A Kinetic Model of Starch Hydrolysis by α- and β-Amylase during Mashing

A. MARC and J. M. ENGASSER, *Laboratoire des Sciences du Génie Chimique, CNRS-ENSIC Institut National Polytechnique de Lorraine, 1 rue Grandville, 54042 Nancy Cedex, France,* and M. MOLL and R. FLAYEUX, *Tepral, Laboratoire de Recherche et Développement BSN, 2, rue Gabriel Bour, 54250 Champigneulles, France*

Summary

Kinetics of malt starch hydrolysis by endogeneous α- and β-amylases has been experimentally investigated in laboratory-, pilot- and industrial-scale reactors. The production rates of glucose, maltose, maltotriose and total extract, and the separate α- and β-amylases deactivation rates are measured at varying mashing temperature and different initial starch concentrations and qualities. Based on the experimental results, a model is proposed that takes into account the initial carbohydrates and enzymes dissolution, the starch gelatinization, the separate hydrolytic action of α- and β-amylases on insoluble and soluble starch and dextrins, and the influence of temperature both on enzyme activities and thermal denaturation rate. The model can predict, at the three scales, the final sugars concentrations in the wort for given initial malt concentrations and enzymatic contents, and for a fixed temperature profile during the mashing process.

INTRODUCTION

The enzymatic hydrolysis of starch is a first step of many industrial conversions of agricultural products into food, beverages, and chemicals. Amylases which catalyse starch hydrolysis are either present in the processed raw materials or are produced separately by fermentation.[1,2]

In the brewing industry, the initial degradation of starch during mashing strongly influences the wort sugar composition and, consequently, the fermented beer quality. During mashing, when ground malt is mixed with warm water, several interrelated chemical and physical changes take place. Essential are the hydrolytic actions of endogeneous enzymes, mainly amylases and proteases, which convert insoluble carbohydrates and proteins into fermentable sugars and amino acids.[3]

Several experimental reports deal with the kinetics of the enzymatic transformation of malt starch during mashing,[4-7] but the quantitative interpretation of the results is quite limited. Some kinetic models have been proposed for the action of α- and β-amylase on insoluble or soluble starch,[8-11] but they do not describe the influence of essential control parameters, such as temperature, nor allow a prediction of the final sugar composition.

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A more complete model of insoluble starch hydrolysis by the simultaneous action of α- and β-amylase is here presented. Based on laboratory-, pilot-, and industrial-scale mashing experiments, the objective of the model is to correlate, for a given process temperature profile, the initial content of carbohydrates and enzymes in the mash to the final fermentable sugars composition in the wort.

MATERIALS AND METHODS

Substrates

On a laboratory- and pilot-plant scale, mashing experiments were performed on different standard varieties of ground malt, with no addition of external enzymes. For the industrial-scale experiments, a mixture of several malt varieties and corn grists were used as sources of starch and hydrolytic enzymes. Substrate granules size is about 250 μm for laboratory-scale plants and 5 mm for pilot and industrial scale plants.

Mashing Reactors

Laboratory- and pilot-plant mashing were carried out in 7- and 1200-L closed, stirred, and thermostated vessels, respectively [Fig. 1(a)]. For industrial-scale experiments, two stirred reactors, a mash copper, and a mash tun, 4×10^4 and 8×10^4 L total volume, respectively, equipped with temperature regulation, were used [Fig. 1(b)].

Operating Conditions

Three sets of experiments were performed on the laboratory scale. The first was at a constant initial malt-to-water ratio of 1/3 (1.575 kg malt mixed with 4.725 kg water) and at constant temperatures of 40, 45, 50, 55, 57.5, 60, 65, and 70°C. In the second set, the initial malt concentration was the same, but temperature rises according to a predetermined profile during the mashing process. The third set was carried out with linearly rising temperature and at four different initial malt-to-water ratios: 1/2, 1/3, 1/4, and 1/5.[12]

For the pilot-plant experiment, 340 kg of malt and 960 kg of water were initially mixed in the vessel, and the temperature rose linearly from 35 to 75°C during the 90-min mashing process.

On an industrial scale, wort was prepared according to the following double-mash procedure. In the mash copper, 1500 kg of malt and 4760 kg of corn grists were mixed with 1.8×10^4 L water, heated to 78°C, maintained 10 min at this temperature to complete starch liquefaction, then heated at 100°C. On the other hand, 9330 kg of ground malt and 3.1×10^4 L water are introduced in the mash tun and the resultant mixture is held at 52°C for 60 min. Then, the liquefied adjunct mash in the mash copper is combined with the main malt-mash in the mash tun. The combined mash is first maintained at 67°C for 20 min, then heated and further maintained at 75°C for 15 min.[13]

