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"Comparison of the amino acids extraction from human and animal hair"

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Abstract: Amino acids are the building blocks of proteins, and they can be extracted from human and animal hair through a process called hydrolysis. Hydrolysis is a chemical reaction that breaks down proteins into their constituent amino acids. In this work, the efficacy of hydrolysis to extract amino acids from human and animal hair was examined. 100 grams of hair samples were hydrolyzed for 4-6 hours at a temperature between 40 and 60 degrees using a 330ml of 6N hydrochloric acid solution. After hydrolysis, sodium hydroxide was used to raise the pH of the resultant solutions to 7. Several assays were used to confirm the presence of amino acids, and then high-performance liquid chromatography analysis was performed for analyzing the amino acid content in the mixture. According to research data, it has been found that human hair has a higher amino acid concentration than that of animals. The work validates the efficiency of hydrolysis for amino acid extraction and offers insightful information for potential future uses. Maximum amino acid content was obtained from both human and animal hair samples after a 6-hour hydrolysis process, demonstrating how human hair is superior in respect of amino acid content.

IndexTerms **- Amino acids, human hair, animal hair, hydrolysis**.

Introduction

Hairs are intricate biological structures that are formed primarily of the protein keratin in both humans and animals. Both human and animal hairs include a variety of amino acids that are essential for hair growth and development, despite the fact that the structure and function of hair vary depending on species and where it is found on the body[1].

It has become more significant to extract amino acids from human and animal hair because of the potential applications in medicine, forensics, and ecology. A person's health, nutritional state, and exposure to toxins from the environment can all be learned from amino acid analyses[2]. Proteins are made up of amino acids, which are necessary for life. All living things, including people and animals, contain them. A number of different amino acids make up the protein that makes up hair. Depending on the animal's species, age, and state of health, the amino acid composition of its hair can change[3]. The comparison of the amino acid makeup of human hair with animal hair was the aim of this investigation. The investigation involved hydrolyzing the amino acids from both human and animal hair. Proteins can be chemically broken down into their individual amino acids through a process called hydrolysis[4]. After that, high-performance liquid chromatography was used to analyze the amino acids.

The hair samples came from a range of places, including animal farms, pet stores, and salons for people. To get rid of any dirt or debris, the hair samples were cleaned with soap and water. After that, the hair samples were weighed and put in a beaker[5]. The beaker was filled with a hydrochloric acid (HCl) solution. To find the ideal circumstances for amino acid extraction, the HCl content was changed. The beaker was then heated to 100 °C for one hour in a water bath. The water bath's temperature dropped from 40 °C to 60 °C after an hour. An additional 4, 5, and 6 hours were given for the beaker to soak in the water bath. The beaker was taken out of the water bath and allowed to cool to room temperature for six hours. After cooling, the solution was transferred to a centrifuge tube and spun for 10 minutes at 10,000 rpm. A fresh centrifuge tube was used to collect and transfer the supernatant. A sodium hydroxide solution was then used to neutralize the supernatant. A pH meter was then used to change the solution's pH to 7. HPLC was then used to analyze the neutralized solution. A sample's amino acids can be separated, recognized, and measured using HPLC, a potent analytical method. Water and acetonitrile were used in a gradient to elute the HPLC column. Using a UV detector, the amino acids were found. Our study's findings demonstrated that the amino acid makeup of human hair and animal hair differed noticeably. The concentrations of the amino acids arginine, lysine, and histidine were shown to be greater in human hair. Glycine, alanine, and valine were discovered to be present in higher concentrations in animal hair.

2. Materials and methods

2.1 Selection process

In the present study, animal (goat) hair was retrieved from a nearby farm in Kolhapur district while human waste hair was collected from a local barbershop. bought sodium hydroxide, a neutralizer, and hydrochloric acid, an analytical-grade acid catalyst, from nearby vendors. Hair from both human and animal waste was pre-treated with cleaner before being rinsed with warm water. This helps to remove impurities, moisture, natural oil, and impure oil.

2.2 Instrumentation

The Agilent 1100 series LC system with a photodiode array detector was used to design and validate the method. Agilent technology and the Chemstation software are used to perform liquid chromatography (LC).

