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Handbook of Indigenous Fermented Foods

edited by
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INTRODUCTION

Two primary fermentation products related to preservation of foods are acids and alcohol. Acid-fermented foods were covered in Section II. A number of the alcohol-fermented foods also involve an acid fermentation; and, of course, all alcoholic foods can become acidic if conditions following alcohol production are aerobic, permitting growth of *Acetobacter*, which produces acetic acid (vinegar). Generally food spoilage and disease-producing microorganisms cannot develop in either an acidic or an alcoholic environment, therefore the combination of acid and ethanol gives double protection.

Fermentations yielding alcohol and/or acid generally offer low-cost ways of preserving food in a world where the majority of the people cannot afford canned, frozen, or dehydrated foods (except those that are sun-dried). Nearly all humans, except those who do not consume alcohol for religious reasons, incorporate such products into the diet with enthusiasm. Alcohol serves as a source of calories; undesirable for the over-fed West but valuable to the calorie-deficient villager.

Primitive wines and beers that will be discussed in this section are not the crystal-clear products known in the Western world. Instead they are cloudy, effervescent slurries containing residues of the substrates and the fermenting yeasts and other microorganisms. Thus, primitive wines and beers provide not only calories but B vitamins as well. Since people subsisting on polished rice are often deficient in thiamine and riboflavin, addition of these vitamins to the diet in the form of a primitive wine or beer can be lifesaving, preventing beri-beri and riboflavin deficiency. Maize diets are generally low in niacin and frequently lead to pellagra, which is generally not found in cultures subsisting on maize if a portion of the diet is consumed as primitive beer or wine with its content of niacin. Most primitive wines and beers contain small amounts of protein and amino acids, which contribute to the protein nutrition of the consumer. Thus, these products are very important wherever consumed in the developing world. In this section we will examine the types and nutritional significance of a number of primitive wines and beers.

ALCOHOLIC FOODS/BEVERAGES IN WHICH SUGARS ARE THE PRINCIPAL FERMENTABLE CARBOHYDRATES

Honey Wines

Honey wine (mead; metheglin) has been an indigenous fermented beverage for thousands of years (Morse and Steinkraus, 1975). Honey was the only concentrated sugar widely available in prehistoric times. Brothwell and Brothwell (1969) suggest that the half-empty honey pot left in the rain may have been the first alcoholic drink. Fruit wines were probably discovered as soon as man tried to collect and store sweet fruits and berries.

It is estimated that about 20 tons of honey are used to manufacture honey wine in New York City each year, mainly for the Kosher trade. Honey wines are also made on a small scale in England and France. In Poland, the honey cooperative at Krakow still makes mead, mainly from dark honeys by traditional processes. *Dwojniack* is made with equal weights of honey and water and is fermented with osmophilic yeast. The ethanol content is about 16% v/v and there is residual sugar, making it quite sweet. Dwojniack is aged 5 to 7 years in 4000-liter wooden vats. *Trojniack* is made from 0.5 kg honey/kg water. Its ethanol content is about 12.5% v/v, it is slightly sweet,

and it is aged 3 years or longer. Cornflower (*Centaurea*) honey is considered best for trojniack (Morse and Steinkraus, 1975).

Methods of analyzing honey have been reviewed by Steinkraus et al. (1971) and applied to honey produced by *Apis dorsata*, the rock bee (Minh et al., 1971). While the average total solids of *A. mellifera*, the European bee, honey is 82.8%, principally sugars (White et al., 1962), *A. dorsata* honey averages 72.19% solids, probably due to moisture absorption in the high humidity of the tropics. The major sugars in honey of *A. mellifera* are fructose (38.19%), glucose (31.28%), maltose (7.31%), and sucrose (1.31%). The pH of *A. mellifera* honey averages 3.9.

Honey, particularly light yellow honey, is deficient in nitrogen and growth factors needed by yeasts. Thus, natural fermentations of light honeys are prolonged, requiring months rather than days for completion. Dark honeys contain more pollen and consequently more growth factors than light honeys and therefore ferment more readily than light honeys. The rates of fermentation of all honeys can be increased by addition of nitrogen and growth factors that stimulate the yeast (Steinkraus and Morse, 1966).

The addition of hops to honey wines is a very old technique. The flavor and aroma of hops are quite popular and, in addition, hops contain tannins that can precipitate proteins causing cloudiness. Hops also contain compounds that help make alcoholic beverages more stable biologically (Prescott and Dunn, 1959). Hops may also contain nutrient factors of value to the yeast metabolism.

Chemical Composition of Honey Wines

Sarin (1921) analyzed 42 honey wines produced in Eastern Europe. The alcohol contents ranged from 6.4 to 16.6% v/v with an average ethanol content of 11.0% v/v. Average acidity (as lactic) was 0.554 g/100 ml (range 0.342 to 1.062); average content of volatile acid (as acetic) was 0.178 g/100 ml (range 0.067 to 0.574); average ash content was 0.169 g/100 ml (range 0.71 to 0.699); and average residual invert sugar content was 15.7 g/100 ml (range 0.71 to 37.44). Patschky and Schone (1970) analyzed 13 samples of mead ranging in alcohol content from 6.6 to 14.2% v/v. The pH values ranged from 3.45 to 4.0. Total acid (as tartaric) ranged from 0.24 to 0.63 g/100 ml and volatile acids (as acetic) ranged from 0.02 to 0.19 g/100 ml. Steinkraus and Morse (1973) analyzed 11 honey wines available in the U.S. market and found that alcohol content ranged from 12.2 to 20.8% v/v. The pH varied from 2.90 to 3.75. Total titratable acidity (as tartaric) ranged from 0.220 to 0.708 g/100 ml, and volatile acids (as acetic) ranged from 0.014 to 0.079 g/100 ml. Acetaldehyde content ranged from 18.2 to 126.5 mg/100 ml. Content of residual reducing sugar (as dextrose) ranged from 2.5 to 27.8%. The residual sugar in all but two samples was 10% or higher, indicating that commercial meads are generally quite sweet.

Ethiopian Tej (Vogel and Gobezie, 1977)

Description

Ethiopian *tej* is a home-processed honey wine found throughout the country. Quality tej is yellow, sweet, effervescent, and cloudy, due to the content of yeasts. The flavor depends upon the part of the country where the bees have collected the nectar and the climate. Hops and spices add distinctive flavors.

Patterns of Consumption

Honey, being expensive, places pure honey tej beyond the reach of most Ethiopians. Thus, tej is used only for special occasions. Originally tej was found only in the homes of royalty and noblemen. A less expensive version of tej is made by replacing part of the honey with sugar and adding a natural yellow food coloring. Honey and honey wines are used for bartering, and they play an important role in feasts, dowries, and marriage ceremonies.

Steps in Production

The only pieces of equipment needed are a covered container and a cloth for straining. The fermentation pot is smoked so that the tej will have a desired smoky flavor. The ingredients are honey, water, and hops (*Rhamnus prinoides*). Spices such as ginger may be added. The honey used for brewing in Africa is either collected from wild nests or produced in traditional barrel-type hives, and thus, contains broken combs, wax, pollen, and bees. The belief persists that crude honey makes a better mead than refined honey. The pollen serves as a yeast nutrient, and residual wax floating on the surface may keep the fermentation more anaerobic. The flow sheet for production of tej is given in Figure 1.

Proportions of honey to water can vary from 1:2 to 1:5 v/v. Since honey is about 80% sugars, the diluted honey will contain from about 13 to 27% sugar. Final alcohol content, if the fermentation goes to completion, will be from 7 to 13 or 14% v/v. Desta (1977) reported ethanol contents in traditional tej ranging from 13.18 to 13.73 v/v. His samples of tej must have been made with the higher concentration of honey.

In the early days, a handful of roasted barley was added to the pot to cause it to ferment. Also, wood or bark from the shrub *Rhamnus tsaddo* was added. Fermentation time was 5 or 6 days (Platt, 1955).

Essential Microorganisms

Yeasts of the genus *Saccharomyces* are responsible generally for conversion of sugars to ethanol: no inoculum is used. Thus, the fermentation depends upon yeasts present in the environment. The fermentation would very likely be improved if desirable strains of fermentative yeasts belonging to genus *Saccharomyces* were isolated and used for inoculum.

