Determining Pasteurization Units From Residual Melibiase Activity in Lager Beer

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ABSTRACT

A method was developed to determine the number of pasteurization units a lager beer has received from measurements of beer pH and residual melibiase (α-galactosidase) activity. A procedure for determining the melibiase activity is given. For a given pH and temperature, the heat inactivation of melibiase was shown to follow an exponential decay. Then 35 heat inactivation constants (k) were determined at seven pH values (pH 3.90–4.50) for five temperatures (60.0–64.1° C) for two lager beers. Based on either the experimental or the computed values for k at pH 4.0–4.5, a method is outlined for assessing the number of pasteurization units of a lager beer. For beer pasteurized at 60° C, it was possible to estimate up to 30–125 PU, depending on the pH of the beer. The melibiase method was judged to be accurate to ± 20%.

Key words: Enzyme, α-Galactosidase, Heat inactivation, Lager beer, Melibiase, Pasteurization

Beer is pasteurized to kill yeast and bacteria that might bring about deterioration in its appearance and taste (5). Lack of adequate pasteurization can lead to such deterioration, which normally develops after prolonged storage of the finished beer. Brewers need methods they can use before the beer leaves the brewery that will indicate whether sufficient pasteurization has been achieved. Accelerated biological methods as well as rapid chemical methods are used for this purpose.

A pasteurization unit (PU) is a measure of the heat received by a beer during pasteurization; 1 PU is 1 min at 60° C (140° F) or the equivalent time-temperature effect on microorganisms. Beer is frequently given 10–100 PU, although 5 PU is sufficient in most cases.

For almost 80 years, methods based on invertase have been used as "all-or-none" pasteurization tests, which indicate whether a given beer has been pasteurized. The invertase test was introduced by Bau (1) in 1902. Since then it has been studied in detail and has undergone several improvements (5,7–11). It is the only chemical method in current use. It is possible to use the invertase test in a quantitative manner, but because the enzyme is rather heat labile, it is not practical to use this method beyond an upper limit of about 5 PU.

The invertase method can be used on lagers and ales, because bottom- and top-fermenting yeasts both contain the enzyme and excrete it into the fermenting beer. Brewing chemists have looked for other yeast enzymes likely to be found in nonpasteurized beer in the hope of finding one more heat stable than invertase. Such attempts led to a method (12) based on acid phosphatase. However, this enzyme appears to be even more heat labile than invertase, and the upper limit of the method based on acid phosphatase is about 3 PU.

In lager beer there is one enzyme of particular interest, i.e., melibiase, an α-galactosidase that hydrolyzes melibiose into galactose and glucose. Melibiase is present in the cell walls of brewer's lager yeasts (*Saccharomyces carlsbergensis*) but not in brewer's ale yeasts (*S. cerevisiae*). The presence of melibiase in nonpasteurized lager beer was demonstrated only recently (3). Although melibiase activity (approximately 40 units/liter [U/L]) is 10–20 times less than that of invertase, melibiase is considerably more heat stable at the conditions (temperature and pH) prevailing for beer pasteurization (3). These findings led to the present study, which was undertaken to develop an improved chemical pasteurization test that would exceed the maximum 5 PU possible with the invertase method. A preliminary account of these studies has been given earlier (4).

The heat inactivation of melibiase at a given pH and temperature follows first-order kinetics (i.e., exponential decay of melibiase activity versus time) (3). This was reported (3) for a lager beer of pH 4.02 at three temperatures (57.1, 60.0, and 63.2° C), and the rate constants, k, and half-life times, $T_{½}$, were determined. At 60.0° C the heat inactivation constant (rate constant, k) and half-life time for melibiase were 0.0688/min and 10.1 min, respectively. Thus, for a given beer of pH 4.02 pasteurized at 60.0° C, the residual melibiase activity (U/L) after t min of heat treatment, is:

$$U/L = e^{-kt} \cdot (U/L)_0, \quad (1)$$

where $(U/L)_0$ is the original melibiase activity of the lager beer before pasteurization.

It follows that if k is known, and the melibiase activities before $(U/L)_0$ and after pasteurization (U/L) can be determined, the time in minutes, t, can be calculated, e.g.:

$$t = \ln [(U/L)_0/(U/L)] \, 1/k. \quad (2)$$

If the beer is pasteurized at 60° C, then by definition (2) the number of PU is equal to the length of time (in minutes) of the heat treatment during pasteurization.

If beer is pasteurized at a temperature (T° C) other than 60° C, the effect of pasteurization is assumed to increase by a factor of $1.393^{(T-60)}$(2,6,8):

$$(PU/min)_{T°C} = (PU/min)_{60°C} \cdot 1.393^{(T-60)}. \quad (3)$$

This means that if k for melibiase has been determined at the known temperature of pasteurization (T° C), the time can be calculated and the number of PU obtained:

$$\text{No. PU} = 1.393^{(T-60)} \cdot t. \quad (4)$$

The strategy then is to determine the heat inactivation constants for melibiase for a range of relevant pH values and appropriate temperatures. Most lager beers have pH values in the range 3.9–4.5; and for tunnel pasteurization of bottled or canned beer, temperatures in the range of 60–65° C are commonly used. Two brands of lager beers were chosen for this study, and the heat inactivation constant for melibiase (k) was determined at seven different pH values and five different temperatures. From the resultant 35 values for k (each averaged from samples of the two beers), the number of PU for a given beer can be derived from the percentage residual melibiase activity, either by computation or from tabulated values for the appropriate pH and temperature.

