Journal of Experimental Research

DECEMBER 2023, Vol 11 No 4

Email: editorinchief.erjournal@gmail.com
editorialsecretary.erjournal@gmail.com

Received: Oct., 2023
Accepted for Publication: Dec., 2023

COMPARATIVE ANALYSIS OF LIQUORS OBTAINED FROM ENZYME AND ACID HYDROLYSES OF RICE MALT THROUGH INFUSION MASHING TECHNIQUE

Ogunbodede, T.T. and Nwobodo, O.M.

Department of Applied Microbiology and Brewing
Enugu State University of Science and Technology, Enugu. Nigeria.

***Author for Correspondence:** titus.ogunbodede@esut.edu.ng

ABSTRACT

The main challenges associated with enzymatic hydrolysis are slow reaction rates, high enzyme costs and poor understanding of enzyme kinetics on malt substrates. Hence, this study was carried out to comparably analyse liquor obtained from enzyme and acid hydrolyses of rice malt using infusion mashing technique. Two conical flask containing fifty grams (50g) of rice grists each were hydrolyzed using enzymes (amyloglucosidase, -amylase, -amylase and proteinase) and dilute acid (H_2SO_4), respectively. After mashing, the resulting worts were analysed for their physicochemical properties. The worts were boiled with isomerised hop extracts, followed by cooling with subsequent pitching with yeast prior to primary fermentation which lasted for 5 days. The green beers were analysed for their physicochemical properties. The result showed that enzyme and acid hydrolyzed rice worts had original gravity of 1.031 and 1.019°, sugar of 7.8 and 4.83°Brix, pH of 5.2 and 2.9, viscosity of 1.94 and 2.6cp with adequate amounts of reducing sugars (glucose and maltose). The result of liquor analysis after primary fermentation gave specific gravity (1.006 and 1.013°ρ), sugar (1.54 and 3.32°Brix) pH (4.2 and 3.1), %alcohol (3.27 and 1.69%v/v) and apparent fermentability (80.64 and 18.75%). The work indicates that enzyme hydrolysis is preferred as it adequately and effectively converts more starch to fermentable sugars than in acid hydrolysis.

Keyword: Rice malts, enzyme hydrolysis, acid hydrolysis, infusion mashing.

INTRODUCTION

Beer can be characterized as an alcoholic beverage produced by the alcoholic fermentation of malted barley, hops, or their components or products, with or without unmalted grains, generally referred to as adjuncts, in potable brewing water. (Ogu, 2016).

The major raw materials used for beer or malt beverages making include: malt, brewer's yeast, hops and water. Malt is obtained through the process called malting. (Ogu, 2006).

Malting can be defined as the controlled germination of cereal grains (barley, sorghum, millet, wheat, rice etc.) to produce enzymes such as alpha-amylase, beta-amylase, lipase, esterase, protease etc. capable to breakdown complex food materials like carbohydrates, proteins, lipids etc. in the grain to the forms such as (glucose, amino acids, fatty acids, limit dextrinase and glycerol etc.) that can be metabolized by the yeast. Malting also involves modification of tissue structure of the grain to produce easily and friable grains. (Okafor, 2007). Malting aids include: Gibberellic (GA_3), Hydrogen peroxide, formaldehyde, ozone.

There are important steps in the manufacturing of beer, these steps could be summarized as follows: malting, grinding (milling), mashing, lautering, wort boiling, wort cooling, fermentation processes, clarification and maturation of beer during storage, chill proofing, carbonation, filtration, packaging, labelling, pasteurization, packaging and stamping of the cases (Wolfgang, 2010).

The greatest potential for ethanol production from starch sources lies in hydrolysis of the starch using enzymes and acids (Belton and Taylor, 2014). Due to the low enzyme content of rice malt, external enzymes and acids are thus required for industrial production of fermentable sugars from the available carbohydrates (Sarkodi *et al.,* 2014). Out of the two methods, acid hydrolysis is least desired as it is often accompanied with the formation of furans (Chamunorwa *et al.,* 2002). Difficulty in removing the acid, also adds to the cost of

production and possible chemical poisoning. The type of sugars produced have to be known since some sugars are not readily fermented by normal brewer's yeast (Etokakpan and Palmer, 2009).

