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RESEARCH ARTICLE

A SIMPLE SPECTROPHOTOMETRIC METHOD FOR QUANTIFICATION OF CASEIN IN MILK AND MILK PRODUCTS

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Abstract

In the food industry, production processes and food products are subjected to the highest possible quality control requirements. In order to safeguard the quality of a certain product along the production chain, it is important to know its composition. This, for instance, especially applies to a natural product like milk and its further processing in order to efficiently control the production of cheese and quark. Milk contains an important natural protein that is responsible for the essential curdling process during the production of cheese: casein. Casein protein provides the body with all the amino acids necessary to help build muscle. Casein protein is digested more slowly than other proteins, so it might be better at reducing appetite and increasing feeling of fullness. Effective control of the production processes of dairy products only becomes possible when the casein concentration is known. In this experiment casein content in milk and curd samples was determined at 524.5nm in visible range against biuret reagent by using simple uv-visible spectrophotometer. The curd and milk samples were collected from the local market. For quantitative determination of casein in milk and curd, classic protein analysis method was used i.e. biuret method. This method is based on biuret reaction in which colored copper based complex was formed. The casein protein concentration in curd sample was found to be 1684ppm. The concentration in milk sample 1 was found to be 2893ppm and in milk sample 2 was found to be 1522ppm.

Introduction:-

Milk has more than 100 different proteins. Most of these proteins are classified into 3 'N' fractions: which are casein (CN)-78%, serum or whey proteins -17%, non-protein nitrogen (NPN)-5% (B. Ribadeau-Dumas and R. Grappin, 1989). Among all the proteins casein are the most abundant. Caseins in turn consist of 4 individual proteins (αs1, αs2, β and k-casein). Serum or whey proteins consist of α-lactalbumin (α-la), β-lacto globulin (β-lg), blood serum albumin (BSA) and immunoglobulin (Ig)(Kumaresan, C. Selma, N. V. Reshma and N. A. Jacinth, 2017) Casein

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contains different degree of phosphorylation (αs1, αs2, and β-CN) and glycosylation (K-CN)(S. Patil, S. S. Hosapete, S. Irkal and S. Rajput, 2019). Casein with the formation of breakdown products will get partly hydrolyzed at milking.(C. P. Kulkarni, 2017, J. Jakubowski, Z. Sienkiewicz and E. Nowak, 1985)

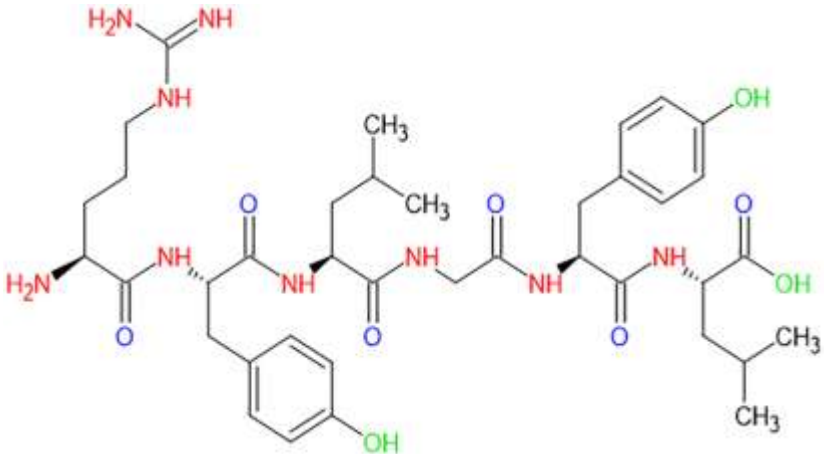

Fig 1:- Structure of casein.

Quantitative determination of casein according to the biuret method is a classic protein analysis method (Kamizake, N. K.; Gonçalves, M. M.; Zaia, C. T; Zaia, D, 2003, Lüthi-Peng, Q.; Puhan, Z, 1999). This method is based on the biuret reaction and enables the determination of proteins in solution via a colored copper based complex (Nakai, S.; Le, A. C, 1970, Kuaye, A. Y, 1994). Proteins react with copper sulphate in an alkaline environment to produce a purple color complex (Webster, G. C, 1970, Gornall, A. G.; Bardawill, C. J.; David, M. M, 1949). This colored complex can be determined spectrometrically at the wavelength of 540nm in the visible range of the spectrum. Complex formation proceeds in sufficient amounts and it is therefore possible to use the biuret method to quantitatively determine the amount of protein in the sample (Gradinaru, R.; Murariu, M.; Dragan, E. S.; Drochioiu, G, 2007, MEHL, J.W, 1949). The relationship between the concentration of a sample and its photometric absorption is found by using the lambert beer law (MOLNAR, MARGIT, 1948, ROBINSON, H. W., AND HOGDEN, C. G, 1940).

