EXPERIMENTAL AND ANALYTICAL STUDY OF THE EFFECT OF TEMPERATURE ON THE SACCHARIFICATION OF BARLEY MALT

A. A. Brandão, adolfobrando@id.uff.br[1]
F. O. Quintanilha, felipe.o.quintanilha@gmail.com[1 2]

[1]Laboratory of Thermal Sciences (LATERMO), Department of Mechanical Engineering – PGMEC/TEM, Universidade Federal Fluminense
[2]Centro Federal de Educação Tecnológica Celso Suckow da Fonseca – CEFET-RJ

Abstract: *This paper presents an experimental study regarding the kinetics of saccharification during the mashing of barley for the production of wort. A simple model for describing the species conservations was adapted from a previous study. The model involves mass balances for starch, dextrins, maltotriose, maltose and glucose, as well as balances for the activity of the enzymes that are responsible for the chemical reactions encompassed in the starch breakdown. These enzymes (α- and β-amylases) work at different rates at different temperatures, and hence the entire control of the hydrolysis reactions that occur within the mashing process is done via temperature. The model is implemented using the Mathematica system, and a parametric analysis is carried-out for demonstrating how different carbohydrate profiles can be obtained by performing the mash at different target temperatures and for different periods.*
Keywords: *Starch hydrolysis, Enzyme activity, Mashing process, Mash efficiency.*

NOMENCLATURE

1. INTRODUCTION

The production of wort is a process known to mankind since the dawn of civilization, as brewing has accompanied the development of modern society for millennia. Mashing is the first step in wort production from malted grains, which encompasses three principal enzymatic reactions: hydrolysis of starch into fermentable parts (mainly maltose, maltotriose and glucose), hydrolysis of proteins into free amino acids, and the degradation of β-glucan chains. The hydrolysis of starch is the most important reaction because it determines the quality of fermentable carbohydrates in the wort.

Predicting the wort composition is extremely important in brewing processes; however, it is under-explored when compared to the others parts of the brewing process. The wort sugar profile determines the characteristic of the final product; as a result, an accurate mathematical model and simulation routine is of extreme importance for brewing industries. Kinetic models have been proposed in the literature for the hydrolysis of starch (Marc and Engasser, 1983; Koljonen *et al.*, 1995; Brandam *et al.*, 2003). Koljonen *et al.* (1995) developed a model describing the hydrolysis of starch catalysed by α- and β-amylase in mashing. The model was employed for selecting an ideal temperature profile for mashing. Laboratory scale mashes with different temperature profiles were performed for estimating model parameters for a two-row barley variety. An estimate for the proportion of gelatinized starch at different temperatures was also obtained. The results showed that the model predicted the concentration of active α- and β-amylase to a sufficient accuracy in all experiments. A mathematical model for the representation of amylase activities with temperature dependency was presented in (Brandam *et al.*, 2003). In this model, parameters were estimated by fitting nine experiments of one malt variety.

Besides looking into starch degradation into simpler carbohydrates, some models have investigated the evolution of other substances during mashing. Durand *et al.* (2009) demonstrated the applicability of dynamic optimization to improve the time-temperature schedule of a mashing process. They utilized the same model proposed by Koljonen *et al.* (1995), but also considered the concentration in wort of β-glucans and arabinoxylans. The authors sugested that a lower temperature average profile of about 51°C should be preferred over typical industrial mashing profiles. Enari and Markkanen (1975) found that the addition of heat stable β-glucanase can decrease the wort β-glucan concentration. Kettunen *et al.* (1996) presented a mathematical model to describe the effect of mashing temperature on the dissolution and hydrolysis of β-glucans, showing that the lowest β-glucan concentration was achieved at a mashing temperature of 48°C. Li *et al.* (2004) developed a model for the degradation of arabinoxylans by endo-xyllanase, and mashing experiments at different temperature profiles were made for identifying model parameters. Li *et al.* (2005) analyzed the effect of different mashing parameters (mash thickness, grist coarseness and stirring) on the solubilization and hydrolysis of arabinoxylans.

