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Microbial Technology

Fermentation Technology

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Preface

In the decade since the first edition of "Microbial Technology" appeared, applied microbiology has changed, expanded, and diversified. As new products were introduced in this period, and greater demand for some of the old ones developed, the total fermentation capacity increased at about the same rate noted in the previous 25 years. The number of fermentation products, it is estimated, has quadrupled, while the volume of products manufactured has increased tenfold. This growth has prompted publication of a second edition, completely revised and enlarged.

To accomplish a worldwide survey of industrial microbiology and to describe its contributions to agriculture, industry, medicine, and environmental control, the editors are indebted to 57 willing and expert contributors. Their comprehensive reviews of traditional fermentations and propagations, as well as newly developed microbe-dependent processes and products, are presented in a two-volume set.

Volume I, subtitled "Microbial Processes," describes the production and uses of economic bacteria, yeast, molds, and viruses, and reviews the technologies associated with products of microbial metabolism.

Volume II, subtitled "Fermentation Technology," deals principally with fermentations and modifications of plant and animal products for foods, beverages, and feeds, while reviewing salient aspects of microbial technology: general principles, culture selection, laboratory methods, instrumentation, computer control, product isolation, immobilized cell usage, economics, and microbial patents.

H. J. Peppler
D. Perlman

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Chapter 1

Beer Brewing

D. H. WESTERMANN
N. J. HUIGE

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I. INTRODUCTION

A. Historical Background

History indicates each society has developed an alcoholic beverage of some type based on the indigenous sources of starch or sugar. Wheat, corn, rye, millet, rice, oats, barley, potatoes, and other vegetables and fruits have been converted to potable alcoholic beverages. It is generally conceded that beer originated in the twin cradles of civilization, Mesopotamia and Egypt, about 6000 years ago.

According to Weeks (1949), the etymology of the word beer, as we know it today, indicates it originated from the Latin verb *bibere,* to drink. Similarly, the Spanish word for beer, *cerveza,* apparently originated from *cerevisia,* which combines the Latin *ceres,* goddess of grain, and *vis,* vigor.

It is not clear from historical records whether this vigor, which can be interpreted as the physiological effect of alcohol, or its preservative contribution to grains was the principal stimulus for long-term growing acceptance by societies. However, it is known that alcoholic beverages became closely associated with religious ritual during the infancy of early civilized societies.

The knowledge that a concoction of grains and water could ferment naturally to produce an alcoholic beverage followed the advance of agriculture across Europe with the Celts. The Teutons, who eventually settled in the Rhine area and became the Germanic Tribe, had followed the Celts in their westward migration. Some of the Teutons eventually settled in England by the fifth century and were followed by the Saxons. At least the possible sequence by which the brewing art could have spread across the continent and England appears historically logical.

The influence of the church on the art and science of brewing grew rapidly over a 1000-year period, reaching a climax in the sixteenth and seventeenth centuries. The influence of religious and civil law on brewing in Europe and England is a fascinating story, well documented by Arnold (1911).

Major brewing centers were eventually established in Pilsen, Czechoslovakia; Munich and Dortmund, Germany; Burton-on-Trent, England; and Dublin, Ireland. The local water supplies of each area gave rise to unique product characteristics associated only with that area.

Although the American Indians were already making a brew from maize, English-type beer was brought to America on the *Mayflower*, according to Weeks (1949). A journal of the *Mayflower* contained the following record: "We could not now take time for further search . . . our victuals being much spente, especially our beer."

English ale brewing traditions accompanied the colonists, and it was not until the 1840s that German lager beer became more widely accepted in the United States (Singruen, 1938).

English ales were fermented at ambient temperatures (21°–27°C) with top-fermenting yeast, while the German lager beers were fermented with bottom-fermenting yeast at the lowest temperatures which could be sustained year-round (10°–15°C). The lager beer was stored in cool caves for several months, resulting in improved physical stability (Baron, 1962). The superior keeping quality and preferred taste of lager beer rapidly led to its domination of the American market.

