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Influence of Thermally Processed Carbohydrate/Amino Acid Mixtures on the Fermentation by *Saccharomyces cerevisiae*

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The production of alcoholic beverages such as Tequila, Mezcal, whiskey, or beer includes the fermentation of a mash containing Maillard reaction products. Because excessive heating of the mash can lead to complications during the following fermentation step, the impact of Maillard products on the metabolism of *Saccharomyces cerevisiae* was investigated. For this purpose, fermentation was carried out in a model system in the presence and absence of Maillard reaction products and formation of ethanol served as a marker for the progression of fermentation. We found that increasing amounts of Maillard products reduced the formation of ethanol up to 80%. This effect was dependent on the pH value during the Maillard reaction, reaction time, as well as the carbohydrate and amino acid component used for the generation of Maillard reaction products. Another important factor is the pH value during fermentation: The inhibitory effect of Maillard products was not detectable at a pH of 4 and increased with higher pH-values. These findings might be of relevance for the production of above-mentioned beverages.

KEYWORDS: Maillard reaction; nonenzymatic browning; yeast; saccharomyces cerevisiae; fermentation; beer; Tequila; Mezcal; whiskey

INTRODUCTION

The starting point for this project was the observation that during the production of Mezcal, excessive heating of the fructose-rich mash led to complications during the following fermentation step. Mezcal is an alcoholic beverage related to Tequila, which is produced from agaves containing the polysaccharide inulin (*1*, *2*). Because yeasts cannot ferment this polysaccharide, the agaves are cooked for a prolonged time (24−36 h, 90 °C) or are even autoclaved (12 h, 110−120 °C). During this procedure, the acidic pH of the agave matrix and the heat lead to hydrolysis of inulin into its fructose monomeres. After a milling step, the liquid is separated from the plant fibers to yield the mash, which then is subject to fermentation by yeasts. This mash displays a dark brown color, an indication that the so-called Maillard reaction has taken place. The Maillard reaction occurs between reducing carbohydrates and amino groups (e.g., of plant proteins) and is strongly accelerated by heat. In the course of this reaction, the unstable Schiff base is formed first, which then undergoes the so-called Amadori rearrangement to form the Amadori product. The Amadori product itself can undergo numerous further reactions that lead to a wide variety of often brown products, the so-called Maillard products (MP). Indeed, it could be shown that MP are generated abundantly during the thermal procession of inulin (*3*). Thus, excessive formation of MP might be the reason for the problems during the fermentation step of Mezcal production. This conclusion would not be surprising, because it is meanwhile well accepted that MP exert effects on living organisms. They have been implicated, for example, in the pathology of artherosclerosis (*4*), Alzheimer's disease (*5*), diabetes, and chronic renal failure (*6*). From that background, it can be expected that MP might influence the fermentation by yeast. In fact, yeasts come in contact with MP not only during the manufacture of Mezcal but also during the production of other alcoholic beverages. For example, beer brewing includes kilning of green malt, a step during which MP are formed. Kiln-dry malt is also used in the course of whiskey production. Therefore, we tried to answer the general question if MP can influence the fermentation by yeast.

MATERIALS AND METHODS

Materials. Lyophilized baker's yeast was obtained from Windhager (Stubenberg, Germany). Other materials or chemicals were purchased from Fluka (Taufkirchen, Germany).

Experimental Procedures. In the following, the experimental procedure for the first experiment (**Figure 1**) is described in detail. For the other experiments (**Figures 2**−**7**), only the changes from this standard protocol are given.

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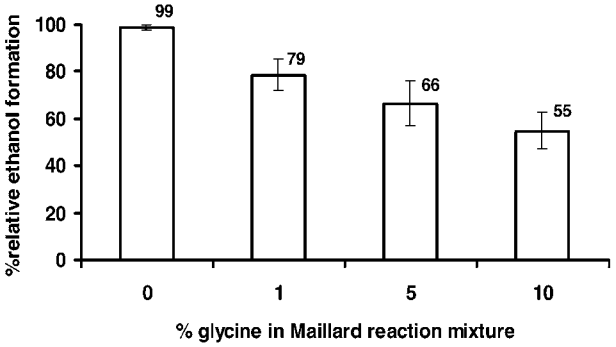

Figure 1. Influence of increasing glycine concentrations on the ethanol formation by yeast. Data (sextuplicate) are displayed as mean ± SEM The mean value of absolute ethanol formation for all control mixtures was 0.9% ethanol.