3. Results and Discussion

3.1 Experimental process

The procedure of acid hydrolysis is used to remove amino acids from both human and animal hair. Both human hair and animal hair samples are first cleaned with distilled water and a mild detergent to get rid of any external impurities. The samples are then chopped into little pieces with a scalpel or scissors after being dried in a desiccator.

fig.1: hair samples of animal and human

A 330 ml solution of 6N hydrochloric acid (HCl) is added to the hydrolysis tube or vial containing the 100 grams of hair samples, covering the hairs. After that, the tube is sealed and heated for four, five, or six hours to denature the proteins into their individual amino acids. Initially, the temperature was held at 100 ℃ until the hairs began to settle. After that, the temperature was held at 50 ℃ or 60 ℃ for the following 4 /5 /6 hours. Each hair sample underwent three sets of 4,5 and 6 hours of tests. The ideal representation of the experimental setup created for the hydrolysis process is shown in fig.2 below.

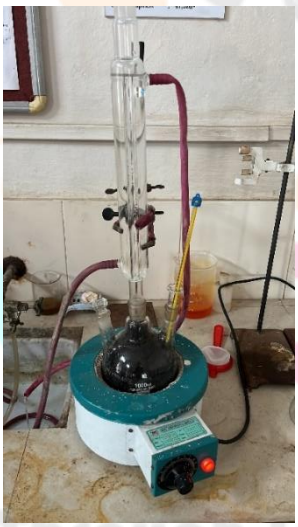

fig.2: experimental setup for the hydrolysis process

The acid solution is neutralized by adding a solution of sodium hydroxide (NaOH) until the pH of the solution is around 7.0 after hydrolysis, after which the hydrolysis tube is allowed to cool to ambient temperature[6]. The neutralization procedure is crucial to stop further amino acid breakdown and to get the sample ready for further testing. Fig. 3 illustrates the same neutralization process.

fig.3: neutralizing the acid solution by adding the desired amount of sodium hydroxide

To get rid of any insoluble components like hair fibers and cell debris, the hydrolysate is filtered via filter paper or a syringe filter[7]. The free amino acids that were isolated from the hair samples and included in the resultant filtrate are depicted in fig. 4.

fig.4: filtration is done using normal filter paper.

TCA (Trichloro Acetic Acid) testing was done after six hours. For this, 4 mL of distilled water was diluted with 1 mL of 20% TCA solution in a test tube containing 5 mL of filtered hydrolyzed solution[8]. No precipitation was seen in the test tube, which suggests that the solution was properly hydrolyzed and that there were amino acids present. High-performance liquid chromatography (HPLC) is used to determine the amino acid profile of the hydrolysate.

3.2 High-performance liquid chromatography analysis

3.2.1 Column selection

Since amino acids are notorious for being hydrophobic, a method development trial using unique column chemistry was launched in place of conventional C8, C18, etc. columns. Merck HILIC (zwitterionic bonded phases) column, Acclaim mixed mode (reverse phase and weak anion exchange property in single column), and finally freeze the Kromasil SIL column with 250 mm length, 4.6 mm diameter, and 5 m particle sizes were among the different column chemistry that was screened.

3.2.2 Mobile phase selection

The procedure was tested utilizing a Silica column and a variety of buffers, including phosphate, ammonium format, and ammonium acetate. The method development used phosphate buffer because amino acids are UV inactive. The different compositions of mobile phase A, 2.5 mM potassium dihydrogen phosphate, and mobile phase B, acetonitrile, were tested, and it was discovered that an increase in the percentage of acetonitrile increased retention time and a tailing factor while a decrease in the percentage of acetonitrile decreased both. The procedure has been refined using 75% acetonitrile, with a tailing factor of 0.8 to 1.2 and a retention duration of 6 to 15 minutes. For each amino acid, the plate number and tailing factor have been calculated. The approach has been tested for several pH ranges (pH from 2.0 to 7.5), and it was discovered that the lower pH is more tailing-prone while the higher pH is less so. Due to the high pH, only Aspartic and Glutamic acids were discovered to have a significant impact on retention time and tailing factor. The remaining 15 amino acids were not detected. In the end, mobile phase A with a pH of 2.85 was selected for routine analysis because the tailing factor is within range and the retention of glutamic and aspartic acids on the column.

