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DETERMINATION OF CASEIN IN MILK

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In the present study the precipitation of casein from the various milk samples such as cow milk, goat milk were studied. The technique of precipitation of casein is used to predict the protein content in the milk samples. It was found that the main components of casein have genetic variants that differ in several amino acid residues. These proteins have a molecular weight of about 20 thousand, an isoelectric point of about 4.7, contain an increased amount of proline (the polypeptide chain has a b-structure) and are resistant to denaturants.

Key words: albumin, enzyme, casein, protein, phosphoprotein.

СҮТТЕГІ КАЗЕИНДІ АНЫҚТАУ

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Зерттеу барысында сиыр сүті, ешкі сүті сияқты әртүрлі сүт үлгілеріндегі казеиннің тұнбасы зерттелді. Сүт үлгілеріндегі ақуыз мөлшерін болжау үшін казеин титрлеу әдісі қолданылады. Казеиннің негізгі компоненттерінің бірнеше аминқышқылдарының қалдықтарымен ерекшеленетін генетикалық нұсқалары бар екендігі анықталды. Бұл ақуыздардың молекулалық салмағы 20 мыңға жуық, изоэлектрлік нүктесі шамамен 4,7, құрамында пролин мөлшері жоғарылаған (полипептидтік тізбек b-құрылымына ие) және денатуранттарға төзімді.

Негізгі сөздер: альбумин, фермент, казеин, ақуыз, фосфопротеин.

ОПРЕДЕЛЕНИЕ КАЗЕИНА В МОЛОКЕ

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В настоящем исследовании изучалось осаждение казеина из различных образцов молока, таких как коровье молоко, козье молоко. Техника осаждения казеина используется для прогнозирования содержания белка в пробах молока. Установлено, что основные компоненты казеина имеют

генетические варианты, различающиеся несколькими аминокислотными остатками. Эти белки имеют молекулярную массу около 20 тысяч, изоэлектрическую точку около 4,7, содержат повышенное количество пролина (полипептидная цепь имеет b-структуру) и устойчивы к денатурантам.

Ключевые слова: альбумин, фермент, казеин, белок, фосфопротеин.

Introduction

Milk is one of the most valuable human foods. The role of milk as a complete food product in supporting the body's vital processes is well known. Of particular value are milk proteins - the most biologically important organic substances. The amino acids formed as a result of the breakdown of proteins are used to build body cells, enzymes, protective bodies, hormones, and more.

Milk and milk products are an important component of food ration of a modern human. Milk contains three types of protein: casein (caseinogen), milk albumin and lactoglobulin. The main protein of milk is casein, which is 2.7 %, or 81.9 from total amount of proteins in milk.

The quality and output of protein dairy products are known to be determined by the fractional composition of casein.

Usually in milk control the mass fraction of proteins (total protein), including casein and whey proteins. Less commonly, only casein content is determined in milk.

Nowadays there is evidence that the milk casein is the source of a variety of significantly important bioactive peptides.

Casein (/ˈkeɪsiːɪn/ KAY-see-in, from Latin caseus "cheese") is a family of related phosphoproteins (αS1, αS2, β, κ).

Casein has a wide variety of uses, from being a major component of cheese, to use as a food additive. The most common form of casein is sodium caseinate.

As a food source, casein supplies amino acids, carbohydrates, and two essential elements, calcium and phosphorus.

High nutritional value of milk is attributable not only to content of protein substances, oil, carbohydrates, mineral salt sand their favorable balance in it, but also to a specific content of above-mentioned components [1].

Although whey protein, casein, soy proteins are good to be of high biological value, but we must consider other ingredients associated with them.

Last days content of milk significantly changes before drying of cows. The content of sodium salt dramatically increases and content of calcium salt decreases, as a result milk becomes salty, quantity of leukocytes in it increase. Acidity decreases. Viscosity and density increase, as well as content of oil, protein, casein and content of milk sugar decreases [2].

Casein has high nutritional value. The main protein of milk - casein (caseinogen), is phosphoprotein, in molecule of which phosphor is connected to hydroxy-amino acids in the form of phosphorus acid, forming ester with serine, threonine. Besides that, casein is connected to calcium of milk and at that forms active casein – phosphate and calcium complex. Casein, which is in milk in the form of calcium salt, is named calcium caseinate.