Preparation of the YP Solution. A 10-g sample of yeast extract and 20 g of peptone were dissolved in 1 L of distilled water. If necessary, the pH was adjusted to 5.8 by addition of 50 μL of 12 N HCl. Finally, the solution was autoclaved for 30−45 min at 120 °C.

Preparation of the Yeast Solution for Inoculation of the Samples. A 500-mg sample of lyophilized yeast powder together with 2 g of glucose were dissolved in 100 mL of YP solution. This solution was shaken at room temperature for approximately 4 h. To ensure that the same amount of yeast was always added to the samples, yeast cells were counted shortly before inoculation of the samples with a microscope using a Neubauer chamber. The mean value of 5 counts was calculated. For inoculation, an aliquot of this yeast solution was used to achieve a final concentration of 1.25×10^9 yeast cells per liter.

Preparation of the Maillard Mixture. A 15-g sample of glucose and 2.61 g of dipotassium hydrogen phosphate were dissolved in a final volume of 150 mL distilled water containing 0, 1, 5, and 10% glycine. The pH of all solutions was adjusted to 7 with phosphoric acid (85%) or concentrated KOH. A 70-mL aliquot of each solution was stored at room temperature, and the rest was autoclaved for 45 min at 120 °C.

Preparation of the Nutrient Solution. A 112-g sample of glucose and 19.05 g of potassium dihydrogen phosphate were dissolved in distilled water to a final volume of 1400 mL. The pH was adjusted to 7.4 with concentrated KOH.

Preparation of the Samples. A 160-mL aliquot of nutrient solution, 60 mL of YP solution, and 60 mL of the heated Maillard mixture or unheated control were mixed in a glass flask with a stirring bar. Before the aliquot of the yeast inoculum was added, the pH was checked and adjusted to 7 with concentrated phosphoric acid or concentrated KOH if necessary. After addition of the yeast inoculum, the flask was sealed with a fermentation tube and temperature was maintained at 25 °C over 18 h.

Determination of the Ethanol Concentration. After 18 h, the samples were well shaken, and the solution was checked for microbiological contaminations with a microscope. In case of a contamination, the experiment was excluded from further analysis. A 5-mL sample of the yeast solution was pipetted into a 25-mL flask. To this solution, 30 μL of *n*-propanol were added as internal standard, and exactly 3 mL were distilled into a plastic tube on ice. The distillate was directly used for the gas-chromatographic quantification of ethanol. The mean coefficent of variation was 0.04. Retention times of ethanol and *n*-propanol were 2.4 or 3.6 min, respectively. A standard curve was obtained analogously by using defined ethanol concentrations for the procedure, including the distillation step. Gas chromatography was performed using a Macherey-Nagel Permabond CW 20 M-DF column (30-m × 0.25-mm). The temperature of the injector and detector was set to 200 °C. Oven temperature was 45 °C for 4 min and then risen to 110 °C with a rate of 40 °C/min. An injection volume of 0.3 μL and a split of 1:100 were used. The mean value of absolute ethanol formation for all control mixtures of each experiment is given in the legends of the figures.

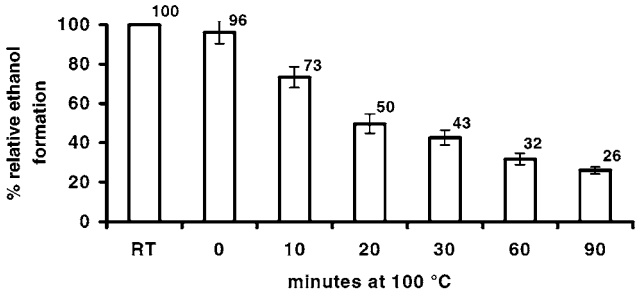

Figure 2. Influence of the duration of Maillard reaction on the ethanol formation by yeast (pH 7). Data (octuplicate) are displayed as mean ± SEM. The mean value of absolute ethanol formation for all control mixtures was 0.9% ethanol.

