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Research Article

PRELIMINARY SCREENING OF AMINO ACID PROFILES IN HUMAN SWEAT USING AGAROSE GEL ELECTROPHORESIS

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ABSTRACT

Agarose gel electrophoresis (AGE) is a versatile analytical technique used to separate and analyze biomolecules. This study aimed to screen and characterize the profiles of essential amino acids in human sweat using AGE. Sweat samples were collected from healthy human volunteers and analyzed using agarose gel electrophoresis. The resulting bands were visualized using Coomassie Brilliant Blue staining, and amino acid identification was attempted by comparing the migration patterns with those of known reference standards. The results showed distinct migration patterns for different amino acid standards, suggesting the potential applicability of the technique for amino acid profiling. However, the presence and concentrations of individual essential amino acids in sweat require further confirmation using validated analytical methods. Additional studies are necessary to establish the reliability of sweat-based amino acid profiling and to determine whether it can provide meaningful information about nutritional status.

Keywords: Agarose gel electrophoresis, Human Sweat, Coomassie Brilliant Blue, Amino Acid Profiling, Biomolecules.

INTRODUCTION

Amino acids are vital building blocks for cell growth, survival, regeneration, and repair (Cowan,1998). There are nine necessary, nine endogenous, and twenty-one human-produced amino acids. The majority of non-essential amino acids, including glycine, proline, glutamine, arginine, asparagine, aspartate, alanine, arginine, and serine, are made from glucose, whereas tyrosine and cysteine are made from the metabolism of phenylalanine and methionine (Katsarou, Efthimadou, Psomas, Karaloita, & Vourloumis, 2008). Since they cannot be produced from scratch, essential amino acids must be obtained through diet. Six amino acids are non-essential, six are conditionally essential, and nine are not synthesized by humans. Pyrrolysine is regarded as non-essential and is not utilized by the majority of organisms. It is only proteinogenic in specific microorganisms (Chen, 2011). Tyrosine, proline, glutamine, glycine, arginine, and cysteine are additional amino acids. Phenylalanine and tyrosine are grouped together because one of them can be synthesized from the other using the enzyme phenylalanine/tyrosine ammonolysis (Adamson & Reynolds, 1997). The 23 primary amino acids that make up proteins are the building blocks of both plant and animal proteins. Peptide bonds bind these amino acids together, and certain proteins have a particular arrangement of amino acids (Armitage, 1998). Protein needs to be broken down into its constituent parts during digestion since the body is unable to absorb it in its natural state. Understanding the function of protein in nutrition requires an understanding of amino acids. Electrophoresis is a technique that separates molecules according to their size, charge, and binding affinity using an electro-electric field and phosis migration. In forensic science and scientific testing, electrophoresis is a technique used to separate and analyze biomolecules like as proteins, nucleic acids, plasmids, DNA, RNA, and fragments as well

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as to determine the source DNA. Though it uses a different separation method, principle, and sample types, electrophoresis is a separation technique that is comparable to chromatography (Brody & Kern, 2004). While proteins are divided by the charge in agarose, shorter molecules migrate more quickly than longer ones. Gels serve as sieving and preventing heat convection and preserving separation for the application of post-electrophoresis stain. Usually, DNA gel electrophoresis is employed for further characterization or for analytical purposes following PCR amplification.

MATERIALS AND METHODS

Equipment

Weighing machine, Sonicator, Microwave oven, Refrigerator, Power pack machine, Electrophoresis chamber, Casting tray, Comb, Connecting wire, Transilluminator, Micropipette.

Chemicals

Agarose high eeo, Tris-buffer, Coomassie Brilliant Blue Dye, Gel drying solution. Using the agarose gel electrophoresis technology, the selected electrophoresis method was used to screen the importance in human sweat.

Selection of samples

Essential amino acids are analyzed by using agarose gel electrophoresis are Arginine, Histidine, Lysine, Aspartic acid, Glutamic acid See Table 1.

Preparation of buffer

The 2.8 gm of Tris powder was weighed and dissolved in 1000 ml of Distilled Water. Then the Buffer solution was Sonicated for 10 minutes. Heated in microwave oven for 5 minutes. Then stored in the well closed storage container.

Table 1. Selection of samples.