Chemical Changes during Fermentation and Changes in Nutritional Value

The principal chemical changes are the production of ethanol and carbon dioxide from the fermentable sugars.

No data are available about changes in nutritional value, but certainly the growth of yeasts in the diluted honey and their consumption in the fermented tej contribute B vitamins to the consumer.

Toxicology

There are reports of headaches following overconsumption of tej. This suggests that the content of fusel oils may be high.

Sugar Cane Wines

Introduction

Traditional sugar cane wines are sweet-sour, effervescent, turbid, alcoholic beverages made by fermentation of sugarcane juice. Generally light tan to

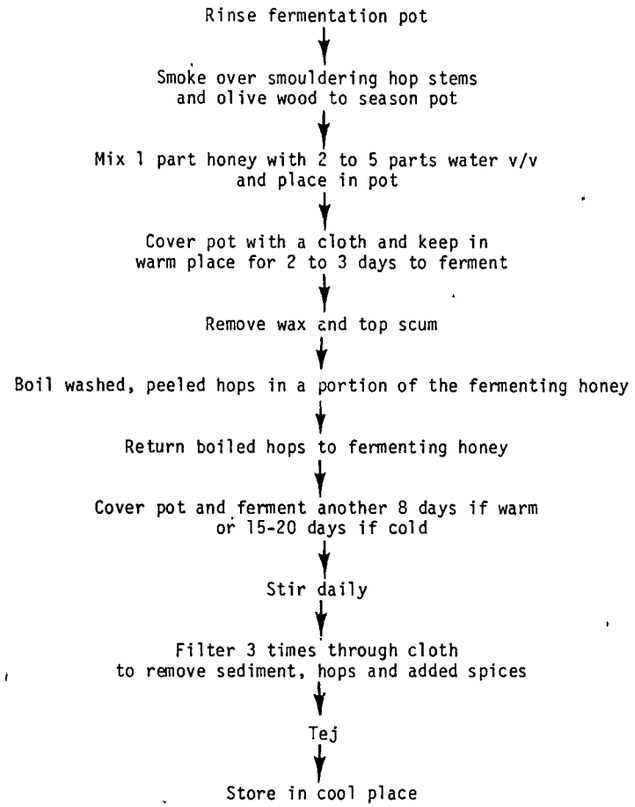

FIGURE 1. Flow sheet: Production of Ethiopian tej. (From Vogel and Gobezie, 1977.)

yellowish in color, they are produced in most developing countries wherever sugar cane is available. Sugar cane wine is also known in Japan as *shoto sake* (Kozaki, 1976). This paper will describe sugar cane wines from two major areas: Philippine *basi* (Sanchez, 1977; Tanimura et al., 1978b) and Kenyan *muratina* (Harkishor, 1977).

Philippine Basi (Sanchez, 1977; Tanimura et al., 1978b)

Description: Philippine *basi* is a traditional alcoholic beverage, particularly among the Ilocanos on Luzon. It is made by fermenting boiled, freshly extracted sugar cane juice with a mixture of yeasts, bacteria, and molds, or with organisms found on samac (*Macharanga tanarius* or *M. gradifolia* Linn.) leaves, bark, or fruit.

Steps in preparation: The method for producing basi using a dried, powdered starter (*bubod*) is given in Figure 2. *Bubod* is a relatively stable form of basi culture used to prepare the final basi starter, *binubudan*. It consists of powdered rice and ginger mixed thoroughly with enough water to

have a consistency that permits rolling the material into a ball and flattening it. The discs are coated with bubod 1 to 3 months old and incubated in rice straw for 36 to 48 hr at room temperature. The bubod is then sun-dried (Figure 3).

Binubudan-activated starter preparation is shown in Figure 4. Rice is cooked with water so that the grains remain separate; any lumps are broken up and the rice is cooled to 40 to 45°C. Then it is inoculated with 300 g powdered bubod for 4 kg of rice. The rice and bubod are mixed in a clean basin and covered with banana leaves and then clean cheese cloth, and fermentation continues for 24 hr.

Sugar cane juice is extracted in a mechanical press operated by a water buffalo (*dadapilam*). It takes 1 hr to produce 170 liters of 26°C Brix juice. The juice is poured into an iron kettle (*saang*), boiled, and the scum is removed. Next, a bamboo basket containing 2.2 kg of powdered year-old duhat (*Sizygium cumini* Linn.) bark, 30 g powdered tangal bark, and 500 g of fresh guava leaves is immersed in the main batch of juice. These materials

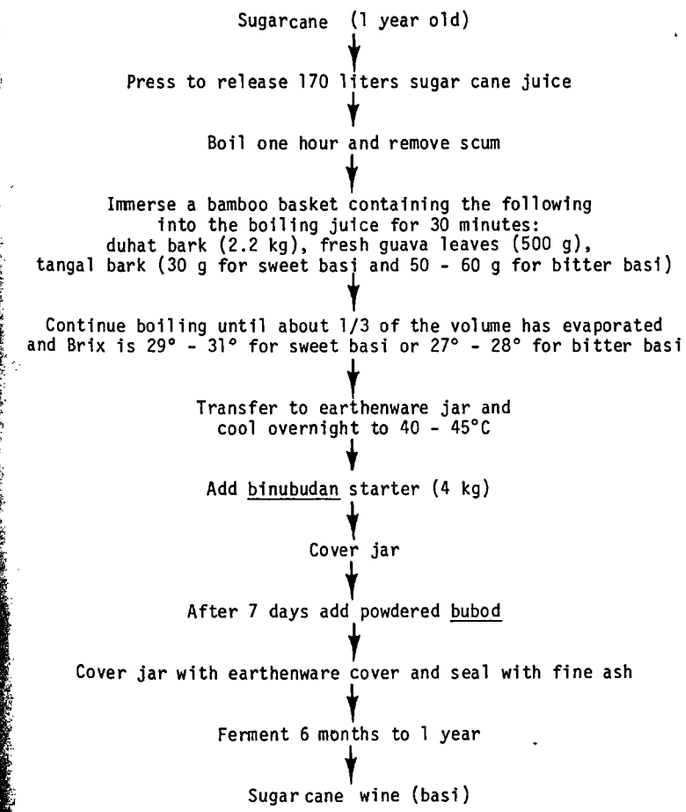

FIGURE 2. Flow sheet: Preparation of Philippine basi using binubudan. (Adapted from Sanchez, 1977.)

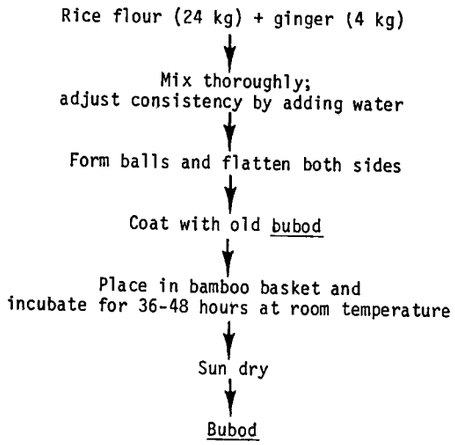

FIGURE 3. Flow sheet: Preparation of Philippine bubod (starter). (Adapted from Sanchez, 1977.)

are added for color and flavor. Boiling continues for 30 min, and then the basket containing the additives is removed. Boiling is continued until the Brix reaches 29 to 31°C. The boiling process takes 2 to 3 hr. The concentrated juice is poured into a clean earthenware jar through a screen to remove particulate matter, a clay lid is placed on the jar, and the juice is allowed to cool 24 hr to 40 to 45°C. The juice is then inoculated with 24-hr binubudan starter.

After inoculating the sugar cane juice with binubudan, fermentation continues for a week before bubod is added to speed up the fermentation. After 1 month of fermentation, the mouth of the earthenware jar is covered with clean cloth or absorbent paper and the earthenware cover is sealed with paste

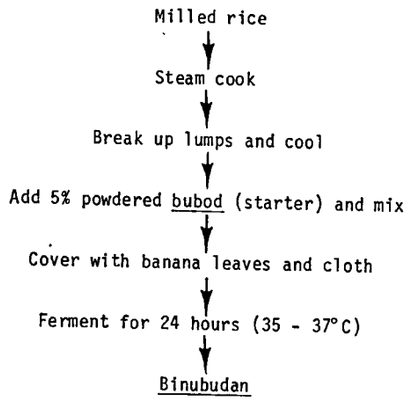

FIGURE 4. Flow sheet: Preparation of Philippine binubudan (activated starter). (Adapted from Sanchez, 1977.)

or fine wood ashes. The product, basi, is then allowed to age for 1 year before consumption.