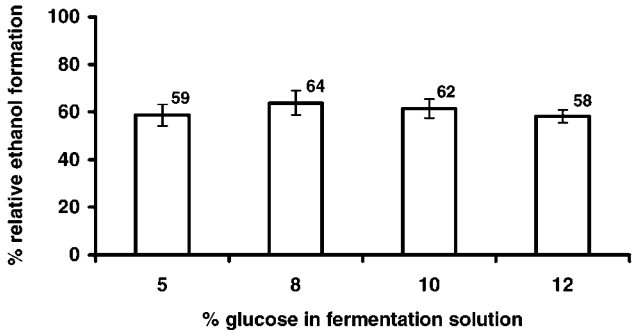

Figure 3. Influence of increasing glucose concentrations on the ethanol formation by yeast in the presence of equal amounts of MP. Data (octuplicate) are displayed as mean ± SEM. The mean value of absolute ethanol formation for all control mixtures was 0.7% ethanol.

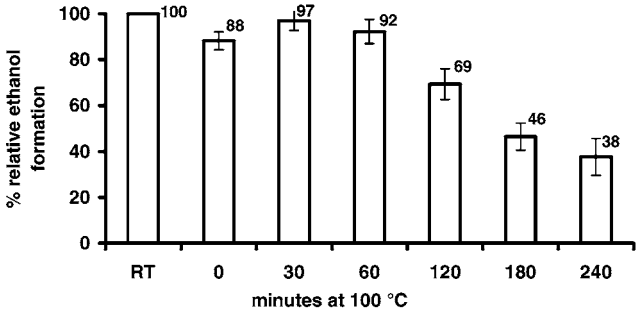

Figure 4. Influence of the duration of Maillard reaction on the ethanol formation by yeast (pH 7). Data (octuplicate) are displayed as mean ± SEM. The mean value of absolute ethanol formation for all control mixtures was 0.9% ethanol.

Statistical Analysis. A paired *t*-test was used. According to common use in the literature, *p*-values from 0.05 to 0.01 were regarded as weakly significant, from 0.01 to 0.001 as significant, and below 0.001 as highly significant. The first experiment was performed in sextuplicate.

Influence of the Heating Time at pH 7 (**Figure 2**). Instead of four different Maillard mixtures, the 4-fold volume of a Maillard mixture with 5% glycine was prepared. A 150-mL aliquot of this solution was stored at room temperature, and the rest was heated at 100 °C under reflux. After 0, 10, 20, 30, 60, and 90 min, samples of 70 mL were obtained. The experiment was performed in octuplicate.

Influence of the Glucose Concentration in the Nutrient Solution (**Figure 3**). Instead of four different Maillard mixtures, the 4-fold volume of a Maillard mixture with 1% glycine was prepared. For the nutrient solutions, 4.76 g of potassium dihydrogen phosphate was dissolved in a final volume of 350 mL containing 5, 8, 10, or 12% glucose. The pH of the solutions was adjusted to 7.4 with concentrated KOH. The experiment was performed in octuplicate.

Influence of the Heating Time at pH 5 (**Figure 4**). Instead of four different Maillard mixtures, the 4-fold volume of a Maillard mixture

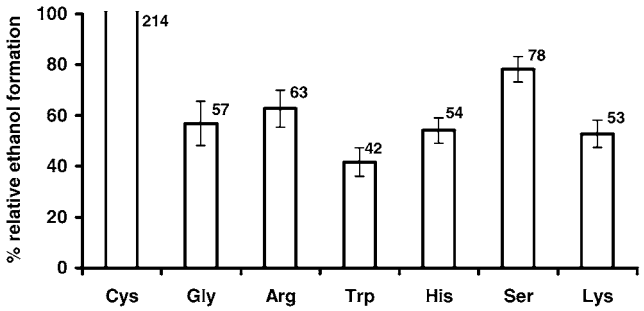

Figure 5. Influence of different amino acid components of the Maillard reaction mixture on ethanol formation by yeast. Data (septuplicate) are displayed as mean ± SEM. The mean value of absolute ethanol formation for all control mixtures was 0.7% ethanol.

