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A mathematical model of the formation of fermentable sugars from starch hydrolysis during high-temperature mashing

Robert Muller*

Brewing Research International, Lyttel Hall, Nutfield, Surrey, RH1 4HY UK

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Abstract

During the mashing process of brewing, activity of the amylolytic enzymes decays due to the high temperatures used to gelatinise the starch. Because the different enzymes produce different sugars, high temperatures can be exploited to modify the fermentability of resulting worts. This is especially useful when producing low alcohol beers. The expression a.exp(b.t)-c.exp(d.t) (where t is the temperature of the mash in °C) provides a simple but useful description of the activity of the amylases. Combining the activities of alpha- and beta-amylases results in a prediction of the resulting fermentability. A simple modification to the expression accommodates changes in mash thickness. The error of prediction is approximately 3° of fermentability. The model is not appropriate for predicting the fermentability of worts produced at the lower standard mashing temperatures. It can be used without the necessity of analytical parameters so analyses that the brewer would not normally perform are not required. If increased accuracy is needed, the results of two previous mashes can be used to modify the parameters used.

Keywords: Amylolysis; Fermentability; High Temperature; Mash; Model

1. Introduction

The mashing stage of the brewing process involves the mixing together of a milled grist (usually barley malt) and a hot liquor (usually water at about 65°C for isothermal mashes). This results in the gelatinization of the starch present, permitting subsequent breakdown to fermentable sugars (i.e. mono-, di-, and tri-saccharides) by the malt enzymes. The spectrum of sugars formed depends on the actual enzyme activities present. Beta-amylase produces the fermentable sugar maltose, whereas alpha-amylase generates both fermentable and non-fermentable sugars [1,2]. Other enzymes in the grist, such as limit dextrinase, may also contribute to the fermentable sugar profile. These enzyme activities are profoundly influenced by such a high mashing temperature as 65°C. Beta-amylase, in particular, is rapidly denatured at temperatures above 55°C. Alpha-amylase is rather more stable and remains active for over an hour at 65°C. Thus the fermentability (attenuation limit) of the resulting sugar solution (wort) can be modulated by the mashing temperature and higher mashing temperatures can be used to produce worts with reduced fermentability [3]. The thickness of the mash, that is the liquor-to-grist ratio, also influences enzyme stability. All malt enzymes show enhanced thermostability in more concentrated mashes. These variables mean that predicting the resulting fermentability from mashing conditions has proven to be difficult.

With a series of defined unit operations the brewing process is ideally suited to mathematical modeling [4–10]. In particular the mashing stage has been the subject of several predictive models [11–21]. Although providing excellent accuracy, the problem with previous models was that they were frequently more complicated than proved useful. Most required several malt analyses as parameters and a computer to manipulate the equations. Because a test mash itself is moderately easy to perform there is limited value in a model that requires analysis of enzymes, starch content and other analytical parameters that are not part of normal brewing analysis. A predictive model should therefore be simple to calculate and require as few novel analyses as possible.

An extreme example of reducing fermentability is high-temperature mashing, an approach that can be taken to prepare a low alcohol beer. In this case the grist is mashed

* Corresponding author. Tel.: +44-0-1737-824 228; fax: +44-0-1737-822747 (R. Muller).

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at unusually high temperatures (for example 85°C). Under these conditions beta-amylase is rapidly inactivated but sufficient alpha-amylase remains to digest and liquefy the starch. However, very little fermentable sugar is formed and the subsequent fermentation is limited to reduced alcohol production [3]. Low alcohol beers are generally produced infrequently and identifying an appropriately high temperature can be a matter of luck. A predictive model would help increase the accuracy with which this is conducted and to obtain the desired value "right first time," reducing the element of trial and error with this procedure. This is critical to the economic production of low alcohol beers by this method especially when they are produced infrequently.

As well as for high-temperature mashing, a prediction of fermentability may also be used to ensure that appropriate levels are being obtained under more usual brewing conditions. With tax levied on alcohol content it is extremely important to control fermentability. When fermentability is higher than desired, it may be a simple matter to reduce this by altering mashing temperature without resorting to the extremes associated with low alcohol beer production. A model may also be useful in predicting the effect on fermentability of changing mash thickness.