In this paper an adaptation of the model proposed by (Koljonen *et al.*, 1995) was used for investigating the kinetics of starch degradation in mashing of barley malt. Preliminary results were carried out for two barley varieties in different single-temperature infusion mashes. The effects of mashing temperature and time were investigated for a variety of test-cases which were plotted in the form of contour plots of mashing efficiency, extract yield and fraction of fermentable sugars. The results show optimal mashing temperature ranges.

2. MATHEMATICAL MODEL

The mathematical model considered in this study is based on the equations developed by (Koljonen *et al.*, 1995). It consist of a species balance for the starch degrading enzymes (α and β amylases), as well as species balances for starch, dextrins and smaller carbohydrates.

All carbohydrate and water concentrations are expressed in mass per total volume of mash.

The simplifying assumptions are stated as:

- The total volume $\mathcal{V}_g + \mathcal{V}$ remains constant throughout the mash.
- The temperature variation is used imposed, such that no energy balance is required.
- Starch is converted into dextrin and maltotriose only by the effect of α-amylase.
- The enzymes in solution are denatured at a rate that depends on temperature and enzyme concentration.

The balance for the enzyme concentration in the mash is assumed to occur in two steps. First, the enzymes contained in the grains (α_g and β_g) are dissolved into the liquid phase (α and β). Once in solution these enzymes are denatured at a rate that depends on temperature. This process can be described by the following equations:

$$\mathcal{V}_g \frac{\mathrm{d}\alpha_g}{\mathrm{d}t} = -H_\alpha M (\alpha_g - \alpha), \tag{1}$$

$$\mathcal{V}_g \frac{\mathrm{d}\beta_g}{\mathrm{d}t} = -H_\beta M (\beta_g - \beta), \tag{2}$$

$$\mathcal{V} \frac{\mathrm{d}\alpha}{\mathrm{d}t} = H_\alpha M (\alpha_g - \alpha) - k_\alpha \alpha, \tag{3}$$

$$\mathcal{V} \frac{\mathrm{d}\beta}{\mathrm{d}t} = -H_\beta M (\beta_g - \beta) - k_\beta \beta, \tag{4}$$

where α_g, α, β_g and β are enzyme concentrations, which is given in terms of activity (U/s).

The temperature-dependent coefficients are given by:

$$k_\alpha = k_{\alpha,0} \exp\left(-E_{\alpha,d}/(RT)\right) \tag{5}$$

$$k_\beta = k_{\beta,0} \exp\left(-E_{\beta,d}/(RT)\right) \tag{6}$$

where $k_{\alpha,0}$ and $k_{\alpha,0}$ is the maximum specific rates of enzyme destruction, for α- and β-amylase.

The starch breakdown into dextrins and maltotriose are given by the hydrolysis reactions:

$$(\mathrm{C_6H_{10}O_5})_n + \frac{n}{3}\,\mathrm{H_2O} \longrightarrow \frac{n}{3}\,\mathrm{C_{18}H_{32}O_{16}} \tag{7}$$

$$(\mathrm{C_6H_{10}O_5})_n \longrightarrow x\,(\mathrm{C_6H_{10}O_5})_{n/x} \tag{8}$$

where the first reaction implies that for each mole of maltotriose produced one mole of water is adsorbed, which in terms of mass fractions implies that one gram of water is absorbed per each 28 grams of maltotriose produced. The second reaction represents the breakdown of starch (a polysaccharide of glucose monomers) into smaller glucose polymers called dextrins.

For the breakdown of dextrins into glucose and maltose, the following hydrolysis reactions are written:

$$(\mathrm{C_6H_{10}O_5})_{n/x} + \frac{n}{x}\,\mathrm{H_2O} \longrightarrow \frac{n}{x}\,\mathrm{C_6H_{12}O_6} \tag{9}$$

$$(\mathrm{C_6H_{10}O_5})_{n/x} + \frac{n}{2\,x}\,\mathrm{H_2O} \longrightarrow \frac{n}{2\,x}\,\mathrm{C_{12}H_{22}O_{11}} \tag{10}$$

implying that one gram of water is absorbed per each 10 grams of glucose produced, and 19 grams of maltose produced.