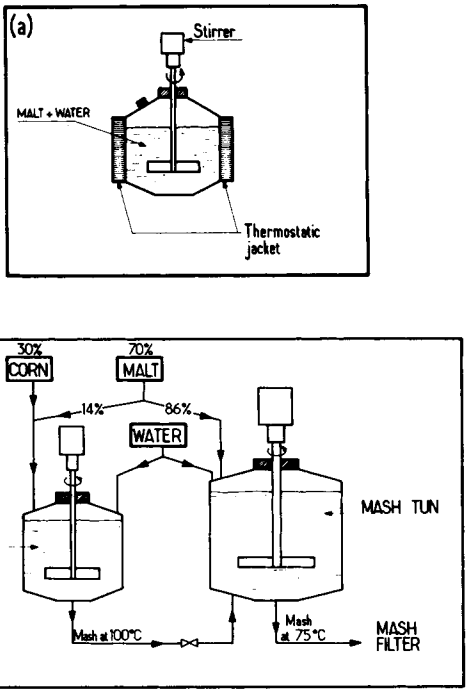


Fig. 1. Schematic representation of the (a) laboratory and pilot plant and the (b) industrial reactors.

Analytical Methods

Samples of the mash were taken at intervals during mashing, immediately cooled to 0°C to prevent further enzymatic conversion, then centrifuged at 0°C before analysis.

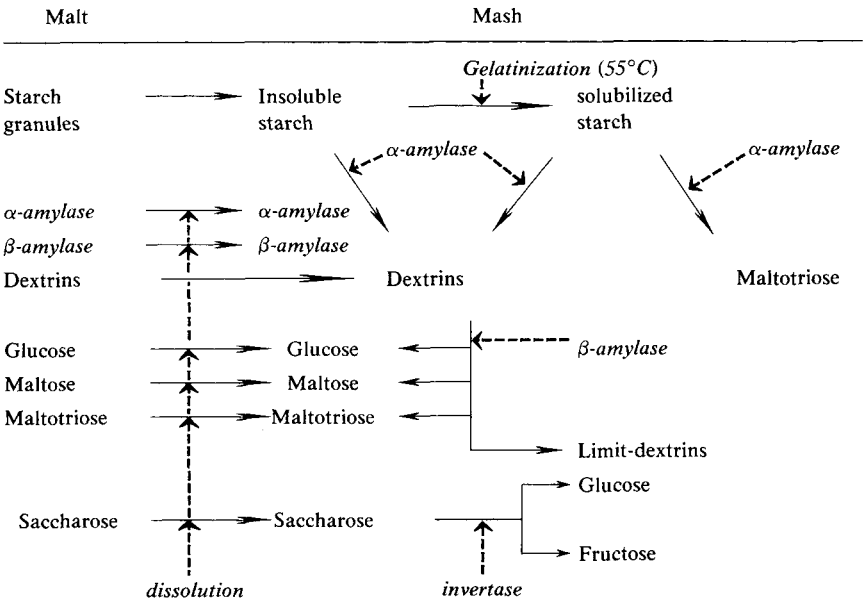
The total extract is determined from the supernatant density, automatically measured at 20°C with an autoanalyzer.[14] It represents the total fermentable sugars and dextrins. Fermentable sugars (glucose, fructose, maltose, saccharose, and maltotriose) are analyzed by HPLC. The α- and β-amylase activities were determined by an immunodiffusion procedure.[15] Starch is analyzed by polarimetry.[16]

THE KINETIC MODEL

Model Description

As indicated in Table I, the model includes the main physical and chemical phenomena determining the rate of starch hydrolysis during mashing. The essential physical factors are the dissolution of hydrolyzed carbohydrates contained in the malt, the initial dissolution of the malt enzymes, the gelatinization of starch around 55°C which strongly enhances its susceptibility to hydro-

TABLE I
Schematic Representation of the Main Phenomena Included in the Model



lytic enzymes, and the thermal denaturation of the enzymes. The model also takes into consideration the specific action of the two amylases. The α-amylase acts both on insoluble and solubilized starch to give mainly dextrins and maltotriose. The β-amylase then hydrolyses the dextrins into glucose, maltose, maltotriose, and limit dextrins. The hydrolysis of saccharose by invertase into glucose and fructose is also included in the model.

Rate Equations

The rate of dissolution of the hydrolyzed carbohydrates (glucose, maltose, maltotriose, fructose, saccharose, and dextrins), which occurs during the first two minutes, is simply taken proportional to the amount of carbohydrate in the malt.