1.1. Aims

The aim of this paper is to provide a simple calculation that is straight forward to use, requiring a minimum of analytical data, that will aid in the prediction of wort fermentability after high-temperature mashing. Clearly such a simple equation is a compromise between accuracy and ease of use. For this reason several ways of exploiting the model will be presented, each requiring a different level of input but each with a different level of accuracy.

The model is not appropriate for predictions of fermentability from standard mashes that are usually limited by starch structure and not by enzyme activity.

2. Materials and methods

2.1. Materials

Malts were supplied by Crisp Maltings Group, Great Ryburgh, UK.

2.2. Methods

Laboratory scale mashing was carried out in a BRI laboratory mashing bath according to the procedure defined in the Recommended Methods of the Institute of Brewing [28].

Fermentability was determined by forced fermentations also after the Recommended Methods of the Institute of Brewing [28].

Modeling was performed using Microsoft Excel (version 7.0)

3. Results

3.1. Model construction

This model assumes that gelatinized starch is degraded by two malt enzymes, the alpha-, and beta- amylases. Alpha-amylase breaks up the high molecular weight starch into smaller molecular weight dextrins. The product of beta-amylase activity is simply the fermentable sugar, maltose, whereas extended alpha-amylase activity yields fermentable sugars such as glucose, maltose, and maltotriose but also non-fermentable sugars, such as the low molecular weight dextrins [22–24]. Other enzymes that generate fermentable sugars are assumed to behave like beta-amylase.

Thus fermentability is the result of a combination of alpha- and beta-amylase activities present in the grist [25, 26]:

$$\%\ \text{Fermentability} = \%\ \alpha\ \text{amylase fermentable products} + \%\ \beta\ \text{amylase fermentable products} \quad (1)$$

The formation of these products will depend on two principal factors: 1) the quantity of enzyme present in the original malt grist (here called enzyme quantity). This quantity may be viewed as the total potential activity with units of % fermentables; and 2) the proportion of that quantity that is effective during the mash (here called enzyme remaining). This is only a multiplier and has no units.

It will be assumed that the quantity of the enzymes is fixed by selection of the malt, but that the high temperatures of the mash will destroy a proportion of these enzymes with a concomitant loss of activity. Then the proportion of remaining enzyme activity against temperature may be modeled as follows.

Increased activity with temperature is given by:

$$\frac{\partial(activity)}{\partial(t)} = k_1(t) \quad (2a)$$

Loss of activity with temperature is given by:

$$\frac{\partial(activity)}{\partial(t)} = -k_2(t) \quad (2b)$$

Combined these can be solved as:

$$\text{activity} = a \cdot \exp(b \cdot t) - c \cdot \exp(d \cdot t) \quad (2)$$

where t is temperature in°C and the parameters a, b, c, and d are different positive constants [17–19,27].

At lower temperatures, the $c \cdot \exp(d \cdot t)$ value is insignificant and enzyme activity then increases almost exponentially with temperature. At higher temperatures the value of $c \cdot \exp(d \cdot t)$ becomes close to the first exponential term and