EXPERIMENTAL

Beer Samples

Danish lagers of 10.7° P were used in this study. Beer A was a Carlsberg Hof with a pH of 4.34 and 40.5 U/L of melibiase before

heat treatment. Beer B was a Tuborg Grøn with a pH of 4.16 and 65.6 U/L of melibiase before heat treatment. Nonpasteurized beers were provided for this study by courtesy of the two breweries.

Determination of Melibiase

Melibiase activity was measured by the release of glucose from melibiose, as described by Enevoldsen (3). One unit of melibiase activity is the amount of enzyme that hydrolyzes 1 μmol of melibiose per minute at pH 5.0 and 30° C. For pasteurized lager beer (2–20 U/L), the following assay was adopted: 2.00 ml melibiose (2.50% w/v) + 1.50 ml acetate buffer (0.2*M*, pH 5.0) + 1.50 ml lager beer (pasteurized) = 5.00 ml at 30° C. Aliquots of 0.5 ml were taken at different times (e.g., at 0, 2, and 4 hr) and transferred to a boiling water bath for 10 min. After cooling to room temperature, 200 μl was taken for determination of glucose (or galactose).

Determination of Glucose

The method of choice for determination of glucose has been the Gluc-DH method (Catalog no. 14 335; E. Merck, Darmstadt, West Germany). Glucose dehydrogenase catalyzes the oxidation of β-D-glucose to D-gluconolactone and the concurrent reduction of nicotinamide adenine dinucleotide, NAD^+, to $NADH,H^+$, which is measured spectrophotometrically at 340 nm. To facilitate the conversion of α-D-glucose to β-D-glucose, mutarotase is added. To 200 μl of sample containing up to 80 μg of glucose, 2.00 ml reagent mixture (0.12*M* phosphate buffer, pH 7.6; 0.15*M* NaCl; 10 U/ml of glucose dehydrogenase; 0.21 U/ml of mutarotase; and 2.2 m*M* NAD) is added. After 7–17 min at room temperature, the absorbance at 340 nm is measured. The increase in absorbance is linear with the amount of glucose. For this study, a standard of 25.0 μg of glucose and a reagent blank were included in each series of determinations. As an example: 25.0 μg glucose ~ $\Delta A_{340} = 0.549 - 0.149 = 0.400$. It is also possible to calculate the amount of glucose from the molar absorption coefficient for NADH [$\epsilon = 6{,}300$ L/(mol · cm)] whereby 0.1 μmol/ml corresponds to $\Delta A_{340} = 0.630$, and the above $\Delta A_{340} = 0.400$ then gives 25.1 μg of glucose in the 2.20-ml mixture, or in the 0.20-ml sample.

TABLE I
Residual Melibiase Activity in a Lager Beer after Heat Treatment at 60.0° C for Varying Times[a]

Time (min)	Melibiase Activity (Units/Liter)	ln (Units/Liter)
6	29.42	3.3817
8	27.20	3.3032
10	24.50	3.1987
12	22.08	3.0951
14	19.87	2.9892

[a] Beer A, pH 4.20, with original activity of 40.5 U/L. Compare with Fig. 2.

TABLE II
Heat Inactivation Constants for Melibiase in Two Lager Beers at Varied pH Values and Temperatures

	Melibiase Heat Inactivation Constants (k/min)				
pH	60.0° C	61.0° C	62.1° C	63.1° C	64.1° C
Beer A					
3.90	0.1293	0.2093	0.3400	0.4714	0.5501
4.00	0.0736	0.1567	0.2593	0.4060	0.5358
4.10	0.0571	0.1113	0.1857	0.3431	0.4613
4.20	0.0497	0.0920	0.1426	0.2696	0.4180
4.30	0.0411	0.0699	0.1181	0.2402	0.3046
4.40	0.0364	0.0602	0.1041	0.1931	0.2488
4.50	0.0345	0.0487	0.0965	0.1746	0.2497
Beer B					
3.90	0.1227	0.1870	0.3438	0.4303	0.5355
4.00	0.0711	0.1346	0.2238	0.3596	0.4777
4.10	0.0548	0.1143	0.1847	0.3286	0.5208
4.20	0.0408	0.0775	0.1352	0.2386	0.3351
4.30	0.0372	0.0735	0.1111	0.2144	0.2471
4.40	0.0313	0.0584	0.0925	0.1689	0.2191
4.50	0.0283	0.0520	0.0843	0.1473	0.2213

Calculation of Melibiase Activity

The formation of 1 μmol of glucose (or galactose) from melibiose per minute at pH 5.0 and 30° C also represents one unit of melibiase activity. Melibiase activities in beer were expressed in units/liter (U/L).