During milk acidification in the process of falling out of clot, calcium caseinat, interacting with lactic acid, degrades to calcium lactate and casein, precipitating in the form of sediment (at that a large portion of calcium lactate remains in liquid part, in serum). Milk has a complex composition.

It has more than a hundred different components. Usually, in widely established practice, chemical composition of milk is characterized by essential substances, quantity of which is not strictly constant.

It changes depending on different factors [3]. Caseinis a compound protein, formed from predecessors of casein – caseinogen at milk caseation. One of the main indicators of suitability of milk for production of milk products is its capability to posset and form thick elastic casein clot under influence of fermenting agent. Many genotypic and paratypic factors influence on these qualities of milk, but specifically - breed, feeding stuff and technology of animal nutrition, genotype of cows, containing casein and its particles, calcium and phosphor, condition of a lacteal gland. Quantity of casein in cow milk ranges from 2.1 to 2.8 %. Basic composition of unfractioned casein (in %) is the following: carbon -53.1; hydrogen – 7.1; oxygen – 22.8; nitrogen – 15.4; sulfur – 0.82; phosphor - 0.8.

Casein is obtained in various ways. An important factor determining the industrial production of casein is its solubility in various solutions. Casein is soluble in dilute solutions of alkalis and in strong acids, but insoluble in dilute

acids, where it precipitates. For the production of casein, fresh skimmed milk is used.

The casein is a protein molecule with elastic properties, high mechanical strength and also insoluble in water. Due to such properties its suitability to be used as a polymer increases. To improve upon the properties of casein it is blended with formaldehyde which serves as a plasticizer and the aerogel is formed. The formed aerogel can serve as a viable substitute for conventional non-biodegradable polymers used for low density, high temperature applications. These materials may have several potential applications including the fabrication of bioscaffolds, foams, contact lenses, drug delivery capsules, and numerous other technological applications. The casein is a protein molecule with elastic properties, high mechanical strength and also insoluble in water. Due to such properties its suitability to be used as a polymer increases. To improve upon the properties of casein it is blended with formaldehyde which serves as a plasticizer and the aerogel is formed. The formed aerogel can serve as a viable substitute for conventional non-biodegradable polymers used for low density, high temperature applications. These materials may have several potential applications including the fabrication of bioscaffolds, foams, contact lenses, drug delivery capsules, and numerous other technological applications.

There are four main methods for determining the mass fraction of protein in milk and dairy products:

–Kjeldahl's Method

–Refractometric method

–Colorimetric method

–Formative titration method

A widespread method for determining the mass fraction of protein in milk is formol titration. The method has limitations and can be used only for raw (unpasteurized) milk with an acidity not exceeding 20° T. However, it is often used at dairy enterprises to control the mass fraction of protein in a normalized mixture after pasteurization and even in finished drinking milk that has undergone various heat treatments (pasteurization or sterilization). To understand why this cannot be done, we consider the chemistry of the method of formal titration.

This is a titrimetric method based on the neutralization of the carboxyl groups of monoaminodicarboxylic proteins with a solution of sodium hydroxide. The amount of alkali spent on neutralization is proportional to the mass fraction of protein in milk.

As is known, protein molecules are characterized by the presence of positively charged amino groups ($–NH_3$ +) and negatively charged carboxyl groups ($–COO^-$) [4]. Being in the same molecule, oppositely charged amino and carboxyl groups are attracted to each other, forming an internal complex salt of a neutral nature. This inhibits the interaction of alkali (sodium hydroxide) with carboxyl groups.

In order to release the carboxyl groups of monoaminodicarboxylic acids of proteins, to make them available for titration with alkali, formaldehyde (formalin) is added to milk, which, interacting with the amino groups of proteins, blocks them. In this case, carboxyl groups are released and become available for titration with sodium hydroxide.

The work investigated the amount of casein, as well as laboratory conditions, an indirect method and methods for determining the absolute refractive index using refractometry.

A refractometer is a laboratory or field device for the measurement of an index of refraction (refractometry). The index of refraction is calculated from Snell's law while for mixtures, the index of refraction can be calculated from the composition of the material using several mixing rules such as the Gladstone–Dale relation and Lorentz–Lorenz equation.