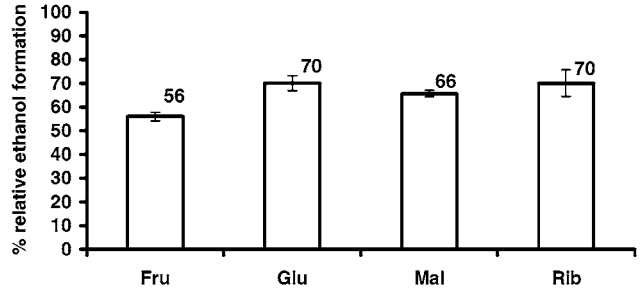

Figure 6. Influence of different sugar components of the Maillard reaction mixture on ethanol formation by yeast. Data (sextuplicate) are displayed as mean ± SEM. The mean value of absolute ethanol formation for all control mixtures was 0.7% ethanol.

with 5% glycine was prepared. The pH of this solution was adjusted to 5 with concentrated phosphoric acid. A 150-mL aliquot of this solution was stored at room temperature, and the rest was heated to 100 °C under reflux. After 0, 30, 60, 120, 180, and 240 min, samples of 70 mL were obtained. The experiment was performed in octuplicate.

Influence of the Amino Acid Component (**Figure 5**). For the preparation of the Maillard mixtures, 1.5 g of glycine, 2.1 g of serine, 2.4 g of cysteine, 4.1 g of tryptophan, 3.5 g of arginine, 2.9 g of lysine, or 3.1 g of histidine were dissolved together with 12.5 g of glucose and 2.61 g of dipotassium hydrogen phosphate in distilled water to a final volume of 150 mL. The pH value of all samples was adjusted to 7 with concentrated phosphoric acid or concentrated KOH, respectively. The experiment was performed in septuplicate.

Influence of the Carbohydrate Component (**Figure 6**). For the preparation of the Maillard mixtures, 15 g of glucose, 15 g of fructose, 30 g of maltose, or 12.5 g of ribose were dissolved together with 2.61 g of dipotassium hydrogen phosphate in distilled water to a final volume of 150 mL. The pH value of all samples was adjusted to 7 with concentrated phosphoric acid or concentrated KOH, respectively. The experiment was performed in sextuplicate.

Influence of the pH During Fermentation (**Figure 7**). Instead of four different Maillard mixtures, the 4-fold volume of a Maillard mixture with 5% glycine was prepared. To obtain nutrient solutions with different pH, 28 g of glucose and 6.09 g of dipotassium hydrogen phosphate were dissolved in distilled water to a final volume of 1400 mL. Aliquots (350 mL) of this solution were adjusted to a pH of 8, 7, 5, or 4 with concentrated phosphoric acid or concentrated KOH. After the addition of the Maillard mixture, the solutions were adjusted to the corresponding pH. The experiment was performed in octuplicate.

RESULTS AND DISCUSSION

General Setup of a Model System to Investigate the Influence of Maillard Products on the Fermentation by Yeast. In this study, a model system was used to assess the effects of MP on the fermentation by yeast. The general principle

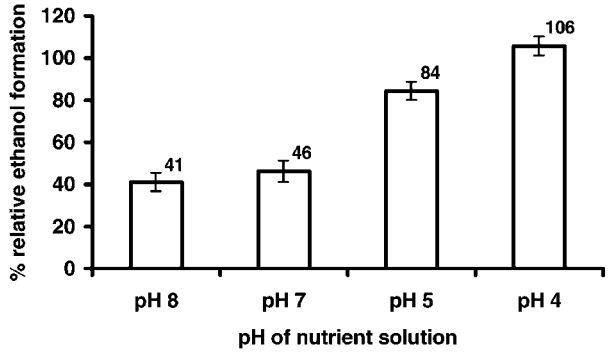

Figure 7. Influence of the pH value during fermentation on ethanol formation by yeast in the presence of MP. Data (octuplicate) are displayed as mean ± SEM. The mean value of absolute ethanol formation for all control mixtures was 1.1% ethanol.

is to compare fermentation in the absence and presence of MP with other conditions unaltered. MP were produced by heating a mixture of a carbohydrate (e.g., glucose) and an amino-component (e.g., glycine), the so-called Maillard mixture, under defined conditions. A part of the carbohydrate−amino acid mixture was stored at room temperature as unheated control without MP. The heated Maillard mixture and the unheated control were then added to buffered solutions of nutrients necessary for yeast growth, such as glucose, yeast extract, and peptone. As a side effect of this operation, differences in glucose concentration between heated and unheated mixtures, a result of the Maillard reaction, are almost fully leveled out. Fermentation was then carried out for 18 h using normal baker's yeast (*Saccharomyces cerevisiae*) as the model organism. To monitor fermentation, ethanol concentrations were determined after 18 h in samples with and without MP. Ethanol formation in the presence of MP was then divided by ethanol formation in the absence of MP to obtain the relative ethanol formation as an indicator of the influence of MP on the fermentation.