3.2.3 Analyzing High-Performance Liquid Chromatography Data

The statistical analysis of amino acids obtained from both human and animal hairs entails a number of procedures, including data cleaning and preparation, descriptive statistics, data visualization, statistical testing, analysis, and interpretation[13]. Three human hair samples, three animal hair samples, and one analysis of a standard amino acid were used in a total of seven HPLC tests on amino acids extracted from human hairs and animal hairs in order to compare the results with those from the other six samples[14]. The standard sample, as seen in fig. 5, displays readings of pure amino acids that may be compared to the data from the human and animal hair samples.

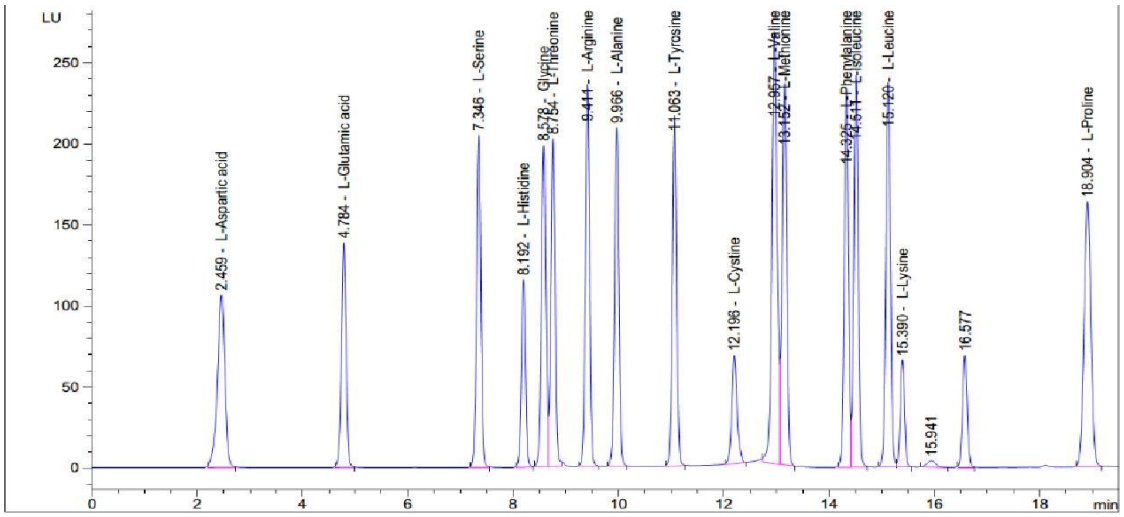

fig.5: hplc chromatogram of amino acids after hydrolysis of standard amino acid

Standard amino acids were used for the HPLC analysis, and several types of amino acids were produced during the testing[9]. These amino acids include phenylalanine, aspartic acid, serine, glutamic acid, histidine acid, glycine, threonine, arginine, alanine, valine, tyrosine, phenylalanine, isoleucine, proline, etc. The percentage of each type of amino acid in the standard sample, along with its absorbance area and percent, are shown in table no. 1 below.

table no.1: the results of hplc analysis of amino acids of the standard amino acid.

Name of Acid	Absorbance	%
L- Aspartic Acid	250	**1.55**
L- Serine	416	**2.58**
L- Glutamic Acid	1230	**7.63**
L- Histidine	339	**2.10**
Glycine	289	**1.79**
L-Threonine	1467	**9.10**
L-Arginine	1675	**10.39**
L- Alanine	1076	**6.68**
L- Tyrosine	276	**1.71**
L- Valine	1598	**9.91**
L- methionine	785	**4.36**
L- Phenylalanine	843	**5.23**
L- Isoleucine	553	**3.43**
L- Lysine	432	**2.68**
L-Leucine	426	**2.17**
L_ Proline	5674	**35.20**
Total	**16118**	100

The six samples were also examined in a similar manner, and the results are shown below. First, the human hair samples are shown in Figures 6, 7, and 8, where the concentration of amino acids is relatively high and the adsorbent area is reflecting the same.