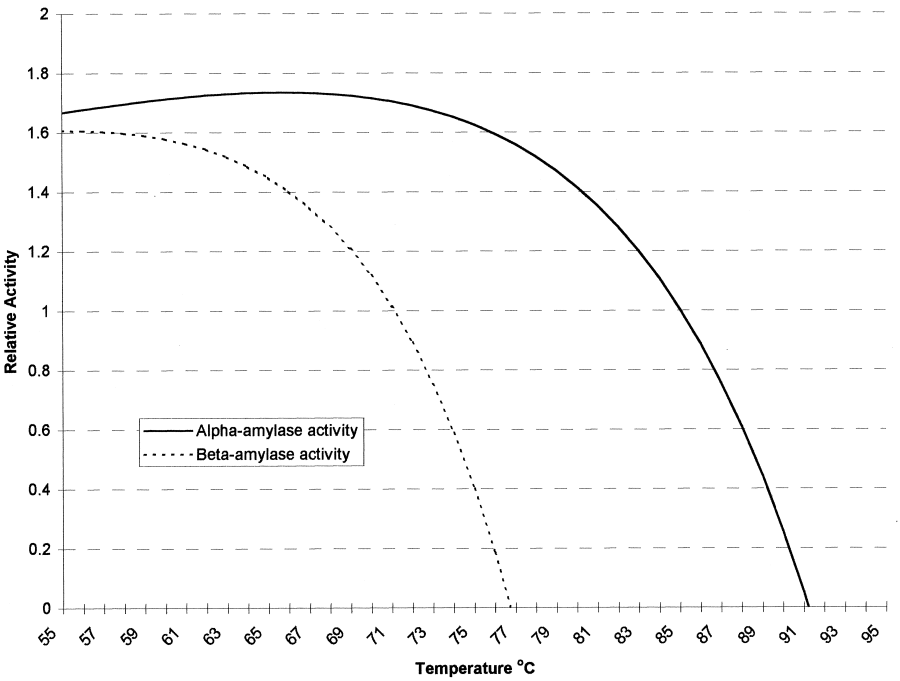

Fig. 1. The relative activity of alpha- and beta-amylase as a function of temperature at constant mash thickness. The parameters chosen to provide for suitable performance of the amylase enzymes are shown in Table 1.

the combined value of the expression rapidly approaches zero corresponding to the thermal decay of the enzyme. This expression accounts for most of the important features observed for the effect of temperature on enzyme activity [27]. Fig. 1 shows the general features of this type of curve for alpha- and beta-amylases over the range of temperatures 55°C and 95°C. The graph shows both the increase and decrease as well as the maximum activity and the point of no remaining activity. Over this range of temperature the increase in activity is very small because the second exponential term is always significant. The range was not extended because at lower temperatures the starch in the mash would not gelatinise and such temperatures would not be used. Of course this expression can take negative values whereas enzyme activity cannot and so the model must be adapted to accommodate this.

Enzymes exhibit enhanced thermostability when used in concentrated sugar solutions such as those encountered in mashes [1,2]. In thinner mashes the amylases become less stable and this can be incorporated into the model as an addition to the negative term.

Thus fermentability may then be described as:

$$\%\text{Fermentability} = \alpha\ \text{quantity} \cdot (\alpha\ \text{remaining}) + \beta\ \text{quantity} \cdot (\beta\ \text{remaining}) \quad (3)$$

with the proportion of enzyme activity remaining calculated as:

$$(\alpha \text{ or } \beta)\ \text{remaining} = a \cdot \exp(b \cdot t) - c \cdot \exp(d \cdot t + e \cdot m + f) \quad (4)$$

Where t is temperature (°C) and m is mash thickness (the ratio of water to grist by weight), a–f are fixed positive parameters.

To simplify this model, it is assumed that after high-temperature mashing, wort fermentability is limited only by sugar content and that the alpha- and beta-amylases are the only relevant enzymes. In fact, although other enzymes may be significant, their activity can be covered by the term for beta-amylase as their performance is effectively the same with regards to temperature (see Section 4). It is also assumed that although the activities of the enzymes change with temperature and mash thickness the type of products they yield does not; notably that the ratio of fermentable to non-fermentable products from the alpha-amylase remains the same and does not change with temperature.

The model is limited to isothermal mashes because high-temperature mashing to produce low alcohol beers requires a single high mashing-in temperature unlike the ramped temperatures frequently used during programmed mashing for standard beers. For this same reason it is not necessary to consider starch gelatinization temperatures because it is anticipated that the mash temperature will always be higher than this, typically in excess of 75°C during low alcohol beer production. The time period of mashing in this model is 1 h. The breakdown of starch (as compared to the for-

mation of fermentable sugars) is not considered here even though ineffective starch degradation may be a significant problem at the higher temperatures associated with reduced enzyme activity and low fermentability worts. Finally, it is assumed that it does not matter how fermentability is determined provided that the method is consistent.

3.2. Model for two input values

If the performance of the enzymes with regard to mash thickness and temperature can be considered to be a property of these enzymes, then the parameters generated by the calculation of remaining activity can be viewed as constants. The performance of different malts then depends only on the starting level of each of these enzymes. That is different malts will perform differently only because they contain different levels of enzyme and not because of any difference in the properties of these enzymes. If two fermentabilities, obtained from these malts are known then these starting levels can be determined as two unknowns in two equations.

