

AMINO ACIDS PRODUCTION THROUGH HUMAN WASTE HAIR

PALETI NAGARJUNA¹, SANJAY VERMA², ASHOK SHARMA³ AND SARITA SHARMA⁴

Department of Chemical Engineering, Ujjain Engineering College, Ujjain, M.P., India

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ABSTRACT

Amino acid is an amphoteric compound of long polypeptide chain derived from human waste hair. Optimum conditions for amino acid based plant booster production from human waste hair through the hydrolysis process were determined. For 1000 g of dried washed hair, 6 N HCL solution, at a reaction temperature 100 °C for until settling down of hair in the round bottom flask, then hydrolysis process continues at 60 °C for 6 hours were observed as optimum conditions for production of amino acids by the analysis of different experimental conditions. The effect of reaction temperature, reaction time, and catalyst concentration was studied to reconfirm optimum conditions. At these optimum conditions 78.60% yield of amino acid obtained and 14 types of amino acids were extracted from human waste hair.

KEY WORDS : Amino acids, Hydrolysis, Human waste hair.

INTRODUCTION

Environmental pollution and shortage of conventional fertilizers are two critical challenges, which need to be addressed. Currently, human waste hair is considered as a waste material in most parts of the world, and its accumulation in waste stream causes various environmental problems (Gupta, 2014). Recovery of useful materials and degradation of human waste hair in the society is needed for the environmental concern. The human waste hair contains twenty kinds of amino acids (Staron *et al.*, 2011), these amino acids are recovered by using hydrolysis process. This amino acid mixture is used as a plant booster for plant, it gives growth, plants metabolism, protein synthesis, and defense against insects (Kumar and Shah, 2017). Conventional fertilizers are not regarded as sustainable and questionable from the economic, ecological and environmental point of views (Chen, 2006). Among alternatives, amino acid based bio fertilizers derived from keratin waste (feathers, hog hair, fish waste and human waste hair) are most promising fertilizer sources because they are renewable and environmentally friendly (Cheng *et al.*, 2008). Amino acid can be defined as any liquid or

powder form that can be produced from keratin waste. Amino acid can be produced by hydrolysis in the presence of an acidic catalyst (Yan *et al.*, 2013). Among the available raw materials, human waste hair is cheaper and easier to collect than other keratin waste; hence this could possibly be the best raw material for amino acid production as cost of production will be reduced.

MATERIALS AND METHODS

In the present experimental work, human waste hair was collected from a local barber shop. Analytical grade acid catalyst and decamol powder was purchased from local the markets. Pre-treatment of human waste hair was done by shampoo and it was then washed with lukewarm water. This helps to remove impurities, moisture, natural oil, and stricken oil.

Hydrolysis process

Hydrolysis has been revealed as a powerful and useful tool for producing hydrolyzed from proteins using human waste hair as raw material. By a stringent control of simple reaction parameters as acid concentration, temperature and time, it is

(¹M. Tech. scholar, ^{2,3,4} Professor)

*Corresponding author's email: paletinagarjuna99@gmail.com

possible to the conversion of proteins into amino acids (Marcet *et al.*, 2016). Hydrolysis could be a reaction involving the breaking of molecule using water. After drying of hairs, 1000 g (30%) of the hair were taken into a round bottom flask (5000 ml). 3300 ml of 6 N/5N HCL solution was poured in the round bottom flask. After fixing the condenser to flask, the flask was heated for hydrolysis for 5/6/7 hours. Temperature was kept at 100 °C initially till the hairs settled down. Then for next 6 hours temperature was kept at 60 °C/40°C. After 6 hours, TCA (Trichloro Acetic Acid) test was performed. For this 5 ml filtered hydrolyzed solution and 1 ml of 20% TCA solution was taken and diluted with 4 ml distilled water in a test tube and mixed thoroughly. No precipitation observed in test tube, this indicate proper hydrolysis of the solution.

Optimum conditions for hydrolysis

Optimum conditions were taken from the ranges found in previous studies. For this 30 to 40 % (weight ranging 1000 to 1500 g) of the hair and 5N HCL to 6 N HCL solutions were taken (30% weight of the hair/volume of the solution). 100 °C hydrolysis temperature was taken for first 1 hour and then at 40 °C to 60 °C for the next 6 hours reaction. Optimum conditions for production of amino acids are presented in Table 1.

