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Research Article

Colorimetric Method for Determination of Proline on Thin Layer Using a Modified Reagent

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Abstract

Amino acids classified nutritionally essential (indispensable) or nonessential (dispensable) for animals and humans. Proline is an essential amino acid and its carbon Skelton cannot have synthesized by human and animal cells and, therefore, must be provided by from the diet. In order to investigate the presence of proline, several techniques have been proposed. In this study, thin layer chromatography technique (TLC), using modified reagent to detect proline in some biological samples, was used. The result of this study Clearfield that proposed reagent was successfully determined proline with color characteristic. thus, formation of yellow color with modified reagent is an added advantage of this method.

Keywords: *Proline; TLC; Colorimetric Method; Ninhydrin Reagent*

Introduction

Proline is one of an essential amino acid. It plays role in protein synthesis and structure, nutrition, anti-oxidative and immune responses. proline and hydroxyproline are most abundant in collagen and milk proteins, and requirements of proline for whole-body protein synthesis are the greatest among all amino acids. Proline is the most widely distributed osmolyte under stress conditions in plant [1], eubacteria. Protozoa, marine invertebrate and algae [2-5]. Therefore, physiological needs for proline are particularly high during the life cycle. For the determination of the presence of proline in biological and pharmaceutical samples various methods have been reported. Some Methods were used to quantify proline in order to determine its concentration [6], other methods used to detect proline as one of amino acids using chromatographic techniques [7-9]. In this study, an attempt to develop chromatographic technique in order to detect proline only without other amino acids has been successfully achieved. The method was sufficient to detect proline with color characteristic.

Material and Methods

Standard Preparation

0.1g of proline (Sigma, USA) dissolved in 2 mL Sulfosalicylic acid (3g of Sulfosalicylic acid dissolved in 100 ml distil water.

Samples preparation

Stressed plant with NaCl

Barely (*Hordeum vulgare*) and bean (*Vicia faba*) plants exposed to NaCl at levels (-3, -10 and -15 bar) for one month to detect then plant were harvested, dried at 70°C for 2 days and ground to a fine powder. 0.1g of plant stressed samples were dissolved in 2 ml Sulfosalicylic acid.

Seaweed samples

Samples of seaweed, *Cladophoropsis gerloffii* (green algae) and *Halopteris scoparia* (brown algae) were collected from the sea of Misurata city - Libya. The samples were oven dried and ground to a fine powder. 0.1g of plant stressed samples were dissolved in 2 ml Sulfosalicylic acid.

Reagent Preparation

Ninhydrin reagent

$SnCl_2$ solution ($SnCl_2.2H_2O$) was freshly prepared: 16 mg of $SnCl_2$ dissolved in 10 ml (0.2M) of citrate buffer solution, 10g of NaOH dissolved in 100 mL distilled water, pH was adjusted to 5.

Reagent preparation

Ninhydrin solution: 1.25g of ninhydrin dissolved in 30 ml glacial acetic acid then 20 ml of sulphuric acid was add.

Separation solution

Toluene : glacial acetic acid : water (12:5:3).

Detection of proline on TLC Paper

Standard amino acids solutions were spotted on the chromatography paper (Silica Gel 60 F254 TLC aluminium sheet 20 cm X 20 cm, thickness 0.1 mm; Merck, Germany) by graduated capillary tubes (capacity: 5 µL volume; Spectrochem). TLC papers were air-dried and subjected to TLC with Toluene: glacial acetic acid: water (12:5:3, v/v) as a mobile phase. After development, TLC were dried, sprayed with the reagent and then heated at 105°C for 6 minutes in an oven. Colors were observed visually.

Results and Discussion

The most striking feature of the proposed reagents (Ninhydrin reagent) and mobile phase solution is detecting proline only, among several amino acids in the samples, and the formation of distinguishable color after final heating. The detection of proline limited. it ranged between 1 to 5 µg [10].

Rotation factor

The profiles of proline from this method was almost identical [10]. Tested proline showed clear distinguished color (Figure 1), and was not influenced by the modification. Results of this study showed that plant extract contain amino acids, with this mothed only proline can be detected.

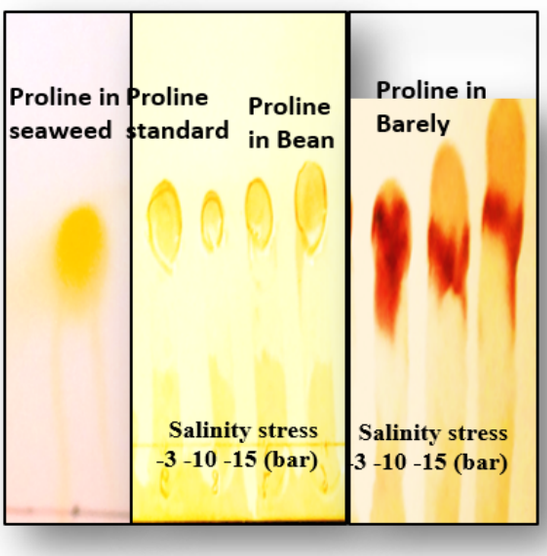

Figure 1: Proline with characteristic color detected in some biological samples

Bean Osmotic pressure (bar)	Rotation factor (R_F)
3-	5.6
10-	5.6
15-	5.5
Barely Osmotic pressure (bar)	
3-	4.5
10-	4.5
15-	4.3
Seased	
Cladophoropsis gerloffii	4.3
Halopteris scoparia	4.3

Table 1: Proline detected in some biological samples.

Conclusions

Modified method used in this study was sufficient general to include the determination proline in samples of biological origin. The method was highly sensitive to determine low concentration of proline on TLC Furthermore, it can be proceeded in the laboratory. It is fairly simple to permit and it can be applied conveniently to large numbers of biological samples. It appeared probable that a Colorimetric method would best fulfil these requirements.

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