Thus, for:

$$\begin{pmatrix} \%F_1 \\ \%F_2 \end{pmatrix} = \begin{pmatrix} \alpha - \text{remaining}_1 & \beta - \text{remaining}_1 \\ \alpha - \text{remaining}_2 & \beta - \text{remaining}_2 \end{pmatrix} \cdot \begin{pmatrix} \alpha - \text{quantity}_1 \\ \beta - \text{quantity}_2 \end{pmatrix}$$

$$\underline{\mathbf{A}} = \underline{\mathbf{B}} \cdot \underline{\mathbf{C}}$$

Then the quantities, $\underline{\mathbf{C}}$ can be obtained from:

$$\underline{\mathbf{C}} = \underline{\mathbf{B}}^{-1} \cdot \underline{\mathbf{A}}$$

The values for enzyme quantity will be called $\underline{\mathbf{C}}$ for later use and may be varied as described below. These values have no physical meaning although they may indicate relative amounts of the two enzymes.

3.3. Parameter estimation

The parameters that define the performance of the amylases with temperature and mash thickness were obtained by curve fitting procedures using MicroCal Origin and an Excel spreadsheet. The values were optimized by using a least squares method.

For:

$$\text{Enzyme remaining} = a \cdot \exp(b \cdot t) - c \cdot \exp(d \cdot t + e \cdot m + f) \quad (5)$$

The following constants have been used in this work (see Table 1).

Again these values have no absolute meaning but have been chosen for ease of calculation. The constant d has units t^{-1}.

Using these parameters in the equations yields enzyme activity profiles shown in Fig. 1. Table 2 lists some features of these profiles.

Table 1
Constant values for activity calculations

Parameter	Value for α amylase	Value for β amylase
a	1	1
b	.01	.01
c	.0001	.0001
d	.01	.13
e	.22	0
f	.36	0

3.4. Model simulation

Fig. 2 shows a comparison between fermentability values obtained in laboratory mashes with those predicted by the model using the parameter values given above. The predicted values of fermentability show the same trends as those obtained during mashing experiments. In particular fermentability decreases with an increase in mashing temperature, the incremental change becoming greater as the temperature becomes higher. Furthermore fermentability falls slightly with thinning mashes, again the effect being most noticeable at the higher temperatures.

Mashes were also compared to modeling data at thickness ratios of 2.5, 3.5, and 4.5, although these data are not presented for clarity. Of the 30 points then considered, the total error was 63° of fermentability being an average of 2.1°, the greatest error in the predicted value was 7° (at 80°C and mash thickness 2:1).

Fig. 3 shows a comparison between fermentability values obtained in laboratory mashes with those predicted by the model for a different malt from that used in Fig. 2. With different starting levels of amylases the performance of the malt was very different to the previous example. In this case the relative quantity of beta-amylase was much greater than for alpha-amylase, the significance of its contribution to fermentability and the sensitivity to temperature would also be different. The fall in fermentability was indeed more rapid than that obtained with the previous malt as would be indicated by the higher level of the more heat sensitive beta-amylase.

Again mashes were also conducted at thickness ratios of 2.5 and 3.5, the data being omitted for clarity. In this case, of 16 points analyzed, the total error was 41° of fermentability being an average of 2.6°, the greatest error in the predicted value was 9° (at 75°C and mash thickness 2:1).

It is evident from the preceding results that malt quality will influence the outcome of a high-temperature mash.