B. Current Practice

Beer retains its unique taste and character because of historical acceptance coupled with legal restraints of composition. In the United States Federal Regulations, beer is defined as a malt beverage resulting from an alcoholic fermentation of the aqueous extract of malted barley with hops. It may include other sources of carbohydrates called adjuncts. Legally, malt liquor, ale, porter, stout, and sake are also malt beverages.

Current American beers are made from malted barley, with corn grits or syrup or rice grits as the adjunct carbohydrate source, and hops. Purified water, to which brewing salts are added, and a cultured yeast complete the five principal ingredients of brewing.

Barley which has been malted comprises the basic raw material for brewing. In the malting process, the barley kernel, separated from the stalk and chaff, is germinated under controlled temperature and humidity to generate enzyme systems and partially degrade endosperm starch and protein. Growth of the germinated kernel is stopped by drying of kilning in hot air, during which time some flavor and color components are also formed. The dried malt is milled before it is mashed with brewing water.

In the mashing process, milled malt and adjunct, such as corn grits or rice, are each made into a mash with brewing water, subjected to individual controlled time–temperature cycles, and combined for further enzymatic digestion and subsequent extraction of sugars and proteins.

The extract produced in the combined mashing process is separated from the nonsoluble husk and grain portions in a lauter tub or filter press. This extract is called wort.

Wort is boiled in the brew kettle, along with hops and syrup, if used as an adjunct. The dried hop cones used for brewing contain lupulin glands which are the source of bitter resins and essential oils that contribute some of the characteristic bouquet to beer.

The boiled wort is cooled, inoculated with a pure yeast culture, and fermented. Each brewery maintains a yeast strain considered to be unique to its own process and product character. The fermentable sugars are converted to ethyl alcohol and trace quantities of flavor ingredients, while the nonfermentable carbohydrates remain in the beer. Carbon dioxide from the fermentation is recovered, purified, and returned to the finished product.

The yeast generated during fermentation is separated from the beer by centrifugation or natural settling. After storage and aging for several weeks, recarbonation, and two or more filtrations, the finished product is packaged.

II. RAW MATERIALS

A. Barley

For centuries barley was the "staff of life" in Europe, as evidenced by barley kernels found in ancient ruins and tombs. Until the fifteenth century it was the principal ingredient of bread. However, because barley flour is not readily leavened, wheat, which makes a lighter bread, became the favorite of bakers.

Botanically, barley is a grass or member of the Graminae family. However, it has unique properties which make it ideally suited for the brewing of beer. The barleys used for brewing possess a very tough husk which is firmly cemented to the kernel. This husk provides protection for the kernel during handling and subsequent germination. Later in the brewing process the husks form a filter bed for the separation of extractable carbohydrates and protein from the mash and contribute a characteristic flavor. Barley can be readily malted, a process in which the events of plant reproduction are simulated and later stopped by the removal of moisture. During the malting process barley produces large amounts of amylases, proteases, and other enzymes which partially degrade starch, proteins, and some types of cellulose. The biochemistry of the malting process is described in Section III.

Two distinct barley types and many varieties of each type have been grown for brewing. The two-row and six-row designations of type describe the number of rows which form around the axis of the kernel head. In two-row barley, one kernel develops at each of two nodes, while three kernels develop at each node in six-row barley. Each type of barley has its own brewing characteristics. Historically, two-row barley produced a more mellow beer and contained less protein and enzymatic potential than six-row barley. This resulted largely from the greater plumpness of the two-row kernel (i.e., less surface to volume ratio). Agronomic crossbreeding during the last decade has tended to minimize this difference.

To produce typical light American beers, barleys must be used which provide ample enzymatic activity to hydrolyze adjuncts. The six-row barley varieties, Larker, Dickson, and Conquest, are typical of those grown in North and South Dakota and Minnesota. These varieties provide about 80% of the barley for the American brewing industry. They have high enzymatic potential and protein contents of up to 13.5%. While higher enzymatic potential and protein content can be generated, brewers usually avoid such barley because of product instability. A western six-row barley with less desirable brewing characteristics is also grown primarily in California.

The two-row barleys, known for their contribution of mellowness and flavor, include varieties such as Piroline, Betzes, Klages, Firlbecks III, Vanguard, Hannchen, and Shabet. They are grown in the western states of Washington, Oregon, Idaho, and Montana.

