Beer, Pulque, and Tequila Quality Are Improved by Study

BEER FERMENTATION

Modern Fermentation Processes

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Brewing will always remain an art, but the universal demand for a mellow, delicately flavored beer has greatly extended the brewer's appreciation of the value of scientific aids and sensitive quality-control methods in a modernized fermentation process. In many instances, as little as 1 p.p.m. of trace constituents can be detected by organoleptic flavor and odor analysis of beer. The brewer may even ascertain the probability of a deviation from raw ingredient specifications while visually observing his fermenting beer.

THE RESEARCH OF PASTEUR in the field of fermentations initiated a series of events which have been mainly responsible for supplementing the age-old art of brewing with innumerable scientific aids. However, the art of brewing, in essence, will always retain its indispensable importance in beer fermentations. An organoleptic flavor and odor analysis of beer is, in many instances, sensitive to qualitative changes of as little as 1 p.p.m. of certain trace constituents. The brewer may, at times, even ascertain the probability of a deviation from raw ingredient specifications while visually observing his fermenting beer—for example, a slight change of protein constituents of malted barley which normally is not detected by routine laboratory analysis.

The organoleptic sensitivity to changes in beer flavor has impressed upon the brewmaster the necessity of minimizing or avoiding deviations from his specified production standards. The universal demand for a mellow, delicately flavored beer has greatly extended the brewer's need for scientific aids and sensitive quality-control methods for a modernized fermentation process.

Beer fermentation is internationally thought of as either "bottom fermentation" or "top fermentation," depending upon whether the yeast utilized settles to the bottom of the fermenting tank or rises to the top upon completion of yeast development. The former is used mainly for the production of lager beer and the latter for the production of ale. Practically all American breweries may be classified in the bottom fermentation category, and this is the only type referred to in this paper.

Plant Facilities

The modern fermenting cellar structure is characterized by massiveness and a scrupulously sanitary appearance. All inner surfaces are completely insulated and are free from ledges, cracks, or crevices capable of harboring biological media. The cellars are provided with ample drainage facilities and streamlined for ease in maintenance and sanitation. Beer wort fermentation, as in antibiotic production, must be conducted under strictly aseptic conditions.

An adequate air-conditioning system should be installed, capable of keeping the cellar uniformly refrigerated regardless of process load or external climatic conditions. The system should also be capable of removing carbon dioxide gas normally formed during fermentation and replenishing the atmosphere with clean filtered air. The use of closed fermenting tanks greatly minimizes the requirements of the air-conditioning system.

Equipment

Practically every conceivable type and shape of fermenting vessel have been manufactured and utilized by the brewing industry. In order of preference, the materials used for fermenting tank construction are stainless steel, pure aluminum, a group consisting of glass-lined steel, Ebon-lined concrete tanks, and phenolic-type plastic-lined steel, and a less desirable group consisting of Mammut-lined steel and varnished steel. Choice of material used is usually predicated upon capital available for fabrication of fermenting tanks. Wood, because of its inherent porosity (even when coated) and subsequent risk of infection, has become the least popular material for tank construction.

The material chosen for fermenting tank fabrication must be nonporous and sufficiently smooth to minimize beer stone (calcium oxalate) deposit. The interior of the fermenting tank must be engineered to eliminate possible crevices for bacterial accumulation, and should be inert to water, beer, or wort, and capable of withstanding the normal cleansing action of suitable detergents.

Fermenting tank accessories include the usual temperature indicators or recorders, sanitary valves, pressure and vacuum safety valves, sampling cocks, liquid level gage, and a heat-exchange coil utilizing brine, glycol, or some other refrigerant as the cooling medium.

The ancient method of controlling fermentation temperatures by use of ice "swimmers" has long since been replaced by the use of attemperating coils, which are normally made of copper, tinned copper, or stainless steel. The rate of heat exchange may be regulated manually by use of valves or automatically by a temperature-controlling cam.

The brewery fermenters are either of an open fermenting or closed fermenting type. The choice between closed and

open fermenters is in reality mainly an argument between the brewers who adhere to the practice of skimming off the "resin-protein" head on fermenting wort and those who tend to avoid skimming procedures. Skimming may be eliminated without adversely affecting the quality of the product, particularly if the fermentation is started in the starting tank, and then decanted or pumped off into the fermenter, leaving the settled resin-protein particles and some yeast, including dead cells, behind. The improvement of working conditions, the economic savings, the elimination of undesirable air-borne bacteria, and the advantages gained by keeping a blanket of carbon dioxide gas over the beer at all times indicate that all fermenting tanks should be closed and the carbon dioxide evolved be withdrawn by means of a centralized gas-exhaust system.