Table 2
Important features of enzyme activity profiles

	α-Amylase	β-Amylase
Maximum activity	67.3°C	55.4°C
Zero activity	91.1°C	76.7°C

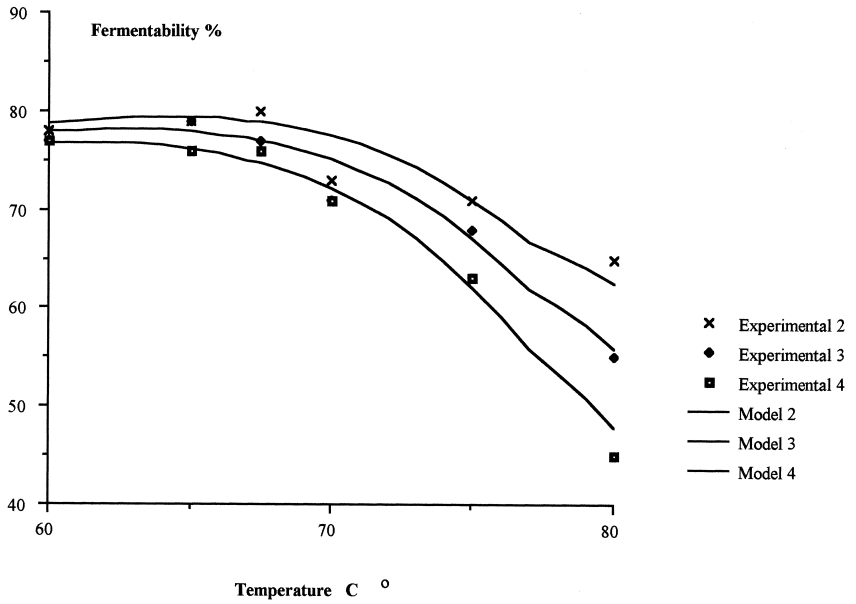

Fig. 2. A comparison between observed and predicted fermentabilities without analytical input. Laboratory scale mashes provided the observed data. The predicted data has been obtained using the values: The values obtained for **C** were 40 for α quantity and 6 for β quantity.

Indeed some malts may be more suitable for low alcohol beer production than others by virtue of their temperature sensitivity. The model presented here would suggest that this is due to a different complement of enzymes in the various malts. So, although the total extract from the starch may be the same, the fermentability obtained at high tem-

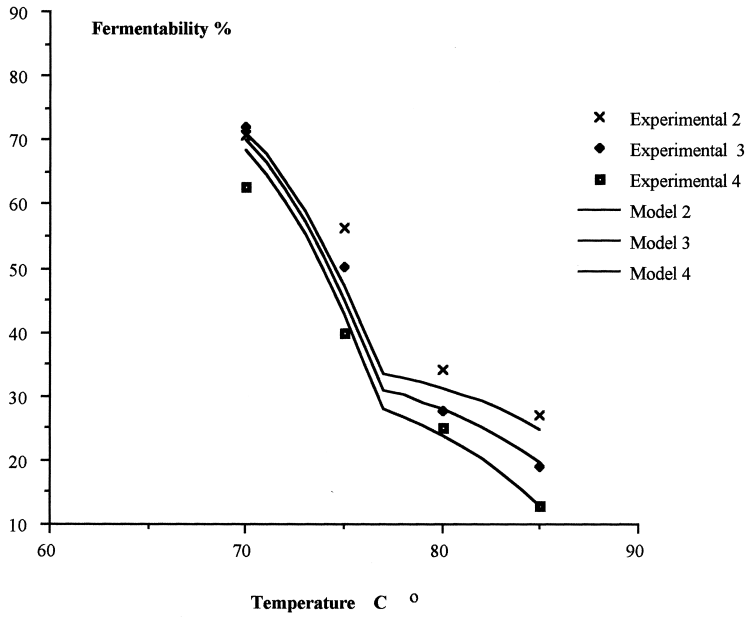

Fig. 3. A comparison between observed and predicted fermentabilities with two input parameters. This figure shows the same experiment as that in Fig. 4 but using a different malt with very different mashing characteristics. The values for α quantity and β quantity were obtained by matrix inversion. The values obtained for **C** in this case were 20 for α and 32 for β.