The species balances are given in terms of groups of carbohydrates. Starch is treated as a single species, whose conservation balance is given by:

$$\frac{\mathrm{d}x_1}{\mathrm{d}t} = -\alpha\,(\gamma_5 A_5 + A_2)\,(x_1 - x_1^0\,u) \tag{11}$$

which represents the starch breakdown into maltotriose (x_5) and dextrins (x_2) by the action of α-amylase. The coefficient γ_3 corresponds the the mass fraction of actual starch consumed ($\gamma_3 = 27/28$) per mass of maltotriose produced.

The mass balance for dextrins is written as:

$$\frac{\mathrm{d}x_2}{\mathrm{d}t} = \alpha\,A_2\,(x_1 - x_1^0\,u) - \beta\,x_2 \left(\gamma_3\,B_3 + \gamma_4\,\frac{B_4}{k_m + x_2} + B_6\right) \tag{12}$$

This reaction has a production term corresponding to the amount of starch that is degraded into dextrins, and a consumption term due to the conversion of dextrins into glucose (x_3), maltose (x_4) and limit-dextrins (x_6) by the β-amylase

enzyme. The coefficients $\gamma_3 = 9/10$ and $\gamma_4 = 18/19$ correspond to the mass fractions of dextrins consumed as glucose and maltose are produced.

Equations (11) and (12) also contemplate the fact that starch needs to be gelatinized prior to its breakdown. This is included in the equations by introducing the quantity $u = u(T)$, which is the mass fraction of ungelatinized starch. This function is defined as:

$$u(T) \;=\; \begin{cases} 1, & T \le T_u \\ (T_g - T)^2(3\,T_u - T_g - 2T)/(T_u - T_g)^3, & T_u < T < T_g \\ 0, & T \ge T_g \end{cases} \tag{13}$$

such that no starch is gelatinized below T_u and hence its breakdown only begins for temperatures above this threshold. The values for these temperatures are $T_u = 315.4$ and $T_g = 336.5$.

The species balance for glucose, maltose, maltotriose and limit-dextrins are given by the following equations:

$$\frac{\mathrm{d}x_3}{\mathrm{d}t} \;=\; B_3\,\beta\,x_2, \tag{14}$$

$$\frac{\mathrm{d}x_4}{\mathrm{d}t} \;=\; B_4\,\beta\,\frac{x_2}{k_m + x_2}, \tag{15}$$

$$\frac{\mathrm{d}x_5}{\mathrm{d}t} \;=\; A_5\,\alpha\,(x_1 - x_1^0\,u), \tag{16}$$

$$\frac{\mathrm{d}x_6}{\mathrm{d}t} \;=\; B_6\,\beta\,x_2, \tag{17}$$

which corresponds to the production of these components as a function of the consumption of starch and dextrins.

The A_i and B_i coefficients are given as Arrhenius-type reaction coefficients:

$$A_2 \;=\; A_2^0\,\exp\left(-\frac{E_\alpha}{RT}\right), \qquad A_5 \;=\; A_5^0\,\exp\left(-\frac{E_\alpha}{RT}\right), \tag{18}$$

$$B_3 \;=\; B_3^0\,\exp\left(-\frac{E_\beta}{RT}\right), \qquad B_4 \;=\; B_4^0\,\exp\left(-\frac{E_\beta}{RT}\right), \qquad B_6 \;=\; B_6^0\,\exp\left(-\frac{E_\beta}{RT}\right), \tag{19}$$

In addition to the previous equation, a mass balance for water could be included. However the water concentration in the mass can be directly calculated from the other components as:

$$x_0 \;=\; \frac{\mathcal{V}\,\rho_0}{\mathcal{V} + \mathcal{V}_g} \;-\; (x_t - x_t(0)), \qquad \text{where} \qquad x_t \;=\; \sum_{i=1}^{6} x_i. \tag{20}$$

where ρ_0 is the density of water at the beginning of mashing.