$$r_{\text{diss}}^{\text{gl}} = k_{\text{diss}}\,(I_{\text{gl}}) \tag{1}$$

$$r_{\text{diss}}^{\text{mal}} = k_{\text{diss}}\,(I_{\text{mal}}) \tag{2}$$

$$r_{\text{diss}}^{\text{mlt}} = k_{\text{diss}}\,(I_{\text{mlt}}) \tag{3}$$

$$r_{\text{diss}}^{\text{fr}} = k_{\text{diss}}\,(I_{\text{fr}}) \tag{4}$$

$$r_{\text{diss}}^{\text{sac}} = k_{\text{diss}}\,(I_{\text{sac}}) \tag{5}$$

$$r_{\text{diss}}^{\text{dex}} = k_{\text{diss}}\,(I_{\text{dex}}) \tag{6}$$

where r_{diss}^{gl}, r_{diss}^{mal}, r_{diss}^{mlt}, r_{diss}^{fr}, r_{diss}^{sac}, and r_{diss}^{dex} are the respective rates of dissolution of glucose, maltotriose, malt, fructose, saccharose, and dextrose (in g/min); k_{diss} is the dissolution constant (min^{-1}); (I_{gl}), (I_{mal}), (I_{mlt}), (I_{fr}), (I_{sac}), and (I_{dex}), are the respective amounts of carbohydrate in the malt (g).

The rate of the two amylases dissolution or leaching, which takes place during the first 20 min, is considered limited by the rate of enzyme transfer and thus taken as equal to the product of a transport coefficient by the difference between the enzyme concentration in the malt grain and in the liquid:

$$r_{diss}^{\alpha} = H\,(\text{malt})\,([\alpha]_g - [\alpha]) \tag{7}$$

$$r_{diss}^{\beta} = H\,(\text{malt})\,([\beta]_g - [\beta]) \tag{8}$$

where r_{diss}^{α} and r_{diss}^{β} are the respective rate of dissolution of α- and β-amylase (g/min); H is the global transport coefficient per unit weight of malt (L min^{-1} g^{-1} malt); $[\alpha]_g$ and $[\beta]_g$ are the concentrations of α- and β-amylase in the grain (g/L), respectively; $[\alpha]$ and $[\beta]$ are the concentrations of α- and β-amylase in liquid (g/L); (malt) is the initial amount of malt (g).

Insoluble and soluble starch hydrolysis by α-amylase into dextrins and maltotriose is assumed a first-order rate process with respect to both enzyme and starch concentration, however, with a different rate constant prior and after gelatinization. No distinction is made between the amylose and amylopectin components of starch:

$$r_{dex}^{\alpha} = A_{dex}^{i}\,[\alpha]\,[I_{starch}] \tag{9}$$

$$r_{dex}^{\alpha} = A_{dex}^{s}\,[\alpha]\,[\text{starch}] \tag{10}$$

$$r_{mlt}^{\alpha} = A_{mlt}^{s}\,[\alpha]\,[\text{starch}] \tag{11}$$

where [starch] is the concentration of soluble starch (g/L); $[I_{starch}]$ is the concentration of insoluble starch (g/L); r_{dex}^{α} and r_{mlt}^{α} are the production rates of dextrins and maltotriose by α-amylase action (g L^{-1} min^{-1}); A_{dex}^{i} is the kinetic constant of dextrins production from insoluble starch (L min^{-1} g^{-1} of enzyme); A_{dex}^{s} is the kinetic constant of dextrins production from soluble starch (L min^{-1} g^{-1} of enzyme); A_{mlt}^{s} is the kinetic constant of maltotriose production from soluble starch (L min^{-1} g^{-1} of enzyme).

Simple first-order rate expressions both with respect to β-amylase and dextrins concentrations are found adequate for dextrins hydrolysis by β-amylase into glucose, maltotriose, and limit dextrins. A more complex Michaelian rate expression with respect to dextrins concentration is, however, necessary for the modelling of dextrins conversion into maltose:

$$r_{gl}^{\beta} = B_{gl}\,[\beta]\,[\text{dex}] \tag{12}$$

$$r_{mal}^{\beta} = \frac{B_{mal}\,[\beta]\,[\text{dex}]}{K_m + [\text{dex}]} \tag{13}$$

$$r_{mlt}^{\beta} = B_{mlt}\,[\beta]\,[\text{dex}] \tag{14}$$

$$r_{Ldex}^{\beta} = B_{Ldex}\,[\beta]\,[\text{dex}] \tag{15}$$

where r^{β}_{gl}, r^{β}_{mal}, r^{β}_{mlt}, and r^{β}_{Ldex} are the respective production rates of glucose, maltose, maltotriose, and limit-dextrins (g L^{-1} min^{-1}); [dex] is the concentration of dextrins (g/L^{-1}); B_{gl}, B_{mlt}, and B_{Ldex} are the respective kinetic constants of production of glucose, maltotriose, and limit-dextrins (L min^{-1} g^{-1} of enzyme); B_{mal} and K_m are the kinetic constants for production of maltose (B_{mal} in min^{-1}; K_m in g/L).

Saccharose hydrolysis, a minor process during mashing, is assumed a first-order process, the invertase being however completely denatured at temperature above 55°C:

$$r^{inv}_{gl} = C\,[sac] \text{ at temperatures} \leq 55°C \tag{16}$$

$$r^{inv}_{fr} = C\,[sac] \text{ at temperatures} \leq 55°C \tag{17}$$

$$r^{inv}_{gl} = r^{inv}_{fr} = 0 \text{ at temperatures} > 55°C \tag{18}$$

where r^{inv}_{gl} and r^{inv}_{fr} are the production rates of glucose and fructose by invertase (g L^{-1} min^{-1}); C is the kinetic constant of production of glucose and fructose by invertase (min^{-1}); [sac] is the concentration of saccharose (g/L).