In a second procedure, bubod and binubudan starters are not used. Instead, the sugar cane juice is inoculated with organisms present on 1-year-old dried fruit, leaves, and bark of the samac (*Macharanga tanarius* or *M. grandifolia* Linn.). The preparation is given in Figure 5. In this process, fresh sugar cane juice is boiled for 2 hr to about three-quarter volume, at which point the concentrate is transferred to an earthenware jug and cooled overnight. Milled rice, dried samac leaves, bark, and dried fruit are added and mixed thoroughly. The mouth of the jug is covered with banana leaves and fermentation continues for 3 or more months. Finally, the beverage is aged about 1 year.

Microbiology: Bubod starter varies widely in its physical dimensions and chemical analyses. Bubod cakes weigh 100 to 200 g and contain approximately 14% moisture. Total carbohydrate is 50 to 90%; reducing sugar is 0.5%; total nitrogen is 1%; and crude ash is 0.7%. Bubod also varies in its content of molds, yeasts, and lactic acid bacteria (Table 1).

Viable cell counts of bubod range from 1×10^7 to 2×10^8/g; single-celled yeasts range from 4×10^5 to 4×10^7/g; filamentous yeasts range from 3×10^6 to 1×10^8/g; lactic acid bacteria range from 1×10^6 to 3×10^7/g; and total bacteria range from 1×10^5 to 6×10^7/g.

Viable cell counts on samac fruits are quite low: yeasts 3×10^2/g; lactics 6×10^2/g; molds 16×10^3; and total bacteria 1×10^4/g (Table 2). Samac bark contained similar low levels of organisms: yeasts 7×10^2/g; lactics 2×10^3/g; molds 9×10^3/g; and total bacteria 3×10^3/g.

Kozaki (1976) reports that the dominant organisms in basi are *Saccharomyces Endomycopsis*, and lactic acid bacteria.

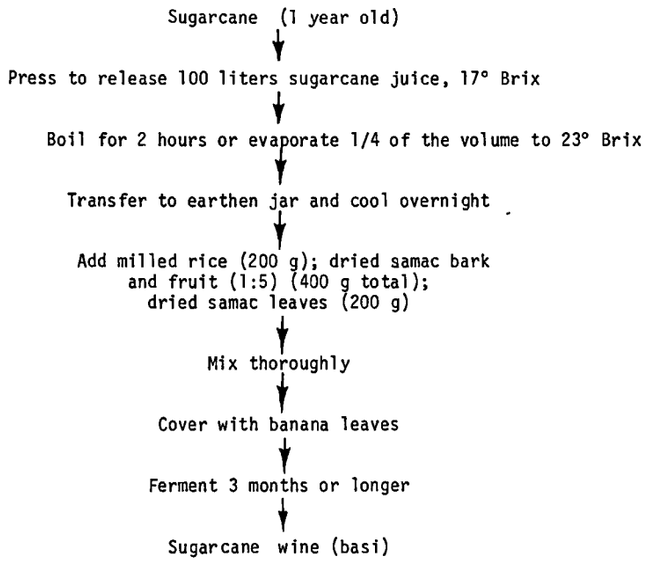

FIGURE 5. Flow sheet: Preparation of Philippine basi using samac (*Macharanga tanarius* Linn.). (Adapted from Sanchez, 1977.)

TABLE 1. Viable Cell Counts of Bubod[a]

Sample number	Molds (× 10^3)	Yeasts (× 10^6)[b]		Bacteria (× 10^5)[b]	
		Single cell	Filamentous	Lactics	Total
1	7.8	41.0	98.0	260.0	500.0
2	17.0	4.2	4.4	4.5	7.4
3	10.0	9.5	27.0	0.1	0.3
4	8.9	0.45	3.0	80.0	230.0
5	38.0	11.0	21.0	27.0	97.0
6	8.0	27.0	26.0	130.0	260.0
7	10.0	7.4	66.0	0.2	1.2
8	20.0	1.6	18.0	0.7	1.5
9	110.0	18.0	14.0	140.0	640.0
10	50.0	3.8	25.0	7.7	18.0

[a]Average of three replicates.

[b]Cells/g.

Source: Sanchez (1977).

Chemical analyses of basi: Soluble solids in eight samples of commercial basi (Table 3) ranged from 6 to 15%, pH from 3.7 to 4.1, reducing sugars from 0.92 to 8.13%, total sugars from 1.54 to 10.16%, total acid (ml 0.1 *N* NaOH/10 ml) from 3.49 to 16.50, and alcohol from 9.4 to 13.4% v/v.

Kenyan Muratina (Harkishor, 1977)

Patterns of consumption and place of muratina in socioeconomic hierarchy of the community: *Muratina*, also called *kathroko*, *neoobi*, and *njohi*, is an indigenous sweet/sour alcoholic beverage produced by the Kikuyus of Kenya. It is consumed as a refreshing beverage in place of Western-style beers, and is drunk in large quantities at festivals and social gatherings. Because of its low cost it is preferred to barley beers.

TABLE 2. Viable Cell Counts of Samac[a]

Sample	Yeasts	Bacteria[b]		Molds
		Lactics	Total	
Fruit	3.3	6.0	100	160
Leaves	57.0	18.0	43	84
Bark	7.0	17.0	26	93

[a]Average of three replicates.

[b]Cells × 10^2/g.

Source: Sanchez (1977).

TABLE 3. Chemical Composition of Basi

Sample	°Brix	pH	Reducing sugar (%)	Total sugar (%)	Alcohol (% v/v)
1	9.0	3.9	2.28	3.08	13.2
2	15.0	4.0	8.13	10.16	12.3
3	12.5	4.1	5.72	6.02	13.4
4	13.4	3.8	6.97	7.19	12.2
5	7.0	3.7	1.96	2.92	9.7
6	8.5	3.9	2.72	3.25	10.6
7	7.0	3.8	0.92	1.67	12.6
8	6.0	3.8	0.92	1.54	9.4

Source: Tanimura et al. (1978b).

Development of semicommercial processes for producing muratina has led to profitable employment for workers who crush sugar to extract the juice for sale to commercial and household manufacturers. Muratina serves as partial payment for services rendered by farm laborers, and often muratina parties are held at the end of a hard day's work. Muratina is socially indispensible; however it is consumed only by the men, women and children are not allowed to drink it.

Methods of production (See Figure 6 for flow sheet): In the home, the sugar cane is shredded with the sharp edges of corrugated iron and the juice is squeezed out by hand. Otherwise, the sugar cane is brought to a commercial crushing mill. After extraction, the juice is placed in a wooden barrel. Honey or sugar may be added to increase the alcohol content of the muratina.

Starter culture: The juice is inoculated with muratina (sausage tree) fruit, from which the beverage gets its name. Only muratina fruit that has fallen upon the ground is used; fruit hanging on the tree is never used. The raw fruit is first sun-dried, then it is cut longitudinally into two halves and the seeds are removed. The fruit is boiled in two or three changes of water, and the fruit pieces are again sun-dried to a brown color. The dried fruit is inoculated into a small quantity of sugarcane juice and incubated in a warm place for 1 or 2 days. The fermented juice is discarded and the fruit is again sun-dried. It is now ready for inoculating large batches of muratina and it can be used successively. However, it must be sun-dried following each new fermentation.

Fermentation: The dried muratina fruit is fastened to the bottom of a barrel with twigs, the sugar cane juice is added, and incubation continues for 2 or 3 days in a warm place. Frequently, the sugar cane juice is diluted, probably to increase the yield. Temperature of fermentation is from 30 to 35°C. The wooden barrels help in this regard by retaining the heat produced by the fermenting microorganisms. The barrel is filled nearly to the top with juice and this, with the blanket of CO_2 gas which is produced during fermentation, keeps the juice anaerobic. Completion of fermentation is determined by taste and observation of the rate of gas evolution.