An example of the calculation follows. The absorbance at 340 nm increased linearly from 0.280 to 1.297 in 240 min; thus $\Delta A_{340} = 1.017$, which corresponds to 63.6 μg of glucose, or 0.353 μmol of glucose in 200 μl of the 5,000-μl digest. Thus, in 240 min, 1.50 ml of pasteurized beer produced 8.83 μmol of glucose or 24.5 μmol of glucose per minute per liter of beer, or approximately 24.5 U/L.

The substrate concentration (1.00% w/v of melibiase) is 29.24 m*M*. It has been shown (3) that K_m at 30° C for melibiase-melibiose is 4.75 m*M*. Thus, if V_{max} values are required, the present units have to be multiplied, with $1.16[V_{max} = v \cdot (So + K_m)/So]$ corresponding to substrate saturation. In the present paper, however, all values are based on 1.00% w/v of melibiase, pH 5.0, and 30° C.

RESULTS

Adjustment of pH

The pH of the two lager beers was adjusted by adding 1*N* H_2SO_4 (0.1–2.0 ml/670 ml of beer, or approximately two bottles) or 1*N* NaOH (0.1–1.4 ml/670 ml of beer) to obtain as accurately as possible the following pH values: 3.90, 4.00, 4.10, 4.20, 4.30, 4.40, and 4.50. All pH values were determined in aliquots at room temperature (20° C).

Heat Treatment of Beer Samples

After the pH was adjusted, lager beers A and B were kept cold (at approximately 0° C to retain the CO_2), and 15-ml portions were transferred to 20-ml serum bottles and sealed. The serum bottles were preheated at 40° C for 3 min, then they were transferred to a thermostated water bath.

In trial experiments it took 6 min for beer to attain the desired temperature (± 0.1° C). Accordingly, two serum bottles (one each of A and B) were removed every 2 min, corresponding to 6, 8, 10, 12, and 14 min, and chilled in an ice bath. The heat treatment for the individual temperatures was performed on separate days, and the following temperatures were recorded: 60.0, 61.0, 62.1, 63.1, and 64.1° C, respectively. The heat-treated beer samples (350 samples in all) were kept in the cold room until analysis for (residual) melibiase activity.

Melibiase Activity

The melibiase activity for 175 samples each of beers A and B were determined as described in Experimental and exemplified in Figure 1 for a sample of beer A at pH 4.20, 60.0° C, and heated for 10 min, which gave 24.5 U/L of melibiase activity.

Determining Heat Inactivation Constants for Melibiase

Table I shows the melibiase activity recorded for the five samples of beer A heated to 60° C for 6, 8, 10, 12, and 14 min. Assuming first-order kinetics for the heat inactivation of melibiase, the natural logarithm of the melibiase activity versus time should give a straight line with a negative slope for k, as shown in Figure 2, and the correlation coefficient of $r^2 = 0.9972$ indicates this to be valid.

In this way 35 values for melibiase k were determined for beers A and B for the seven pH values and five temperatures in question. These values are given in Table II. Except for the values for 64.1° C,

which were based on the 6-, 8-, and 10-min samples only, all values were based on five time intervals. The correlation coefficient in each case (49 of 70 were above 0.99, the lowest being $r^2 = 0.9633$) indicated that the heat inactivation of melibiase for a given pH value and temperature follows first-order kinetics. Although it appears that the 35 k values for beer A in general are somewhat higher than those for beer B, the average of the two sets, given in Table III, is used in the following. As expected, the rate of heat inactivation increases with higher temperatures and lower pH.

Half-Life Times for Melibiase

The half-life time, $T_{½}$, is the time it takes to reduce any given melibiase activity to half of its value; this is a convenient and somewhat more useful term than k. Although $T_{½}$ is simply calculated,

$$T_{½} = \ln 2/k = 0.6931/k, \qquad (5)$$

it describes in a simple manner a characteristic feature of an exponential decay, such as that of melibiase activity versus time. Half-life times for melibiase corresponding to the average k values in Table III have been calculated and given in Table IV.

At pH 4.20 and 60° C, $T_{½}$ for melibiase is 15.30 min. Accordingly, if a beer of this pH and temperature has 40 U/L of melibiase, after 15.3 min the activity will be reduced by 50% (20 U/L) of its original value. After a second $T_{½}$ has elapsed (at 30.6 min), melibiase has declined to 10 U/L, or 25% of its original activity, and after $3 \times T_{½}$ (45.9 min), the activity has dropped to 5 U/L, corresponding to 12.5% of the original activity. Table IV shows that the half-life time for melibiase ranges from 1.28 min at pH 3.9 and 64.1° C to as high as 22.07 min at pH 4.5 and 60° C.