As classically done in enzyme kinetics thermal denaturation of the two amylases are represented by first-order rate processes:

$$r^{\alpha}_{d} = k^{\alpha}_{d}\,[\alpha] \tag{19}$$

$$r^{\beta}_{d} = k^{\beta}_{d}\,[\beta] \tag{20}$$

where r^{α}_{d} and r^{β}_{d} are the rates of denaturation of α- and β-amylase (g L^{-1} min^{-1}); k^{α}_{d} and k^{β}_{d} are the kinetic constants of denaturation of α- and β-amylase (min^{-1}).

The essential influence of temperature both on enzyme hydrolytic activities and enzyme deactivation is described by Arrhenius type relationships as:

$$A_j = A^0_j \exp\,(-E^{\alpha}_j/RT) \tag{21}$$

$$B_j = B^0_j \exp\,(-E^{\beta}_j/RT) \tag{22}$$

$$k^{\alpha}_{d} = k^{\alpha}_{d0} \exp\,(-E^{\alpha}_{d}/RT) \tag{23}$$

$$k^{\beta}_{d} = k^{\beta}_{d0} \exp\,(-E^{\beta}_{d}/RT) \tag{24}$$

where A_j and B_j are the kinetic constants of production of the substance j, respectively, by α- and β-amylase (L min^{-1} g^{-1} enzyme); A^0_j and B^0_j are the preexponential factors corresponding to A_j and B_j (L min^{-1} g^{-1} enzyme); E^{α}_j and E^{β}_j are the activation energies for the production of the substance j, respectively, by α- and β-amylase (cal/mol) k^{α}_{d0} and k^{β}_{d0} are the preexponential factors corresponding to k^{α}_{d} and k^{β}_{d} (min^{-1}); E^{α}_{d} and E^{β}_{d} are the activation energies for the denaturation of α- and β-amylase (cal/mol); R is the gas constant (cal mol^{-1} K^{-1}); T is the temperature (K).

The whole set of mass balance differential equations, representing the time variation of the different species in the mash, is given in the Appendix. The

system was numerically solved with appropriate initial conditions on a MITRA 15 computer by the Runge-Kutta procedure.

Parameter Values

The initial carbohydrates and enzyme concentrations in the mash are experimentally determined and their values given in Table II for the different laboratory- , pilot- , and industrial-scale experiments. They account for variable malt qualities and initial malt concentrations. The rate constants of the model were evaluated from the laboratory scale results[17] and are given in Table III. In a first step, enzyme deactivation kinetics were determined from mashings with linearly rising temperature profiles. Specific assays of α- and β-amylase allows the evaluation of the two denaturation rate constants at different temperature, and, as a result, the calculation of the corresponding activation energies E_d^α and E_d^β. The values of the rate constants and of activation energies for the different enzymatic hydrolysis have then been determined from experimental results at constant malt concentration and constant or variable temperatures. Carbohydrate dissolution constants were taken equal for all substances and evaluated from the initial rise in soluble carbohydrate concentrations. Transfer coefficients accounting for enzyme dissolution were obtained by fitting the experimental results at different initial malt concentrations. The gelatinization temperature, which may vary between 53 and 65°C depending on the raw materials, is, as a first approximation, taken equal to 55°C. The same values of the rate constants and activation energies were then used to model pilot and industrial scale mashing processes.

RESULTS AND DISCUSSION

Laboratory Scale

Experimental and theoretical results are shown in Figure 2 for four constant temperature mashings at 40, 50, 57.5, and 65°C. Except for some deviations at 50°C, the model is seen to satisfactorily describe the initial dissolution of carbohydrates from the malt grains, and the subsequent increase in total extract, maltose, glucose, and maltotriose as a result of enzymatic starch and dextrins hydrolysis.

Figure 3 shows the resulting influence of temperature on the final total extract, maltose, maltotriose, and glucose concentrations after 150 min of mashing. The model at least qualitatively predicts the observed fall in maltose concentration due to fast β-amylase deactivation at temperatures above 60°C.