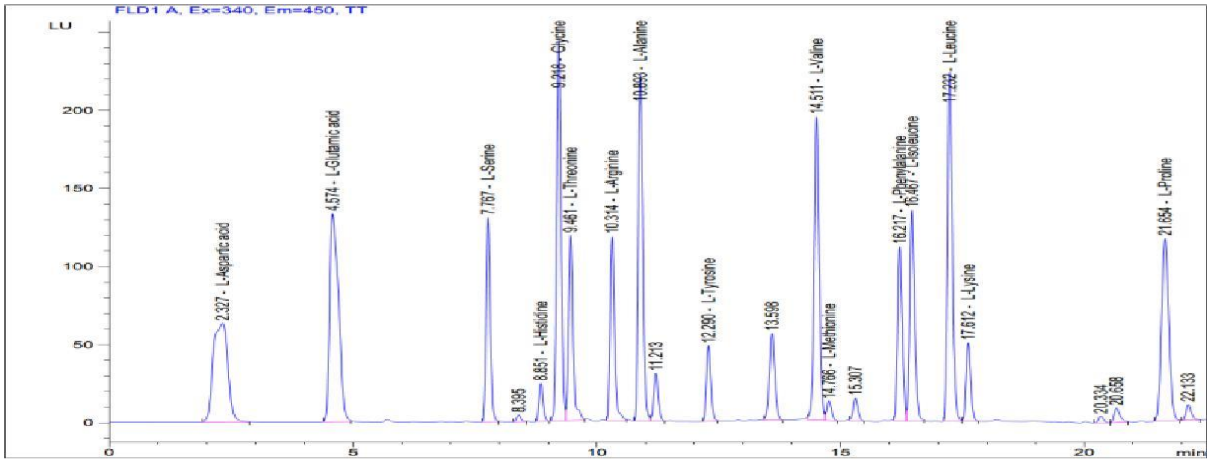

fig.6: hplc chromatogram of amino acids after hydrolysis of human hair sample (4hr)

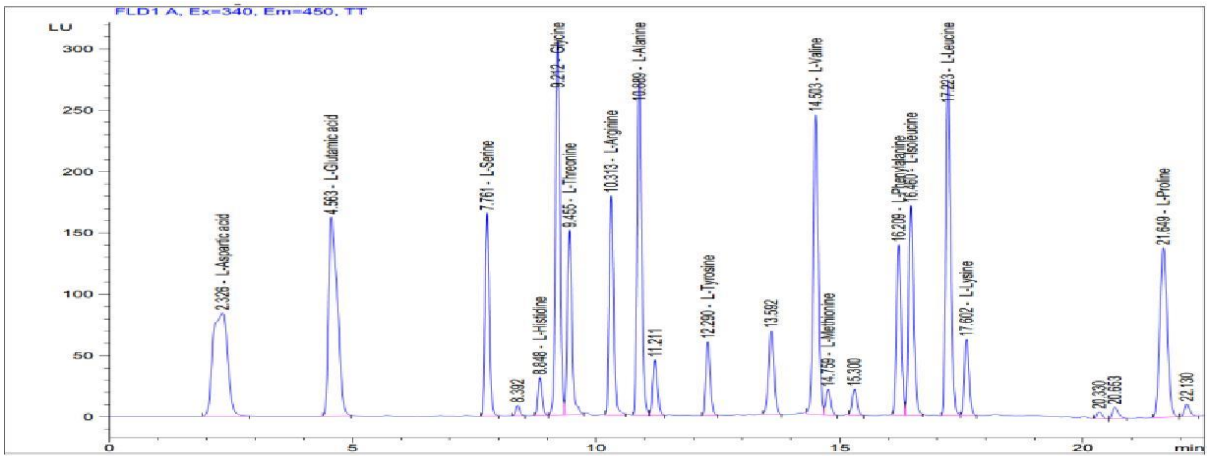

fig.7: hplc chromatogram of amino acids after hydrolysis of human hair sample (5hr)