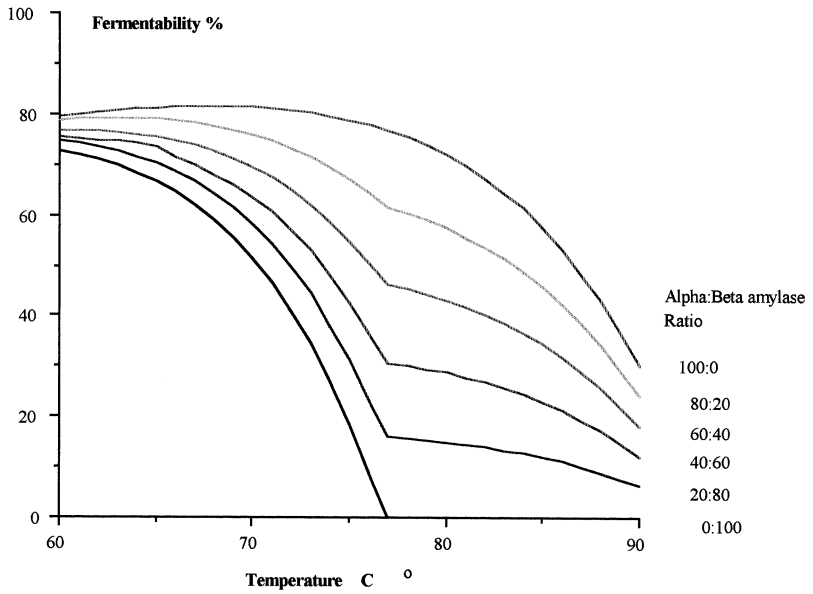

Fig. 4. A comparison of the predicted performance of malts with different amylase ratios. The values for the parameters alpha-amylase quantity and beta-amylase quantity were varied between 0 to 100%. The effect on the predicted fermentability was examined in thick mash (Liquor: Grist Ratio 2:1).

peratures may be different because of the differences in the products of the amylases. There are several reasons why the ratio of alpha- to beta-amylase may vary. The latter is widely recognized as a quality trait in malting barley and there has been a tendency for breeders to select barleys that attain high levels beta-amylase relative to alpha. Furthermore beta-amylase is synthesized in a repressed form on the ear of the plant and is quickly activated during malting. On the other hand, alpha-amylase is formed de novo and at later stages in the malting process. Thus, as commercial pressures on maltsters predisposes them to use shorter malting times, it is likely that some malts contain a lower ratio of alpha- to beta-amylase. It would, in practice, be very difficult to obtain a range of malts with a predetermined range of amylase ratios. This is, however, very easily tested using the model system.

The supposition that the ratio of alpha- to beta-amylase will influence the outcome of a mash was tested by varying the parameters alpha-amylase quantity and beta-amylase quantity. Fig. 4 compares the predicted fermentabilities from mashes with varying levels of alpha- and beta-amylases at a range of temperatures that might be used for high-temperature mashing to produce low alcohol beers. When the performance of the malt was dominated by one of the enzymes the changes in fermentability were dependent only on the stability of that enzyme. In addition, as MacGregor and colleagues [29] also found, one enzyme can compensate for the absence of the other.

When there is a balance of the two enzymes then the changes in fermentability become biphasic. The model suggests that there will be a rapid change in fermentability followed by a slower decrease as the temperature increases. This is especially helpful in a brewing environment where it is necessary to produce a wort fermentability of about 25 to 30% to make a low alcohol beer [3]. A malt with the appropriate balance of enzymes will achieve this over a more flexible range of temperatures then a malt that is predominated by one or the other enzyme.

4. Discussion

This model has been developed to aid in the prediction of wort fermentability during high-temperature mashing for the production of low alcohol beers.

The model uses two variables and two calculated constants to predict the fermentability of a wort. The constants are defined by a calculation of the remaining activity of alpha- and beta- amylase at different mash temperatures and thickness. These are combined with terms that relate to the initial quantity of these enzymes in the malt grist. The model can then be used with the parameter values provided or can be adjusted in the light of known results. In the latter case the best estimates are obtained if two known results are used and at least one of these is related to the temperature sensitivity of the enzymes. In this case the equations predict the fermentability of worts produced at high temperatures with an average error of $\pm 3°$. The model also predicts that some malts will be more suitable for low alcohol beer production than others.

The model is not sufficiently accurate to predict changes with normal mashing conditions because the error is too large to be useful. Furthermore under standard mashing conditions starch structure, which is not incorporated into this model, plays an important part in determining wort fermentability. The fermentabilities of standard worts are

relatively insensitive to changes in amylase activity [19]. It is only at higher temperatures, where the thermal decay of these enzymes becomes significant, that fermentability becomes closely linked to enzyme activity.