Flavor, texture, and biochemical changes: Muratina has a sour alcoholic flavor. It is turbid because of its content of fermenting

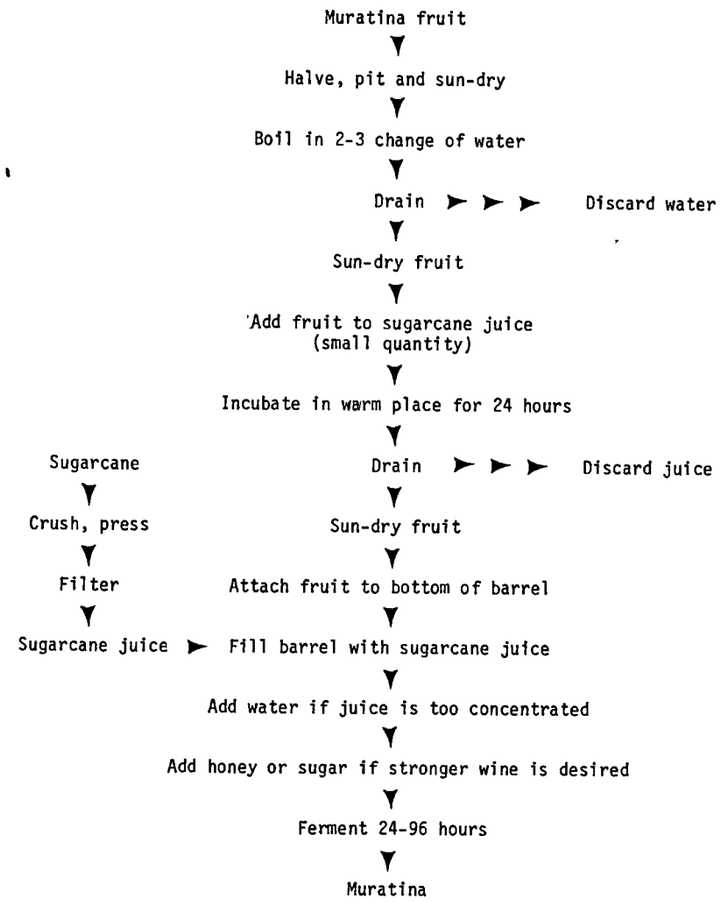

FIGURE 6. Flow sheet: Home production of Kenyan muratina. (From Harkishor, 1977.)

microorganisms and is effervescent due to being bottled while carbon dioxide is still being produced.

The pH of the freshly pressed sugar cane juice is between 5.2 and 5.7; it falls to pH 3 at the end of the fermentation. Ethanol, organic acids, and carbon dioxide are produced from the sucrose in the cane.

Microbiology and nutritive value: No systematic studies have been made but it has been observed that the fermentation is dominated by yeasts. *Sarcina* spp. also appear to be present.

Muratina is not filtered. It is a turbid beverage containing the fermenting organisms. Thus, based on other studies, it would be expected that muratina is rich in B vitamins. The ethanol serves as a good source of calories in the diet.

Toxicological problems: Raw, undried muratina fruit can cause diarrhea, and if such fruit is used in the fermentation, the product also may cause diarrhea.

Stability of muratina on the market: It will likely remain a staple in the sugar cane districts as long as its price is one-fourth to one-third of the cost of lager and pilsner beers.

Palm Wines (Toddys) (Okafor, 1977a; Odeyemi, 1977; Faparusi, 1977; Theivendirarajah et al., 1977a,b; Samarajeewa, 1977; Nyako, 1977; Merican, 1977; Shuaib and Azmey, 1977; Wong and Jackson, 1977; Ekmon and Nagodawithana, 1977)

Names in Various Countries

Palm wines include Nigerian *emu*; Nigerian *ogogoro*; Philippine *tuba*; Ceylonese *ra*; Ceylonese *panam culloo*; Ghanian *nsafufuo* from *Elaeis guineensis*; *doka* from *Raphia hookeri*; *yabra* from *Boraaus aethiopum*; Malaysian *tuak*, *niva* (Malay), and *kallu* (Indian).

Description

Palm wine is fermented palm sap. Apparently, most palm saps can yield a wine. Among the palms most frequently tapped are the coconut palm (*Cocos nucifera*), the oil palm (*E. guineensis*), the wild date palm (*Boraaus flabellifer*), the nipa palm (*Nipa fruticans*), the wild date palm (*Phoenix sylvestris*), the raphia palm (*Raphia hookeri* or *R. vinifera*), and the kithul palm (*Caryota urens*). A complete listing of palm species used to make wine is given by Swings and DeLey (1977).

Fresh palm sap is generally a dirty brown, but as yeasts multiply in it, it becomes pale and eventually milky white and opalescent. Thus, palm wines are generally sweetish, heavy, milky white, vigorously effervescent, alcoholic beverages. In effect, they are a suspension of living microorganisms in fermenting palm sap (Ayernor and Mathews, 1972; Okafor, 1975a,b). Alcohol content varies but generally is about 1.5 to 2.1% when consumed (Bassir, 1962). A faint sulfurlike odor may also be present.

In Ghana, the wine is usually sold in pint bottles or large earthenware or calabash (gourd) pots. If distributed in large containers, it is ladled out with a small calabash.

Areas of Production

Palm wines are produced wherever palm trees are grown, including the forest zone of southern Nigeria; the swampy, deciduous and derived savannah forests of West Africa; two-thirds (roughly 60,000 mi^2) of Ghana, mainly the southern and central areas; almost all coconut-growing areas in Sri Lanka, mainly on the western coast from Puttalam to Tangalle, in Jaffna peninsula, around Batticoloa on the eastern coast, and the coconut triangle of northern Sri Lanka, Chillaw, Kalutara, and Rathnapura; and large areas of the Philippines. Coconuts are major crops in Sri Lanka and the Philippines. Sri Lanka has 1.2 million acres of coconut plantations of which nearly 10,000 acres are used for toddy production.

How Consumed and Place in Diet

Palm wine is consumed as a mildly alcoholic beverage similar to beer. Some consumers drink palm wine in place of water at meals.

Palm wine has a special place in traditional celebrations and ceremonies such as marriages, burials, and settling disputes. In Nigeria, particularly

or canned. In such a form, palm wine may interest those with more purchasing power (Okafor, 1977b).

Recently, the Nigerian federal government established a Nigerian Council of Science and Technology which has been charged with the responsibility of setting up research centers, one of which will deal with the preservation of palm wine.

Mexican Pulque (Sanchez-Marroquin, 1977; Herrera et al., 1977; Goncalves de Lima, 1977)

Description

Pulque is the national drink of Mexico where it was inherited from the Aztecs (Goncalves de Lima, 1975). Pulque (*ocotli* or *huitztle*) marketed under the trademarks Miel-Mex, Xochitl, Jicara, Malinche, and Magueyin is a white, viscous, acidic alcoholic beverage of less than 6° GL made by fermentation of the juice of *Agave*, mainly *A. atrovirens* or *A. americana* (the century plant). By official definition, its refractometer reading is 25 to 30 at 20°C and its refractive index is 1.3365 to 1.3380. Additional characteristics include: pH 3.5 to 4.0; alcohol 4.0 to 6.0% v/v; total acidity (as lactic) 400 to 700 mg/100 ml; fixed acid (as lactic) 200 to 400 mg/100 ml; density (20°C) 0.9960 to 1.000; reducing sugars (as glucose) 200 to 500 mg/100 ml; protein content (N × 6.25) 300 to 500 mg/100 ml; total solids 2.0 to 3.0 g/100 ml; ash 200 to 500 mg/100 ml; esters (as ethylacetate) 20 to 30 mg/100 ml; aldehydes (as acetaldehyde) up to 2.5 mg/100 ml; and higher alcohols (fusel oils) 80 to 100 mg/liter.

Places Where Produced

Agave spp. grow on very poor soil, making consumption of pulque of particular importance in the diets of the low-income people. The most extensive production is in the Hidalgo State and the states on the plateau surrounding Mexico City.