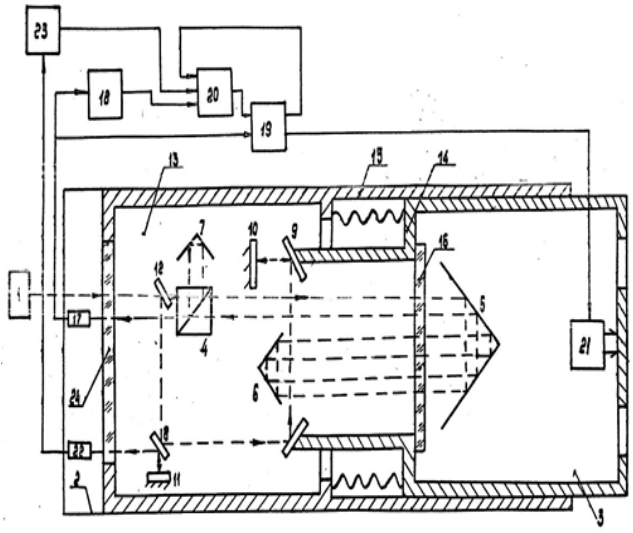


Figure 1. Refractometer with a double interferometer

The refractometer contains a monochromatic light source 1, housing 2, a working chamber 3 with a test medium, a main interferometer made according to the Michelson scheme, which consists of a beam splitter 4, a mirror in the form of an angular reflector 5, a retroreflector 6, and a mirror in the form of an angular reflector 7 in a non-working shoulder, an auxiliary interferometer made according to the Michelson scheme, which consists of a beam splitter element 8, a rectangular reflector 9, mirrors 10 and 11 [5].

Materials and research methods

Cow milk with different content of casein was chosen the object of research. Research was conducted in the University of Storage Technology, Plovdiv city, Bulgaria. The following reagents were taken for conduction of experiment: whole defatted milk; 10% acetic acid; 1% sodium hydroxide solution; 5% copper sulphate solution.

Equipment: test tubes, filtering funnel, paper filter.

Sequence of procedures.

1. Extracting of casein.

Put 2cm^3 of milk into the tube, add the same amount of distilled water and combine resultant mix.

Add 10% acetic acid to the solution in drops until formation of sediment. Avoid excess of acid.

Sediment shall be filtered on the paper filter and washed several times by distilled water (on the filter).

Dissolve sediment by 1% alkali solution. Resultant solution shall be filtered through water-lubricated filter.

2. Qualitative reaction to protein

Add 1 drop of copper sulphate solution to 1cm3 of filtered solution. We can see formation of red-purple coloring, which is typical for proteins.

Results and their discussion

Cleaned casein, extracted from milk with the help of acetic acid, is white amorphous powder without smell and taste, almost insoluble in water, soluble in diluted alkali solutions, alkali salts and alkaline-earth metals and mineral acids. It can be divided into particles, distinguished by content and properties.Earlier chemical analysis of milk was investigated, which shows that content of milk sugar, ashes, calcium and phosphor increased as far as part of casein increased to 2.8 %. After this decrease of content of these components was observed consequently on 0.03 – 0.08; 0.01 – 0.03; 1.22 – 3.04; 2.29 – 4.08. Analysis of received data shows that changes, connected to content of protein in milk, had important influence on its technological properties[6].

Therefore, decrease of content of casein in milk leads to increase of duration of coagulation under influence of fermenting agent, degeneration of quality of casein clot, increase of loss of nutritional substances during processing of clot, decrease of intensiveness of biochemical processes.

3. The isoelectric point (pI, pH(I), IEP), is the pH at which a molecule carries no net electrical charge or is electrically neutral in the statistical mean. The standard nomenclature to represent the isoelectric point is pH(I), although pI is also commonly seen, and is used in this article for brevity. The net charge on the molecule is affected by pH of its surrounding

environment and can become more positively or negatively charged due to the gain or loss, respectively, of protons (H^+).

Surfaces naturally charge to form a double layer. In the common case when the surface charge-determining ions are H^+/OH^-, the net surface charge is affected by the pH of the liquid in which the solid is submerged.