The Substrate, Wort

The fermentation substrate, generally known as "wort" in the brewing industry, is a carefully prepared and enzymatically converted extract of malted barley, hops, and a specially prepared cereal or carbohydrate adjunct such as sugar, rice, or corn grits. The extract should be of approximately 11.8° to 12.3° Plato and sufficiently converted to produce with the aid of brewer's yeast a final gravity of 2.8° to 3.6° Plato and alcohol content of 3.10 to 3.90% by weight. The pH of a normal wort may vary from 5.2 to 5.8 and will mainly depend upon the strong buffer systems in malted barley and adjuncts and upon the mineral salt constituents of the brewing water. The major buffering systems of malt include phosphates, carbohydrates, and amino acids. In water the bicarbonates tend to increase the substrate pH, and the calcium ions by virtue of their influence upon malted barley phosphates will tend to decrease the pH.

The extract must contain the nutrients necessary for proper development of the yeast and the substances necessary for the enzymatic conversion to the final product. Extreme care must be exercised to produce a wort containing degradation products of cereal carbohydrate and cereal protein constituents that are properly balanced for optimum development of yeast cell and beer flavor. A deficiency of mineral salts, especially phosphates and other yeast nutrients, will result in the degeneration of the yeast with subsequent abnormal beer flavor. In the absence of available phosphates, brewer's yeast may not ferment sugar and if the phosphate content falls appreciably below 0.04%, the fermentation may become sluggish and abnormal. A surplus of wort constituents such as certain gums, nitrogenous substances, and many of the metallic salts is also capable of adversely affecting final beer quality. The inhibitive effect of the residues remaining after the yeast has assimilated the nitrogen fraction is a relatively important factor to consider when selecting nitrogenous substances for wort preparation.

The amount of air injected into the wort at the hot wort cooler for yeast propagation purposes varies from 0.1 to 0.4 cubic foot per barrel of wort. The quantity of air actually retained by the cold wort varies from 0.035 to 0.070 cubic foot per barrel of wort and depends largely upon the location and dispersive efficiency of the air-injection nozzle as well as the temperature and gravity of the wort. Excessive amounts of air, although encouraging production of large yeast crops, will affect flavor adversely. This may be attributed to the fact that as yeast propagation is increased by excessive aeration during cooling, an increase occurs also in production of higher alcohols and organic acids which are formed through the assimilation of amino acid nitrogen by the brewer's yeast. The complexity of beer flavor production is better understood when we realize the vast number of compounds, including vitamins, enzymes, water minerals, and innumerable hop, malt, and adjunct ingredients, that find their way into the packaged beer. Such complexity indicates how difficult it becomes for the brewer to maintain a narrow deviation from adopted flavor and odor standards. The usual range in composition of American wort and beer is shown in Tables I and II.

Table I. Range in Composition of Substrate Wort

(Fluctuates with variable trend of American palate)

Sp. gr. at 20°/20° C.	1.04755	1.04965
Extract, degrees Plato	11.80	12.30
Reducing sugar, as maltose	7.0	8.5
Sugar degree, % reducing sugar	64.0	72.0
pH	5.2	5.8
Total acidity, as lactic	0.11	0.12
Color, 0.5-inch cell, Lovibond series 52	3.0	5.0
Protein (nitrogen × 6.25)	0.38	0.50
Iodine reaction	None	

Table II. Range in Composition of Packaged Lager Beer

(Fluctuates with variable trend of American palate)

Sp. gr. at 20°/20° C.	1.01071	1.01410
Extract apparent, degrees Plato	2.74	3.60
Extract real, degrees Plato (calcd.)	4.08	5.45
Alcohol by weight	3.10	3.90
Reducing sugar, as maltose	0.90	1.55
pH	4.10	4.50
Total acidity, as lactic	0.13	0.17
Color, 0.5-inch cell, Lovibond series 52	2.50	3.50
Protein (nitrogen × 6.25)	0.24	0.38
Carbon dioxide, % by weight	0.50	0.57

The Microorganism, Saccharomyces cerevisiae

The brewers' yeast chosen will depend a great deal upon its ability to attenuate properly and quickly and to flocculate rapidly out of suspension as soon as the fermentation cycle is completed, its resistance to autolysis, and its influence on flavor development characteristics. Brewer's yeast exhibits relatively high fermentation activity and relatively low aerobic respiration activity, which differentiates it from many other yeasts. Both activities proceed simultaneously and may be proportionately controlled by the extent of wort aeration and influenced by wort composition and temperature.