In Figures 4(a), 4(b), and 4(c) are presented experimental and theoretical results for mashings carried out with three different temperature profiles. They include the time variation of the amount of active α- and β-amylase, measured as the variation of the residual enzyme activity in the mash at 20°C. Whereas the modelling of the change in β-amylase activity is excellent for all three experiments, deviations exist for the deactivation of α-amylase at least in

TABLE II
Initial Carbohydrates and Enzyme Concentrations in the Mash

Mashing Experiment		Liquid phase total volume (L)	Glucose (g/L)	Maltose (g/L)	Maltotriose (g/L)	Saccharose (g/L)	Fructose (g/L)	Dextrins (g/L)	Starch (g/L)	α-Amylase (g/L)	β-Amylase (g/L)
Laboratory scale with constant temperature		4.8	6	12	1	7.5	2	26.5	170	12.6×10^{-3}	12.6×10^{-3}
Laboratory scale with variable temperature		4.8	7	14	1	11	1	28	163	12×10^{-3}	12×10^{-3}
Laboratory scale with variable malt concentration	1/2	4.9	11	14.5	1.4	8.6	2.8	40.4	244	19.2×10^{-3}	20×10^{-3}
	1/3	4.8	7	10	1	6	1.6	28	176	12×10^{-3}	12×10^{-3}
	1/4	5.1	7	8	0.5	4.5	1	17	140	9.7×10^{-3}	8.5×10^{-3}
	1/5	5.1	2	5.5	$\simeq 0$.	3.3	1.3	12	130	8.2×10^{-3}	9.6×10^{-3}
Pilot scale		980	7	10	1	6	3.5	41	161	12×10^{-3}	12×10^{-3}
Industrial scale		3.4×10^4	6	12	1	7.5	2	26.5	170	12.6×10^{-3}	12.6×10^{-3}

TABLE III
Values of the Model Parameters

Enzyme	Model Parameters: α-Amylase before gelatinization	α-Amylase after gelatinization	β-Amylase	Invertase
Hydrolysis				
Kinetic constants (L min^{-1} g^{-1})	$A^{i,0}_{dex} = 9.1 \times 10^{33}$	$A^{s,0}_{dex} = 2 \times 10^{14}$ $A^{s,0}_{mlt} = 3 \times 10^{13}$	$B^0_{gl} = 9 \times 10^{12}$ $B^0_{mal} = 12 \times 10^{25}$ min^{-1} $K_m = 40$ g/L $B^0_{mlt} = 5 \times 10^{12}$ $B^0_{Ldex} = 2 \times 10^{13}$	$C = 0.67 \times 10^{-2}$ min^{-1}
Activation energy (cal/mol)	$E^{\alpha}_{dex} = 5 \times 10^5$	$E^{\alpha}_{dex} = 2 \times 10^4$ $E^{\alpha}_{mlt} = 2 \times 10^4$	$E^{\beta}_{gl} = 2 \times 10^4$ $E^{\beta}_{mal} = 3.5 \times 10^4$ $E^{\beta}_{mlt} = 2 \times 10^4$ $E^{\beta}_{Ldex} = 2 \times 10^4$	
Dénaturation	$k^{\alpha}_{d0} = 2.16 \times 10^{11}$		$k^{\beta}_{d0} = 2.35 \times 10^{28}$	
Préexponential constants (min^{-1})				
Activation energy (cal/mol)	$E^{\alpha}_d = 2 \times 10^4$		$E^{\beta}_d = 4.5 \times 10^4$	
Dissolution rate constant	$k_{diss} =$ L min^{-1}			
Transfer rate constant	$H = 5.1 \times 10^{-3}$ L min^{-1} kg $malt^{-1}$			

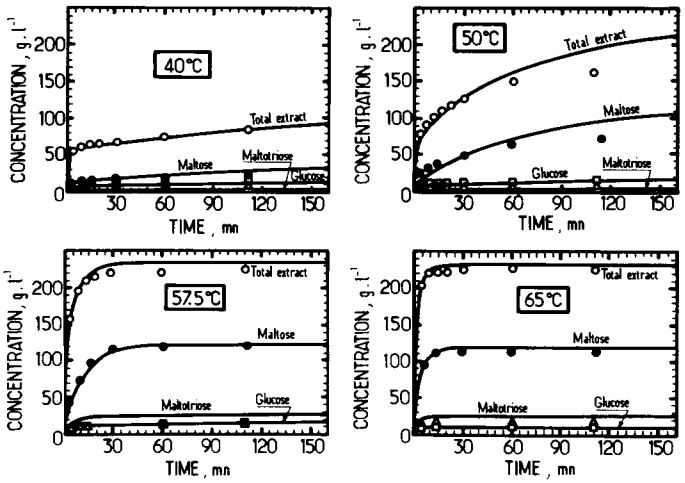


Fig. 2. Experimental and theoretical results for four constant-temperature mashings (40, 50, 57.5, and 65°C) in laboratory-scale reactors.

two of the results. Except for the rise of maltose prior to gelatinization, the model is seen to precisely describe the increase in glucose, maltose, maltotriose, and total extract during the three mashings.