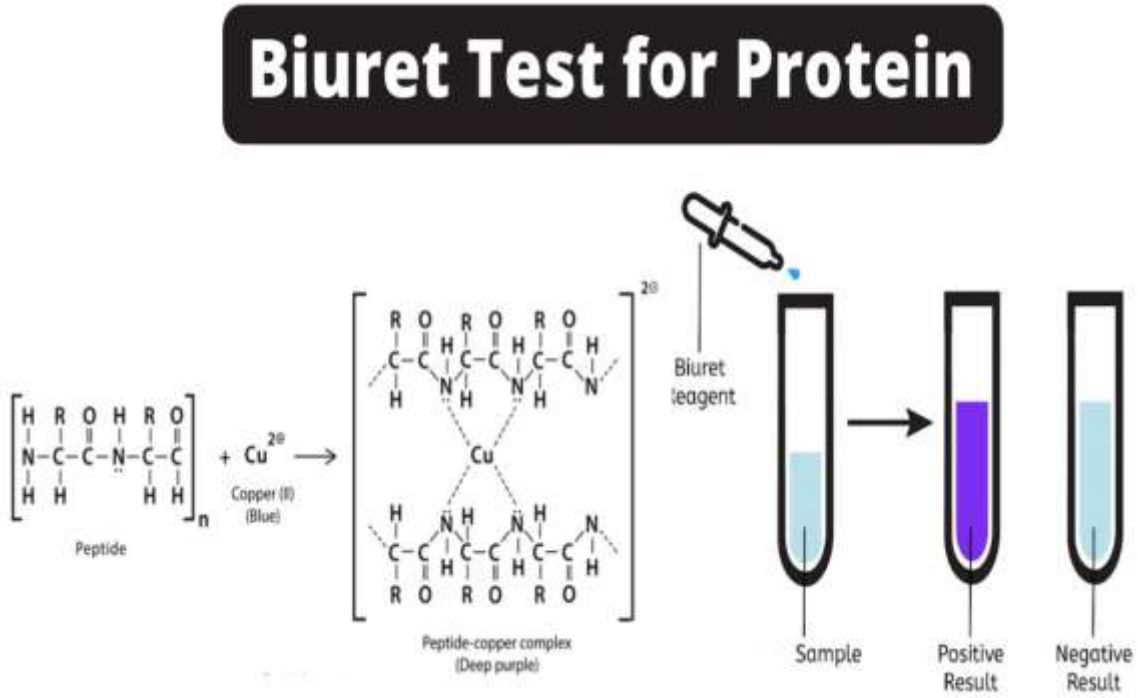

Fig 2:- Reaction that takes places between biuret reagent and the protein when biuret reagent is added to it.

Materials And Methods:-

Samples

Skimmed milk sample was collected from the local market. Homemade curd was taken.

Reagents

Biuret Reagent: 1.5grams of copper sulphate and 6 grams of potassium tartarate are dissolved in 500ml of distilled water.300ml of 10%NaOH are added (3grams of NaOH was dissolved in 100 ml of distilled water). All the chemicals used were AR grade from SDFCL(s d FINE-CHEM LIMITED).

Standard Solution Preparation

Casein was dissolved in 3% NaOH solution. Stock solution of casein (1000 ppm) was prepared by dissolving 10mg of casein in 10ml of 3% NaOH solution. Further dilutions of 1500, 2000, 2500, 3000, 3500 and 4000ppm were prepared with 3%NaOH solution. 1ml of standard solutions was taken and to it 4ml of biuret reagent was added, shaken and kept aside. Their absorbance was read in uv-visible spectrophotometer by taking 1ml of 3%NaOH and 4ml of biuret reagent as reference. Calibration curve was plotted.

Sample Solution Preparation

Samples used for this experiment were milk and curd. Milk was taken from the local market and homemade curd was used. 2g [±0.001] of sample was weighed and transferred to 25ml volumetric flask. To the weighed sample 4ml of biuret reagent was added and made up to the mark with 3%NaOH. The solutions were filtered and the filtrate absorbance was measured in uv-visible spectrophotometer. From calibration curve the concentration of casein in curd and milk is read.

Results:-

The data present in table 1 is the absorbance of standard preparations and the samples. Figure 3 is the calibration curve of the standard preparations.