Yeast growth and propagation during the fermentation cycle are characterized by three phases. The first or the lag phase is the initial period, during which time the yeast remains dormant and the specific gravity is unchanged. The lag phase time may be reduced to practically nil by elevating the starting temperature. Increasing the substrate air content and slight rousing (agitation) will also tend to diminish the lag phase period. The second, or logarithmic growth phase, is characterized by a rapid logarithmic growth of yeast cells, an increase in temperature which is controlled by cooling with the aid of attemperating coils, a rapid decrease of pH value, and a rapid reduction in specific gravity accompanied by formation of carbon dioxide and alcohol. The carbon dioxide gas formed during this phase rises through the surface and may be collected, compressed, and stored until required for final reinjection into the fully aged beer. The third phase of diminishing growth occurs as a result of a combination of factors involving the assimilation of nutrient substances and accumulation of fermentation-inhibiting by-products. The retarded growth rate continues until eventually growth completely ceases.

The most widely practiced method of yeast addition is to inject it proportionately into the cold wort flowing into the fermenting vessel. The pitching rate may vary from 0.15 to 0.35 pound of dry yeast per barrel of wort, which is equivalent to approximately 6000 to 14,000 yeast cells per cubic millimeter of wort.

The number of yeast cells obtained on completion of fermentation per unit volume of the fermented wort appears to be independent of the yeast pitching rate employed. The absolute number will be approximately the same ir-

respective of whether large or small pitching rates are used. Under similar conditions, the smaller the pitching rate, the larger will be the reproduction percentage. As the pitching rate is increased, the smaller will become the reproduction percentage.

The yeast cell displays many interesting behaviorisms. For example, yeast pitched into a wort containing slightly different characteristics from the medium in which it had been developed will require a certain number of repeated pitchings before becoming adapted to its new environment. As the yeast adapts itself to a new environment it undergoes fundamental physiological changes which could perceptibly affect the final beer flavor. It has been demonstrated that variable beer flavors may be obtained from the same substrate by different varieties of yeast. The study of flavor control from worts containing mixed nitrogenous substances becomes rather complex when the resultant flavor profile could be due to either the residual substances left after assimilation of the protein amino nitrogen or to the yeast excreting the flavor substances as a normal part of its metabolism.

Good consistent yeast crop recovery for subsequent batch pitchings is important to the brewer. The average recovery normally amounts to three or four times the weight required for an initial pitching.

The effect of wort aeration upon yeast crop recovery is shown in Figure 1. As the aeration rate approaches the wort saturation point of about 0.07 cubic foot per barrel of 50° F. 12° Plato wort, an increase in the yeast crop is noted.