Enzymatic hydrolysis of starch is still considered as one of the main limiting steps in beer production. The main challenges associated with enzymatic hydrolysis are slow reaction rates, high enzyme costs, and poor understanding of enzyme kinetics on malt substrates. There are limited studies on the use of acids in hydrolyzing starch used in brewing, hence, this study intend to compare the starch and acid hydrolysis of malt obtained from sorghum and their application in beer production.

Therefore, this work is aimed at comparative analysis of liquors obtained from enzyme and acid hydrolysis of rice malt through infusion mashing.

MATERIALS AND METHODS

Materials

Rice malt, hops, yeast (*Saccharomyces cerevisiae*), water and enzymes (amyloglucosidase, fungamyl-amylase), thermamyl (-amylase) and neutrase (proteinase)) and diluted Acid (H_2SO_4).

Analytical method

The methods of analyses employed in the evaluation of malt, wort and beer were based on the recommended methods of analyses of the Institute of Brewing (IOB) and American Society of Brewing Chemists (ASBC) (Agu and Palmer, 2005).

The rice malts were milled using milling machine to obtain coarse grits used for mashing. Two conical flasks were washed properly and labelled. The flasks were labelled as follows: Sample A = 50g of rice grist for enzyme hydrolysis and Sample B = 50g of rice grist for acid hydrolysis. The rice grists were weighed into the two well labelled conical flasks; 360ml of distilled water was added, 0.5ml of each of the enzymes were added to the enzyme flask and about 1ml of diluted Acid (H_2SO_4) was added to the acid flask. The content of the flasks were stirred properly and covered with foil. With the help of a water bath, mashing process commenced immediately. After mashing it was allowed to cool for 30 minutes. The mashes were filtered using sterile muslin cloth to obtain the worts and spent grains were removed. At the end of the mashing, the resulting worts were analysed for original gravity using hydrometer, pH with pH meter, flow rate through titrimetric method, other parameters determined included viscosity, reducing sugars and temperature. The worts were pitched with *Saccharomyces cerevisiae* in readiness for primary fermentation which lasted for five days. At the end of primary fermentation, the beers were analysed for specific gravity, pH, apparent fermentability, % alcohol and temperature, following the methods of analysis of the Institute of Brewing.

RESULTS

The result of the physicochemical analysis of wort obtained from mashing studies presented in Table 1 showed that enzyme and acid hydrolyzed rice worts had original gravity of 1.031 and 1.019°, sugar of 7.8 and 4.83°Brix, pH of 5.2 and 2.9, viscosity of 1.94 and 2.6cp with adequate amounts of reducing sugars (glucose and maltose).

Table 1: Result of the physicochemical analysis of the wort samples

Samples	Original gravity ($^0\rho$)	Sugar (°Brix)	pH	Temperature (^{0}C)	Flow rate (s)	Viscosity (cp)	Reducing sugars(mg/l) Glucose	maltose
A	1.031	7.8	5.2	29	43.23	1.94	60.15	50.21
B	1.019	4.83	2.9	29	58	2.6	98.1	81.17

KEY: A = Wort from enzyme hydrolysis, B = Wort from acid hydrolysis

Table 2 showed result of liquor analysis after primary fermentation and the result gave specific gravity (1.006 and 1.013$^0\rho$), sugar (1.54 and 3.32°Brix) pH (4.2 and 3.1), % alcohol (3.27 and 1.69%v/v) and apparent fermentability (80.64 and 18.75%). The result gave the quality of acceptable liquor produced through enzyme hydrolysis with lesser quality liquor produced through acid hydrolysis.

Table 2: Result of properties of Liquors after primary fermentation

Samples	Specific gravity (^{0}p)	Sugar (°Brix)	pH	%Alcohol (v/v)	Apparent fermentability (%)
A	1.006	1.54	4.2	3.27	80.64
B	1.013	3.32	3.1	1.69	18.75

KEY: A = Liquor from enzyme hydrolysis, B = Liquor from acid hydrolysis

DISCUSSION

Liquors were obtained from enzyme and acid hydrolyses of rice malts from infusion mashing. Comparatively, from the analysis, the results obtained generally showed that enzyme hydrolysis produces liquor of acceptable properties than acid hydrolysis using rice malts. This could be attributed to the fact that enzymes hydrolysis substantially degraded the rice malts to fermentable sugars readily and rapidly to achieving saccharification. This enable the viable yeast strain introduced into the wort to achieve faster fermentability rate. This result is in accordance with **Yonkova** *et al.* (2007). The result of the study showed lesser production of sugar from the liquor produced from acid hydrolysis whereas higher sugar production of sugar was seen in liquors produced from enzyme hydrolysis. The above observation was probably due to substrate inhibition. This confirms that enzyme preferably attack carbohydrates in solution than acid. This is in accordance with the results of Evans (2013).