Determining PU from Experimental k Values

For pasteurization of beer at a fixed temperature, the number of PU is determined by equation 4. The melibiase method, in turn, shows how the time, t (in minutes) can be derived from equation 2. Accordingly, the number of PU can be expressed by equations 6 or 7:

$$\text{No. PU} = 1.393^{(T-60)} \cdot \ln[(U/L)_0/(U/L)] \cdot 1/k_{(pH,T)} \qquad (6)$$

$$\text{No. PU} = 1.393^{(T-60)} \cdot \frac{T_{½(pH,T)}}{\log 2} \log \frac{(U/L)_0}{(U/L)}. \qquad (7)$$

For the melibiase method, the pH and melibiase activity of the beer must be determined. It is preferable to know the original melibiase activity before pasteurization $(U/L)_0$; otherwise a typical value, e.g., 40 U/L, must be assumed. The pH is important in selecting the appropriate k value ($k_{(pH,T)}$, Table III) or $T_{½}$ (pH, T) (Table IV), which also depends on the temperature. To substantiate the method and illustrate the procedure, the following experiment was performed.

Example. A nonpasteurized lager beer of pH 4.22 was pasteurized at 60° C for 10, 20, 50, and 100 min in our pilot brewery.

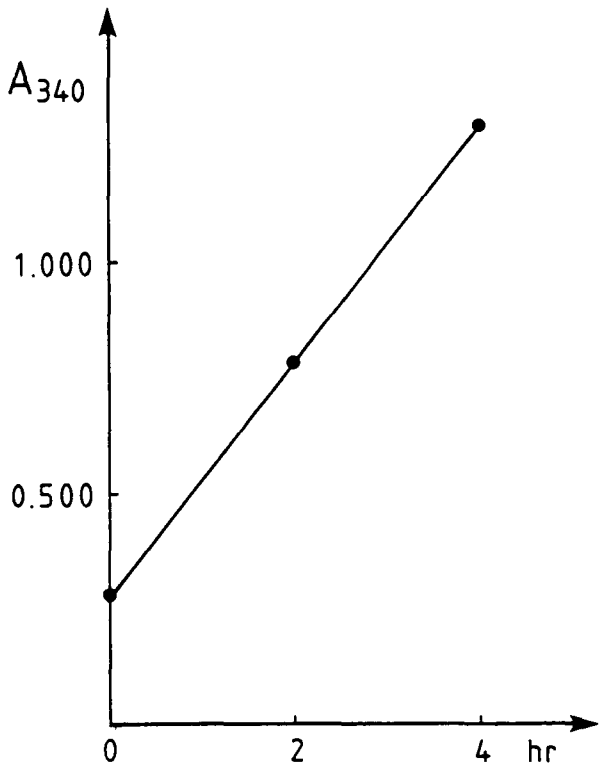

Fig. 1. Determination of residual melibiase activity in lager beer A (pH 4.20, 60.0° C, 10-min sample). Melibiose (1.00% w/v) is used as substrate, and the amount of glucose released over time at pH 5.0 and 30° C is determined by the glucose dehydrogenase method. The time zero absorbance at 340 nm of 0.280 is caused partly by the reagent blank (approximately 0.150) and partly by the color of the beer sample (0.130). The absorbance was 0.780 after 2 hr, and 1.297 after 4 hr, corresponding to the formation of 63.6 μg of glucose per 200 μ of digest, or approximately 6% conversion of the substrate. This is clearly within the linear range whereby enzymic activities are measured by their initial rates of reaction.

TABLE III
Values for Heat Inactivation Constants for Melibiase Averaged for Two Lager Beers, A and B, at Varied pH Values and Temperatures

	Melibiase Heat Inactivation Constants (k/min)				
pH	60.0° C	61.0° C	62.1° C	63.1° C	64.1° C
3.90	0.1260	0.1982	0.3419	0.4509	0.5428
4.00	0.0724	0.1457	0.2416	0.3828	0.5068
4.10	0.0560	0.1128	0.1852	0.3359	0.4911
4.20	0.0453	0.0848	0.1389	0.2541	0.3766
4.30	0.0392	0.0717	0.1146	0.2273	0.2759
4.40	0.0339	0.0593	0.0983	0.1810	0.2340
4.50	0.0314	0.0504	0.0904	0.1610	0.2355

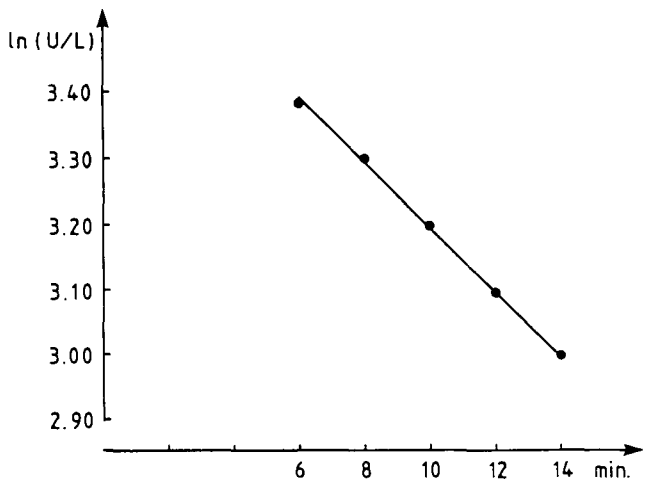

Fig. 2. The melibiase heat inactivation constant, k, for a given pH and temperature (beer A, pH 4.20, 60.0° C) is determined from the slope of the line obtained by plotting the natural logarithm (base e) of the melibiase activities (U/L) against time (in minutes). The correlation coefficient for the straight line was $r^2 = 0.9972$, and the slope was −0.04966; thus, k = 0.0497/min. Beer B gave k = 0.0408/min, and the average value for the two beers was k = 0.0453/min.