Patterns of Consumption

Children at or under school age receive pulque three times a day. This provides 2.2 to 12.4% of their calories and 0.6 to 3.2% of their protein requirements each day (Instituto Nat. de Nutricion, 1976).

Pulque is distributed commercially in 250-liter wooden or fiberglass barrels by car, truck, and railroad to the pulquerias, which are special bars where the beverage can either be consumed immediately or taken home for consumption.

Consumption is similar to that of draft beer. In Mexico City, with a population of 10,000,000 people, about 1000 kiloliters of pulque are consumed each day. Drinkers consume from 3 to 10 glasses to several liters per day, mainly at lunch and during leisure periods. Peasants are the prime consumers, but the middle-class also consumes pulque on birthdays, at weddings, on picnics, and as an accompaniment to local foods. It is consumed as a low-alcohol beverage and as a nutritional supplement because of its content of vitamins and protein. Medicinal qualities have also been attributed to it by tradition, and it is consumed as a medicine for gastrointestinal disorders, anorexia, asthenia, renal infections, and decreased lactation.

Religious Significance

In ancient times, pulque had a religious significance as an offering to the gods, particularly Mayahuel, the Aztec goddess of pulque. Upon the collapse of the Aztec empire, the beverage lost its preeminence for religious rituals, but it remains a popular beverage.

Steps in Production

The substrate for pulque is *Agave* juice, called *aguamiel.* It is extracted from mature (8- to 10-year-old) plants (Figure 11). The floral stem (primordium) (Figure 12) is cut by means of a special knife, an operation described as "castration" of the floral bud. Juice gradually accumulates in the cavity left by its removal. The accumulated juice is removed daily by oral suction through a large, dried gourd (or squash) plant called an *acocote* (Figure 13). The juice is then carried to the *tinacales*, where it is fermented in open wooden, leather, or fiberglass tanks whose capacity is usually about 700 liters.

In the usual fermentation, uncontrolled natural inoculum from a previous pulque fermentation is added to the tank. The fermentation lasts 8 to 30 days, depending upon the temperature, seasonal changes, and other uncontrolled factors. Tank volume is kept constant by removing quantities equal to those being added to allow a semicontinuous production. Because neither the optimum amount of fresh juice that should be added each time nor the desirable time interval between additions is ever determined, the product quality,

FIGURE 11. Agave plant. (Courtesy of A. Sanchez-Marroquin, Centro de Estudios Economicos y Sociales del Tercer Mundo A. C., San Jeronomo, Lidige, Mexico.)

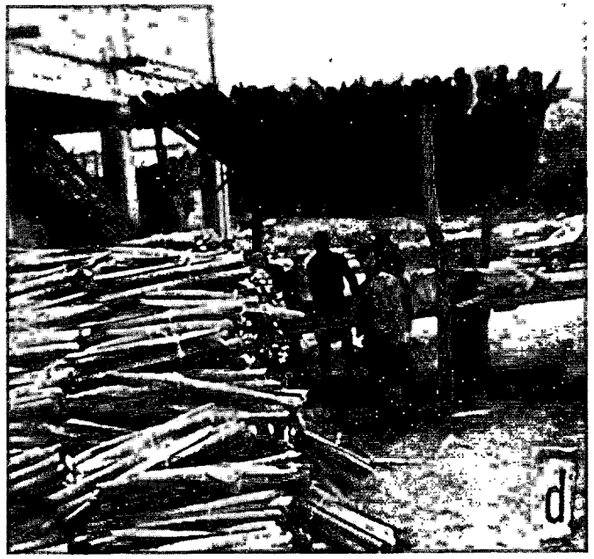

FIGURE 12. Agave stems. (Courtesy of A. Sanchez-Marroquin, Centro de Estudios Economicos y Sociales del Tercer Mundo A. C., San Jeronomo, Lidige, Mexico.)

the degree of fermentation obtained, and other characteristics vary. Therefore, pulque producers frequently resort to adulteration of the final product. It is impossible to bottle the spontaneously fermented pulque untreated because it never ferments completely under the above conditions and the bottles burst. In a series of papers Sanchez-Marroquin (1953, 1957, 1967, 1970; Sanchez-Marroquin and Hope, 1953) studied the microbial flora of pulque and developed a pure-culture process. Using a mixed inoculum (5 to 10% v/v) containing *Saccharomyces cervisiae*, a homofermentative *Lactobacillus* sp., *Zymomonas mobilis*, and a *Leuconostoc* sp., pasteurized *Agave* juice with 8° Brix sugar concentration, a pH of 6.0 to 7.0, and a temperature in the range of 15 to 28°C (optimum temperature is 28°C), fermentation time was markedly shortened to 48 to 72 hr. Settling, racking, and blending required another 12 to 24 hr.

A pilot plant (Figure 14) with a capacity of 1500 liters/day followed by a commercial 50,000 liters/day plant were built. A series of 5000-liter fiberglass fermentation tanks assure semicontinuous operation. Settling and blending tanks hold 10,000 liters. Pasteurization of the fresh *Agave* juice is accomplished in plate heat exchangers, and the juice then passes through presteamed lines to the fermentation tanks. The tanks are inoculated and fermentation continues 48 to 72 hr or until the sugar has been metabolized. Next, the pulque is piped to the settling, blending, and viscosity adjusting tanks. The product is then bottled or canned. This process markedly shortens fermentation time, allows optimization of operational parameters, and yields a standardized product.

Essential Microorganisms

Bacteria closely related to *L. mesenteroides* and *L. dextranicum* are present in the pulque fermentation and play a vital role through their production of bacterial polysaccharides (dextrans) in the development of the traditional viscosity in pulque (Sanchez-Marroquin and Hope, 1953). It would be also expected that these organisms would increase the acidity of the aguamiel very rapidly in the initial stages of the fermentation.

In addition, homo- and heterofermentative lactobacilli produce lactic acid, increasing the acidity of the pulque. The lactobacilli are closely related to *Lactobacillus plantarum* and *L. brevis*. (Sanchez-Marroquin and Hope, 1953).

The primary ethanol-producing yeast is *S. cerevisiae* (formerly classified as *S. carbajali*). Other yeasts are generally present in traditional pulque but probably play a less important role than *S. cerevisiae*. Among those found are *Endomycopsis*, *Pichia*, and *Torulopsis* spp. (Sanchez-Marroquin and Hope, 1953).

Zymomonas mobilis subsp. *mobilis* is considered by some (Swings and DeLey, 1977) to be the causal organism for the pulque fermentation. It is a Gram-negative, catalase-positive, nonsporulating, polar-flagellated, actively motile rod 4 to 5 μm by 1.4 to 2.0 μm in size. The cells occur as diplobacilli. *Zymomonas* is facultatively anaerobic, and under anaerobic conditions, the organism uses the Entner-Douderoff pathway for the catabolism of glucose. Forty-five percent of the glucose is transformed to

FIGURE 13. *Left*: Acocote, instrument for collecting the agave juice. *Right*: Tinacal, one of the fermentation tanks. (Pulque has been produced by this method for more than 400 years.) (Courtesy A. Sanchez-Marroquin, Centro de Estudios Economicos y Sociales del Tercer Mundo A. C., San Jeronomo, Lidige, Mexico.)