The proximate composition of the three major barley types, described by Winton and Winton (1932), is shown in Table I, and the analysis of malts from these barleys in Table II.

B. Adjuncts

Approximately 70% of the malted barley kernel is potentially available as soluble carbohydrate. It is this carbohydrate, principally starch, which is converted to fermentable sugar and nonfermentable dextrins. The fermentable sugars are ultimately converted primarily to ethyl alcohol. The maximum alcohol and remaining solids content of an all-malt beer is therefore established by the milled malt solids concentration which can conveniently be handled in an all-malt mash. The ratio of protein to carbohydrate and amount of alcohol in the finished beer is primarily a fundamental characteristic of the malted barley.

All-malt beers historically were more satiating and had poor physical

TABLE I. Proximate Composition of Three Barley Types

Analysis	Midwestern six-row	California six-row	Western two-row
Kernel Weight (mg)	36	44	40
Husk (%)	12	14	10
Protein (%)	12	11	10
Fat (%)	2	2	2
Starch (%)	58	58	60
Fiber (%)	5.7	6.6	5.2
Ash (%)	2.7	3.0	2.5
Enzyme potential after malting	High	Low	Medium

TABLE II. Typical Analyses of Malts from Three Barley Types

Malt analysis[a]	Midwestern six-row	California six-row	Western two-row
Kernel weight, dry basis (mg)	30.0	39.0	37.0
Growth; length of acrospire			
0–¼ (%)	0	1	1
¼–½ (%)	1	5	1
½–¾ (%)	5	9	6
¾–1 (%)	92	85	91
Overgrown (%)	2	0	1
Assortment			
On 7/64 screen (%)	32	68	85
On 6/64 screen (%)	53	26	10
On 5/64 screen (%)	14	6	1
Through screen (%)	1	6	1
Moisture (%)	4.2	4.4	4.2
Extract, fine grind, dry basis (%)	76.8	77.0	80.5
Extract, coarse grind, dry basis (%)	74.8	75.0	79.0
Difference (%)	2.0	2.0	1.5
Wort color, ½ inch cell, °Lovibond	1.6	1.4	1.2
Protein (N × 6.25), dry basis (%)	12.3	11.0	10.5
Soluble protein as percentage of total	40.0	35.0	38.0
Diastatic power, dry basis, °L	135	65	90
α-Amylase, dry basis, 20° units	38	25	30

[a] From American Society of Brewing Chemists (1976).

stability due to their high concentration of soluble protein. American malts had three properties which were different from European malts. Protein content and enzymatic activity were substantially higher, and the husk was thicker. Early American brewers recognized that these properties could be exploited to use other sources of starch as a supplemental brewing material. The higher enzymatic activity would allow hydrolysis of gelatinized starch from other cereal grains, and the heavy husk would provide an efficient filter bed for extraction of solubles from mashes containing adjuncts. The limited residual proteins from these supplemental materials, such as corn and rice, were not readily hydrolyzed by malt proteases. Therefore, beers produced with these supplemental adjuncts had lower protein content for a given alcohol content, resulting in better physical stability with a lighter character.

With the exception of specialty products, all American brewers use an adjunct for brewing, as described by Matz (1970). While wheat, milo, and unmalted barley have been used, corn grits, corn syrup, and rice grits are dominant. Of the 155,000,000 barrels of beer currently produced, two-thirds are brewed with corn grits or corn syrup adjunct.

When corn grits, refined grits, flakes, or rice are used by the brewer, they are pregelatinized by boiling in a "cooker" before adding to the malt mash. Cooker mashes generally contain about 10% malt to provide the enzymes which partially hydrolyze the gelatinized starch to reduce viscosity.

Corn syrups produced for the brewing industry by the corn wet milling industry are made from acid and enzymatic hydrolysis of starch slurries. Dependent upon the method of hydrolysis, it is possible to produce syrups with 0–100% fermentability. Typical analyses of brewing adjuncts are shown in Table III.