AMINO ACID TYPE	ESSENTIAL AMINO ACIDS
Hydrophilic: Acidic	Aspartic acid Glutamic acid
Hydrophilic: Basic	Arginine Histidine Lysine

Preparation of gel

The Agarose powder was taken in various concentration in the trial preparation. The process was described briefly in below:

0.5 % Concentration

0.5 mg of Agarose powder was weighed and taken. After the 30 min of process, the Density and thickness of the gel was obtained very slightly. So, the expected gel formation was non-ideal.

1% Concentration

1mg of Agarose powder was weighed and taken. After the 30 min of process, the density and thickness of the gel was obtained perfectly. So, the expected gel formation was Ideal.

1.5% Concentration

1.5mg of Agarose powder was weighed and taken. After the 30 min of process, the density and thickness of the gel was obtained too hard. So, the expected gel formation was Non-Ideal for the present study. Therefore, the 1gm of Agarose was weighed and dissolved in 100 ml in tris buffer solution. Then the gel solution was sonicated for 10 minutes and heated in microwave oven for 5 minutes. It was poured into the casting tray, and it was then placed in refrigerator, for the formation of gel (Chen, 2011).

Preparation of dye

A dye (COOMASSIE BRILLIENT BLUE) 0.2mg of dye powder was mixed in ethanol, to make the volume up to 1ml.The prepared dye was mixed with the sample solution, to identify the movement of the sample in the gel (Dolník, 1997).

Casting of gel

Before the addition of gel solution, the gel platform was fully sealed by the tape. The gel solution was poured into the casting tray. Then it might be shifted to the refrigerator to settle down of the gel. After the gel settlement, the tape surrounded by the gel platform was removed. The electrophoresis chamber was connected to the power pack machine. Prepared gel tray was placed into the electrophoresis chamber. Then the prepared buffer solution was filled into the electrophoresis chamber up to the gel platform was completely dipped into the buffer solution (Hegg & Burstyn, 1998).

Preparation of Standard

The standard method involves preparing essential amino acids at various concentrations for experimental trials. The

process is briefly described as follow. In 0.1% of concentration, 0.1mg of standard amino acid was taken. After 15mins of process, the stain was NOT DEVELOPED. In 0.2% of concentration, 0.2 mg of standard amino acid was taken. After 15mins of process, the stain was DEVELOPED. In 0.3% of concentration, 0.3mg of standard amino acid was taken. After 15mins of process, the stain was NOT DEVELOPED.

Essential amino acids used

Acidic amino acid - ASPARTIC ACID and GLUTAMIC ACID, Basic amino acid - LYSINE, HISTIDINE and ARGININE, Taken 0.2 mg of essential amino acids was weighed and mixed with 1ml of tris buffer solution, and it was taken in a storage container. Then added the 1ml of dye (Coomassie brilliant blue) in the prepared standard essential amino acid. To identify the movement of the standard in the gel by the colour of blue.

Preparation of sample

The sample (sweat) was taken from the human with the help of cotton buds. Then the sample was mixed with 1ml of tris buffer solution. Then add 1ml of dye (Coomassie Brilliant Blue) in the prepared sample solution. To identify the movement of the sample in the gel by the colour of blue. The gel was taken to the transilluminator to identify the moments of sample and standard as bands.

Spotting of sample

The electrophoresis chamber was connected to the power pack. Through the help of connecting wires and this contains positive and negative charge wires. Red was considered as Positive, and the Black was considered as Negative. In the separation process, the voltage should be produced by the power pack to the electrophoresis chamber at the volt of 150V. That can produce the less efficient of separation of amino acids. Therefore, the voltage produced to the electrophoresis chamber was slightly increased at the volt of 230V in order to produce the less efficient of the separation of amino acids. Further, the voltage was slightly increased at the volt of 270V and now it produced the efficient form of amino acid separation. The electrophoresis chamber was run by current through the power pack at the point of 270 V. At the point of running, the positive charged ions moved towards negative charged ions. The sample and standard were taken by the help of micropipette, then it was placed in the gel surface in the well present in gel platform one by one. But the sample and the standard in the well that should not mixed with the buffer solution, but the gel should be present inside the buffer solution.