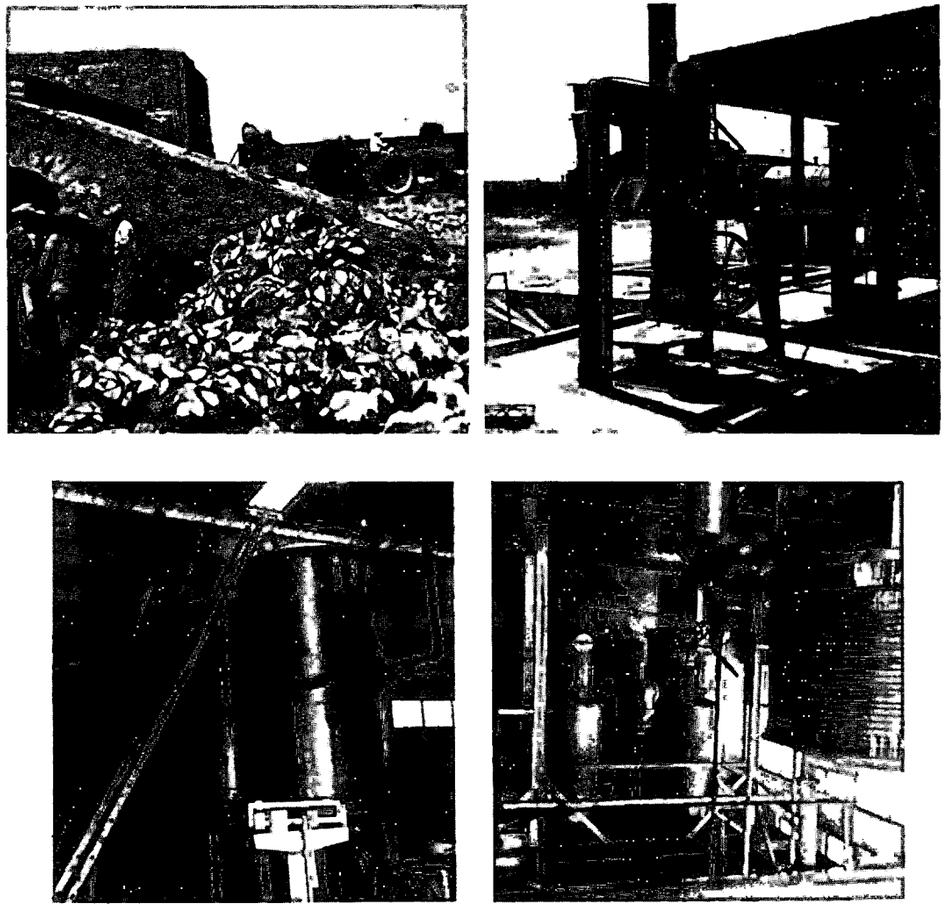

FIGURE 14. *Top*: Collection of agave "stems" and pressing to get the juice. *Bottom*: Chemical treatment of juice and evaporation. (Courtesy A. Sanchez-Marroquin, Centro de Estudios Economicos y Sociales del Tercer Mundo A. C., San Jeronomo, Lidige, Mexico.)

ethanol and 45% carbon dioxide anaerobically. The organism also produces some lactic acid and acetylmethylcarbinol. Glucose, fructose, and sometimes sucrose are fermented. Gums are produced and the cultures are slimy. Optimum growth temperature is 30°C. The organism tolerates glucose concentrations as high as 25% w/w.

It is likely that *Zymomonas mobilis* does play a key role in the fermentation of *Agave* juice to pulque. Along with *S. cerevisiae*, it is responsible for the ethanol and carbon dioxide production.

Herrera and Ulloa (1975) reported the presence of *Kloeckera apiculata* in fermenting pulque. Herrera et al. (1972) reported nitrogen-fixing microorganisms in pulque, but the organisms were not identified.

Chemical Changes during Pulque Fermentation

The century plant (*Agave atroviriens*) is a plant 1.5 to 2.5 m high and weighing from 900 to 1500 kg. The plants have about 15 to 30 leaves, 2 to 4 m in length and 30 to 40 cm in width. The leaves have a sugar content of 2 to 10%. The inflorescence is 6 to 9 m in height and weighs 70 to 150 kg. Each plant can produce from 150 to 300 liters of juice per month with a sugar content of 7 to 14% (Sanchez-Marroquin, 1977).

Changes occurring in sterilized *Agave* juice during fermentation with mixed pure cultures are summarized in Table 6. Soluble solids (°Brix) decrease from 11.0 to 6.0; pH falls from 7.0 to 4.6; total acid (as lactic) increases from 0.018 to 0.348 g/100 ml; sucrose decreases from 7.6 to 0.42%; and ethanol increases from 0 to 5.43% (Sanchez-Marroquin, 1977).

Table 7 compares the chemical analyses of traditional pulque with that produced in a modern pilot plant. The most striking differences are the wider range of ethanol contents and higher acetic acid and reducing sugar contents in the traditional pulque. Content of ethyl acetate and fusel oils as determined by gas chromatography are given in Table 8. These probably add smoothness and aroma to the product.

TABLE 6. Changes Occurring in Sterile *Agave* Juice during Fermentation with Mixed Pure Cultures[a]

Determinations	Sterile aguamiel	Final product
°Brix	11.0	6.0
Specific gravity	1.042	0.978
pH	7.0	4.6
Refractive index at 20°C	—	1.338
Total acidity as lactic acid[b]	0.018	0.348
Direct reducing sugars, as glucose[b]	2.40	0.06
Total reducing sugars, as glucose[b]	10.00	0.48
Sucrose[b]	7.6	0.42
Gums as glucose[b]	0.60	0.33
Crude protein[b]	0.17	0.17
Dry residue[b]	15.29	2.88
Ash[b]	0.31	0.29
Ethanol (°GL at 20°C)	0.00	5.43
Higher alcohols as amyl[b]	—	0.51
Volatile acidity as acetic acid[b]	—	0.02

[a]Averages.

[b]g/100 ml.

Source: Sanchez-Marroquin (1977).

TABLE 7. Comparison of Traditional (Tinacales) and Pilot Plant Pulque

Analysis	Traditional[a] (tinacales)	Pilot plant[a]
Ethanol	2.9-6.5	4.1-5.1
Acetic acid	0.2-1.9	0.07-0.10
Lactic acid	0.02-1.3	0.06-1.22
Fusel oil	0.07-0.21	0.01-0.05
Total acidity (as lactic)	0.001-0.96	0.04-1.25
Reducing sugars	0.1-2.3	0.05-0.15
Esters (mg/100 ml)	0.06-21.6	0.04-22.1
Viscosity (cps)	1.20-5.30	3.10-5.10

[a]g/100 ml, except where otherwise designated.

Source: Sanchez-Marroquin (1977).

Nutritional Considerations

The vitamin contents of traditional and pilot plant pulque are compared in Table 9. There is considerable overlapping of the quantities of B vitamins found in pulque produced by the two processes; pulque adds important amounts of these vitamins to the diets of the consumers.

The amino acid contents of traditional and pilot plant pulque samples (minimum and maximum contents) are given in Table 10. The amino acids are significant in the nutrition of the poor who consume pulque as an important part of the diet. While the pilot plant process offers important improvements in the technical aspects of pulque processing, the end products of both procedures are similar.

Pulque has a very special place in the diet of peasants and other low-income people in the poorest semiarid areas of Mexico. *Agave* is the only plant that can grow in the poor soil with the severe shortage of water. For the people in these areas, pulque is a major source of nutrients. The ethanol is calorically significant and one would expect that, like other unclarified yeast-fermented products, pulque constitutes a good source of the B vitamins.

TABLE 8. Esters and Fusel Oils in Pulque[a]

Compound	Concentration (ppm)
Ethyl acetate	121
n-Propyl alcohol	13
Isobutyl alcohol	22
Amyl alcohol (active)	19
Isoamyl alcohol	76

[a]Determined by gas chromatography.

Source: Sanchez-Marroquin (1977).

TABLE 9. B Vitamins in Traditional and Pilot Plant Pulque

Number of samples	Vitamins	Traditional[a,b] (tinacales)	Pilot plant[a,b]
10	Thiamine	5.2-29.0	10.2-38.0
10	Niacin	54.0-515.0	115.0-635.0
10	Riboflavin	18.0-33.1	19.4-35.6
10	Pantothenic acid	60.0-355.0	72.0-392.4
5	*p*-Aminobenzoic acid	12.3-20.7	15.9-32.2
5	Pyridoxine	14.2-33.4	19.1-36.4
5	Biotin	9.1-32.2	11.0-35.3

[a]Range of values obtained.

[b]µg/100 ml.

Source: Sanchez-Marroquin (1977).

Unfortunately, per capita consumption of pulque is decreasing due to competition with other beverages, most of which are less nutritious than pulque. In the Hidalgo State, children receive 2.2 to 12.4% of their calories and 0.6 to 3.2% of their protein from pulque. Even though the school children receive pulque three times a day, they are still poorly nourished (Sanchez-Marroquin, 1977).

Toxicology

No serious toxicological problems have been reported by those consuming large quantities of pulque beyond those observed in heavy consumption of other alcoholic beverages.