Fermenting cellar containing 315-barrel capacity, plastic-lined steel tanks

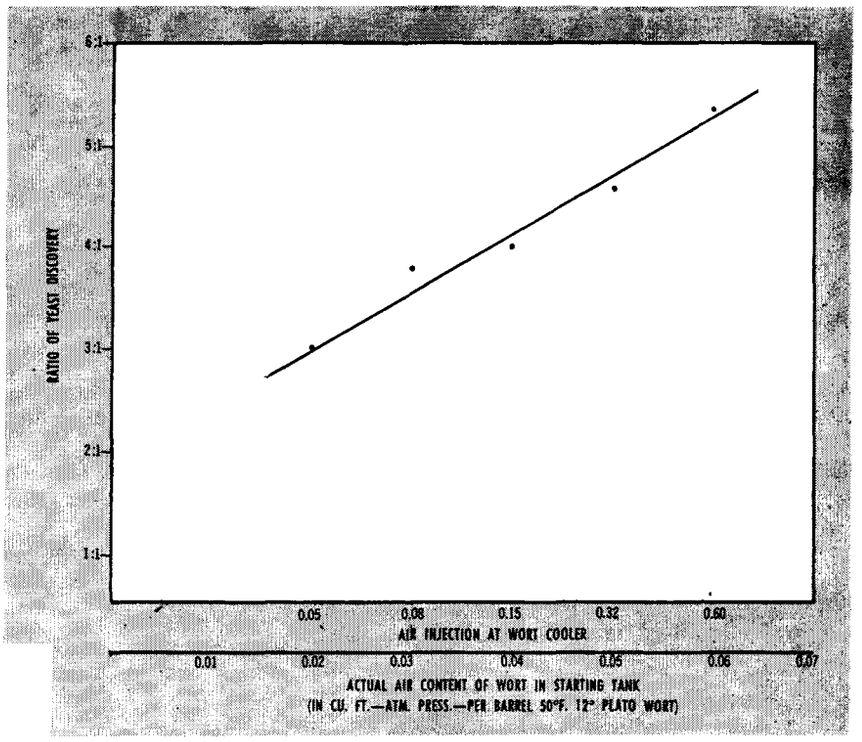

Figure 1. Cold wort aeration vs. yeast recovered from fermenting tank (pitching rate equivalent to 0.2 pounds dry yeast per barrel of wort)

This is probably due to the removal of carbon dioxide caused by the purging effect of air during the initial fermentation period. The amount of air in wort and the carbon dioxide evolved are shown for 7-day and 11-day fermentations in Figure 2. Observations indicate that a yeast strain with poor flocculating power will have strong attenuating tendencies. However, the yeast that settles slowly in the fermenting tank may affect beer quality as well as overload filtration facilities. Suspended yeast densities of two fermentations started at different temperatures are compared in Figure 3.

Hansen in 1883 isolated a single yeast cell and developed the first pure yeast culture method and apparatus. He thereby made available to the brewing industry a means of brewing with pure uncontaminated yeast. The utilization of a pure yeast culture apparatus has in the past saved many a brewery from the disastrous effect of pitching with a yeast highly contaminated with undesirable microorganisms. The popular present-day practice of maintaining an extremely high degree of asepsis in the fermenting cellar during fermentation has resulted in culture purity on a par with that obtained from the average pure culture apparatus. The average brewer considers his entire fermenting cellar as a pure culture apparatus. He maintains strict cleanliness and proper ventilation, and utilizes well designed fermenting facilities and equipment, all for the protection of his invaluable yeast culture and the elimination of all other microorganisms. The more rigorous the adherence to a sterile operation, the more closely pure culture fermentation is achieved.

Fermenting Cellar Operation

The fermenting cellar operation as practiced by the majority of American breweries is conducted as a relatively simple batch procedure.

The properly prepared, chilled, and aerated wort is injected with fluid brewer's yeast as it is transferred into a starting tank. A sample of the wort is obtained for routine quality control analysis in accordance with standard American Society of Brewing Chemists procedure. The average settling time of 4 to 20 hours is normally sufficient for deposition of most of the undesirable suspended solids and weak or dead yeast cells. The fermenting wort is then decanted from the starting tank deposit and transferred to a fermenting tank. Temperature and extract of the wort are observed daily, to ascertain if fermentation is proceeding in accordance with the brewmaster's specifications. The carbon dioxide gas released by the fermentation is checked for purity and transferred to high-pressure gas storage tanks for future carbonation of beer. As fermentation approaches completion, the attemperator valve is adjusted for maximum yeast flocculation and to achieve 34° to 39° F. by the time of transfer to the storage cellar for aging. The fermented and chilled beer is decanted off of the yeast layer and transferred to the storage cellar, and a sample is obtained for routine quality control analysis. The yeast crop is removed,

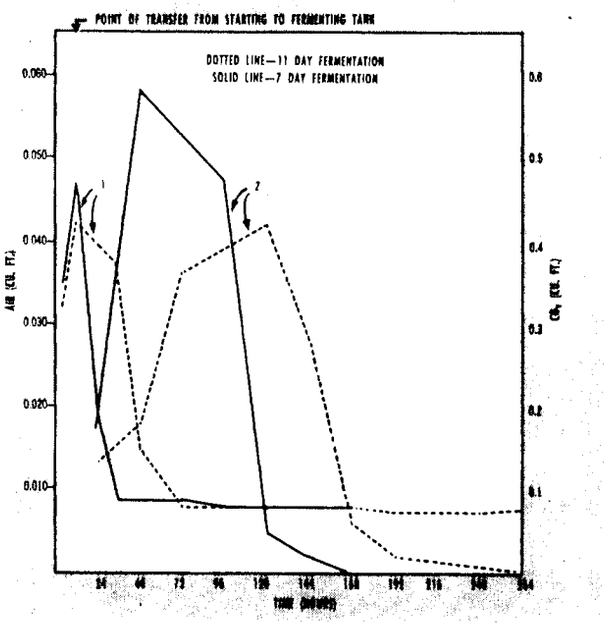

Figure 2. Relationship of wort air content during fermentation to carbon dioxide formed

(1) Air content of fermenting beer in cubic feet per barrel
(2) Carbon dioxide evolved in cubic feet per hour per barrel of beer (calcd.)