Comparison with other models is difficult because they have not addressed the specific problem of high-temperature mashing. Other models have dealt with programmed mashes that use ramped temperatures. These are fundamentally different from the single high-temperature system modeled here so this model is not suited to predicting the outcome of programmed mashes. In addition other models have considered many aspects of mashing including, for example dissolution of enzymes, gelatinization of starch, starch hydrolysis as well as the formation of fermentable sugars [13,19,20]. For this reason these models are much more complicated than that presented here. Marc et al. used 17 equations, Einsiedler et al. used 18 equations whereas Koljonen used 15 although their models accommodated time as a variable and predicted several results. All dealt with programmed mashes.

Previous models have also required several malt characteristics as input parameters. These have included alpha- and beta-amylase activities, starch contents and gelatinization temperatures [13,19,20], none of which are routinely measured by brewers or maltsters. The present model avoids these measurements but with a resulting loss of accuracy. If improved accuracy is required then only previous mashing results are used as inputs. Because most brewers would have previous mashing experience of their malts, this does not constitute a major difficulty.

In summary, although versatile, the complexity of previously published models makes them difficult for brewers to use. With only three equations and a minimum of analytical information, the model presented here is much simpler to use but provides information on only one aspect of brewing. Nevertheless the error obtained is similar to that obtained with previous models.

The model considers only two enzymes and improved accuracy could be obtained by incorporating the role of other enzyme activities into the model. Other enzymes, such as limit dextrinase, may be relevant [30–34]. Furthermore both alpha- and beta-amylases show iso-forms with different thermostabilities [35,36]. The two equations used here must be viewed as a simplification of the more complex situation.

5. Conclusion

The model presented here provides a relatively simple procedure for predicting the fermentability of brewing worts produced by high-temperature mashing. The model requires only temperature and the mash thickness parameters. The calculation requires, however, an estimate of the relative quantities of alpha-amylase and beta-amylase. This estimate can be obtained from the knowledge of the outcome of previous mashes.

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References

[1] Briggs DE, Hough, JS, Stevens R, Young TW. The chemistry and biochemistry of mashing. p. 254. In: Malting and brewing science, 2nd edition. London: Chapman and Hall, 1971.
[2] Briggs, DE. Some use of barley malt. p. 561. In: Barley. London: Chapman and Hall, 1978.
[3] Muller RE. The effects of mashing temperature and mash thickness on wort carbohydrate composition. J Institute Brewing 1991;97:85–92.
[4] Coulter PR, Potter OE. A kinetic study of the susceptibility to enzyme hydrolysis of starch in malt. J Institute Brewing 1973;79:212–8.
[5] Engasser JM, Marc I, Moll M, Duteurtre B. Kinetic modeling of beer fermentation. Proceedings of the European Brewing Convention Congress. Copenhagen, 1981. p. 579–86.
[6] Evans LB, Field RP. Bioprocess simulation: a new tool for process development. Bio/Technology 1988;6:200–3.
[7] Holmberg J, Hamalainen JJ, Reinikainen P, Olkku J. A mathematical model for predicting the effects of steeping program on water uptake during malting. J of the Institute of Brewing 1997;103:177–82.
[8] Johnson CR, Burnham J. Use of bilinear structures for modeling a brewery fermentation process. Measurement and Control 1996;29: 262–5.
[9] Moll M, Flayeux R, Marc A. Study of the kinetics of brewing. Proceedings of the European Brewing Convention Congress. Copenhagen,1981. pp. 241–7.
[10] Warnes MR, Glassey J, Montague GA, Kara B. On data-based modeling techniques for fermentation processes. Process Biochemistry 1996;31:147–55.
[11] Koljonen T, Hamalainen JJ, Sjoholm K, Pietila K. Simulation of the degradation of starch and beta-glucans during mashing. Proceedings of the European Brewing Convention Congress. Oslo, 1993. p. 525–30.
[12] Buhler TM, McKechnie MT, Wakeman RJ. A model describing the lautering process. Monatsschrift fur Brauwissenschaft 1996;49:226–33.
[13] Einsiedler F, Schwill–Miedaner A, Sommer K, Hamalainen J. Reaction kinetic studies on the modeling of starch hydrolysis during mashing. Brauwelt 1997;137:1598–603.
[14] Einsiedler F, Schwill–Miedaner A, Sommer K. Experimental investigations and modeling of complex biochemical and technological processes using the example of mashing. Part l: Proteolysis. Monatsschrift fur Brauwissenschaft 1997;50:164–71.
[15] Einsiedler F, Schwill–Miedaner A., Sommer K, Hamalainen J. Experimental investigations and modeling of complex biochemical and technological processes using the example of mashing. Part 2: Amylolysis. Monatsschrift fur Brauwissenschaft 1997;50: 202–9.
[16] Einsiedler F, Schwill–Miedaner A, Sommer K, Hamalainen, J. Experimental investigations and modeling of complex biochemical and