Brewing technologists generally acknowledge that to produce presently accepted beers, yeast requires 120–140 mg/liter of free amino nitrogen for proper nutrition. Since the nitrogen is available only from malt, this limits the malt to adjunct ratio theoretically to approximately 50/50 for lager beers. American industry beers vary from 100% malt for the specialty products to about 60/40, malt/adjuncts, for lager beers.

C. Hops

Since medieval times herbs have been used to flavor beers, but only hops are used commercially today. The characteristic aroma and bitterness imparted to beer by the oils and resins of hops make beer and similar malt beverages quite unique.

According to Arnold (1911), records exist indicating hops were grown in the seventh or eighth century, but the first recorded use in brewing was made by a nun, St. Hildegard (1098–1197), in a convent at Rupertsberg, Bingen-on-Rhine. The record states: "The hop is of a heating and drying nature, but does contain some little moisture; is, however, of slight benefit to man, since it promotes melancholy and creates in man a sad mood, and also it affects his bowels unpleasantly by reason of its heating properties. Its bitterness, though, when added to beverages, prevents in the latter putrification, and gives to them a longer durability." (Sic)

While the preservative value of hop resins is limited, it is known to contribute some microbiological stability during beer processing. Whether this attribute or its uniquely acceptable flavor contribution stimulated its use may never be put in proper perspective. Both attributes were important.

About 20% of the world's hops are grown in the United States, in the states of Washington, Oregon, California, and Idaho. The Old World famous centers of hop growing still prevail in Kent, England; Saaz, Bohemia; and Hallertau, Bavaria.

The natural family of Cannabinaceae consists of two genera, *Humulus* and *Cannabis*. *Cannabis* is represented by *C. sativa,* which includes Indian Hemp, marihuana, and hashish, while *Humulus* consists of *H. lupulus* and *H. japonicus*. The *H. lupulus* provides resins for brewing,

TABLE III. Typical Analyses of Brewing Adjuncts

Composition	Amount
Cereal Adjuncts	
Corn Grits	
Moisture (%)	10.9
Extract, dry basis (%)	91.4
Oil, dry basis (%)	0.76
Corn Flakes	
Moisture (%)	9.0
Extract, dry basis (%)	92.4
Oil, dry basis (%)	0.50
Refined Grits	
Moisture (%)	9.6
Extract, dry basis (%)	103.3
Oil, dry basis (%)	0.03
Rice	
Moisture (%)	12.0
Extract, dry basis (%)	93.0
Oil, dry basis (%)	0.86
Liquid Adjuncts	
Corn Syrup	
Extract, as is (%)	82.0
Extract, dry basis (%)	100.0
Fermentable extract, as is (%)	60.2
Reducing sugars (as dextrose), as is (%)	50.5
Ash, as is (%)	0.19
pH (10% solution)	4.95

whereas the *H. japonicus* is an ornamental plant devoid of brewing value. It is the dried fruit or flower of the female hop plant which is used for brewing.

The hop plant possesses an extensive root system, which each year sends out a large number of shoots which grow very rapidly to a length of 20–25 ft. These climbing vines are supported on a wire network, and in late August or early September the flowers or cones of these female plants are picked, dried to 9–10% moisture, and baled. Extensive effort is taken to eliminate or minimize the growth of male plants to prevent pollination and seed production in the female flowers. The typical composition of a dried hop cone is given in Table IV, from Hough *et al.* (1971).

The structure of the hop cone is shown in Fig. 1. The cone consists of bracts and seed-bearing bracteoles which are attached to a supporting structure or strig. Lupulin glands, small resinous beads which contain the materials of brewing value, develop along with seeds at the base of the bracteole as the hop cone ripens. The lupulin glands contain the

TABLE IV. Proximate Composition of Hops[a]

Component	Percent of total weight
Water	10.0
Total resins	15.0
Essential oils	0.5
Tannins	4.0
Monosaccharides	2.0
Pectin	2.0
Amino acids	0.1
Proteins (N × 6.25)	15.0
Lipids and Wax	3.0
Ash	8.0
Cellulose, lignin, etc.	40.4
Total	100.0

[a] From Hough *et al.* (1971).