TABLE 10. Amino Acids in Traditional and Pilot Plant Pulque

Amino acids (28 samples)	Traditional[a,b] (tinacales)	Pilot plant[a,b]
Aspartic acid	0.013-0.017	0.011-0.035
Glutamic	0.009-0.023	0.038-0.041
Arginine	0.002-0.003	0.006-0.009
Phenylalanine	0.002-0.006	0.009-0.011
Leucine	0.005-0.008	0.006-0.009
Lysine	0.002-0.004	0.005-0.010
Methionine	0.001-0.002	0.003-0.007
Tryptophane	0.001-0.004	0.008-0.010
Tyrosine	0.016-0.034	0.026-0.038
Threonine	0.003-0.005	0.005-0.007
Valine	0.002-0.004	0.004-0.006

[a]Range of values obtained.

[b]mg %.

Source: Sanchez-Marroquin (1977).

Recent Improvements in Pulque-processing Technology

Sanchez-Marroquin (1977) has recently studied the effects of mechanically pressing the whole *Agave* plant, including stems and leaves, to improve the recovery of the sugary juice used for the fermentation. Once the juice is filtered and clarified, it can be adjusted for optimum sugar concentration and pH prior to fermentation.

Although the viscosity of traditional pulque is one of the unique and typical characteristics of this beverage, the younger generation prefers a low viscosity product. Thus, in the modern pilot plant, pulque is manufactured without the use of *Leuconostoc* spp., which produce the dextran responsible for the viscosity. *Leuconostoc* is grown in separate tanks and the viscous culture is added to a portion of the pulque for those consumers who prefer the viscous beverage. The final product is also enriched with additional yeast which raises both the B vitamin and protein contents.

In addition to the above modifications and improvements of the process, pure starters and concentrated *Agave* syrup are being distributed to manufacturers in order to shorten the fermentation and improve the quality of the product. Yeast that settles from the fermenters is recovered and is either added to the pulque as a supplement or used for cattle feed. The *Agave* plant residues are combined with urea, phosphate, and yeast to produce a fodder for ruminants.

Economics of Production

The Mexican pulque industry produces nearly 500 million liters per year with a selling price of more than 630 million pesos (U.S. $28 million), (0.79 peso; U.S. $0.035/liter). One hundred thousand people are employed in the production. Aguamiel producers can earn about U.S. $198 from a hectare of *Agave* plants.

While industrializing the process might yield a more sophisticated product, there are some dangers involved. First, the cost of modern production is likely to result in a more expensive product out of reach of those who need its nutritional benefits most. Second, if modern processing involves clarification, it is likely that the nutritional value (primarily B vitamins) conferred by the yeast and by other microbial cells may be reduced.

Miscellaneous Mexican Alcoholic Beverages

Colonche, or *nochoctli*, is a product very similar to pulque. It is made from the juice of prickly pear cacti: *Opuntia streptacantha* Lem., *O. robusta* Weld., *O. leucotricha* DC., and *O. orbiculata* Salm-Dyck. Diguet (1928) estimates that this beverage is probably more than 2000 years old. It is sweet and alcoholic with a red color. *Colonche* a few hours old is still effervescent with a slight butyric acid odor; after a few days it becomes acidic. The primary colonche-producing areas are in northeastern Mexico—Chihuahua, Sonora, San Luis Potosí, and Zacatecas (Diguet, 1928; Santamaría, 1959).

The juice is carefully collected and simply fermented using some previously fermented colonche or pieces of the prickly pears which harbor a slimy mass of bacteria and yeast. A *Torulopsis* sp. has been isolated from colonche and given a new name, *T. taboadae*, by Ulloa and Herrera (1978).

Miscellaneous Alcoholic Beverages from Sugary Substrates

Mexican *mezcal* is a tan-colored, sour beverage with an alcoholic content similar to beer. The substrate is the core of the *Agave* plant which is

stripped of its leaves, cooked, and filtered to yield a sugary pulp. The pulp is inoculated with a starter from a previous fermentation (Pennington, 1969).

Indian Jackfruit Wine (Dahiya and Prabhu, 1977)

Description, Places of Production, and Patterns of Consumption

Jackfruit wine is an alcoholic beverage made by fermentation of jackfruit pulp. A social and tribal beverage, it has a characteristic pungent aroma and flavor. Jackfruit (*Artocarpus heterophyllus*) is a large fruit-bearing tree cultivated in moist tropical climates and thick forests.

Nagaland, Tripura, and other eastern hilly areas of India are the major wine-producing areas.

Steps in Production

The steps in production are outlined in Figure 15. Earthen pots, wooden vessels, bamboo baskets and sieves, stone slabs, and flat stones are the only equipment used in traditional procedures. The juice is extracted from sections and pulp with a bamboo basket sieve and a small amount of water and is allowed to ferment in earthen pots, using a small amount of fermented juice as inoculum. The liquid portion is removed by decantation. Temperature of fermentation is between 18° and 30°C, and the fermentation lasts about 1 week.

Microbiology and Chemical Changes

The yeast inoculum is previously fermented juice obtained from earlier natural fermentations. The yeasts involved resemble *Endomycopsis*.

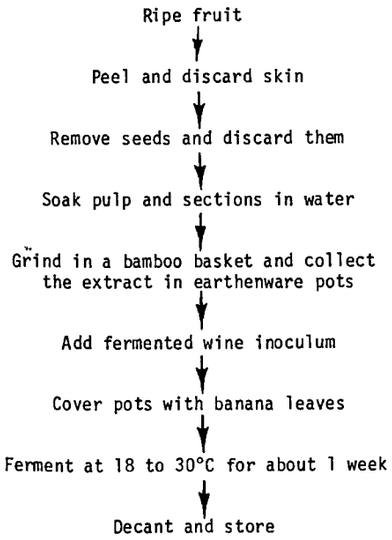

FIGURE 15. Flow sheet: Production of Indian jackfruit wine. (From Dahiya and Prabhu, 1977.)

The fruit sections and pulp yield 53 to 60% of juice by weight with 12 to 12.4% Brix (total soluble solids). Sucrose content is 4 to 4.9%; reducing sugars are 4.5 to 5.2%; pectin content is about 3.5 to 4%. The pH of the wine is between 3.5 and 3.8, suggesting that an acid fermentation accompanies the conversion of carbohydrates to alcohol. The alcohol content in the wine attains 7 to 8% v/v within a fortnight.

Pectin and protein interfere with sedimentation. During storage, pectin is slowly hydrolyzed. The quality of the product is masked by a pungent aroma that increases during storage due to side reactions.

Ways of Improving the Process

The isolation of pectin-utilizing strains of yeast will improve the quality of the product and also remove the pungent odor. The controlled use of metabisulfite will help in the control of the wild yeast.

Ripened jackfruit often burst while on the trees, and they are then contaminated by birds and other animals. Airborne contamination from toxic molds, generally *Aspergillus flavus*, has also been observed. Therefore, burst fruit should be avoided in wine making.

Economics of the Process

The inaccessible location of the trees makes collection and transportation of the fruit difficult. Furthermore, ripening occurs during the rainy season, increasing the chances of contamination and spoilage. Economical methods of recovering starch and other by-products from the seeds might improve the economics of the process. Also, the fruit skin may be marketable as animal fodder.

Kenyan Urwaga (Harkishor, 1977)

Description and Patterns of Consumption

Urwaga is an effervescent, slightly sour alcoholic beverage made from bananas, sorghum, millet, or maize. It is brownish in color and has a consistency similar to porridge. A similar product called *mwenge* is made in Uganda with only bananas and sorghum.

Urwaga is produced in the banana-growing region of Rwanda and is consumed as a refreshing beverage. Very large quantities are consumed at social gatherings and festivals where it is indispensable. Mwenge is consumed primarily in the Buganda Province of Uganda. It is used by hill dwellers to barter for fish caught by people living near lakes.

