism, and accounted for the increment of the specific growth rate of a *Lactobacillus* strain, being a source of minerals or non-thermo-sensitive growth factors. It did not have any effect on a yeast strain (Escamilla-Hurtado *et al.* 1993). Florets of this catalyst were also identified as a source of *Candida guilliermondii*.

Compositionally, tesgüino contains 73.9 percent moisture, 2 percent protein, 0.21 percent crude fibre, 2.5 mg/100 g iron, 0.03 mg/100 g thiamine, 0.03 mg/100 g riboflavin, and 0.29 mg/100 g niacin.

During tesgüino fermentation, protein content increased by 58 percent and lactic acid, acetic acid, and ethanol concentrations were 0.41, 0.11, and 3.73 percent, respectively. At the

end of the process total protein concentration approached 13.2 percent (Wacher-Rodarte, 1995).

Atole

Atole is a sour porridge-type product prepared from maize by members of the Tzotzil ethnic group in Southern Mexico. It is produced by steeping maize grains in water for four days, milling and allowing to stand for one day.

During atole production, lactic acid fermentation commences during steeping and continues in the milled product (Escamilla-Hurtado *et al.* 1993). Lactic acid bacteria present in the atole form diacetyl, which contributes to the characteristic sensory properties of the product.

CONCLUSIONS

This review was begun with the expectation of finding several references to research projects and results obtained in Latin American laboratories working on traditional cereal fermentations. It was however surprising to find that over the past 15 years relatively little experimental work has been conducted in this field. Furthermore, over the past five years, there has been practically no published data on the subject. There is however a need to study in greater detail, the physicochemical and functional changes that occur during the fermentation of cereals in order to improve the methodologies used in their production.

The reasons for the loss of interest in the development of cereal fermentations in Latin America are unclear, since cereal products are still produced and consumed by several million people living the region.

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Cereals are consumed in all regions of the world. The techniques
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This bulletin reviews the fermentation of cereals to produce
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and improvement of fermentation process.