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Analysis of Tryptophan by Colorimetric and HPLC Techniques- A Comparative Study

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Abstract: ***Tryptophan is an essential amino acid present in most protein based foods for brain functions. In the present paper an attempt was made to analyse the tryptophan in curds by colorimetric and HPLC techniques and a comparative study was done. For colorimetric studies proprietry proteins were used for test development. Standard Reference materials were used for method development. The protein was treated with 2 hydroxy -5 nitrobenzyl bromide. Colorimetric analysis was done and the absorbance measurements were done at 550nm. HPLC procedure was also applied to determine the total tryptophan content in several samples of curds. The nutritional quality of the protein could be extrapolated to the expected tryptophan content by using a regression curve. A comparative study of colorimetric and HPLC techniques was done .The results were found to be accurate and reproducible.***

Keywords: Tryptophan, colorimetry, HPLC.

I. INTRODUCTION

Tryptophan is present in most protein based foods and is plentiful in chocolates, oats and in curds. Curds is the only food which can release a chemical called tryptophan in brain. It calms down the brain and brings a cool thinking and the neurons are recharged with a mild rest. On the contrary yoghurt has sugar in it which may be dangerous for the balance of neural activity. It triggers hyperactivity(1,2) . The word tryptophan was derived from the Sanskrit word 'thrupth' meaning satisfaction. Curd is the best brain food which activates the brain in a balanced manner for a tropical climate. Tryptophan is the main constituent of serotonin(3) . Serotonin is a neuro chemical and a natural mood regulator which makes us feel happy and emotionally stable .and has a wide variety of functions in the body. Low levels of serotonin may cause mood disorders. Serotonin is a precursor for melatonin which is a sleep inducing chemical. Serotonin cannot cross the blood brain barrier , so it has to be produced in the brain(4).Here is where curds comes in. Many foods contain tryptophan but what makes the brain to take up tryptophan to create serotonin is curds. That is why eating foods rich in tryptophan might not create the same happiness and satisfaction.

Tryptophan , the building block of serotonin could get into the brain only after sweet or starchy carbohydrates were eaten. The carbohydrate rich foods create production of insulin in the body which clears the amino acids from the blood stream so that the brain can take up tryptophan .In the absence of insulin the brain prefers the competing amino acids , so eating tryptophan rich foods without carbohydrates does not have the same impact that curd rice has (5,6).

II. METHODOLOGY

A. Colorimetry

Tryptophan was determined by colorimetry. It was oxidized by sodium nitrite and coupled to pink coloured leucodye N-1-(naphthyl) ethylene di amine dihydrochloride . Hydrochloric acid, sodium nitrite, sulphamic acid, sulphuric acid and other chemicals used in this study were of analytical grade. Deionized water was used to prepare all solutions. Colorometric method sample (0.1g/L) was prepared in deionised water with a few drops of 1mol/L sodium hydroxide, and the tryptophan solution was suitably diluted before using as standard. Solutions of 1 mol/L of sodium nitrite and 25 g/L of sulphamic acid were prepared in deionized water, stored in refrigerator avoiding direct light and used within one week. A solution of 2 mol/L of sulphuric acid was also prepared. The standard solution was taken in sets of 25 ml calibrated flasks, cooled at 5 degrees centigrade. Sulphuric acid was added to each flask, mixed and sodium nitrite and sulphamic acid , added and placed in ice bath .Tryptophan solutions were then added to the flasks volume made to 25 ml. The absorbance of the pink coloured solution was measured at 550nm. Dipeptides containing tryptophan are less reactive than free tryptophan. Absorption measurements were made at 550nm.At this wavelength there were no interferences by carbohydrates or amino acids or neutral salts. In colorimetry, the light absorptive capacity of a system (coloured solution) is measured and this measurement is related to the concentration of the coloured substance in the solution .When monochromatic light passes through a transparent medium (coloured solution) the rate of decrease in intensity with the concentration and thickness of the medium is directly proportional to the intensity of the light. Series of experiments were done