In the brewing industry it is common to express the amount of soluble sugars as a mass concentration in the liquid phase. Considering that starch is insoluble, this is calculated from the previous concentrations as:

$$e \;=\; \frac{x_e}{x_0 + x_e}, \qquad \text{where} \qquad x_e \;=\; \sum_{i=2}^{6} x_i. \tag{21}$$

The amount of fermentable sugars are the sum of the glucose, maltose, and maltotriose:

$$x_f \;=\; \sum_{i=3}^{5} x_i, \tag{22}$$

such that the percentage of fermentable sugars is calculated by:

$$\phi_f \;=\; \frac{x_f}{x_t}, \tag{23}$$

The brewhouse efficiency η is generally defined as the actual amount of extracted sugars related to its maximum possible value. It can be expressed as a product of individual efficiencies:

$$\eta \;=\; \eta_m\,\eta_l\,\eta_o, \tag{24}$$

where η_m is the mashing efficiency, η_l is the lautering efficiency and η_o is the efficiency of other processes (e.g. boiling and wort transfer to fermenter). The mashing efficiency is defined as:

$$\eta_m \;=\; \frac{x_e}{x_t}, \tag{25}$$

which will be equal to 100% when all starch is converted to dextrins and simpler sugars.

The non-linear system of ordinary differential equations was solved using Mathematica's NDSolve routine with a user prescribed relative tolerance of 10^{-6}.

3. RESULTS AND DISCUSSION

The model parameters experimentally estimated by Koljonen *et al.* (1995) are the enzyme dissolution coefficients (H_α, H_β), the activation energies ($E_\alpha, E_\beta, E_{\alpha,d}, E_{\beta,d}$), the Michealis constant k_m, the gelatinization temperatures (T_u, Tg) and the coefficients that control the maximum rate of hydrolysis ($k_{\alpha,0}, k_{\beta,0}, k_{\alpha,0}, A_2^0, A_5^0, B_3^0, B_4^0, B_6^0$). Table 1 present the parameter values.

Tabela 1: Model parameters for starch hydrolysis

α-Amylase	β-Amylase
	$B_3^0 = 1.62 \times 10^{40}$ l/min/g
	$B_4^0 = 1.05 \times 10^{42}$ l/min^2/g
$A_2^0 = 3.77 \times 10^{10}$ l/min/g	$B_6^0 = 1.09 \times 10^{41}$ l/min/g
$A_5^0 = 6.42 \times 10^{9}$ l/min/g	k_m = 2.8 (g/l)
$E_\alpha = 1.03 \times 10^{5}$ J/mol	$E_\beta = 2.93 \times 10^{5}$ J/mol
$k_{\alpha,0} = 3.86 \times 10^{34}$ min^{-1}	$k_{\beta,0} = 9.46 \times 10^{67}$ min^{-1}
$E_{\alpha,d} = 2.377 \times 10^{5}$ J/mol	$E_{\beta,d} = 4.539 \times 10^{5}$ J/mol
$H_\alpha = 9.72 \times 10^{-5}$ l/g/min	$H_\beta = 7.57 \times 10^{-5}$ l/g/min

The system of differential equations was solved using the initial conditions presented in table 2, which considere the mashing of two different types of barley malts: *Kymppi* and *Kustaa*.