Table 1:- Absorbance of standard preparations of casein and samples.

Concentration	Absorbance
1000	0.1779
1500	0.3014
2000	0.4931
2500	0.6119
3000	0.7851
3500	0.9358
4000	1.0923
Milk sample	0.7494
Concentration	2893.52291
Milk Sample 2	0.3271
Concentration	1522.20015
Curd Sample	0.377
Concentration	1684.23899

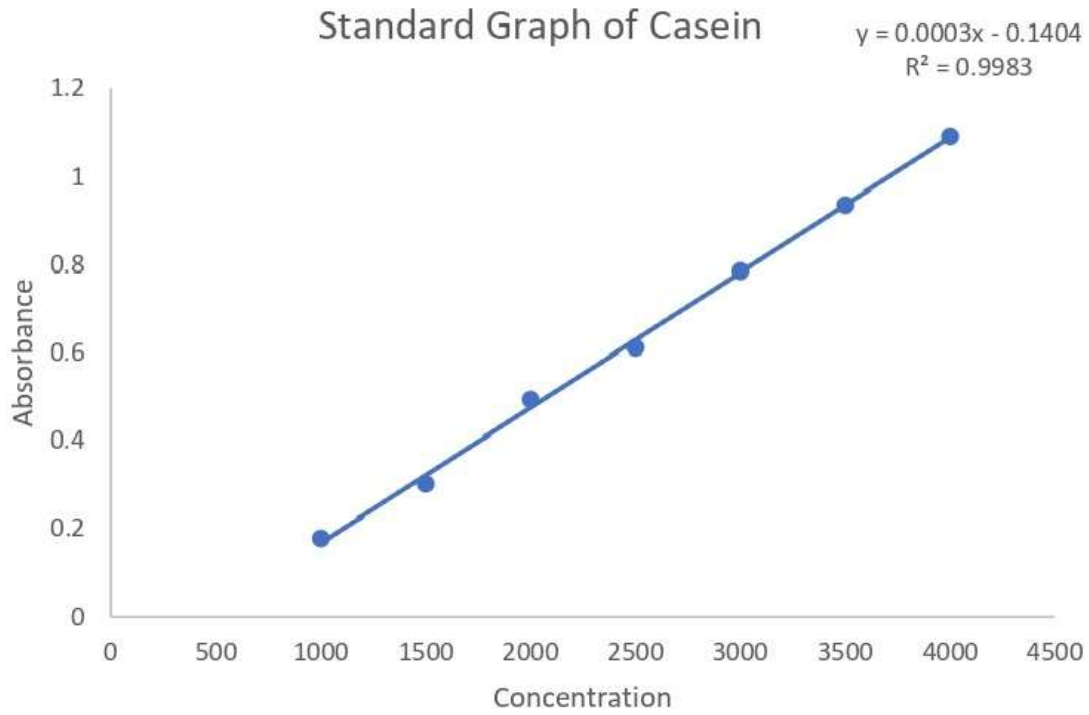

Fig 3:- Standard calibration curve of casein.

Discussion:-

Protein contains peptide bond between the amino acids. Peptide bond is unique in protein. The biuret test was done to show the presence of peptide bonds. These bonds will make the blue biuret reagent turn purple. Cupric ions bind with the peptide bonds of the protein in alkaline medium results in formation of violet copper-peptide complex. The intensity of violet color is directly proportional to the number of bonds which in turn is proportional to the amines of protein in the sample.

Simple uv-visible spectroscopy was used for the determination of casein protein in milk and curd. Standard casein solutions were prepared of concentrations 1000, 1500, 2000, 2500, 3000, 3500, 4000ppm. By addition of biuret reagent to the standard casein color was produced. The absorbance of the concentrations was measured using glass cuvette in the visible range at wavelength 524.5nm.

Calibration curve of absorbance v/s concentration was plotted with standard solution concentrations. Sample solutions of curd and milk were prepared by addition of biuret reagent and filtered. Sample solution absorbance was known by measuring at 524.5nm. Concentration of casein in the samples i.e. milk and curd was calculated from the calibration graph.

Conclusion:-

Casein is the protein found in milk and curd. Casein and copper ions interaction was explored by uv visible spectrophotometer. Casein can be directly detected in uv region as biuret complex which is formed by the reaction of casein with copper ions which are produced from the insoluble copper phosphate or can be detected indirectly by the extracted ions absorption. During the experiment relationship of casein with copper ions was found to be more complex and should be studied. Research should be done to completely understand the relationship of casein and copper ions and to determine the protein in diluted samples.

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