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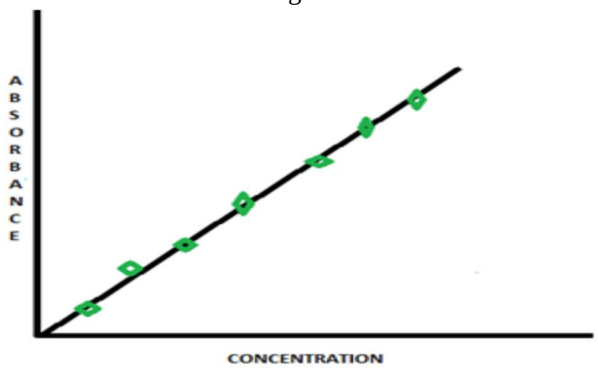
varying the reagent concentrations and hydrolysis time. The colorimetric method is based on the reaction of the sample with certain reagents and the measurement of the optical density of the coloured compound which absorbs maximally at 550m. All measurements were made at 550 nm. Absorbance of the chromophore is directly proportional to the amount of tryptophan present.
L-Tryptophan , sodium hydroxide , acetonitrile and other chemicals used were of analytical purity. Representative samples of curds were obtained directly from manufacturers.
HPLC (high performance liquid chromatography) is a form of chromatography . Sample mixture in the solvent was pumped at high pressure through the column with chromatographic packing material (7,8). Sample collection was performed by an auto sampler with a loop and a cooling unit. . Elution buffer was a degassed potassium phosphate solution . Analyses were carried out at a flow rate of 1.0ml/min and at room temperature. Different samples of curds were diluted with 200µL of potassium phosphate buffer and precipitated with trichloro acetic acid. Samples were dissolved in sodium hydroxide and methyl tryptophan was used as internal standard. Samples were hydrolysed in an oven and the hydrolysates were aerated and cooled and acidified with HCl. The samples were then centrifuged and supernatant filtered into HPLC vials. Tryptophan contents were calculated by dividing the area of the peak by the area of the internal standard and multiplying by the weight of the internal standard. Tryptophan was detected by a fluorescence detector at an excitation wavelength of 365nm.

III. RESULTS AND DISCUSSION

The samples were analysed and concentration of each of the samples was calculated and plotted against absorbance as shown in Figure-1. There is a linear relationship between concentration and absorbance. Beer Lambert ‘s law verified which states that when monochromatic light passes through a transparent medium (coloured solution), the rate of decrease in intensity with the concentration and thickness of the medium is directly proportional to the intensity of the light(9,10).The colorimeter works on the principle - A low voltage lamp forms the light source. This light passes through a selected filter. The light transmitted by the filter passes through the cell containing the coloured solution, and falls on a sensitive photocell. An amplifier amplifies the current generated by the photocell. The amplifier output drives a current meter calibrated in optical density % transmittance(11). Absorbance of the chromophore is directly proportional to the amount of tryptophan present . Values obtained were in close agreement with values reported in the literature. This method produced reproducible and stable results for the concentrations of tryptophan and was validated by comparison with the data obtained and was validated by comparison with data obtained using a glyoxylic acid, sulphuric acid and ferric chloride treatment producing a coloured compound which absorbs at 550nm.

Figure-1



The repeatability of the quantification by HPLC technique was observed by performing several determinations. Accuracy of the analytical procedure was evaluated. The relationship between tryptophan content and protein content of the samples was studied. A regression analysis was carried out (12,13).

IV. CONCLUSION

The objective of this work was to develop an accurate, reliable and less expensive method for tryptophan analysis. Several chemical methods, spectrophotometric analysis methods were used for determining tryptophan in curds. Some of these methods lacked accuracy and reproducibility because of the interferences from common substances (14). An attempt was made in the present paper to compare the colorimetric and HPLC techniques for the analysis of tryptophan. It could be concluded from the comparative study , that the results from both the techniques were comparable and were found to be the same.