Steps in Production (Figure 16) *and Control of the Process*

A pit is dug in the ground approximately 70 cm in diameter by 70 cm in depth. Dry banana leaves are burnt in the pit and the ash formed, as well as the sides of the pit, are covered with green banana leaves. Bananas are placed in the pit and covered with a mixture of soil and banana leaves where they ripen for 5 to 7 days. Alternatively, the bananas are smoked via a tunnel adjoining the pit. The bananas are then peeled and placed in canoe-shaped containers hollowed from tree trunks. The peeled bananas are covered with grass and, following hand washing, the banana-grass mixture is squeezed until the bananas are pulped and forced through a crude wooden filter to remove the juice, which is further filtered through grass in a calabash filter. The juice is finally collected in earthenware pots.

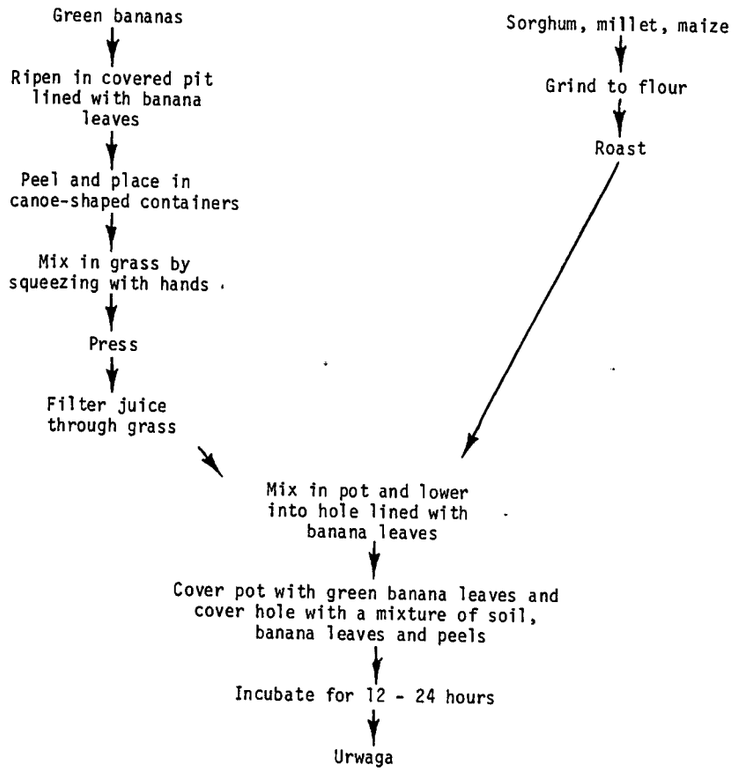

FIGURE 16. Flow sheet: Household preparation of Kenyan urwaga. (From Harkishor, 1977.)

Sorghum, millet, or maize are ground by hand to flour in a crude stone mill, and roasted over a fire. Flours from different grains are mixed, if desired, and the flour mixture is added to the banana juice. The mouth of the pot is covered with green banana leaves.

The pit is now lined with fresh banana leaves, and the pot containing the banana juice and flours is placed in the pit, which is covered with a mixture of soil, green banana leaves, and banana peels. Incubation continues for 12 to 24 hr. Burning of the dry leaves in the pit raises the temperature and hastens ripening of the bananas, while insulation of the pit holds the temperature above the surrounding area. The green banana leaves help maintain a high humidity in the pit, and the pit keeps the fermentation more anaerobic. The finished urwaga is removed and placed in smaller pots or jugs. It is drunk with a straw.

Microbiology

Although both yeasts and lactic acid bacteria are involved, no systematic studies have been made of the process. Certainly fermentation begins as

TABLE 42. Effect of Different Concentrations of $(NH_4)H_2PO_4$ on Nata Formation[a]

Concentration $(NH_4)H_2PO_4$ (%)	Average yield of raw nata from 400 ml culture after 15 days (g)[b]
0.0	110.0
0.1	128.70
0.2	135.50
0.3	148.65
0.4	168.20
0.5	210.35
0.6	178.90
0.7	174.85
0.8	170.45
0.9	163.90
1.0	150.40

[a]All cultures adjusted to pH 5.0.

[b]Wet weight.

Source: Adapted from Lapuz et al. (1967) and Ramos (1977).

tear-resistant paper. Synthesis of cellulose by *Acetobacter* spp. has been studied by a number of workers (Brown, 1886; Hibbert and Barsha, 1931; Tarr and Hibbert, 1931; Muhlethaler, 1949; Kaushal et al., 1951; Hestrin and Schramm, 1954).

Hibbert and Barsha (1931) demonstrated that the polysaccharide produced by *A. aceti* subsp. *xylinum* behaved chemically the same as cellulose. Muhlethaler (1949) using electron microscopy showed that the bacterial cells are first covered with a slime, followed by formation of cellulose strands that gradually thicken to distinct cellulose fibrils within the slime. The fibrils are completely outside the cells and, in fact, the bacterial cells do not contain any cellulose. Kaushal et al. (1951) studied the X-ray diffraction patterns of the bacterial product and concluded that it was definitely cellulose. They suggested that the amorphous material observed at the early stages is carbohydrate at a polymerization level below cellulose. Tarr and Hibbert (1931) found that it was necessary to have a small amount of ethyl alcohol present for synthesis of the cellulosic membrane.

One of the most interesting studies on the bacterial cellulose was that of Hestrin and Schramm (1954). They demonstrated that cellular matter which might contribute some protein is apparently washed out. At best, nata is a source of calories, depending upon the digestibility of the cellulosic matter; in candied form, nata has a high calorie content.

Toxicological Considerations

There have been no reports of any toxic reactions from consumption of nata.

Stability of Nata in the Diet

Nata is a highly acceptable delicacy and it is likely to remain an important confection in the Philippines. It may become of interest to people in other countries as they are introduced to it.

TEA FUNGUS (Hesseltine, 1965; Kozaki et al., 1972)

Description

Japanese or Indonesian tea fungus, *teeschwamm, kombucha, wunderpilz, hongo, cajnij, fungus japonicus,* and *teewass* are names for a beverage consumed widely in Russia, Japan, Poland, Bulgaria, Germany, Manchuria, and Indonesia. In the production of the beverage, tea fungus or zoogloeal mats form on extracts of tea. The films are apparently produced by *Acetobacter* sp. with two yeasts (Hesseltine, 1965) growing together on the surface of tea containing relatively high concentration of sucrose. The film reaches a thickness of 2.5 to 5 cm.

The film produced appears to be very closely related to Philippine nata both in regards to its essential chemical nature and the essential microorganisms that produce the film. Unlike, Philippine nata, however, in which the film itself is the desired food product, it is the liquid on which the film has formed that is consumed.

Steps in Production

Hesseltine (1965) grew the tea fungus on a medium consisting of 34 g dry tea leaves placed in 1 liter of water. After steeping, the tea leaves were removed and 100 g of sucrose were added along with the inoculum; 300 ml amounts of tea extract were placed in 2-liter Fernbach flasks. The flasks were sterilized, dried, cooled, and inoculated with the tea fungus fragments (a tough, brownish pad similar to dried mother of vinegar). Incubation was at 25°C for 10 days. Following growth of the microorganisms, the film is either discarded or used to inoculate new fermentations. The underlying liquid is the product.

Essential Microorganisms

Hesseltine (1965) reported that the essential organisms were *Acetobacter* sp. (NRRL B-2357) and two yeasts (NRRL Y-4810 and NRRL Y-4882). The film formed only when the three organisms were present together.

When *Acetobacter* was used alone, gas was produced and the film was not formed. When the three organisms were combined no gas was produced and the film formed rapidly. Kozaki et al. (1972) reported that *A. xylinum* was the principal organism. This is the Philippine nata microorganism also, and substantiates the close relationship of the two fermentations. Kozaki et al. (1972) isolated several types of yeast: *Saccharomyces* sp., *Torulopsis famata, Pichia membranaefaciens,* and *Candida guilliermondii* from Japanese tea fungus. Formosan tea fungus contained *Candida obtuse* and *Kloeckera apiculata.*

Biochemical Changes

The *Acetobacter* sp. produces a cellulosic pellicle, oxidizes ethanol weakly, produces gluconate and dioxyacetone, and utilizes ammonium sulfate. The beverage (liquid) contains an antibiotic active against *Agrobacterium tumafaciens.* The antibiotic nature of the fermentation has been reviewed by Stadelmann (1961).

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