The pI value can affect the solubility of a molecule at a given pH. Such molecules have minimum solubility in water or salt solutions at the pH that corresponds to their pI and often precipitate out of solution. Biological amphoteric molecules such as proteins contain both acidic and basic functional groups. Amino acids that make up proteins may be positive, negative, neutral, or polar in nature, and together give a protein its overall charge. At a pH below their pI, proteins carry a net positive charge; above their pI they carry a net negative charge. Proteins can, thus, be separated by net charge in a polyacrylamide gel using either preparative gel electrophoresis, which uses a constant pH to separate proteins or isoelectric focusing, which uses a pH gradient to separate proteins. Isoelectric focusing is also the first step in 2-D gel polyacrylamide gel electrophoresis.

In biomolecules, proteins can be separated by ion exchange chromatography. Biological proteins are made up of zwitterionic amino acid compounds; the net charge of these proteins can be positive or negative depending on the pH of the environment. The specific pI of the target protein can be used to model the process around and the compound can then be purified from the rest of the mixture. Buffers of various pH can be used for this purification process to change the pH of the environment. When a mixture containing a target protein is loaded into an ion exchanger, the stationary matrix can be either positively-charged (for mobile anions) or negatively-charged (for mobile cations). At low pH values, the net charge of most proteins in the mixture is positive - in cation exchangers, these positively-charged proteins bind to the negatively-charged matrix. At high pH values, the net charge of most proteins is negative, where they bind to the positively-charged matrix in anion exchangers. When the environment is at a pH value equal to the protein's pI, the net charge is zero, and the protein is not bound to any exchanger, and therefore, can be eluted out.

A number of algorithms for estimating isoelectric points of peptides and proteins have been developed. Most of them use Henderson–Hasselbalch equation with different pK values. For instance, within the model proposed by Bjellqvist and co-workers the pK's were determined between closely related immobilines, by focusing the same sample in overlapping pH gradients. Some improvements in the methodology (especially in the determination of the pK values for modified amino acids) have been also proposed. More advanced methods take into account the effect of adjacent amino acids ±3 residues away from a charged aspartic or glutamic acid, the effects on free C terminus, as well as they apply a correction term to the corresponding pK values using genetic algorithm. Other recent approaches are based on a support vector machine algorithm and pKa optimization against experimentally known protein/peptide isoelectric points.

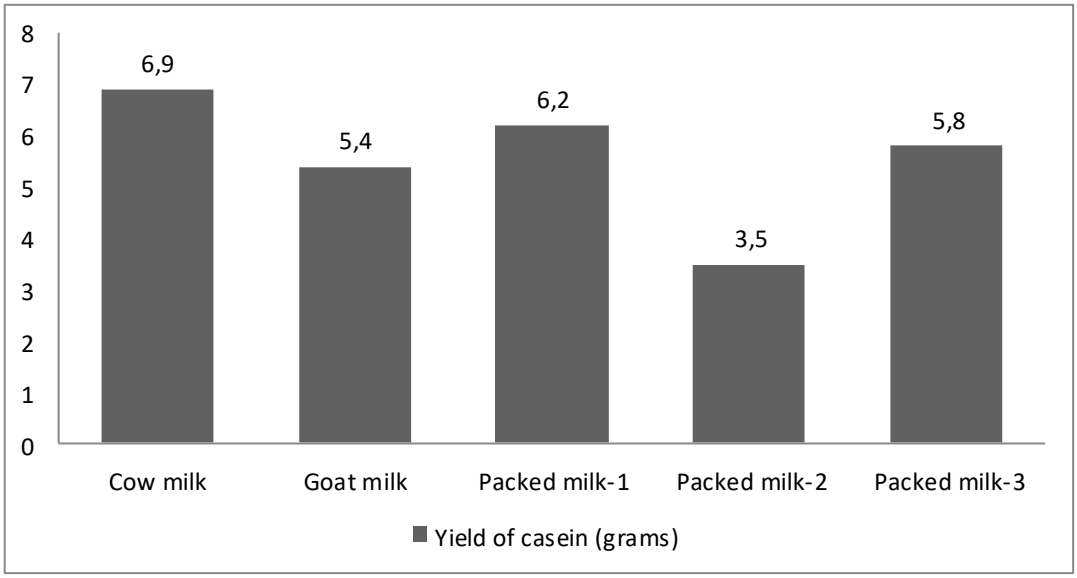
More over, experimentally measured isoelectric point of proteins were aggregated into the databases. Recently, a database of isoelectric points for all proteins predicted using most of the available methods had been also developed.