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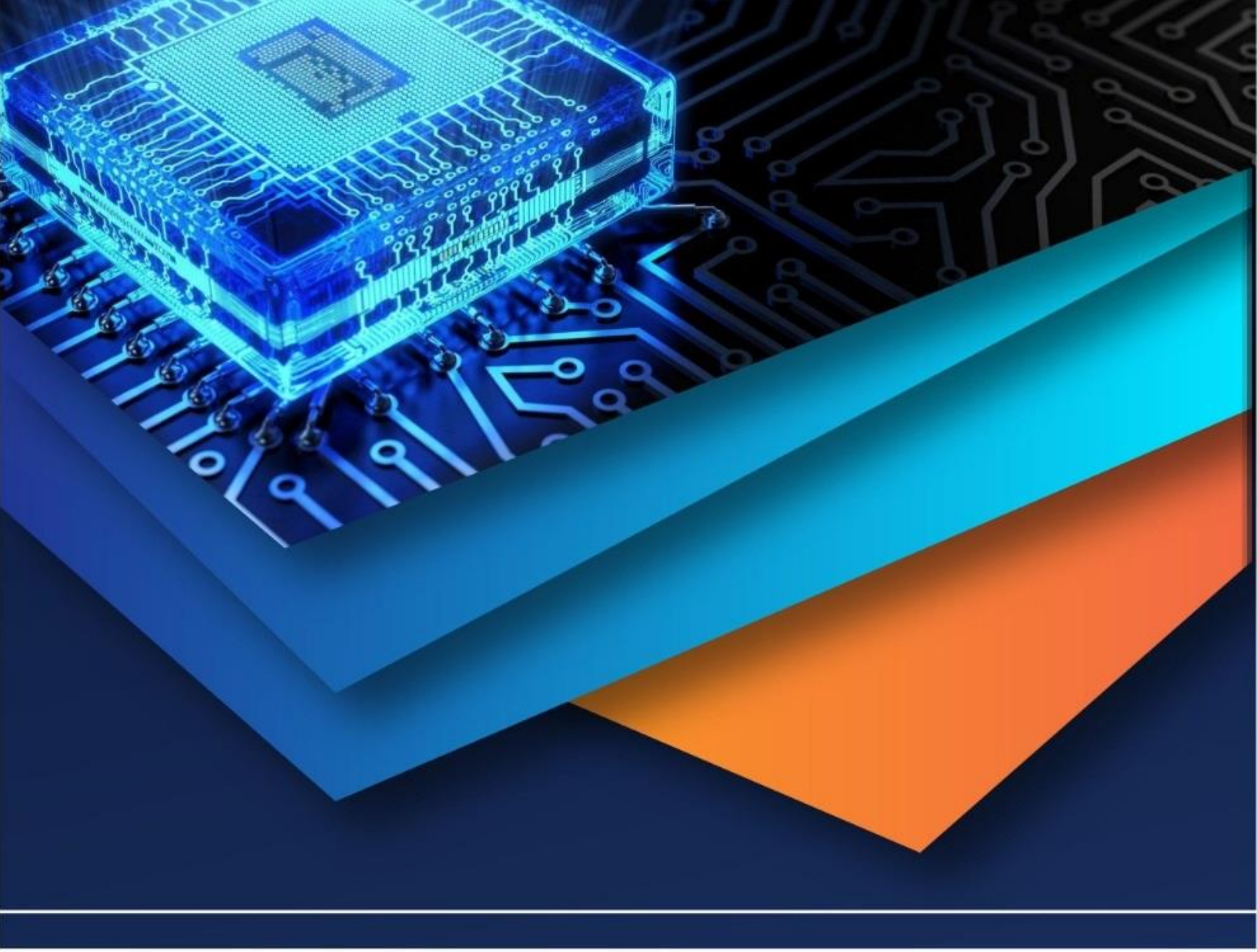

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