Inhibitory Effect of Maillard Products on the Fermentation by Yeast. Fermentation by yeast was carried out in the presence of different amounts of MP. Increasing amounts of MP were realized in two different ways: by increasing amounts of the amino component glycine (**Figure 1**) or by increasing duration of the Maillard reaction (**Figure 2**).

Figure 1 shows the ethanol production in the model system containing 0−10% glycine in the Maillard reaction mixture. With 0% glycine, in the absence of an amino component, the Maillard reaction cannot take place, and only sugar degradation, also called caramelization, occurs. Higher amounts of glycine increase the intensity of the Maillard reaction. After 18 h of fermentation, ethanol formation in the presence of the Maillard reaction mixture with 10% glycine was decreased by 50% compared to the mixture with 0% glycine ($p < 0.01$). Mixtures with 1 and 5% glycine lie between these two extremes. Therefore, it seems that an increasing formation of MP leads to a significant decrease in ethanol formation. Caramelization of glucose per se (0% glycine) is not capable of inducing any significant decrease in ethanol formation.

Figure 2 shows the result of an experiment in which the Maillard mixtures were heated for different periods of time: a Maillard reaction mixture with 5% glycine (pH = 7) was heated up to 90 min at 100 °C. Time measurement was started when the final temperature of 100 °C was reached (RT = sample at room temperature). In the unheated mixture, no MP are present, whereas increasing amounts of MP are formed during prolonged heat treatment. After 0, 10, 20, 30, 60, and 90 min, samples

were drawn and examined for fermentation as described above. A decrease in ethanol formation can be clearly observed with ongoing formation of MP. After 90 min of Maillard reaction, ethanol formation is reduced by 70% in comparison to $t = 0$ min ($p < 0.001$). A significant decrease ($p < 0.01$) in ethanol formation could be observed already after 10 min. Consequently, this experiment confirms that MP could inhibit ethanol formation by yeasts.

Because glucose is consumed during the Maillard reaction, glucose concentrations decrease. The lower glucose concentrations could be the reason for low ethanol formation in mixtures with high concentrations of MP. To exclude this possibility, the sensitivity of ethanol formation with regard to glucose concentration was assessed. Fermentation was carried out under standard conditions with glucose concentrations ranging from 5 to 12% in the nutrient solution (**Figure 3**). From this experiment, it can be concluded that the inhibitory effect of MP on yeast does not depend on the glucose concentration in a wide concentration range. Therefore, it can be excluded that diminished concentrations of glucose caused by Maillard reaction or sugar degradation are responsible for the observed effect. A similar experiment (data not shown) was performed to exclude the influence of different phosphate concentrations resulting from pH-adjustment. Because neither phosphate nor glucose concentration influenced fermentation noticeably, it is very unlikely that the observed effects were caused by changes in osmolarity. This is important, because osmolarity can increase during the Maillard reaction by fragmentation.

Parameters Influencing the Inhibitory Effect of Maillard Products on Yeast. The following experiments were performed to investigate the effect of parameter changes in the model system on the observed inhibitory effect.

pH of the Maillard Reaction. A time course experiment was performed in which the Maillard mixture was heated at pH 5 for different periods of time. This experiment is particularly important for Tequila or Mezcal production, because in these processes, Maillard reaction takes place at pH 4−5. The Maillard reaction mixture contained 5% glycine. Similar to the experiments at pH 7 (**Figure 2**), intensified Maillard reaction leads to a decrease in ethanol formation (**Figure 4**). However, at a weak acidic pH, the inhibitory effect develops much slower: Only after 120 min of heating could a significant inhibitory effect be noted ($p < 0.01$). After 240 min, inhibition was highly significant ($p < 0.001$). The results of this experiment are well in line with the known fact that the intensity of the Maillard reaction strongly increases with higher pH values. At the same time, we were able to show that even at a pH of 5 after a longer duration of heat treatment, inhibitory amounts of MP can accumulate, which might be relevant for the production of Tequila or Mezcal. However, it must be pointed out that Maillard products derived from fructose and not from glucose are formed in these beverages (see below, effects of different sugar components).

