Sérgio Luiz de Lucena, et. al. International Journal of Engineering Research and Applications
www.ijera.com
ISSN: 2248-9622, Vol. 14, Issue 4, April, 2024, pp: 06-08

RESEARCH ARTICLE OPEN ACCESS

Effects of pH and Temperature on the Enzymatic Hydrolysis of Barley Malt

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ABSTRACT

Malt barley (*Hordeum vulgare)* is the brewer's preferred grain for making beer. Malted grains contain starch and amylase enzymes that were naturally produced during the grain germination process under controlled conditions of temperature, aeration and humidity (malting). The enzymes, mainly a and b-amylases, are the catalysts that transform the starch into fermentable sugars, and the sugars will be transformed to ethanol during fermentation process by yeast. The total reducing sugars were measured using Miller´s colorimetric methodology reading the absorbances at 540nm. The temperature and pH play a very important role on the enzymatic hydrolysis. The aim of this work was to evaluate the effects of pH and temperature on the enzymatic hydrolysis of barley malt. It was observed that both pH and temperature affect the concentration of total sugars released during the hydrolysis. It was concluded that by applying the pH=5.5 and temperatures ranging from 65 to 80°C yielded higher absorbances meaning more sugars were released from the barley malt starch (maximum hydrolysis).

Keywords **-** amylases, brewing, enzyme, malt barley, mashing, fermentation

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Date of Submission: 02-04-2024 Date of acceptance: 13-04-2024

I. INTRODUCTION

Malted grains contain substances such as starch and amylase enzymes that were naturally produced during the grain germination process under controlled conditions of temperature, aeration and humidity (malting). The enzymes, mainly a and b-amylases, are the catalysts that transform the starch into fermentable sugars, and the sugars will be then transformed into carbon dioxide CO_2 and ethanol during fermentation process by yeast. Many grains that contain starch can be transformed in malt such as wheat, corn, sorghum, rice, etc. The main use of malted grains as a powder or coarse flour is in the food and beverages industries for producing alcoholic beverages as beers due to its diastatic properties (transformation of the malt´s starch into sugars by enzymatic hydrolysis under proper conditions of pH and temperature when mashing. Also, the malt flour can be used for baking breads helping in the development of texture, flavor and the golden color. Malt barley is the preferred one when producing beers with a classical flavor. In the context of brewing, barley malt starch is hydrolysed by the endogenous malt amylases during mashing [1].

Enzymes are biomolecules (typically proteins) that present a very specific catalytic activity transforming sub-strates in products [2]. Proteins are formed by peptide bonds between amino acids resulting in a long polypeptide chain that presents a primary, a secondary and, a tertiary structure. Some proteins exhibit a quaternary structure depending on the solution´s pH. The structures are originated from chemical interactions between the amino acid residues along the polypeptide chain. The enzyme´s structures play a very important role on the enzyme activity, more specifically the structure of the active site. It is in the active site where the enzyme-substrate interaction effectively occurs to form the products [3]. The three-dimensional 3D structure of the enzymes produces a suitable and specifically shaped catalytic active site for interacting with a substrate and forming products under appropriate physicochemical conditions. The temperature and the pH are important factors that can affect the enzyme structures and, consequently, the enzyme-substrate interaction. The rate of enzyme catalyzed reaction increases with temperature up to a certain limit. Above a certain temperature the enzyme activity decreases due to enzyme denaturation. There is an optimal temperature where the enzyme activity

is maximum [4]. The pH affects the net electrical charge of the enzyme molecule and, consequently, the 3D structure of the active site when ionic groups are present. The changes in the active site affect its kinetics properties and enzyme activity. Most of the enzymes have a pH condition that maximizes the enzyme activity (optimum pH). The knowledge of the enzyme´s optimum pH is paramount for any enzymatic process.

This study aimed to evaluate the net effects of pH and temperature on the enzymatic hydrolysis of barley malt when analyzing the total reducing sugars produced by the barley malt amylases.

II. Material and Methods

2.1 *Malt barley*

Malt barley, as a coarse flour, was kindly supplied by a local brewing company.

2.2 Enzymatic hydrolysis of the malt barley

The raw malt barley was sieved for removing most of the barleys' straw and the resulting flour were submitted for testing out the effects of pH and temperature.

2.2.1 Effect of temperature: 3g of the malt barley flour were added and mixed in an 250mL Erlenmeyer flask containing already 97g of 50mM pH 5.5 acetate buffer at room temperature. The flask was incubated at the selected temperature for 20min using a laboratory water bath under constant manual agitation. Temperature range from 30^{o}C up to 95^{o}C. A sample of 1mL was collected from the solution at 20min of incubation time and mixed with 9mL 0.1M NaOH in a testing tube for stopping the enzymes' activity in this high pH solution. The resulting 10mL solution contains the sugars released by the enzymatic hydrolysis from the malt barley at the tested temperature and pH5.5. The total reducing sugars (TRS) released during hydrolysis were measured according the colorimetric methodology described by Miller [5] by reading the absorbance at 540nm. Higher absorbances mean more sugars were produced during the hydrolysis.

2.2.2 Effect of pH: All hydrolysis experiments were conducted in a similar fashion way as those realized for testing the temperature effect. For testing out the pH effect, hydrolysis temperature was selected as 70^{o}C and a proper 50mM buffer solution was used with pH=3.0, pH=4.5, pH=5.5, pH=6.5, pH=7.5 and, pH=9.0 as necessary.