The melibiase activity at time zero was 46.0 U/L, and the residual activity levels were as shown in Table V. By interpolation, the heat inactivation constant at pH 4.22 and 60.0°C was found to be $k_{(pH\ 4.22,\ 60°C)} = 0.0441/min$, and the number of PU was calculated from equation 6:

$$\text{No. PU} = 1 \cdot \ln[(U/L)_0/(U/L)] \cdot 1/0.0441.$$

The results given in Table V are in close agreement with the anticipated values of 10, 20, 50, and 100 PU.

The data presented in Table VI illustrate a range of values to facilitate the calculation of PU valid for pasteurization at 60°C. Because nonpasteurized lager beers typically have melibiase activity of 30–70 U/L and because melibiase activities down to about 1 U/L can be determined with adequate accuracy, Table VI gives a range of residual melibiase activities from 90 to 2% of the original melibiase activity. For each pH value, the corresponding number of PU can be read. If the pasteurization temperature is 60°C, the melibiase method permits the number of PU to be determined up to 30 PU for lager beers of pH 3.9 and to 125 PU for lager beers of pH 4.5.

In a similar manner, a semilogarithmic diagram (Fig. 3) was produced. Provided the temperature of 60°C is used for pasteurization, it is possible to find the number of PU based on the percent residual melibiase activity (the ordinate) by choosing the appropriate straight line for which the pH value is indicated.

If the pasteurization is known to take place at temperature T°C, above 60°C, the appropriate k value ($k_{(pH,T)}$) or $T_{½\,(pH,T)}$ must be applied, and the derived time multiplied by $1.393^{(T-60)}$ to obtain the number of PU. Tables and diagrams corresponding to Table VI and Figure 3 can be constructed for the appropriate temperatures. If information about the temperature of pasteurization is not available, it is recommended that the values for 60°C be used and this assumption be noted.

Estimating PU from Computed k Values

Up to this point, the melibiase method for determining PU has been based on the 35 tabulated values for the experimentally determined heat inactivation constants (Table III) or the corresponding half-life times (Table IV). Interpolation between nearest neighbors gives the k or $T_{½}$ for the pH and temperature in question. It is, however, tempting to examine whether it is possible to express the k for melibiase as a mathematical function of pH and temperature, either on theoretical grounds or on an empirical basis. This would mean that computed k values could replace the experimental values, so that extrapolation beyond the framework of tabulated experimental k values might be possible.

As a result of a series of operations (details presented in Discussion), it has been possible to express the pH and temperature dependence of $k_{(pH,T)}$ as follows:

$$k_{(pH,T)} = k_{(pH\ 4.50,\ 60°C)} \cdot 1.65^{(T-60)} \cdot 1.18^{10(4.50-pH)}, \quad (8)$$

where $k_{(pH\ 4.50,\ 60°C)} = 0.0314/min$, and the term for $k_{(pH,T)}$ is applicable in the pH range of 4.0–4.5 and for temperatures from 60.0–64.1°C. This in turn gives:

$$T_{½\,(pH,T)} = \frac{22.07\ \text{minutes}}{1.65^{(T-60)} \cdot 1.18^{10(4.50-pH)}}. \quad (9)$$

When this value for $T_{½\,(pH,T)}$ is incorporated into the equation, the following expression is obtained for the number of PU:

$$\text{No. PU} = 73.3\ \frac{1}{1.18^{(T-60)} \cdot 1.18^{10(4.50-pH)}}\ \log\frac{(U/L)_0}{(U/L)}. \quad (10)$$

TABLE IV
Half-Life Times for Melibiase in Lager Beer at Varied pH values and Temperatures[a]

	Half-Life Times				
pH	60.0°C	61.0°C	62.1°C	63.1°C	64.1°C
3.90	5.50	3.50	2.03	1.54	1.28
4.00	9.57	4.76	2.87	1.81	1.37
4.10	12.38	6.14	3.74	2.06	1.41
4.20	15.30	8.17	4.99	2.73	1.84
4.30	17.68	9.67	6.05	3.05	2.51
4.40	20.45	11.69	7.05	3.83	2.96
4.50	22.07	13.75	7.67	4.30	2.94

[a] Values are averaged in beers A and B.

TABLE V
Check of Residual Melibiase Activity (RMA) Method for Determining Pasteurization Units[a]

Pasteurization at 60°C Time (min)	Melibiase Activity (Units/Liter)	Residual Melibiase Activity (%)	Pasteurization Units Received, Determined by RMA Method
0	46.0	"100"	"0"
10	31.2	67.8	9
20	20.9	45.4	18
50	4.6	10.0	52
100	0.67	1.5	96

[a] Pilot pasteurization of bottles of lager beer of pH 4.22 at 60°C for varied times after the External Bath Temperature Reached 60°C.