Tabela 2: Initial conditions for two different mashes

Mash	water (m^3)	Malt (kg)	Starch (g/l)	Dextrins (g/l)	Glucose (g/l)	Maltose (g/l)	Maltotriose (g/l)	α-Amylase (U/l)	β-Amylase (U/l)
	$\mathcal{V}$	M	$x_1(0)$	$x_2(0)$	$x_3(0)$	$x_4(0)$	$x_5(0)$	$\alpha_g(0)$	$\beta_g(0)$
Kymppi	2.0×10^{-4}	0.05	112.1	20.6	5.1	10.3	0.0	3.97×10^{5}	1.21×10^{6}
Kustaa	2.0×10^{-4}	0.05	105.0	24.6	4.8	8.8	0.0	3.34×10^{5}	9.12×10^{5}

The first set of results involve simulations that were all carried out with the *Kymppi* malt. They are intended to demonstrate the features in kinetics of mashing, as calculated using the model selected for this study.

Figure 1 displays the enzyme concentration (in terms of activity) with time for mash temperatures of 60°C and 80°C. As one can observe, the enzyme dissolution from the grain into the mash is slightly dependent on temperature, being more intense for warmer mashes. The rate of enzyme destruction, on the other hand, is much more affected by temperature. In order to take a further look into the evolution of the α- and β-amylases after dissolution, their activities are plotted for different mash temperatures in figure 1. As can be seen, the temperature has a clear degrading effect on the enzyme acti-

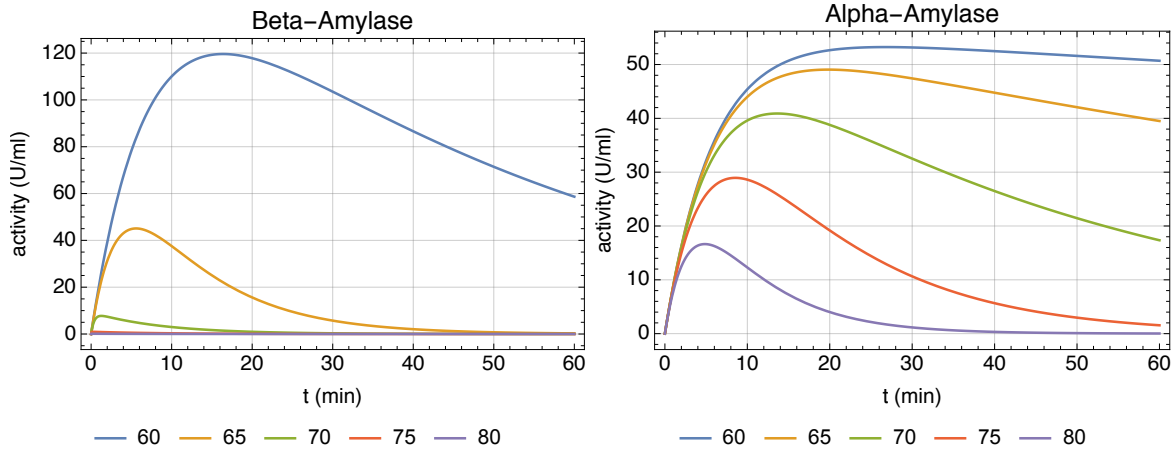

Figura 1: Evolution of dissolved α- and β-amylases for different mash temperatures.

vity. For temperatures of 75° and over, there is practically no β activity, which implies that in mashed with temperatures in this range all starch conversion will be performed solely by the α-amylase.

Next, figure 2 displays the evolution of the concentration of the different carbohydrates in the mash with time for single-infusion mashes at 60°C and 80°C. As observed in these graphs, the 80°C mash leads to a faster starch degradation. For the 60°C mash, not only slower is the degradation os starch, but it is incomplete, as this temperature is a few degrees