RESULTS AND DISCUSSION

At the point of running, the sample should be separated as the several bands. Then gel was transfer into petri dish and poured 5ml of gel drying solution and the gel get shrinked. After that, the gel was kept into the transilluminator for the clear vision of bands present in the gel. Evaluation of essential amino acid present in human sweat by agarose gel electrophoresis determined by using Rf value (Li & Yang, 2009).

Formula: Rf = Distance travel by sample / Distance travel by tracking dye

In 1% concentration, 1mg of agarose powder was weighed and taken. After the 30 min of process, the density and thickness of the gel was obtained perfectly. So, the expected gel formation was observed to be ideal See Table 2.

Table 2. Selection of gel concentration.

CONCENTRATION	TIME (min)	IDEAL / NON- IDEAL
0.5 %	30	Non-Ideal
1.0 %	30	Ideal
1.5 %	30	Non-Ideal

In 0.2% of concentration, 0.2 mg of standard amino acid was taken. After 15mins of process, the stain was well developed See Table 3.

Table 3. Selection of standard concentration.

CONCENTRATION	TIME (min)	DEVELOPMENT
0.1	15	Not developed
0.2	15	Developed
0.3	15	Not developed

Screening of essential basic amino in human sweat shown in Figure 1 and Table 4.

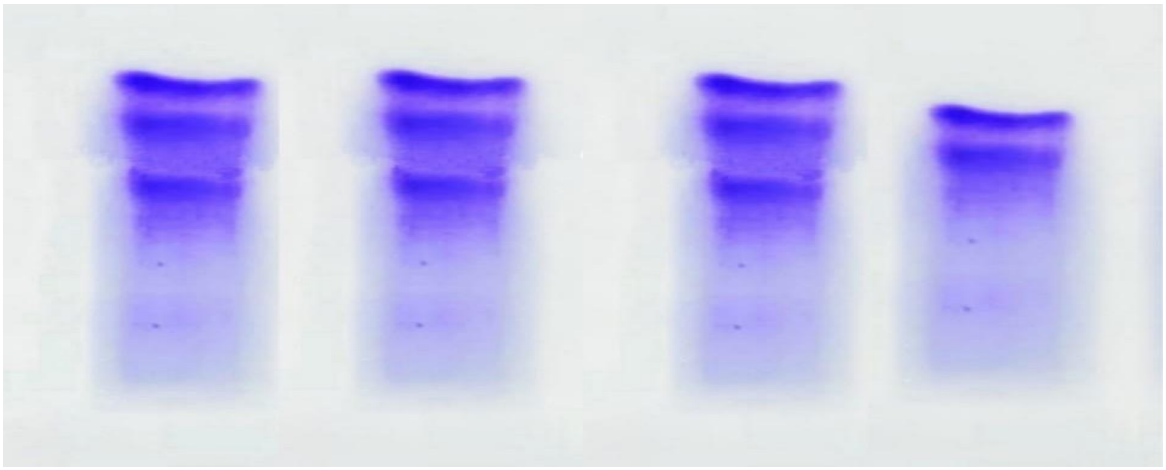

Figure 1. Gel electrophoresis for Basic amino acid.

Table 3. Basic amino acid.

Amino Acids	Subject A	Subject B	Subject C	Subject D
Lysine	+	+	+	+
Histidine	+	-	+	+
Arginine	+	+	+	-

[A]Lysine, [B]Histidine, [C]Arginine.

Data from the Table 3 revealed that the Subject B was lack of Histidine (Basic essential amino acid). It affects the function of the brain. It causes appetite suppression. Enlarged liver and high cholesterol in the body. Furthermore, the Subject D was lack of Arginine (Basic essential amino acid), thus, it causes an abnormal tensing of the muscle and slower than normal growth. Intellectual disability and seizures may prone to affect these subjects. Therefore, these subjects should be supplemented to get enough arginine for specifically for Subject D. The following foods are natural source of arginine, Nuts (peanuts, almonds), Seeds (pumpkin seeds, sunflower seeds), Dairy products (milk, cheese), Meat and Whole grains (oats, corn). Screening essential acidic amino acid in human sweat are shown in Figure 2 and Table 4.