Figure 5 summarizes the results obtained with linearly rising temperatures at varying initial malt to water ratios. As shown using first-order rate expressions for enzyme and carbohydrate dissolutions, glucose, maltotriose, and dextrins productions and Michaelian expression for maltose formation yields

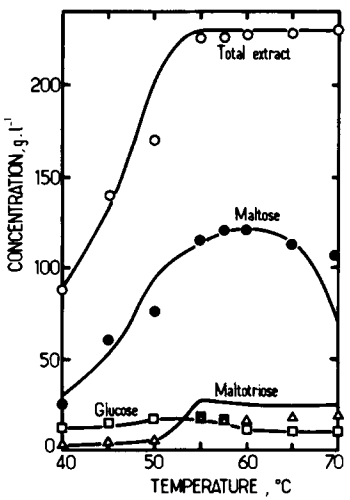


Fig. 3. Experimental and theoretical influence of temperature on the final concentrations after 150 min of constant-temperature mashings.

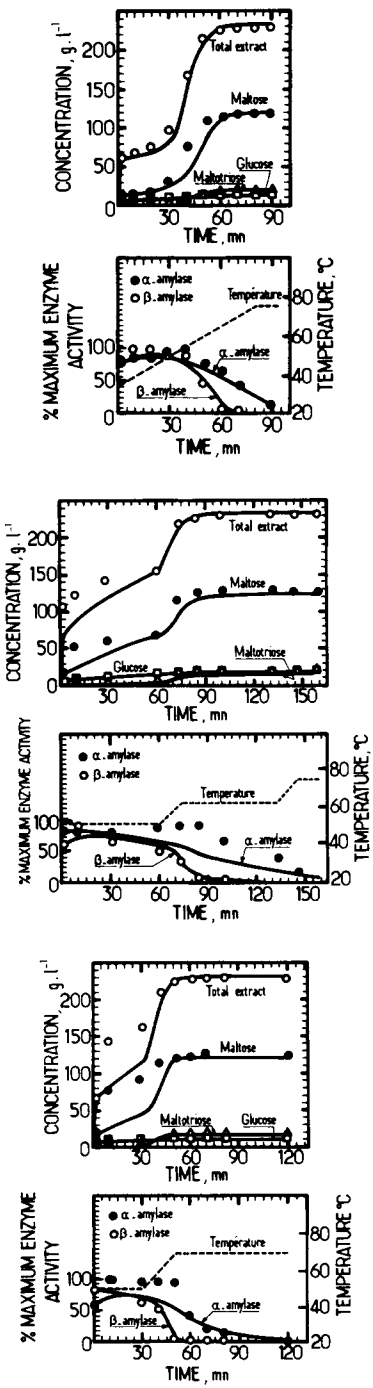


Fig. 4. Experimental and theoretical results for mashing carried out with three different temperature profiles.

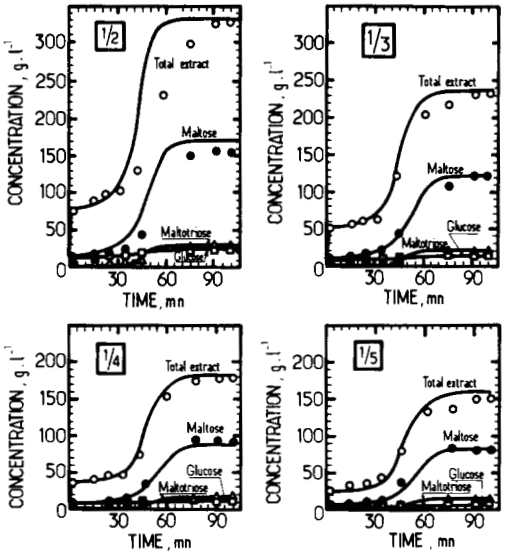


Fig. 5. Experimental and theoretical results with linearly rising temperature, at varying initial malt-to-water ratios.

a very satisfactory modelling of the hydrolysis process at different initial starch concentrations.

Pilot-Plant Scale

For a 1200-L pilot plant mashing with linearly rising temperature, the measured increase with time of glucose, maltose, and maltotriose concentrations are represented in Figure 6. The theoretical curves have been obtained with the same rate constants as for the laboratory experiments but with different initial concentrations corresponding to a different malt quality. The good fit between experiments and theory demonstrate the extrapolation capabilities of the proposed model.

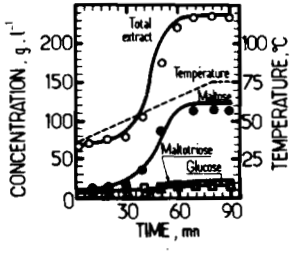


Fig. 6. Experimental and theoretical results for pilot-scale mashing with linearly rising temperature.

Industrial Scale

Figure 7 shows the results for a mashing process carried out under industrial conditions in a 60,000-L reactor. The temperature profile, the carbohydrate and enzyme concentrations are those measured in the mash tun. The strong drop in amylase concentration after 35 min corresponds to the mixing of the contents of the mash tun and the mash copper, which results in an enzyme dilution. Except for some deviation for the initial maltose dissolution, which may be caused by differences in malt particle sizes, and by the progressive addition of malt, the model previously established for laboratory experiments is seen to adequately describe the time variation of carbohydrate and enzyme level for the industrial mashing.