In the course of our work, we compared the content of casein in cow's milk, goat's milk and three other types of pasteurized milk. The results are shown in Table 1. Also we made a diagram based on this table (Diagram 1).

Table 1. Yield of casein in different kinds of milk

№	Milk samples	Yield of casein (grams)
1	Cow milk	6.9
2	Goat milk	5.4
3	Packed milk-1	6.2
4	Packed milk-2	3.5
5	Packed milk-3	5.8

Diagram 1. Yield of casein



Casein is the main protein of milk. It belongs to reserve proteins and is a mixture of several phosphoproteids (as-, b-caseins). The casein fraction also includes g-casein (2.5% of all casein), a product of partial proteolysis of b-casein catalyzed by milk proteinase. The main components of casein have genetic variants that differ in several amino acid residues. These proteins have a molecular weight of about 20 thousand, an isoelectric point of about 4.7, contain increased amounts of proline (the polypeptide chain has a b-structure), and are resistant to denaturants. Casein includes all the amino acids necessary for the body (including those essential), is the main component of cottage cheese and cheese.

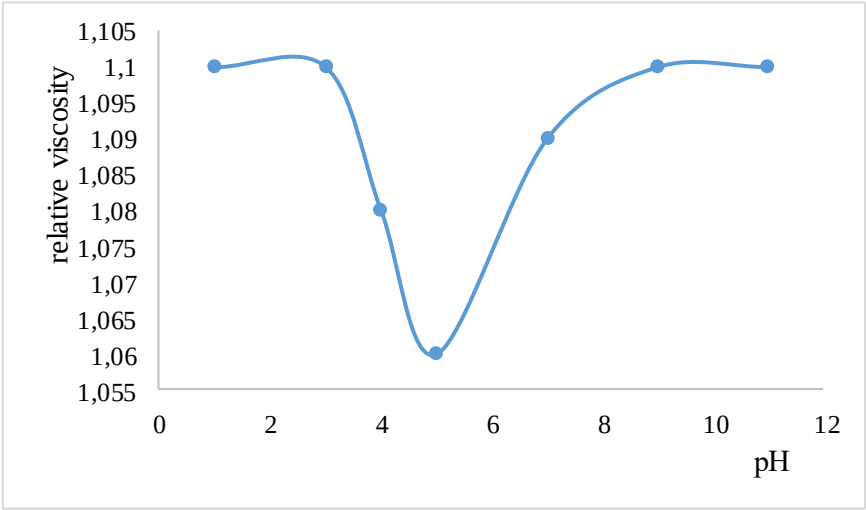


Figure 2. Isoelectric points of proteins

The study of the casein protein content in milk at various pH values, the following tasks determine the isoelectric point (IET) of proteins.

The rice shows the dependences of the relative viscosity of casein on the pH of the aqueous phase.

The method of refractometry is based on measuring the refractive index of a ray of light during the transition from one medium to another. If the test solution is a complex mixture of several components, each of them retains its refractive power, which gives the main

consideration of the total refractive index as an additive value [7].

The object of the study was casein samples in milk. The results are shown in the table 2:

Table 2. Casein samples in milk

Components	The content in milk,%	refractive indices
Lactose	1,49	1,47
Casein	2,8	1,38

Conclusions

Using experiments, you can determine that the composition of milk includes fat and protein. They are easily absorbed even by the child's body. The main protein in milk is called casein. Also, the milk contains carbohydrates that give it a sweet taste. Milk fat and the carbohydrate lactose provide energy.

Mineral salts (phosphorus, calcium) strengthen bones and produce fresh blood. Milk is a very useful and valuable food product, especially for a growing body. Different samples of milk contain different percentage of Casein.

A decrease in the casein content in milk leads to an increase in the clotting time under the action of the enzyme, to a deterioration in the quality of the casein clot, to an increase in the loss of nutrients during the processing of the clot, and to a decrease in the intensity of biochemical processes.

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