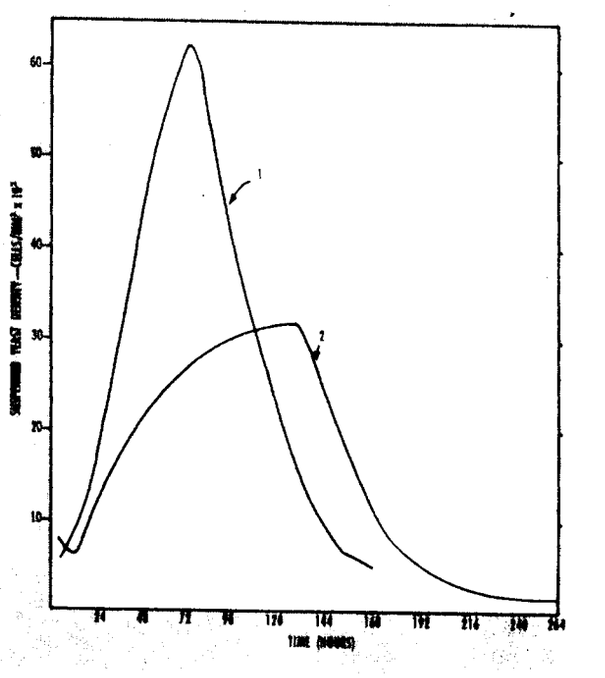

Figure 3. Fermenting time vs. suspended yeast density—in cells per mm³.

(1) Cooler aeration rate of 0.12 cubic feet per barrel wort—starting temperature 54° F.
(2) Cooler aeration rate of 1.12 cubic feet per barrel wort—starting temperature 48° F.

microscopically examined for purity and appearance, and stored in special small vessels at temperatures of 33° to 38° F. until required for the pitching of subsequent brews, which is usually not over 24 hours. A chronological comparison of 7-day and 12-day fermentation cycles is shown in Table III. Changes occurring in pH, temperature, and extract during a typical fermentation cycle are shown in Figure 4, and a flow diagram for the fermentation of 1000 barrels of beer is shown in Figure 5.

Fermentation and Control

The beer fermentation process is usually considered in two mutually related phases. In the first the yeast cells develop and grow and provide the necessary enzymes for the second system, which consists of the actual enzyme-catalyzed production of beer. Experimental evidence and current theories show that wort fermentation normally takes place within the yeast cell. The yeast cell absorbs its nutrient requirements, such as amino acids and sugar, from the wort and yields various by-products and carbon dioxide and alcohol. The enzymes responsible for this catalyzed production of beer act and remain within the yeast cell.

The fermenting temperature influences the attenuating rate and quality of beer flavor. Refrigerated fermentation will minimize the formation of flavor-impairing agents such as various organic acids, higher alcohols, and ketones which are common to high temperature fermentations. Low temperature fermentation will normally encourage the formation of a pleasant bouquet and aroma through the enhanced production by the yeast of desirable aromatic esters and other trace flavor substances. Fermentation temperatures above normal will tend to impair the physiological characteristics of yeast. When this is accompanied by a lack of proper nutrients in the substrate, yeast autolysis will usually occur. The yeast cells will release by autolysis polysaccharides, nitrogenous compounds, glutathione, fatty substances, and inorganic salts which may adversely affect beer flavor and foam. Above-normal temperatures will also encourage the development of undesirable microorganisms.

Starting temperatures may vary from 45° to 60° F. with the optimum at about 48° F., depending upon equipment, substrate, and yeast characteristics. The maximum temperature during fermentation is normally held between 55° and 70° F. with the optimum at about 57° F., depending upon the individual brewery. The temperature of the fermenting cellar is usually kept between 41° and 46° F., which in itself tends to hold the normal fermentation rise within the range for healthy fermentations. After the energetic yeast log growth phase, the fermentation tends to subside and the attemperators are turned on and regulated for rapid cooling. The subsequent decrease in temperature will accelerate yeast sedimentation and condition the freshly fermented beer for aging in the storage cellar.