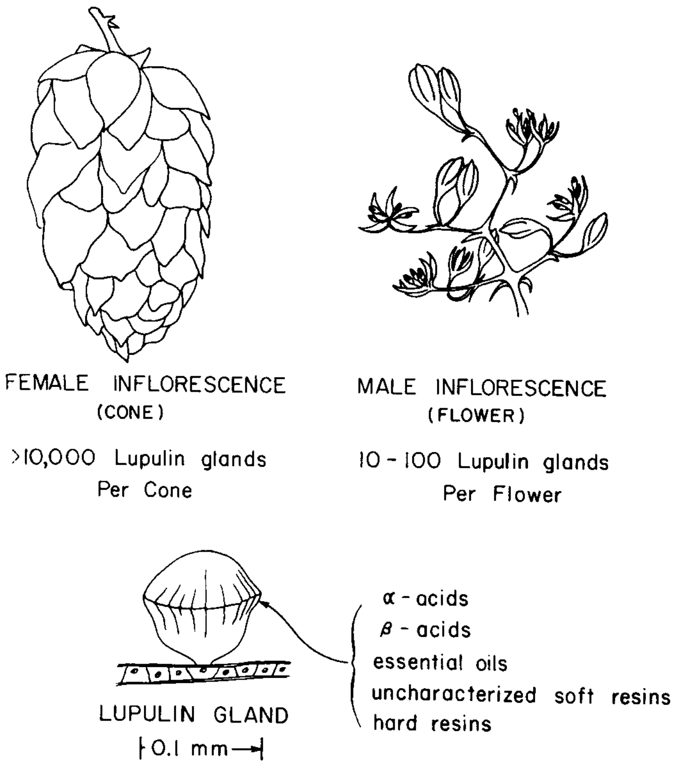

FIGURE 1. Hop cone, male flower, and lupulin gland.

resins and essential oils which characterize the hop variety and determine its value in brewing.

The hop resins are broadly classified by their solubility, as indicated by Findlay (1971), and the specific compounds which comprise the approximate 15% total resins are shown by Burgess (1964) in Table V. The major constituents of hop oil are the hydrocarbons myrcene, farnesene, humulene, and caryophyllene, which account for 50–80% of the oil and the 80 or more oxygenated compounds which are currently identified.

The lupulin of hop varieties differs largely in its volatile oil composition and percentage of cohumulone in the α-acids. The European hop varieties Hallertau and Saaz contain about 20% cohumulone in α-acids, while the American varieties Bullion and Brewer Gold contain about 40%. These hops also contain more humulene and less myrcene than American hops.

When the hop cones are added to the brew kettle the water-soluble resins and oils are dissolved in the wort. The α-acids are isomerized, providing 85–90% of the characteristic hop bitterness. Some additional bitterness is derived from oxidation products of α-acids, with a very minor contribution from oxidized β-acids. Methods of analyzing hops and beer for α-acids and iso-α-acids, respectively, are given in "The Methods of Analysis" (American Society of Brewing Chemists, 1976). An excellent review of hops and hop chemistry is given by Hough *et al.* (1971). The iso-α-acids not only contribute the principal bitterness to beer but are a major reactant, along with protein, for the formation of stable foam with good adhesion.

The yield of iso-α-acids in beer from α-acids in hops added to the

TABLE V. Proximate Composition of Hop Resins[a]

	Percent
Total hop resins	15.0
Soft resins	11.5
α-Acids	5.0
Humulone	3.0
Cohumulone	1.5
Ad-, pre-, posthumulone	0.5
β-acids	3.0
Lupulone	1.5
Colupulone	1.2
Ad-, pre-, postlupulone	0.3
Uncharacterized resins	3.5
Hard resins (humulinone, hulupones)	3.5

[a] From Burgess (1964).

brew kettle is about 25–30%, so the financial incentive for improvement is substantial. One simple method of increasing yield is to grind the hop cones, thus increasing surface exposure of fractured lupulin glands to wort in the brew kettle. This increases yield to 35–40%. Solvent extracts of hops are also made with hexane or dichloromethane. These extracts added to the brew kettle give yields of about 40–45%.