CONCLUSIONS

Based on an experimental investigation of malt mashing, a kinetic model of starch hydrolysis by endogeneous α- and β-amylases is proposed. It quantifies the main physical and biochemical factors controlling the time course of the hydrolytic process, namely the carbohydrates and enzymes dissolution, the starch gelatinization, the catalytic action of α- and β-amylases, the temperature influence on enzyme activities and thermal denaturation. It only entails simple first-order or Michaelian type rate expressions.

As demonstrated by the good agreement between the theoretical predictions and a large number of experimental data, the model can take into account varying temperature profiles during mashing as well as different initial malt concentration and qualities. In addition, when using the same enzymatic and physical rate constants values, the model shows excellent extrapolation capabilities from 7 to 60,000 L, especially for the prediction of the final wort sugar composition.

In order to be used for the optimization and automation of the mashing

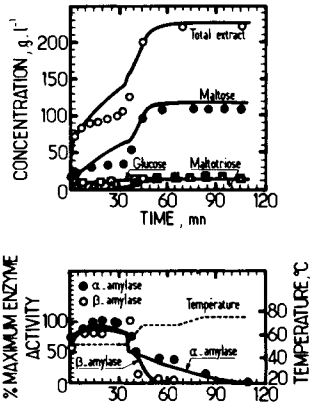


Fig. 7. Experimental and theoretical results for industrial-scale mashing.

process, this model is now being completed to include other essential enzymatic and chemical reactions, such as those involved in protein hydrolysis. Moreover, the applicability of the proposed model to the now widely used process of starch liquefaction by amylases of microbial origin is also being investigated.

APPENDIX: MASS BALANCE DIFFERENTIAL EQUATIONS

Initially Undissolved Substances

The time variation of the different species in the initial malt takes only account their dissolution in the liquid phase:

glucose: $$d(I_{gl})/dt = -k_{diss}(I_{gl}) \tag{25}$$

maltose: $$d(I_{mal})/dt = -k_{diss}(I_{mal}) \tag{26}$$

maltotriose: $$d(I_{mlt})/dt = -k_{diss}(I_{mlt}) \tag{27}$$

dextrins: $$d(I_{dex})/dt = -k_{diss}(I_{dex}) \tag{28}$$

saccharose: $$d(I_{sac})/dt = -k_{diss}(I_{sac}) \tag{29}$$

fructose: $$d(I_{fr})/dt = -k_{diss}(I_{fr}) \tag{30}$$

α-amylase: $$d([\alpha]_g)/dt = -\frac{H}{V_h}(\text{malt})([\alpha]_g - [\alpha]) \tag{31}$$

β-amylase: $$d([\beta]_g)/dt = -\frac{H}{V_h}(\text{malt})([\beta]_g - [\beta]) \tag{32}$$

Dissolved Substances

The time variation of the different species in solution depends on both the initial solution in the liquid and the hydrolytic activities of the enzymes:

glucose: $$\frac{d([\text{gl}])}{dt} = \frac{k_{diss}}{V}(I_{gl}) + \frac{C}{1.9}[\text{sac}] + B_{gl}[\beta][\text{dex}] \tag{33}$$

maltose: $$\frac{d([\text{mal}])}{dt} = \frac{k_{diss}}{V}(I_{mal}) + \frac{B_{mal}[\beta][\text{dex}]}{K_m + [\text{dex}]} \tag{34}$$

maltotriose: $$\frac{d([\text{mlt}])}{dt} = \frac{k_{diss}}{V}(I_{mlt}) + A^{s}_{mlt}[\alpha][\text{starch}] + B_{mlt}[\beta][\text{dex}] \tag{35}$$

limit-dextrins: $$\frac{d([\text{Ldex}])}{dt} = B_{Ldex}[\beta][\text{dex}] \tag{36}$$

starch: $$\frac{d([\text{starch}])}{dt} = -[\alpha][\text{starch}](0.964\,A_{mlt} + A^{i(\text{or } s)}_{dex}) \tag{37}$$

dextrins: $$\frac{d([\text{dex}])}{dt} = \frac{k_{diss}}{V}(I_{dex}) + A^{i(\text{or } s)}_{dex}[\alpha][\text{starch}] - [\beta][\text{dex}]\cdot(0.9\,B_{gl} + 0.947\,B_{mal} + 0.964\,B_{mlt} + B_{Ldex}) \tag{38}$$

saccharose: $$\frac{d([\text{sac}])}{dt} = \frac{k_{diss}}{V}(I_{sac}) - C(\text{sac}) \tag{39}$$

fructose: $$\frac{d([\mathrm{fr}])}{dt} = \frac{k_{\mathrm{diss}}}{V}(I_{\mathrm{fr}}) + \frac{\mathrm{C}}{1.9}(\mathrm{sac}) \tag{40}$$

α-amylase: $$\frac{d([\alpha])}{dt} = \frac{H}{V}(\mathrm{malt})([\alpha]_g - [\alpha]) - k_d^{\alpha}[\alpha] \tag{41}$$

β-amylase: $$\frac{d([\beta])}{dt} = \frac{H}{V}(\mathrm{malt})([\beta]_g - [\beta]) - k_d^{\beta}[\beta] \tag{42}$$

In the above equations, Arrhenius relationships of eqs. (21)-(24) are used to calculate the rate constants A^{i}_{dex}, A^{s}_{dex}, A^{s}_{mlt}, B_{gl}, B_{mlt}, B_{Ldex}, k_d^{α}, and k_d^{β} at varying temperatures.