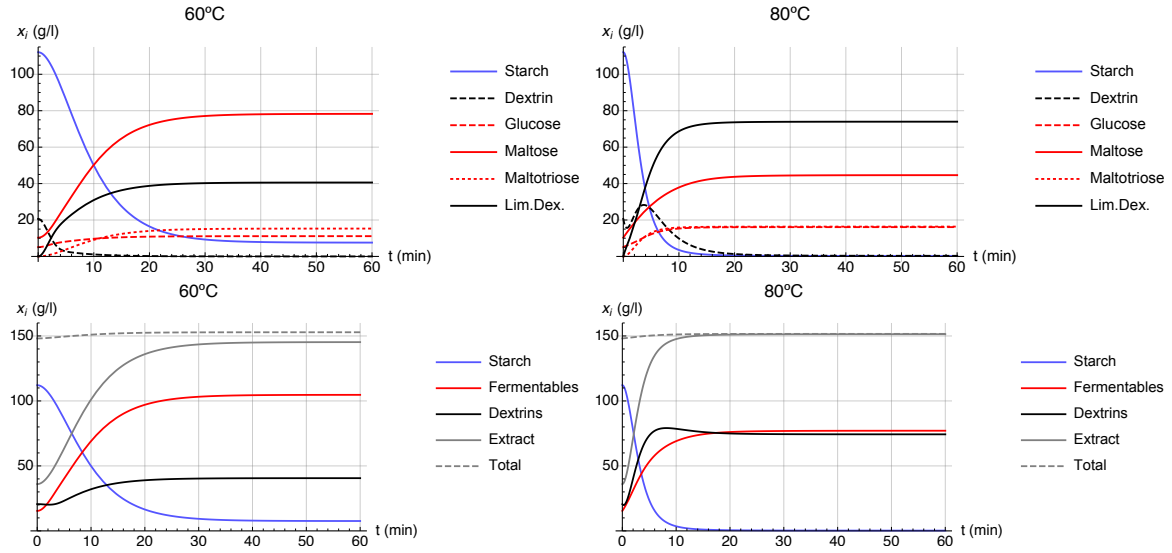

Figura 2: Evolution of carbohydrate concentration with time for different mashing temperatures.

below the upper bound for starch gelatinization. This incomplete starch gelatinization results in an extract concentration x_e that does not reach the maximum possible value x_t and hence in efficiency values lower than 1. Another point worth mentioning is that the higher temperature yields a much higher amount of dextrins, and hence lead to a less fermentable wort. This is expected as at 80°C the hydrolysis is performed practically by the α-amylase enzyme alone.

The final group of figures (3, 4, 5), involve data from the simulation of several combinations of temperature and mash duration, presented as contour plots, calculated for both *Kymppi* and *Kustaa* malts. These figures show that there is a

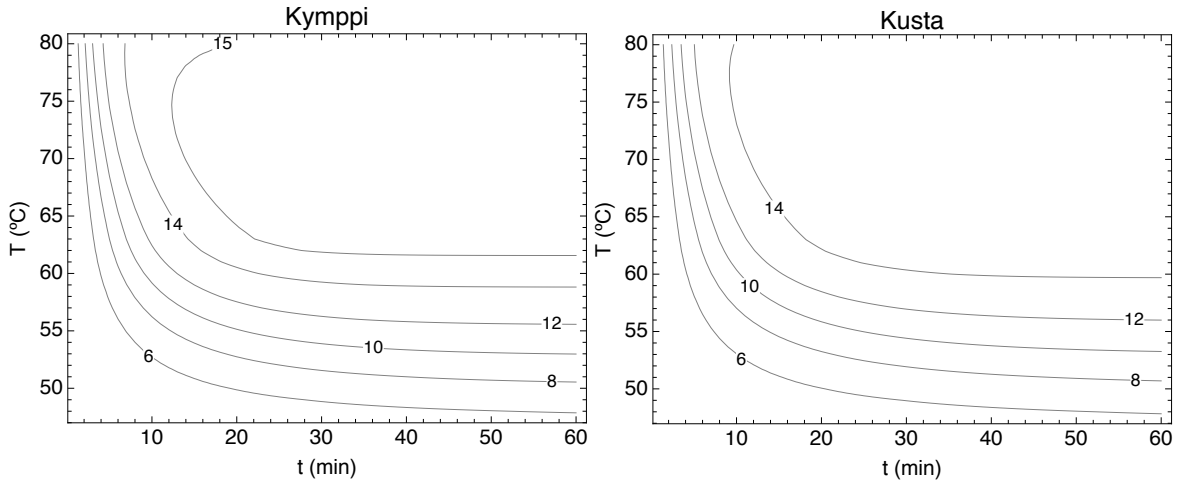

Figura 3: Extract (°P) isolines in single-temperature infusion for different mash times and temperatures.