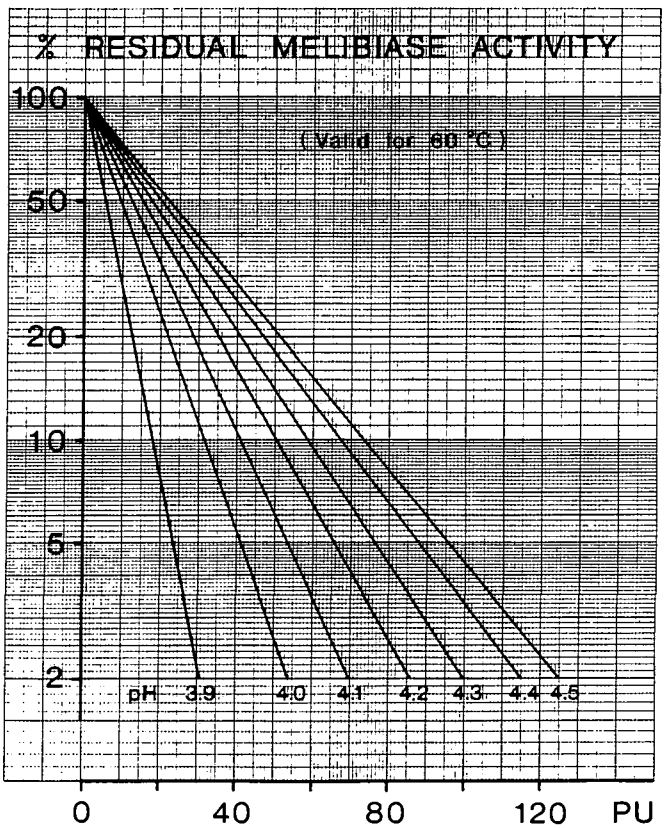

Fig. 3. The diagram enables the number of pasteurization units (PU) to be read from the percentage residual melibiase activity, using the appropriate pH for the lager beer in question. If residual melibiase activity is 10.0%, the pH is 4.20, and the beer has been pasteurized at 60.0°C, the beer has received 50 PU. As an approximation, it is possible to use this diagram for higher temperatures if the pH range is within 4.0–4.5 by reducing the abscissae values by a factor of $1.18^{(T-60)}$.

This is an explicit term, which can be calculated or programmed for estimating the number of PU when $(U/L)_0$, U/L, T° C, and pH values are known. For example, when $(U/L)_0 = 46.0$, U/L = 12.7, T° = 62.1, and pH = 4.22, the estimated number of PU is 18.

It is important to know the original melibiase activity in lager beers before pasteurization. For 30 Danish lager beers representing four brands and four production sites the melibiase activity ranged from 12–73 U/L, with an average of 39.3, or approximately 40 U/L (3). Since that data was collected, a number of lager beers have been examined. At one production site where the dominant brand was examined each Tuesday for eight consecutive weeks, the original melibiase activity ranged from 38.0–45.8 U/L, with an average of 42.9 U/L, which indicates a fairly constant level before pasteurization. However, this may be an oversimplification. It is recommended that each brewery determine its own levels of melibiase activity and range of variation.

DISCUSSION

Comparison of Heat Inactivation Constants for Beers A and B

It would be preferable to have experimental k values for the two beers in question (and ideally for all lager beers) be the same for a given pH and temperature. This is not quite so, because the heat inactivation constants for beer A are in general somewhat higher ($10 \pm 9\%$) than the corresponding values for beer B. Nevertheless, the average k values (Table III) are used throughout this study and are taken as typical values for any given lager beer. Furthermore, the difference between the values for beer A and beer B at a given pH and temperature is considered to be minor compared to the overall (17-fold, or 1,730%) change in the k values with changes in pH and temperature. As already indicated, the heat inactivation constants increase an average of 18% per tenth of a pH unit and about 65% per degree Celsius, thus it seems justified to disregard the fact that the k values for beer A are $4 \pm 4\%$ higher than the average values given in Table III.

Effect of Temperature on Heat Inactivation Constants

With respect to the effect of temperature, it has been shown that an Arrhenius plot (i.e., log k versus the reciprocal absolute temperature [Kelvin]) gives a straight line, providing a mathematical expression for the temperature dependence of heat inactivation for melibiase (3). This has also been shown in the present study; straight lines with correlation coefficients in the range of 0.970–0.997 were obtained for the seven pH values tested.