Amino Components of the Maillard Reaction. To assess the impact of different amino-components of the Maillard reaction on the inhibitory effect on yeasts, Maillard mixtures with 0.13 M of different amino acids were prepared. In detail, glycine (Gly) was investigated to maintain comparability with previous results, cysteine (Cys) was included as a sulfur-containing amino acid and serine (Ser) as hydroxy-amino acid. Lysine (Lys), arginine (Arg), and tryptophan (Trp) were examined because their MP are known to possess physiological relevance. Finally, histidine (His) was also investigated because its side chain can be involved in Maillard reactions.

It can be deduced that all MP derived from amino acids (except cysteine, see below) cause an inhibition of ethanol formation (**Figure 5**). Even for serine, the least effective amino acid, a significant inhibition could be measured ($p < 0.01$). MP derived from tryptophan are stronger inhibitors of ethanol formation than serine ($p < 0.001$). In addition, significant differences could be found between serine−histidine, serine−lysine and arginine−tryptophan ($p < 0.01$). Differences between glycine−tryptophan and serine−glycine were only weakly significant ($p < 0.05$). The variations in the inhibitory potential of the amino acids could be due to different spectra of MP derived from them. On the other hand, it is also possible that amino acids with more than one amino group, such as lysine or arginine, are more potent promoters of the Maillard reaction. Similarly, different p*K*s values of the amino acids could lead to a decreased or increased intensity of Maillard reaction. Thus, the observed differences could be caused either by different intensities of the Maillard reaction or by strong effects of specific Maillard products. Surprisingly, a relative ethanol formation of 214% was found in the presence of Maillard products from cysteine. However, analysis of the underlying absolute ethanol formation (data not shown) revealed that instead of an increased ethanol formation in the presence of cysteine-derived MP, a decreased ethanol formation in the unheated Maillard mixture with cysteine is the reason for the observed augmentation of relative ethanol formation. Thus, this effect must be ascribed to inhibitory effects of cysteine on yeasts rather than to a promoting effect of MP of cysteine. In summary, it can be stated that Maillard mixtures with different amino acids lead to different degrees of inhibition of ethanol formation. The exact reason for this effect still remains to be elucidated.

Sugar Components of the Maillard Mixtures. In the following experiment, the sugar component of the Maillard mixture was varied. In addition to a Maillard mixture with 10% glucose (Glu), equimolar concentrations of fructose (Fru), maltose (Mal), and ribose (Rib) were used. Fructose was investigated because MP of fructose rather than of glucose play a role for Tequila and Mezcal. Maltose was included because of its relevance for beer, and ribose was investigated as an example for pentoses. As usual, glycine was used as amino component. MP derived from all four sugar components showed inhibitory effects on ethanol production (**Figure 6**). Between the sugar components, only a weakly significant difference between fructose and glucose or fructose and maltose was detected ($p < 0.05$).

pH During Fermentation. Finally, the influence of the pH during fermentation on the inhibitory effect was investigated. This study was conducted because, for the preparation of alcoholic beverages, fermentation usually takes place at a weakly acidic pH to reduce microbiological contamination. For this experiment, a standard Maillard mixture with 5% glycine was used. Before fermentation, pH values were adjusted to 8, 7, 5, and 4. Relative ethanol formation after fermentation is shown in **Figure 7**.

It is clearly visible that the inhibitory effect is strongly dependent on the pH during fermentation. At pH of 7 and 8, ethanol formation is reduced by 55 and 60%, respectively. However, the same amounts of MP only lead to a reduction of 20% at pH 5, and at pH 4 no inhibitory effect was observed anymore. Accordingly, the reduction in ethanol formation at pH 7 and 8 was highly significant ($p < 0.001$) and weakly significant at a pH 5. This means that the pH during fermentation is of high importance for the intensity of the inhibitory effect and that under conditions of the production of alcoholic beverages (pH during fermentation between 4 and 5) the

inhibitory effect may occur, yet is weaker. However, the reason for this pH-dependence remains unknown. It is possible that chemical reactions or protonization/deprotonization processes transform MP into less active forms. On the other hand, it is known that the optimal pH for fermentation processes ranges around 4. Other pH values thus could be a stress situation for yeasts and may therefore elevate their susceptibility to other disturbing influences such as MP.