technological processes using the example of mashing. Part 2: cytolysis. Monatsschrift fur Brauwissenschaft 1998;51:11–21.
[17] Hamalainen JJ, Koljonen T, Stenholm K, Pietila, K. A simulation model for planning of the mashing process. Tech Quarterly Master Brewers Association Am 1997;34:313–9.
[18] Kettunen A, Hamalainen JJ, Stenholm K, Pietila, K. A model for the prediction of beta-glucanase and beta-glucan concentration during mashing. J Food Engineering 1996;29:185–200.
[19] Koljonen T, Hamalainen JJ, Sjoholm K, Pietila K. A model for the prediction of fermentable sugar concentrations during mashing. J Food Engineering 1995;26:329–50.
[20] Marc A, Engasser JM, Moll M, Flayeux R. A kinetic model of starch hydrolysis by alpha and beta amylase during mashing. Biotechnol Bioeng 1983;25:481–96.
[21] Moll M, Baluais G, Dodds J, Leclerc, D. A process engineering and technological approach to mash separation. Tech Quarterly Master Brewers Association Am 1991;28:12–7.
[22] Allen WG, Spradlin JE. The amylases and their properties. Brewers Digest 1974;49:48–64.
[23] Enari TM, Sopanen T. Mobilization of endospermal reserves during the germination of barley. J Institute Brewing 1986;92:25–31.
[24] Manners DJ. Starch degradation during malting and mashing. Brewers Digest 1974;49:56–62.
[25] Preece IA, Shadaksharaswarmy M. Joint action of alpha- and beta-amylases. III Further observations and conclusions. J Institute of Brewing 1949;50:373–83.
[26] Preece IA, Shadaksharaswarmy M. Joint action of alpha- and beta-amylases. II. Influence of beta:alpha ratio and of temperature on reducing group production from starch. J Institute Brewing 1949;50: 298–306.
[27] Gareth Morris J. The kinetics of enzyme catalysed reactions. p. 278. In: A Biologist's Physical Chemistry, 2nd edition. London: Edward Arnold, 1974.
[28] In: Recommended methods of the Institute of Brewing. London: Institute of Brewing.
[29] MacGregor AW, Bazin SL, Macri LJ, Babb, JC. Modeling the contribution of alpha-amylase, beta-amylase and limit dextrinase to starch degradation during mashing. J Cereal Sci 1999;29:161–9.
[30] Stenholm K, Home S. A new approach to limit dextrinase and its role in mashing. Proceedings of the Convention of the Institute of Brewing (Asia Pacific Sect). Perth, 1998. pp. 38–42.
[31] MacGregor AW. Alpha-amylase, limit dextrinase, and alpha-glucosidase enzymes in barley and malt. CRC Critical Rev Biotechnol 1987;5:117–28.
[32] Sissons MJ, Taylor M, Proudlove MO. Barley malt limit dextrinase: its extraction, heat stability and activity during malting and mashing. J Am Soc Brewing Chemists 1995;53:104–10.
[33] Lee WJ, Pyler RE. Barley malt limit dextrinase: varietal, environmental, and malting effects. J Am Soc Brewing Chemists 1984;42: 11–7.
[34] Sjoholm K, Macri LJ, MacGregor AW. Is there a role for limit dextrinase in mashing? Proceedings of the European Brewing Convention Congress 1995 Brussels. pp. 277–84.
[35] Evans E, MacLeod LC, Eglinton JK, et al. Measurement of beta-amylase in malting barley (Hordeum vulgare L.). 1. Development of a quantitative ELISA for beta-amylase. J Cereal Sci 1997;26: 229–39.
[36] MacGregor AW. Alpha-amylase 1 from malted barley—physical properties and action pattern on amylose. Cereal Chemistry 1978;55: 754–65.