Nomenclature

A^{i}_{dex}, A^{s}_{dex} the kinetic constants of dextrins production from insoluble and soluble starch respectively, by the α-amylase (L min^{-1} g^{-1} enzyme)

A^{s}_{mlt} the kinetic constant of maltotriose production from soluble starch, by the α-amylase (L min^{-1} g^{-1} enzyme)

A_j the general kinetic constant of production of the substance j, by α-amylase (L min^{-1} g^{-1} enzyme)

A_j^0 the preexponential factor corresponding to A_j (L min^{-1} g^{-1} enzyme)

B_{gl}, B_{mal}, B_{mlt}, B_{Ldex} the kinetic constants of glucose, maltose, maltotriose, and limit dextrins production, respectively, by the β-amylase (L min^{-1} g^{-1} enzyme), (B_{mal} in min^{-1})

B_j the general constant of production of the substance j, by β-amylase (L min^{-1} g^{-1} enzyme)

B_j^0 the preexponential factor corresponding to B_j (L min^{-1} g^{-1} enzyme)

C the kinetic constant of production of glucose and fructose by invertase (min^{-1})

[dex] the concentration of dextrins (g/L)

E_d^{α}, E_d^{β} the activation energies for the denaturation of α- and β-amylase (cal/mol)

E_j^{α} the activation energy for the production of the substance j, by α-amylase (cal/mol)

E_j^{β} the activation energy for the production of the substance j, by β-amylase (cal/mol)

[fr] the concentration of fructose (g/L)

[gl] the concentration of glucose (g/L)

H the global transport coefficient of α- and β-amylase (L min^{-1} g^{-1} malt)

$[I_{\mathrm{starch}}]$ the concentration of insoluble starch (g/L)

(I_{gl}), (I_{mal}), (I_{mlt}), (I_{fr}), (I_{sac}), (I_{dex}) the respective amount of glucose, maltose, maltotriose, fructose, saccharose, and dextrins in the malt (g)

k_{diss} the dissolution constant of malt carbohydrates (min^{-1})

k_d^{α}, k_d^{β} the kinetic constants of denaturation of α- and β-amylase (min^{-1})

k_{d0}^{α}, k_{d0}^{β} the preexponential factors corresponding to k_d^{α} and k_d^{β} (min^{-1})

K_m the michaelis constant for production of maltose by β-amylase (g/L)

[Ldex] the concentration of limit-dextrins (g/L)

[mal] the concentration of maltose (g/L)

[mlt] the concentration of maltotriose (g/L)

(malt) the initial amount of malt (g)

r_d^α, r_d^β the rate of denaturation of α- and β-amylase (g L^{-1} min^{-1})

r_{diss}^{gl}, r_{diss}^{mal}, r_{diss}^{mlt}, r_{diss}^{fr}, r_{diss}^{sac}, r_{diss}^{dex} the rate of dissolution of glucose, maltose, maltotriose, fructose, saccharose, and dextrins, respectively (g/min)

r_{diss}^α, r_{diss}^β the rate of dissolution of α- and β-amylase (g/min)

r_{dex}^α, r_{mlt}^α the rate of production of dextrins and maltotriose, respectively, by the α-amylase (g L^{-1} min^{-1})

r_{gl}^β, r_{mal}^β, r_{mlt}^β, r_{Ldex}^β the rate of production of glucose, maltose, maltotriose, and limit-dextrins, respectively, by the β-amylase (g L^{-1} min^{-1})

r_{gl}^{inv}, r_{fr}^{inv} the rate of production of glucose and fructose respectively, by the invertase (g L^{-1} min^{-1})

R the gas constant (cal mol^{-1} K^{-1})

[sac] the concentration of saccharose (g/l)

[starch] the concentration of soluble starch (g/L)

t the time (min)

T the temperature (K)

V the total volume of liquid phase (L)

V_h the volume of humidity phase of the malt (L)

$[\alpha]$ the concentration of α-amylase in total liquid phase (g/L)

$[\alpha]_g$ the concentration of α-amylase in grain liquid phase (g/L)

$[\beta]$ the concentration of β-amylase in total liquid phase (g/L)

$[\beta]_g$ the concentration of β-amylase in grain liquid phase (g/L)

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