Our experiments showed that MP derived from a glucose/glycine model system are able to suppress ethanol production by yeast. Because other factors such as influence of osmolarity or differences in glucose concentration were widely excluded, it is likely that MP are cytotoxic for yeast. Indeed, it has been published that MP induce mitotic gene conversion in *Saccharomyces cerevisiae* (*7*), and it was shown that the Amadori product (*8*) inhibits amylases in yeasts. This is also supported by our observation that the yeast cell number strongly correlated with ethanol formation ($r = 0.8739$, $p < 0.0001$, data not shown) in our experiments; therefore, it can be concluded that the decreased ethanol formation is due to inhibited yeast growth. Furthermore, Pfeifer et al. (*9*) showed that hydroxymethylfurfural, furfural, and methylglyoxal, which are formed in considerable amounts during the acidic hydrolysis or hydrothermolysis of sugars and polysaccharides, inhibited the fermentation of yeast decisively. However, it is likely that the effects which were observed in our experiments were caused by MP derived from sugars and amino acids. Control mixtures, where glucose was heated in the absence of amino acids and where thermolysis of sugars instead of Maillard reaction takes place, did not show inhibition of yeast fermentation under the conditions applied here (**Figure 1**). Looking back to the starting point of this project, our results clearly show an inhibitory effect exerted by MP, which can cause fermentation problems during the production of Mezcal. In addition, the conditions during beer brewing are similar to the conditions chosen in our model system; therefore, further research on the inhibitory effects of MP in the brewing process will follow.

However, it must be noted that nature and concentrations of MP in mash used for Mezcal or beer production can differ from those applied in our model systems. On one hand, amino acid concentrations in foods are clearly lower. On the other hand, in mash, sugar concentrations could be higher, and harsher heating conditions are applied. Furthermore, proteins that are abundant in food can also promote the Maillard reaction. Therefore, it still remains to be investigated if MP from mash show similar effects on yeast fermentation as observed in these studies.

Because MP are important components for the sensoric quality of almost all alcoholic beverages produced from a mash containing MP, a simple reduction of the Maillard reaction cannot solve the problems that may occur during fermentation. Therefore, detailed investigations are necessary on the parameters during Maillard reaction and fermentation, which influence the inhibitory effect without reducing the sensoric properties of the product. Our studies indicate that the pH value during fermentation can be one interesting approach.

LITERATURE CITED

(1) Perez Zamora, M. C. M. El Mezcal en el Altiplano Potosino-Zacatecano. *Bebidas Mexicanas* **1997**, *3*, 41−44.

(2) Cedeno, M. C. Tequila Production. *Crit. Rev. Biotech.* **1995**, *15* (1), 1−11.

(3) Mancilla-Margalli, N. A.; Lopez, M. G. Generation of Maillard Compounds from Inulin during the Thermal processing of Agave tequilana Weber Var. Azul. *J. Agric. Food Chem.* **2002,** *50*, 806−812.

(4) Schmidt, A. M.; Stern, D. Atherosclerosis and diabetes: the RAGE connection. *Curr. Atheroscler. Rep.* **2000,** *2*, 430−436.

(5) Munch, G.; Thome, J.; Foley, P.; Schinzel, R.; Riederer, P. Advanced glycation endproducts in aging and Alzheimer's disease. *Brain Res. Brain Res. Rev.* **1997,** *23*, 134−143.

(6) Niwa, T.; Katsuazaki, T.; Miyazaki, T.; Ishizaki, Y.; Hayase, F.; Tatemichi, N.; Takei, Y. Immunochemical detection of imidazolone, a novel advanced glycation end product, in the kidneys and aortas of diabetic patients. *J. Clin. Invest.* **1997,** *99*, 1272−1280.

(7) Rosin, M. P.; Stich, H. F.; Powrie, W. D.; Wu, C. H. Induction of mitotic gene conversion by browning reaction products and its modulation by naturally occurring agents. *Mutat. Res.* **1982,** *101*, 189−197.

(8) Schumacher, D.; Kroh, L. W. The decomposition of Maillard reaction products by amylolytic enzymes. 1. Reversible inhibition of alpha- and glucoamylase and alpha-glucosidase by oligosaccharide Amadori compounds. *Z. Lebensm.-Unter. Forsch.* **1994,** *199*, 270−274.

(9) Pfeifer, P. A.; Bonn, G.; Bobleter, O. Influence of biomass degradation products on the fermentation of glucose to ethanol by saccharomyces carlsbergensis W34. *Biotechnology Lett.* **1984**, *6*, 541−546.

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