Although the Arrhenius equation represents basic theory on temperature dependence of the rate of chemical reactions and can be applied to heat inactivation of enzymes (and of microorganisms), this approach is not further pursued in the present context. This is because the effect of temperature on pasteurization as outlined has, by convention, been expressed as an exponential function:

$$PU_{T°C} = PU_{60°C} \cdot p^{(T-60)} = PU_{60°C} \cdot 1.393^{(T-60)}. \quad (11)$$

where p (~1.393) is the factor by which the pasteurization effect increases per 1°C increments. Thus, to obtain a related mathematical expression, the experimental k values were subject to "curve fitting" to an exponential function:

$$k_{T°C} = k_{60°C} \cdot a^{(T-60)}, \quad (12)$$

where $k_{60°C}$ is the computed value for the heat inactivation constant at 60° C. This appeared to give a good correlation, quite compatible with those from Arrhenius plots, as shown in Table VII. The result of curve fitting shows that k for a given pH increases by a constant factor, a, in the temperature range 60.0–64.1° C. This factor is about 1.44 for pH 3.90 and, with good approximation, about 1.65 for the pH range 4.0–4.5 (Table VII).

This further means that the heat destruction of melibiase is more rapid than the generally accepted rate for microorganisms, and increasingly more so as the temperature rises, as indicated by the ratio a/p = 1.65/1.393 = 1.18 for the pH range 4.0–4.5. For a given pH this equation expresses the effect of temperature on the heat inactivation constant:

$$k_{(pH,T°C)} = k_{(pH,60°C)} \cdot 1.65^{(T-60)} = k_{(pH,60°C)} \cdot 1.393^{(T-60)} \cdot 1.18^{(T-60)}. \quad (13)$$

Incidentally, this means that the values in Table VI and Figure 3 can be used for temperatures other than 60° C provided they are divided by $(a/1.393)^{(T-60)}$ to calculate the correct number of PU.

As an example, if a lager beer of pH 4.20 retains 25% of its original melibiase activity after pasteurization, and if heat treatment occurred at 60° C, the beer has received 31 PU. However, if the temperature of pasteurization was actually 62.1° C, the beer has received 31 PU divided by $1.18^{2.1} = 22$ PU.

TABLE VI
Number of Pasteurization Units Valid for 60.0° C

Residual Melibiase Activity (%)	pH Values						
	3.90	4.00	4.10	4.20	4.30	4.40	4.50
90	1	1	2	2	3	3	3
80	2	3	4	5	6	7	7
70	3	5	6	8	9	11	12
60	4	7	9	11	13	15	16
50	5	10	12	15	18	20	22
45	6	11	14	18	20	24	26
40	7	13	16	20	23	27	29
35	8	15	19	23	27	31	33
30	10	17	22	27	31	36	38
25	11	19	25	31	35	41	44
20	13	22	29	36	41	47	51
18	14	24	31	38	44	51	55
16	15	25	33	40	47	54	58
14	16	27	35	43	50	58	63
12	17	29	38	47	54	63	68
10	18	32	41	51	59	68	73
9	19	33	43	53	61	71	77
8	20	35	45	56	64	75	80
7	21	37	48	59	68	79	85
6	22	39	50	62	72	83	90
5	24	41	54	66	76	88	95
4.5	25	43	56	68	79	92	99
4	26	45	58	71	82	95	103
3.5	27	46	60	74	86	99	107
3	28	48	63	77	89	103	112
2.5	29	51	66	81	94	109	118
2	31	54	70	86	100	115	125

TABLE VII
Results of Curve Fitting of Experimental k Values for Melibiase to an Exponential Function

pH	Correlation Coefficient r^2	a^y	p^z	Ratio a/1.393
3.90	0.9678	1.4394	1.393	1.0333 ~ 1.03
4.00	0.9781	1.6031	1.393	1.1508 ~ 1.15
4.10	0.9913	1.6947	1.393	1.2166 ~ 1.22
4.20	0.9951	1.6782	1.393	1.2047 ~ 1.20
4.30	0.9800	1.6341	1.393	1.1731 ~ 1.17
4.40	0.9884	1.6221	1.393	1.1645 ~ 1.16
4.50	0.9968	1.6557	1.393	1.1886 ~ 1.19
4.0–4.5	···	1.6480	1.393	1.183 ~ 1.18

[y] $k_T = k_{60} \cdot a^{(T-60)}$.
[z] $PU_{T°C} = PU_{60°C} \cdot p^{(T-60)}$.

Effect of pH on the Heat Inactivation Constants

There is no basic theory to account for the heat inactivation of an enzyme as an explicit function of pH. Therefore, it has been attempted (by trial and error) to formulate a simple mathematical expression that will mimic the experimental k values and permit the computation of the heat inactivation constant for melibiase as a function of pH.

It is apparent that the role of pH is pronounced; the lower the pH, the less stable the enzyme. Even a change of one tenth of a pH unit has a remarkable effect on the heat inactivation constant. If the effect of pH is assumed to be independent of the temperature, then the k values for any given temperature (60.0–64.1°C) should follow the same trend as a function of pH.

If $10 \cdot (4.50 - \text{pH})$ is chosen as the variable, and only the pH range 4.0–4.5 is considered, it seems possible to obtain a reasonable prediction of the k values as a function of pH:

$$k_{(pH,T)} = k_{(pH\,4.50,\,T)} \cdot 1.18^{10(4.50-pH)}. \qquad (14)$$

This term has the advantage over other perhaps more precise formulations (such as other exponential functions and polynomial approximations) in that it is directly compatible with the term for temperature dependence, equation 13.