Determination of amino acid content

The amino acid content of the hydrolyzed was determined by (HPLC) reverse phase high performance liquid chromatography (Esteban *et al.*, 2010). Three-hundred micro liter of hydrolyzed mixture were mixed with 15 µl of internal standard (2.5 mM amino butyric acid) and dried under vacuum. The sample was dissolved in 300 µL of 20 mM HCL and 10 µL were coupled with AccQ-Fluor (aminoquinolil-N-hidr- oxysuccinimidyl carbamate) for further analysis by reverse-phase HPLC using a C18 column (WATERS 600) and UV detector (WATERS 2487). In this case 14 kinds of amino acids

were determined from hair hydrolyzed mixture.

RESULTS AND DISCUSSION

The effects of catalyst concentration, reaction temperature, and reaction time on amino acid yield were seen. Percentages of amino acids obtained from human waste hair at optimum condition are also presented.

Amino acids yield from human waste hair

Maximum yield of amino acid was obtained at 1000 g of hair and 3300 ml of 6 N HCL were taken (30 % weight of hairs/volume of the solution) and at temperature combination of 100 °C initial and 60°C later during hydrolysis for 6 hours. On combination of hydrolysis volume of mixture obtained was 4100 ml. This mixture filtered twice to get final product of 3220 ml (78.60%) of amino acid mixture.

Effect of lower and higher acid concentration on amino acid yield

Amino acid production was done with higher and lower acid concentrations. For higher acid concentration 1701 ml of concentration HCL was diluted with 1599 ml water to get 3300 ml of 6 N HCL. Similarly for lower acid concentration 1417 ml concentration HCL was diluted with 1883 ml water to get 3300 ml of 5 N HCL. Keeping other variables constant, 30% hairs (1000 g weight of hairs) were taken as per volume of the solution (30% w/v). Initially temperature was kept at 100°C for settling of hair and then 60°C for the next 6 hours for hydrolysis. The lower acid concentration decreased the yield of amino acids. Both increase and decrease of initial acid quantity have a negative influence on the amino acid production (Esteban *et al.*, 2010). The observed behaviour seems to be due to the reduced solubility of keratin in water. In case of lower acid quantity 11 types of amino acids are extracted from hair. Asparatic acid has the highest yield and Methonine is the lowest yield among amino acids.

Table 1. Optimum conditions for amino acids production from human waste hair (Yadav and Katekar, 2015).

Process factors	Suggested range	Used in present study	Optimum obtained in present study
Hair percentage	30% to 40% (1000 to 1500 g weight of hairs)	30% (1000 g weight of hairs)	30% (1000 g weight of hairs)
Acid catalyst	5N HCL to 6N HCL	5N HCL, 6N HCL	6N HCL
Temperature	40 °C to 110 °C	40 °C-100 °C, 60 °C-100 °C	100 °C- 1 hour 60 °C – 6
hours			
Time	5 to 7 hours	5, 6 , 7 hours	6 hours

Threonine, glutamic acid and lysine were not yielded in the present experiment. The minimum yield of amino acid was obtained by low quantity of acid, because of absence of proper reaction between hair and solution. The higher acid quantity increased the yield of amino acid. In case of higher acid quantity 14 types of amino acids were extracted from hair. Aspartic acid has the highest yield and Threonine is the lowest yield among amino acids. The maximum yield of amino acid was achieved by use of high quantity of acid, because of hard surface of the long polypeptide bonds were separated by the higher acid concentration conditions only. The results of higher and lower concentrations are presented in the Fig. 1.

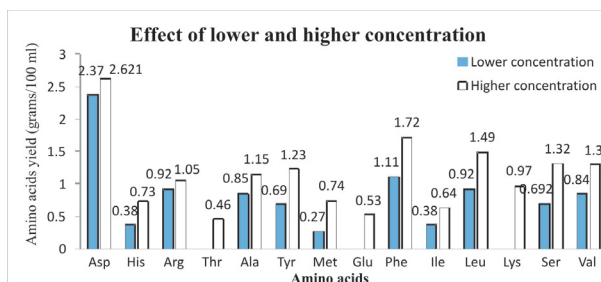


Fig. 1. Effect of lower and higher acid concentration on amino acid yield.