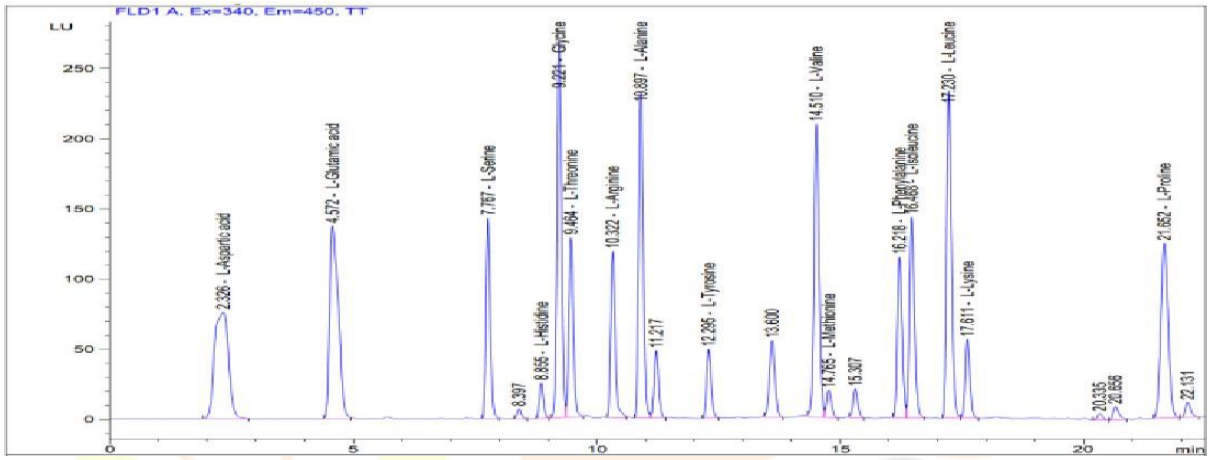

fig.8: hplc chromatogram of amino acids after hydrolysis of human hair sample (6hr)

The investigations below were carried out on human hairs in a similar manner. The information in table no. 2 shows the percentages of the various amino acids in each of the three samples of human hair as well as their absorbance area and percent.

table no.2: the results of hplc analysis of amino acids of human hair samples

	4		5		6	
Name of Acid	**Absorbance**	**%**	**Absorbance**	**%**	**Absorbance**	**%**
L- Aspartic Acid	1568	**8.66**	1526	**8.33**	1534	**8.29**
L- Serine	1230	**6.79**	1212	**6.61**	1245	**6.73**
L- Glutamic Acid	1469	**8.11**	1439	**7.85**	1459	**7.88**
L- Histidine	930	**5.14**	897	**4.89**	942	**5.09**
Glycine	1785	**9.86**	1782	**9.72**	1784	**9.64**
L-Threonine	1126	**6.22**	1106	**6.03**	1125	**6.08**
L-Arginine	1135	**6.27**	1129	**6.16**	1139	**6.16**
L- Alanine	1746	**9.64**	1789	**9.76**	1788	**9.66**
L- Tyrosine	1024	**5.65**	1025	**5.59**	1019	**5.51**
L- Valine	1705	**9.41**	1898	**10.36**	1899	**10.26**
L- Phenylalanine	845	**4.67**	843	**4.60**	843	**4.56**
L- Isoleucine	1201	**6.63**	1325	**7.23**	1326	**7.17**
L- Lysine	1022	**5.64**	1001	**5.46**	1012	**5.47**
L- leucine	1756	**9.56**	1759	**8.98**	1689	**8.69**
L_ Proline	1325	**7.32**	1356	**7.40**	1389	**7.51**
Total	**18111**	100	**18328**	100	**18504**	100

When compared to human data, the amino acid composition of animal hairs in HPLC is low. In fig. 9, the absorbance area is small. Over time, it gradually grows and then stays constant, as shown in figs. 10 and 11.

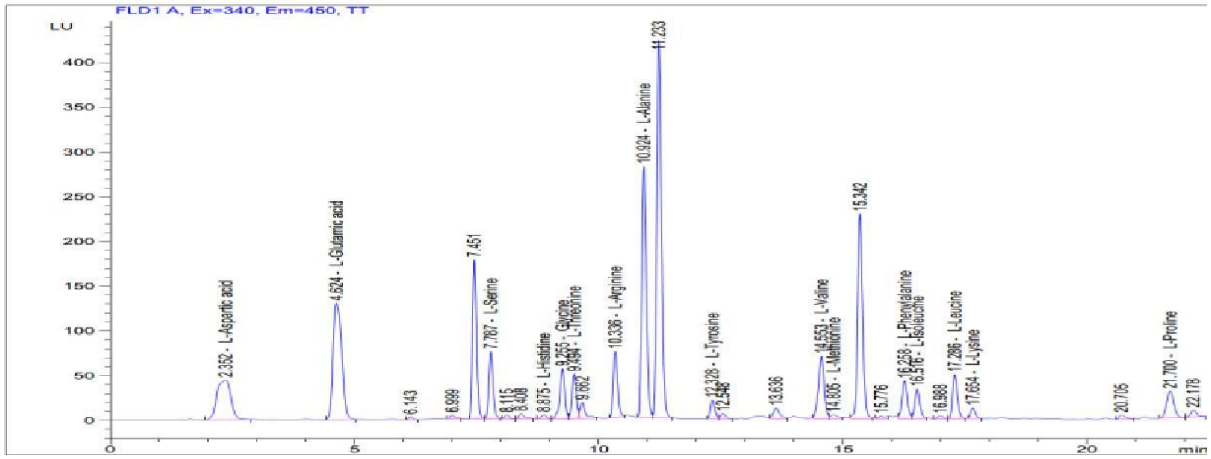

fig.9: hplc chromatogram of amino acids after hydrolysis of animal hair sample (4hr)