common region where a maximum mash efficiency can be obtained from 65°C to 75°C. However, more fermentable sugars are obtained if the mash is performed near the 65°C limit. The lower efficiency for higher temperatures (around 80°C and above) occur because on incomplete conversion due to a quick enzyme denaturation that occurs at these temperature levels.

4. SUMMARY AND CONCLUSIONS

This paper presented a preliminary analysis for the hydrolysis of starch by α- and β-amylases, through the adaptation of a simple model for describing the species conservation for different carbohydrate forms as well as enzymes. The variation of α- and β-amylases activity was analyzed for different temperatures, showing that for temperatures over 75°C the starch hydrolysis is performed by the α-amylase enzyme alone. The concentration of carbohydrates was studied in mashes with different temperatures, and it was seen that mashing at 80°C leads to a faster starch degradation, but also to a higher concentrations of non fermentable sugars (limit dextrins). An investigation of mashing efficiency for *kymppi kustaa* malts was conducted for different mash temperatures and durations. The results showed that a maximum mash efficiency was obtained in the range from 65°C to 75°C; however, performing the mash near the lower limit of this range (65°C) leads to more fermentable sugars. Finally, it was shown that higher temperatures lead to a faster increase in extract, and that the extract is slightly lower for higher mashing temperatures due to the fact that smaller amounts of water are

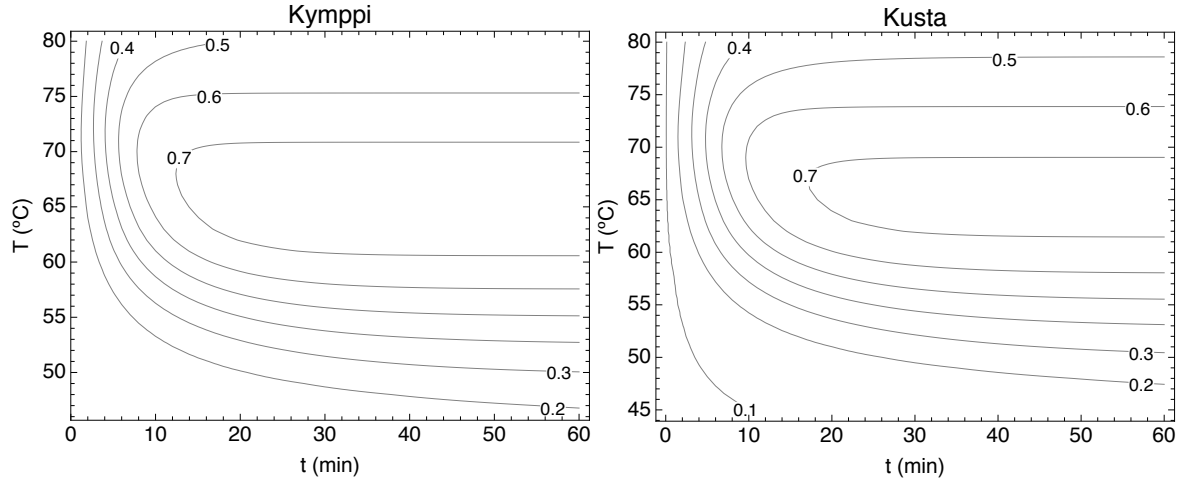

Figura 4: Fraction of fermentable sugars isolines in single-temperature infusion for different mash times and temperatures.