2.3 Buffer solutions

It was used 50mM buffer solutions being citrate buffer for pH=3, acetate buffer for pH4.5, pH=5.5 and pH=6.5, and phosphate buffer for pH=7.5 and pH=9.0. A pH meter was used for all necessary pH measurements as usual.

III. Results and Discussions

3.1 Effects of the temperature on the enzymatic hydrolysis of barley malt. The figure 1 shows the results when different temperatures (ranging from 30°C to 95°C) were applied during 20min for the enzymatic hydrolysis of the barley malt flour by using 50mM acetate buffer at pH=5.5

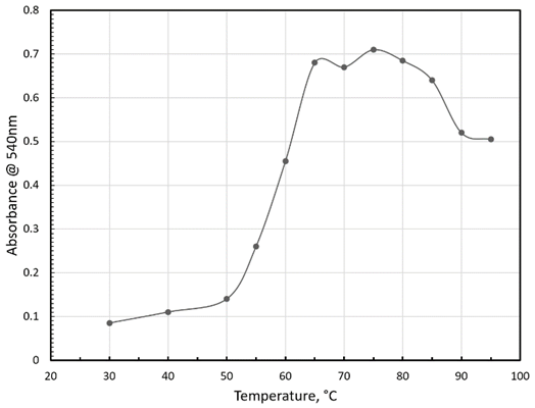

Figure 1. Effect of temperature on the enzymatic hydrolysis of barley malt using 50mM acetate buffer at pH=5.5.

We can observe that as the temperature increases there is a favorable effect by gradually increasing the enzymes activity (thermal activation). Also, as the temperature increases, the starch substrate becomes a soluble gel more susceptible for the amylases action.

The barley malt hydrolysis reaches higher values when temperatures range from 65°C to 80°C being observed absorbances around 0.700. Higher absorbance values mean that more sugars (TRS) were produced from the barley malt starch by the enzymes. Hence, there is a thermal activation of the enzymes in the barley malt and temperature about 70°C yields more total sugars. As the temperature increase above 80°C we observe a negative effect on the enzymatic hydrolysis of the barley malt as the enzyme molecules are being deactivated due to higher temperatures and then less sugars are released from the barley malt starch.

3.2 Effects of the pH on the enzymatic hydrolysis of barley malt. The figure 2 shows the results when different pH values (ranging from 3 to 9) were applied during 20min for the enzymatic hydrolysis of barley malt at 70°C.

The results show that the pH also is an important factor that affects the barley malt hydrolysis. We can observe in the figure 2 that there is a narrow range of pH where the enzymatic hydrolysis of the barley malt is more pronounced. At

pH=5.5 peaks and more sugars are released from the barley malt starch. Enzymes are amphoteric molecules containing a large number of acid and basic groups. The charges on these groups will vary, (according to their acid dissociation constants), with the pH of their environment. This will affect the total net charge of the enzyme molecules. Hence, the pH can affect the ionic interactions that exist in the active site and then sustain a favorable configuration for the barley malt hydrolysis releasing sugars from the starch.

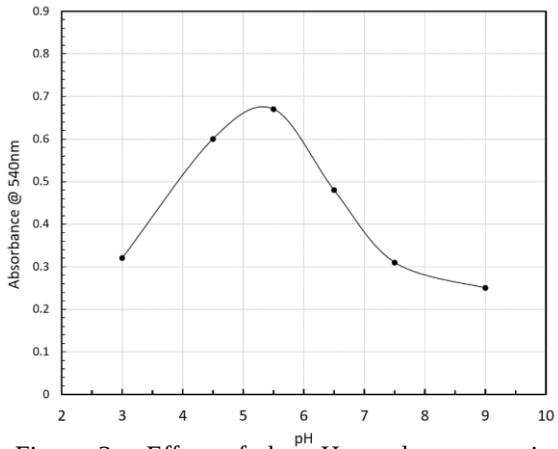

Figure 2. Effect of the pH on the enzymatic hydrolysis of barley malt using 50mM buffers at 70°C.

IV. CONCLUSION

We concluded that both the temperature and the pH are important factors that affect the hydrolysis of the barley malt. It was observed that the temperatures around 70°C and the pH=5.5 yielded higher enzymatic hydrolysis of the barley malt by producing more sugars (TRS).

ACKNOWLEDGMENT

The authors wish to thank "Colônia" a brewing Co. in Toledo, PR, Brazil by providing the barley malt raw flour.

REFERENCES

[1] C. F. De Schepper, P. Michiels, C. Buvé, A. M. Van Loey, C. M. Courtin, Starch hydrolysis during mashing: A study of the activity and thermal inactivation kinetics of barley malt α-amylase and β-amylase, Carbohydrate Polymers, 255, 2021.

[2] P. Gacesa, J. HUBBLE, Tecnologia de las Enzimas. Editorial Acribia, S. A. Zaragoza, Spain, 1990.

[3] J. E. Bailey, D. F. Ollis OLLIS, D. F. "Biochemical Engineering Fundamentals" Ed. McGraw Hill, 2nd Ed., 1986.

[4] M.L.Shuler, F. Kargi. Bioprocess Engineering Basic Concepts. Prentice Hall International, pp.77, 2001.

[5] G.L. MILLER, Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, n.31, pp.426-428, 1959.