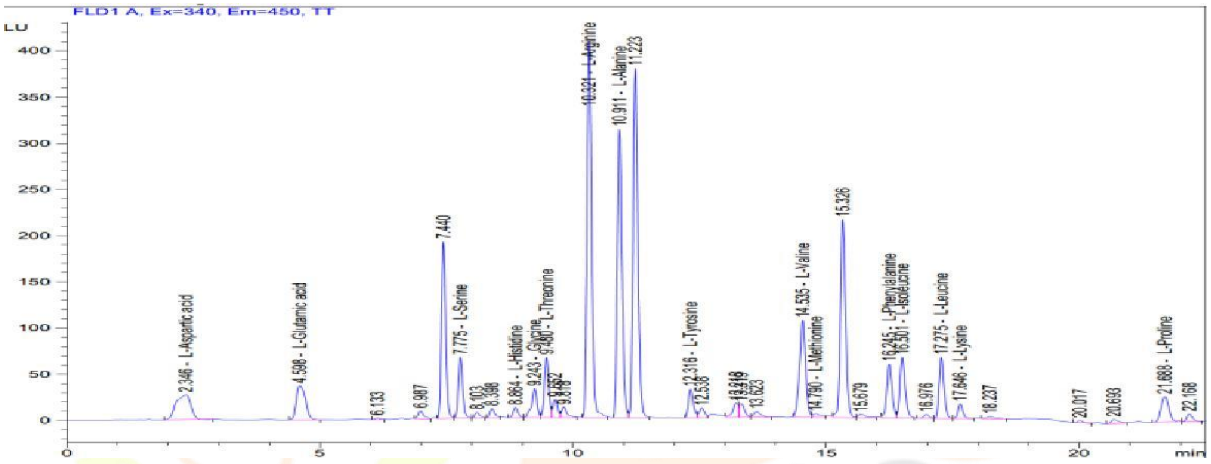

fig.10: hplc chromatogram of amino acids after hydrolysis of animal hair sample (5hr)

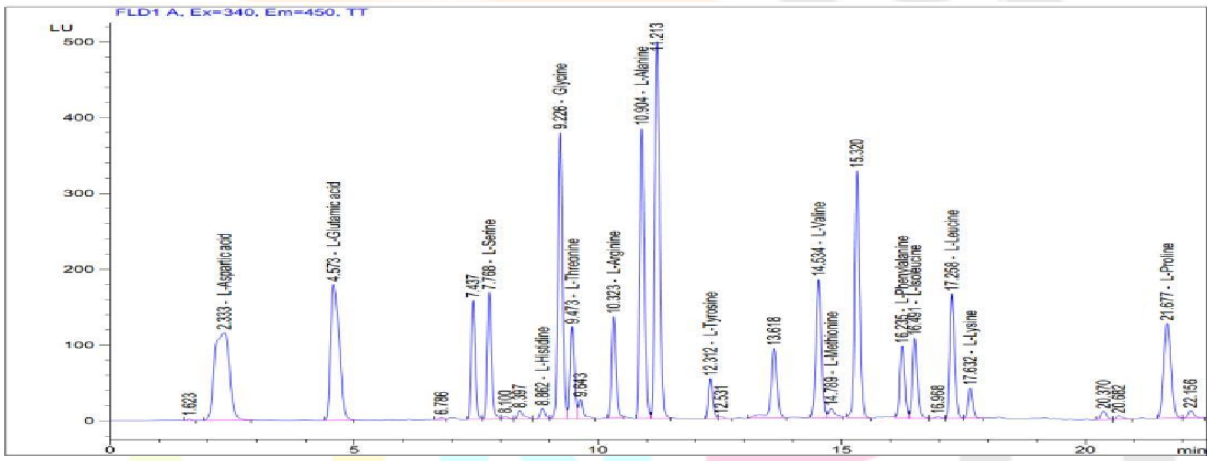

fig.11: hplc chromatogram of amino acids after hydrolysis of animal hair sample (6hr)