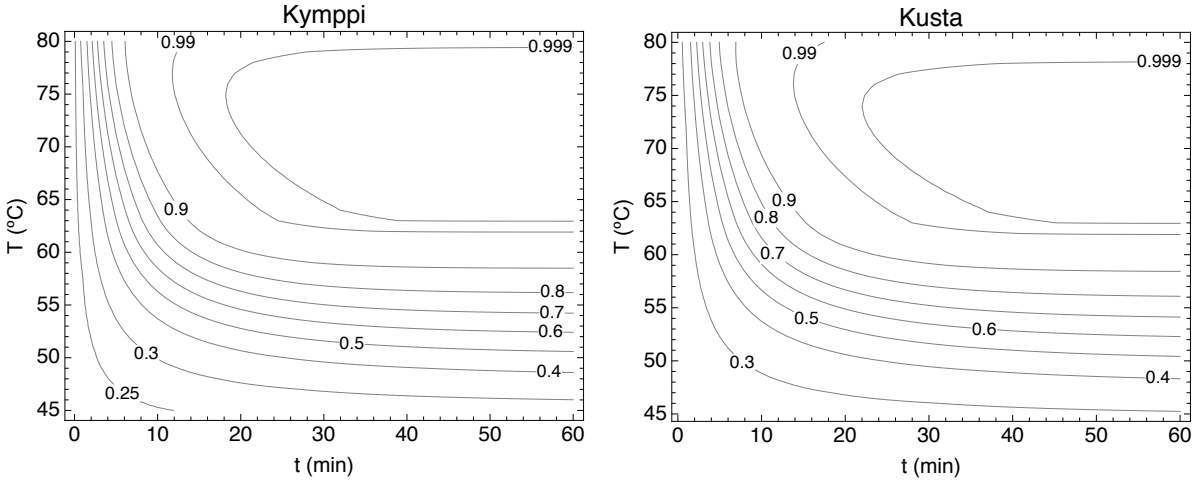

Figura 5: Mashing efficiency isolines in single-temperature infusion for different mash times and temperatures.

consumed in the hydrolysis process.

5. ACKNOWLEDGEMENTS

The authors would like to acknowledge the financial support provided by CNPq, FAPERJ and CAPES.

6. REFERENCES

Brandam, C., Mever, X.M., Proth, J., Strehaiano, P. and Pingaud, H., 2003. "An original kinetic model for the enzymatic hydrolysis of starch during masing". *Biochemical engineering journal*, Vol. 13, pp. 43–52.

Durand, G.A., Corazza, M.L., Blanco, A.M. and Corazza, F.C., 2009. "Dynamic optimization of the mashing process". *Food Control*, Vol. 20, pp. 1127 – 1140.

Enari, T.M. and Markkanen, 1975. "Microbial beta-glucanases in brewing". *Proc. Amer. Soc. Brew. Chem.*, Vol. 33, pp. 13–17.

Kettunen, A., Hämäläinen, J.J., katharina Stenholm and Pietila, K., 1996. "A model for the prediction of beta-glucanaseactivity and beta-glucan concentration during mashing". *Journal of Food Engineering*, Vol. 29, pp. 185–200.

Koljonen, T., Hämäläinen, J.J. and Sjöholm, K., 1995. "A model for the prediction of fermentable sugar concentrations during mashing". *Journal of Food Engineering*, Vol. 26, pp. 329–350.

Li, Y., Lu, J. and Gu, G., 2005. "Control of arabinoxylan solubilization and hydrolysis in mashing". *Food Chemistry*, Vol. 90, pp. 101–108.

Li, Y., Lu, J., Gu, G., Shi, Z. and Mao, Z., 2004. "Mathematical modeling for prediction of endo-xylanase activity and arabinoxylans concentration during mashing of barley malts for brewing". *Biotechnology Letters*, Vol. 26, pp. 779–785.

Marc, A. and Engasser, J.M., 1983. "A kinetic model of starch hydrolysis by apha and beta-amylase during mashing". *Biotechnology and Bioengineering*, Vol. 25, pp. 481–496.

7. RESPONSIBILITY NOTICE

The authors are the only responsible for the printed material included in this paper.