Effect of lower and higher temperature on amino acid yield

Temperature was a crucial parameter for the yield of amino acid (Esteban *et al.*, 2010). Amino acids production was done with higher and lower temperature conditions (60 °C and 40 °C). Keeping the variables constant, 1000 g of hair and 3300 ml of 6 N HCL solution were taken (30% w/v). Initially temperature was kept at 100°C for settling of hair and then 60 °C and 40 °C for next 6 hours for hydrolysis in two experiments. The lower reaction temperature decreased maximum yield on amino acids. The reaction rate was slow at low temperature due to the diffusional resistance. In case of lower temperature condition 12 types of amino acids were extracted from hair. Arginine has the highest yield of and methonine is the lowest yield among amino acids. Histidine and valine are not yielded in the present experiment. The less yield of amino acids was obtained at lower temperature condition, this may be because absence of proper reaction between hair and solution at low temperature. The higher reaction temperature caused a high yield of amino acid. In case of high temperature condition 13 types

of amino acids were extracted from hair. Arginine and glutamic acid are the highest and lowest yield of amino acids. The higher temperature which gave the higher yield of amino acids shows a good agreement to that for the maximum dissociation constant of the water. In the presence of hydronium and hydroxide ions, peptide bonds are broken down into smaller molecules of soluble protein or amino acids. The effects of lower and higher temperature on amino acid yield are presented in Fig. 2.

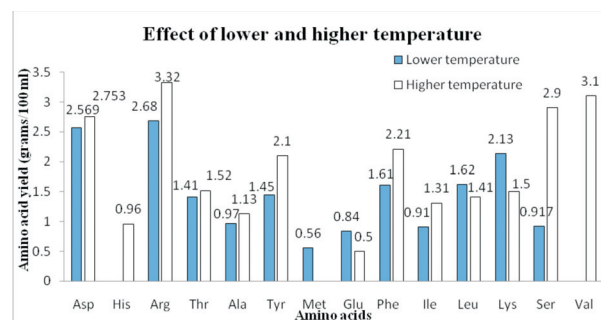


Fig. 2. Effect of lower and higher temperature on amino acid yield.

Effect of lower and higher reaction time on amino acid yield

Reaction time of hydrolysis process was an essential parameter for the yield of amino acids (Esteban *et al.*, 2010). To observe the effect of higher and lower reaction time 5 and 7 hours reaction time were taken for the experiment. 1000 g of hairs and 3300 ml of 6 N HCL solution were taken (30% w/v). Initially temperature was kept at 100 °C for settling of hair and then 60 °C for next 5 and 7 hours for hydrolysis in two sets of experiments. At lower reaction time the yield of amino acids was less. This may be because of reaction rate was slow in the entire process. In case of lower reaction time 13 types of amino acids were extracted from hair. Aspartic acid has the highest yield and threonine has the lowest yield of amino acids. Glutamic acid was not yielded in present experiment. At higher reaction time the yield of amino acids was higher. In case of higher reaction time 14 types of amino acids were extracted from hair. Arginine, serine, valine were among the highest yield of amino acids and lowest yield of amino acid was glutamic acid. Higher reaction time increases the yield of amino acids, because of increasing time has an greater effect on extraction of more amino acids. If the reaction time of hydrolysis process increase above 7 hours, then higher rate of production and destruction of amino acids may take

place. The effects of lower and higher reaction time on amino acid yield are presented in Fig. 3.

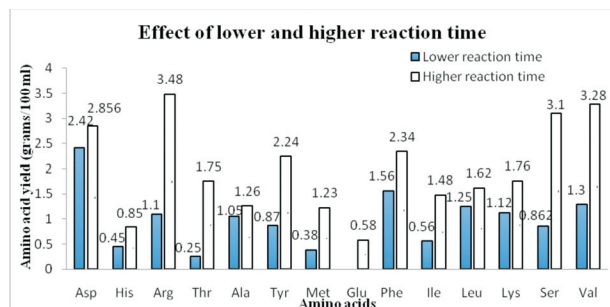


Fig. 3. Effect of lower and higher reaction time on amino acid yield.

CONCLUSION

In present study a range of optimum conditions were taken from previous studies and effects of different variables on yield of amino acids was seen. Hydrolysis is an effective process for the conversion of human waste hair into amino acids. It is found that at optimum conditions, human waste hair gives a maximum percentage yield of amino acid along with yield of different types of amino acids. Moreover, this study also presents the effect of catalyst concentration, reaction temperature, and reaction time on yield of amino acid to reconfirm the maximum yield at optimum conditions.

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