The information about the percentages of various amino acids in table no. 3 of the three animal hair samples, as well as their absorbance area and percent, is provided below.

table no.3: the results of hplc analysis of amino acids of animal hair samples

	4		5		6	
Name of Acid	**Absorbance**	**%**	**Absorbance**	**%**	**Absorbance**	**%**
L- Aspartic Acid	342	**3.35**	288	**1.78**	486	**2.94**
L- Serine	583	**5.71**	432	**2.67**	945	**5.72**
L- Glutamic Acid	133	**1.30**	659	**4.08**	1273	**7.70**
L- Histidine	488	**4.78**	289	**1.79**	318	**1.92**
Glycine	391	**3.83**	544	**3.37**	2982	**18.04**
L-Threonine	543	**5.32**	598	**3.70**	873	**5.28**
L-Arginine	5376	**52.63**	6327	**39.15**	578	**3.50**
L- Alanine	301	**2.95**	3879	**24.01**	943	**5.70**
L- Tyrosine	283	**2.77**	370	**2.29**	3532	**21.36**
L- Valine	503	**4.92**	788	**4.88**	485	**2.93**
L- Phenylalanine	122	**1.19**	486	**3.01**	943	**5.70**

L- Isoleucine	243	**2.38**	523	**3.24**	632	**3.82**
L- Lysine	254	**2.49**	509	**3.15**	672	**4.06**
L- Leucine	345	**3.52**	586	**3.59**	356	**2.35**
L_ Proline	652	**6.38**	467	**2.89**	1872	**11.32**
Total	**10214**	100	**16159**	100	**16534**	100

3.3 Optimum Conditions for Hydrolysis

According to the aforementioned statistics, the ideal parameters for improved hydrolysis are between 30 and 40% (100 to 150 grams of hair weight), and 6 N HCL solutions were used to treat this (30% hair weight/volume of solution), which required 330 ml of HCl. For the first hour, the hydrolysis temperature was set at 100 °C, and for the following five hours, it was between 40 °C and 60 °C. table no. 4 shows the ideal conditions for amino acid production and contrasts them with earlier findings from studies on the hydrolysis of amino acids.

table 4. optimum conditions for amino acids extraction from human and animal waste hair samples.

Process factors	Suggested range	Used in the present study	The optimum in present study
Hair percent	30% to 40%(150 grams weight of hair)	30 % (100 grams of hair)	30 % (100 grams of hair)
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,40-50°C for next 5 hours
Time	5 to 7 hours	4,5,6 hours	6 hours

Conclusion

In summary, the goal of this study was to examine the hydrolysis method's extraction of amino acids from human and animal hair. Based on prior research, the ideal conditions were established using 100-gram hair samples that were subjected to 330 ml of 6N HCl solution. For both human and animal hair samples, the hydrolysis procedure was carefully regulated, with temperatures ranging from 100 °C for the first hour to 40 °C-60 °C for the following 4, 5, and 6 hours. Three of the six trials were focused on each type of hair. Following hydrolysis, the resultant solutions were brought to a pH of 7 using sodium hydroxide, and trichloroacetic acid was used to confirm the presence of amino acids. The amino acids present in the samples were then separated, identified, and quantified using high-performance liquid chromatography (HPLC). The HPLC study consistently showed that human hair has a higher amino acid content than animal hair[10].

The hydrolysis process is successfully used in this research study to extract amino acids from both human and animal hair samples[11]. Additionally, the study shows that both human and animal hair samples generate the highest levels of amino acids after a 6-hour hydrolysis process. For current research and applications, the study's findings have a number of ramifications. According to the research, human hair is a more promising source of amino acids than animal hair, which is the first conclusion. This is so because human hair is easier to hydrolyze and has a larger amount of amino acids. Second, the study shows that the best hydrolysis time for removing amino acids from hair samples is six hours. Third, the research offers insightful information about the amino acid makeup of hair. This knowledge might be put to use to create new items and uses for hair-derived amino acids.

Overall, the results of this study significantly advance our knowledge of the potential of hair as an amino acid source. The study offers useful information that could be applied to the creation of novel hair-derived amino acid-based goods and services.

Conflict of interest

All authors declare no conflict of interest.

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