Combined Effect of pH and Temperature on the Heat Inactivation Constant

The combined effect of pH and temperature was outlined in equation 8, which appears to be valid for pH 4.0–4.5 and 60.0–64.1°C. With respect to pH 3.9, the tabulated values for k have to be used, because the heat inactivation constants for pH 3.9 do not follow the trend for pH 4.0–4.5 either for temperature (the increase is less dramatic than expected) or for pH (the increase is greater than expected). For conditions in the range of pH 4.0–4.5 and temperatures of 60.0–64.1°C either the experimental or computed values for k can be used in equation 8:

$$k_{(pH,\,T)} = 0.0314 \cdot 1.65^{(T-60)} \cdot 1.18^{10(4.50-pH)}.$$

When the 30 experimental values are compared in equation 8 with the corresponding computed values for k, the relative standard deviation is ± 10%, which is considered quite acceptable.

Flash Pasteurization

A further point to be discussed and a separate aspect of the effect of temperature relates to flash pasteurization and the possibility of extrapolating the data beyond the temperature range of 60–65°C. The melibiase method has not yet been investigated under the conditions for flash pasteurization, 71–79°C with holding times less than 1 min (15–60 sec). Heat inactivation constants for melibiase have not been determined in this temperature range, and their determination might not even be possible. Del Vecchio et al (2) limited their investigation to the temperature range of 45–66°C; others have extrapolated their time-temperature findings up to 80°C. Calculating from extrapolation of such magnitude for 1 min at 80°C = (1.393^{20}) 757 PU, or 720 PU (6). As an example, this would mean that if 20 PU were required, instead of 20 min at 60°C, 1.6 sec at 80°C would be used. There is no experimental evidence for this relationship. More likely, the empirical observation that microorganisms are destroyed more effectively as temperatures increase, which allows shorter holding times to be used, explains why flash pasteurization is successful in many breweries.

The Role of pH in Pasteurization

By definition, the number of pasteurization units applied to a given beer relates only to the time and temperature; the role of pH in the thermal destruction of microorganisms is not considered. In their original work, Del Vecchio et al (2) did not measure the pH of their medium (95% beer, 5% wort) nor did they consider the effect of pH.

Nevertheless, it is widely accepted among brewers that for a given number of PU (e.g., 10 min at 60°C) the pasteurization is more effective the lower the pH of the beer. From this observation one can extrapolate the following. If heat inactivation of microorganisms depends on pH in a manner that parallels the pH dependence of melibiase heat inactivation, the residual melibiase activity is a more accurate reflection of the effect of pasteurization than if pH dependence were counterbalanced in calculating the number of PU. In other words, say 20 PU (20 min at 60°C) is sufficient treatment for a beer of pH 4.20; under these conditions melibiase activity falls to 40% of its original value. If this reduction in melibiase activity reflects a sufficient degree of pasteurization, then only 13 PU might be required for a beer of pH 4.0 and 27 PU for a beer of pH 4.4 to achieve a residual melibiase activity of 40%.

Other Factors Affecting Pasteurization

Factors other than pH, temperature, and time influence the effectiveness of pasteurization. This probably goes for the medium, where the amount of ethanol in the beer, the amount of extract, CO_2, the level of contaminants, and other factors all play a part.

One important aspect, of course, is that any surviving microorganism stands less of a chance to develop if the beer is a fully end-fermented beer rather than a beer that contains residual fermentable sugars or to which priming sugar has been added. This is the principle reason that some special types of beer are pasteurized more extensively than the 5–20 PU normally recommended for lager beers.

CONCLUSION

The melibiase method as presented here is a novel method that permits determination of the number of PU a given lager beer has received. The method can be used for up to 30–125 PU, depending on the pH of the beer. It is the only method that can provide information after the beer has left the brewery about how many PU the beer received during pasteurization.

The method requires measurement of the pH of the beer and of its residual melibiase activity. Thus, it can only be applied to lager beers. It is preferable to know the original melibiase activity of the nonpasteurized beer; if this is not known, a typical value, e.g., 40 U/L, is used. The method is primarily applicable to lager beers pasteurized (by tunnel pasteurization) at 60–65°C. It relies upon tabulated values for the melibiase heat inactivation constants given in this paper (Table III) or the computed k values according to equation 8. Then the number of pasteurization units can be expressed as follows:

$$\text{No. PU} = 73.3 \quad \frac{1}{1.18^{(T-60)} \cdot 1.18^{10(4.50-pH)}} \cdot \log \frac{(U/L)_0}{(U/L)}.$$

The melibiase method is fairly simple. It requires a pH meter, a thermostated water bath, an ultraviolet spectrophotometer, and standard laboratory equipment. This method is neither more complicated nor more time-consuming than the majority of brewing analyses according to ASBC, EBC, and MEBAK. Nevertheless, work is in progress to simplify the measurement of melibiase activity, on which the method for determining the number of PU depends. At present, the method is judged to be accurate to within ± 20%.

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