Some commercially available extracts contain isomerized α-acids which have been prepared in aqueous systems with sodium or potassium hydroxide, calcium carbonate, or magnesium carbonate. Isomerization rates are very rapid at higher pH compared to the pH of 5.0–5.4 which prevails in the brew kettle.

The yield of purified potassium salts of α-acids properly added to beer after fermentation and filtration, as described by Westermann (1976), reaches 95+%. These salt extracts are void of the essential oils and β-resins normally contained in whole hops or whole hop extracts. The residual portion of extract from which the purified salts are made is added to the kettle to retain the contribution of remaining hop ingredients to beer character.

D. Water

The chemical composition and concentration of salts in brewing water has a profound effect on the brewing process and resulting beer characteristics. Two ions, calcium and bicarbonate, are critical because of their impact on pH during the brew house operations. The pH of the water and resulting malt extract governs the activity of numerous enzyme systems of the malt, the malt yield, the solution of tannins and other coloring ingredients, the precipitation of proteins in the brew kettle, the rate of browning reactions, isomerization rate of humulone, and the physical and foam stability of the beer. Indeed, brewing water composition is of vital importance. Siebel (1950) has described the composition of water as suitable for brewing lager beer as shown in the tabulation below.

Composition	Amount
Sodium carbonate	None
Calcium and magnesium carbonate	Up to 50 ppm
Sodium and magnesium sulfate	Up to 50 ppm
Calcium sulfate	240 to 400 ppm
Sodium chloride	Up to 100 ppm
Calcium and magnesium chloride	Not over 75 ppm
Iron, as Fe^{2+}	Less than 0.2 ppm
Silica, as SiO_2	Less than 10 ppm

The composition of brewing waters of the world famous brewing centers has been compiled by Scholefield (1956), as shown in Table VI. The high permanent hardness of water used for Burton bitter ales should be noted. The sweeter, darker beers of Dublin, London, and Munich contain less calcium sulfate and more calcium carbonate. Pilsen pale lager is brewed with very soft water.

An ideal water for American lager beers would contain a hardness level midway between Dortmund and Pilsen water, i.e., about 100–125 ppm of Ca^{2+} with generally less than 20 ppm of bicarbonate hardness. At these levels the mash would have a pH of less than 5.6, minimizing the extraction of silicates, tannins, and other harshly flavored materials from the malt. Similarly, the extract in the brew kettle (wort) would ultimately have a pH of 5.1–5.2, producing a beer with potentially good foam and physical stability.

Since waters theoretically ideal for brewing lager beer are not naturally available, two corrective actions can be taken when appropriate. When temporary hardness (bicarbonate) is too high, a mineral acid is added to the water, followed by heating and gas stripping to remove CO_2 from the decomposition of carbonic acid. In addition, gypsum ($CaSO_4$) is added to grains sparge water and to the brew kettle to raise the Ca^{2+} content of the resultant wort.

The desired pH in both mashing and the brew kettle result from the following reactions. The carbonate ion, bicarbonate ion, and CO_2, or carbonic acid, exist in equilibrium, depending upon pH of the water. At high pH the carbonate ion prevails, at neutral pH the bicarbonate, and at low pH, dissolved CO_2 exists. When heating water which contains the bicarbonate ion, an undesirable rise in pH occurs as follows:

$$Ca(HCO_3)_2 \rightleftharpoons Ca^{2+} + 2\,HCO_3^-$$

$$HCO_3^- + H_2O \rightleftharpoons H_2CO_3 + OH^-$$

$$H_2CO_3 \rightleftharpoons H_2O + CO_2 \uparrow$$

Therefore, as previously mentioned, bicarbonate ion concentration is usually reduced before water is used for brewing.

The calcium ion from gypsum ($CaSO_4$) is used to lower the pH in mashing and the brew kettle due to its reaction with secondary phosphates, phytate, and some proteins, peptides, and nucleic acids.

$$3\,Ca^{2+} + 2\,HPO_4^{2-} \overset{\Delta}{\rightleftharpoons} 2\,H^+ + Ca_3(PO_4)_2 \downarrow$$

A similar reaction occurs with phytic acid. The calcium salts tend to precipitate on boiling, exceeding the concentration allowed by virtue of their solubility product constant. If an increase in sweetness or palate