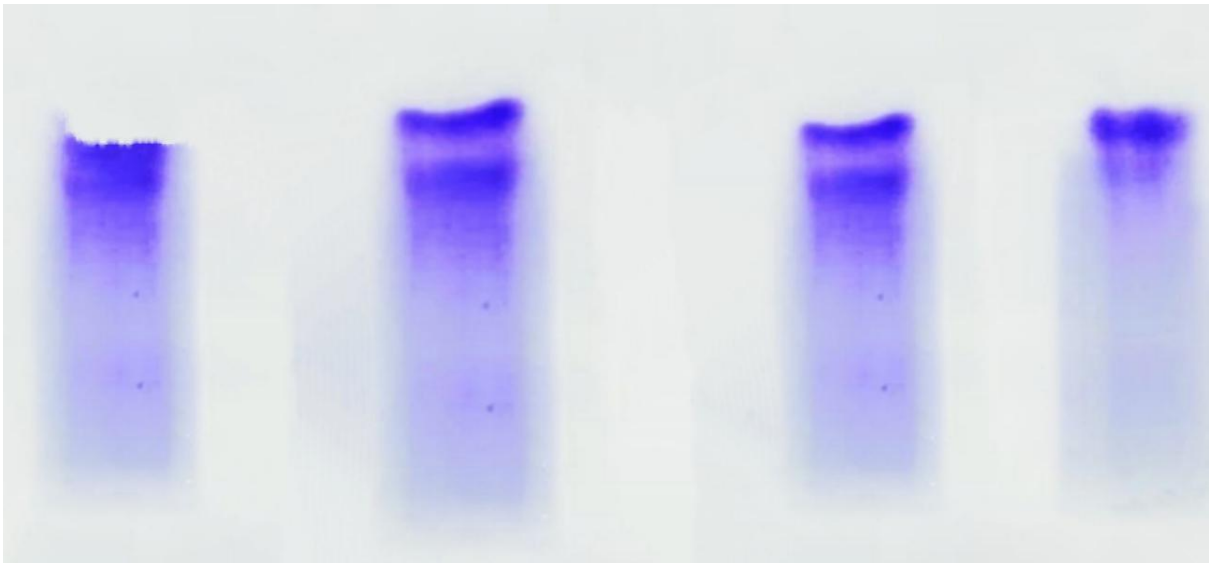

Figure 2. Gel electrophoresis for Acidic amino acid.

Table 4 . Acidic amino acid.

Amino Acids	Subject A	Subject B	Subject C	Subject D
Aspartic Acid	-	+	+	+
Glutamic Acid	+	+	+	-

[A]Aspartic acid, [B] Glutamic acid.

The Subject A was lack of Aspartic acid (essential acidic amino acid). It causes damage to brain, nervous system and liver, It decreases cellular energy, Chronic fatigue, Reduced stamina., whereas the Subject D was lack of Glutamic acid (essential acidic amino acid). It causes low protein diet, Diarrhoea, Insomnia, Brain dullness and Ulceration in gastrointestinal tract. These are the supplements to get enough Apartic acid for Subject A. Raw legumes, Nuts, Soya beans and Peanuts. These are the supplements to get enough Glutamic acid for Subject B. The following foods are natural source of glutamic acid, Dairy products, Beans, Vegetable sources like cabbage, wheat grass, wheat. It also includes Meat, Fish, Eggs.

CONCLUSION

Agarose gel electrophoresis (AGE) was selected as a preliminary technique for investigating amino acid profiles in human sweat. The study examined amino acids, including arginine, histidine, lysine, aspartic acid, and glutamic acid. A 1% agarose gel was prepared, and the samples were subjected to electrophoresis at 272 V using a sample concentration of 0.2 mg, as described in the experimental procedure. Following electrophoresis, several bands were observed in the gel. The gel was subsequently transferred to a Petri dish containing 5 mL of gel-drying solution, which resulted in gel shrinkage. The gel was then examined using a transilluminator to visualize the bands. The migration patterns were evaluated by calculating the retention factor (Rf) values. These findings provide preliminary observations on the migration behaviour of the tested samples under the experimental conditions. However, further method validation and comparison with appropriate reference standards using a suitable analytical technique are necessary to confirm the identity of individual amino acids in human sweat. Additional studies are also required before this method can be considered reliable for assessing amino acid status or nutritional deficiencies.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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