But unlike the acid hydrolysis of rice malts the effects of acidity on acid hydrolyzedworts, affected its rate of fermentability noticeably there was a substantial fall in the gravity of enzyme hydrolyzed wort, the fall in gravity of the enzyme hydrolyzed wort also necessitated the increase in percentage alcoholic. Even with the addition of sodium sulphate which is a salt of sulphuric acid to acid hydrolyzed wort, the wort solution partially show a clear solution. This observation is contrary with the findings of Shewale and Pandit (2009) who observed that acid hydrolysis showed great potential in converting starch in sorghum to a simple fermentable sugars.

The low effectiveness of acid hydrolysis in this study might be as a result of the type of acid used or the concentration of the acid applied. As Łukajtis *et al.,* (2018) concluded that an increase in sugar yield was proportional to both acid concentration and the temperature employed.

CONCLUSION

It can be concluded that rice has a great potential for producing beer of acceptable quality when supplemented with hydrolytic enzymes. The result of this study indicated that acid hydrolysis cannot effectively hydrolyze starch but use of enzyme can effectively convert starch into simple fermentable sugars as seen in this study. Use of acid is therefore not advisable. Use of enzyme hydrolysis is preferred as it adequately and effectively converts more starch to fermentable sugars than in acid hydrolysis. The problem of low wort pH from acid hydrolysis could be managed by adding Na_2CO_3 solution.

REFERENCES

Agu, R.C. and Palmer, G.H. (2005). "Enzymic breakdown of endosperm proteins of sorghum at different malting temperatures. Journal of the Institute of Brewing, 102(8): 415-418.

Belton, P.S. and Taylor, J.R.N. (2014). Sorghum and Millet/Protein sources for Africa. Trends Food Science and Technology, 15: 94-98.

Chamunorwa, G., Neelakantan, S., Mohnaty, A, Jai, K. and Kaushik, T. (2002). Production and use of microbial enzymes. Journal of Current Science,195: 22-27.

Etokakpan, O.U, and Palmer, G.H. (2009). A simple diamylase procedure for the estimation of alpha and beta amylase. Journal of Institute of Brewing, 96: 89-91.

Evans, E. (2013). The impact of the thermostability of alpha-amylase, beta-amylase, and limit dextrinase on potential wort fermentability. Journal of the American Society of Brewing Chemists, 61: 210-218.

Łukajtis, R., Kucharska, K., Hołowacz, I., Rybarczyk, P., Wychodnik, K., Słupek, E., Nowak, P. and Kaminski, M. (2018). Comparison and optimization of saccharification conditions of alkaline pre-treated triticale straw for acid and enzymatic hydrolysis followed by ethanol fermentation. Energies, 11: 639-662.

Ogu, E. O. (2006). Brewing Adjuncts. In: Fundamentals of Brewing Science and Technology, (Ogu ed). JohnBaz Publishers, Enugu. pp: 119-124.

Ogu, E. O. (2016). Technology of Malting: Beer. In: Trends in Brewing Science. JohnBaz Publishers, Enugu. pp: 1-47.

Okafor, N. (2007). Production of Beer. In: Modern Industrial Microbiology and Biotechnology, Science Publishers, Enfield, New Hamsphire 03748, USA. pp: 237-261.

Sarkodi, P. A., Agyapong, D., Dawuona, S., Dwomoh, A. J. and Owusu-Ansah,E. (2014). Improving the saccharification of sorghum mash with supplementary enzymes from local crops. Industrial Journal of Science Resources and Technology, 2(3): 71-78.

Shewale, S. D. and Pandit, A.B. (2009). Enzymatic production of glucose from different qualities of grain sorghum and application of ultra sound to enhance the yield. Carbohydrate Research, 344: 52-60.

Wolgang, K. (2010). Wort Production. In: Technology Brewing and Malting. VLB Publishers, Berlin. pp: 224-412.

Yonkova, G., Georgieva, N., Ginova, T. and Terzi, A. (2007). Biochemical processes by mashing and characterization of the fermentation of feed Barley during brewing. Journal of the University of Chemical Technology and Metallurgy, 42(4): 407-412.