Table III. Comparison of 7-Day and 12-Day Fermentation Cycles

	Temperature, ° F.		Extract, Plato		Alcohol, % by Weight		pH	
Time, Hours	7 days	12 days	7 days	12 days	7 days	12 days	7 days	12 days
Start	54.0	48.5	12.12	11.91	0.00	0.00	5.30	5.35
24	55.5	48.0	10.97	11.30	0.48	0.25	4.60	4.75
48	59.0	50.0	8.20	10.45	1.65	0.61	4.35	4.50
72	60.0	52.0	5.73	8.75	2.69	1.33	4.15	4.40
96	59.8	54.0	3.51	6.90	3.61	2.10	4.06	4.40
120	51.0	56.5	3.24	4.89	3.73	2.95	4.10	4.32
144	45.0	56.6	3.15	3.58	3.77	3.40	4.15	4.28
168	38.0	50.0	3.13	3.30	3.78	3.61	4.16	4.25
192		46.0		3.19		3.66		4.30
216		42.0		3.17		3.67		4.20
240		39.0		3.12		3.69		4.16
264		36.0		3.08		3.70		4.16
288		36.0		3.07		3.71		4.16

The injected air content of wort quickly diminishes as it is utilized during yeast propagation and washed out of solution by the evolution of carbon dioxide gas. In the course of normal fermentation approximately 80 to 95% of the air is removed within the first 48 hours.

The pH in beer fermentation is controlled by the initial selection of substrate nutrients containing a substantial number of strong buffer systems. Both pH and acidity are capable of influencing the development of bouquet and flavor during fermentation. The greater the resultant beer acidity, the more probable the development of an excessively prominent beer bouquet and flavor through fermentation. At the same time, the formation and retention of volatile acids, acetaldehyde, and glycerol in the beer are thought to be inhibited.

Even though approximately 50 to 65% of the nitrogenous ingredients of wort are assimilable by brewer's yeast, only about 15 to 25% of the nitrogen is absorbed during fermentation. The utilization of amino acids by brewer's yeast in the presence of fermentable sugar involves both hydrolytic deamination and decarboxylation, which is a combined function rather uncommon to most other microorganisms.

Of the sugar utilized in wort fermentation, only approximately 94% can be accounted for as converted into alcohol and carbon dioxide. The greatest portion of the remaining 6% may occur as substances such as glycerol and minute amounts of organic acids, esters, aldehydes, and fatty materials.

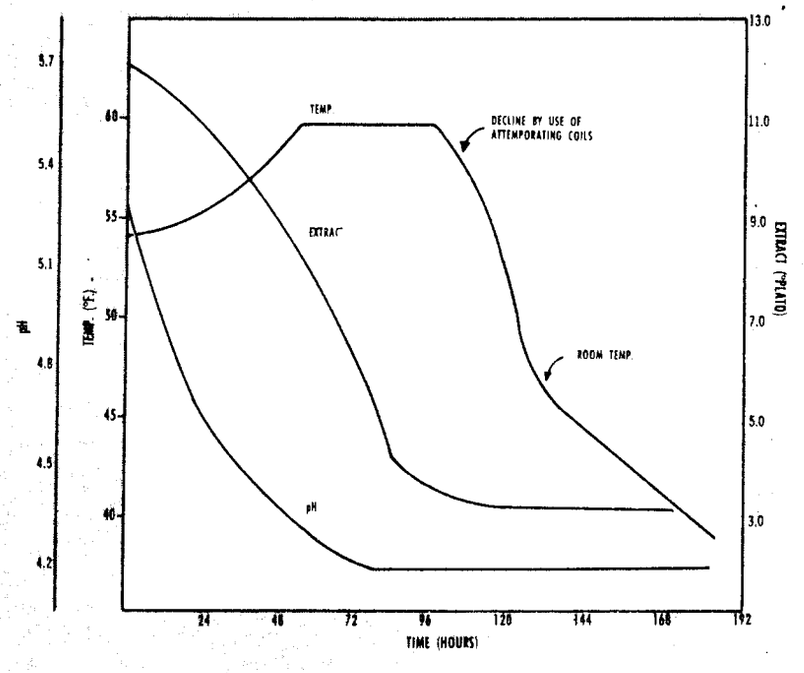

Figure 4. Time *vs.* temperature, pH, and extract

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Figure 5. Flow diagram for the fermentation of 1000 barrels of beer