ISOLATION OF CASEIN FROM BOVIN AND HUMAN MILK

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Abstract: Milk is a multineutrient fluid and it is the primary source of nutrient for human. It consist of 80% of proteins. The protein in the milk is classified into casein and whey protein. Milk proteins consist of 80% of casein and 20% whey protein. The function of casein is to provide energy to human body. The name of casein is related to the family of phosphoproteins. These proteins are commonly found in the mammalian milk. This study deals with the precipitation of casein from the various milk sample such as cow milk, goat milk, buffalo milk, human milk, sheep milk and also the samples that availed from the market. The technique of precipitation of casein is used to predict the protein content in the milk sample.

Keyword: casein, lipid, fat, carbohydrates

Introduction
Milk is a complex physiological liquid that simultaneously provides nutrients’ and bioactive components that facilitate the successful postnatal adaption of the new born infant by stimulating cellular growth and digestive maturation, the establishment of symbiotic micro flora and the development of gut associated lymphoid tissues the help benefits of milk and fermented milk products have been known of since medieval times. Drinking milk has taken the advantage of the expensive nutritional value not only to the child, but also to the adult and the elder. In recent year milk constituent have become recognized as functional food, suggestive there use has a direct and measurable effect on help out comes (Gill et al. 2000a). Milk is composed of water fat proteins (mainly casein micelles and whey proteins), carbohydrates (mainly lactose) and qualitatively Mainner bio active components: minerals, vitamins and enzymes. It contents to measure protein types: casein and whey protein.
Milk composition

Milk contents specific proteins, fats design to be easily digested carbohydrates, minerals, vitamins and other components. Their composition reflects the nutritional requirement for the growth and development of each species. Bovine milk is composing of protein (3.5 %), lipid (5%), carbohydrates (4.9%) and minerals salts (0.7%). Were as human milk consists of protein (1.4%), lipid (4%), carbohydrate (4.9%) and minerals salt (1%). Milk protein has a high biological value there for milk is a good source of essential amino acids. However, a wide array of milk proteins has biological activities that range from anti microbial functions to the facilitation of nutrients absorption and others act as growth factors, enzymes antibodies and immune stimulants. Milk proteins can be broadly classifies into three categories: casines, whey proteins and mucins which are present in the milk fat global membrane. In milk, CASEINs interact with calcium phospaate forming large stable colloidal partical termed as micells. Theses micells make it possible to maintain a super satuared calcium phosphate concentration in milk, providing the new born with sufficient calcium phosphate for the mineralization of calcifying tissues. Milk proteins also facilitate the uptake of neutrants such as trace elements and vitamins that provide a protective function indicating there importance as multifunctional substance.

Bovine whey protein comprises immunoglobulin’s, alpha- lacta albumin, beta- lactoglobuline, serum albumin, immune globulin, lacto pharing, protios peptones fractions. lower amounts of other minor proteins and peptides also exist for e.g hormonal or other physiological activities. In humal milk the wey proteins found in significant quantities are alpha lacta albumin, lacto phaerin, igA,osteopontin and lyzoyme.

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>WATER (%)</th>
<th>MINERAL (%)</th>
<th>PROTIEN (%)</th>
<th>FATS (%)</th>
<th>LIPIDS (%)</th>
<th>CARBOHYDRATES (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COW</td>
<td>87.0</td>
<td>0.7</td>
<td>3.5</td>
<td>3.9</td>
<td>5.0</td>
<td>4.9</td>
</tr>
<tr>
<td>HUMAN (A)</td>
<td>87.3</td>
<td>0.3</td>
<td>1.4</td>
<td>4.1</td>
<td>4.0</td>
<td>4.9</td>
</tr>
<tr>
<td>GOAT</td>
<td>87.0</td>
<td>0.6</td>
<td>1.5</td>
<td>4.2</td>
<td>4.9</td>
<td>4.7</td>
</tr>
<tr>
<td>SHEEP (B)</td>
<td>82.6</td>
<td>0.9</td>
<td>3.5</td>
<td>6.7</td>
<td>5.1</td>
<td>4.6</td>
</tr>
<tr>
<td>BUFFALO</td>
<td>82.0</td>
<td>0.5</td>
<td>3.1</td>
<td>6.1</td>
<td>4.6</td>
<td>4.1</td>
</tr>
</tbody>
</table>

FIG.2.: MILKS SAMPLES
**ISOLATION CASIN FROM MILK**

**PROCEDURE**

- Dilute 100 ml of milk with 100 ml of water. Centrifuge for 10 min.
- Remove the fat layer which floats on top with spatula.
- Add 2% HCL drop by drop with constant stirring.
- Check the PH with bromocresol green indicator.
- Addition of HCL is continued until bromocresol green gives green color (color changes from blue to green, if acid is added in excess it changes to yellow).
- Observe the visible precipitate of casein.
- Allow the precipitate to settle and filter.
- Wash the precipitate in funnel with water and finally with ethanol.
- Collect, dry the precipitate and report yield.
- Confirm with chemical test.

**CHEMICAL TEST FOR CASINE**

- **SOLUBILITY TEST**: check solubility of casein by adding 0.5gm of casein in 2 ml 0.1NaOH, 2ml 0.1N HCL and 2ml of distilled water separately. Keep the tubes in water bath for 10 min and observe casein is completely soluble in 0.1N NaOH.
- **PREPARATION OF SAMPLE SOLUTION**: Dissolve 1gm of casein in 100 ml of 0.1 N NaOH to prepare solution.
- **BIURET TEST**: (General test for proteins) To 2ml protein solution add 2ml 10% NaoH solution and then 3 to 4 drops of 1% copper sulphate solution and mix. Purple color is produced.
- **NINHYDRIN TEST**: To 3 ml of protein solution add 3 drops of ninhydrin solution heat to boil and cool. A bluish purple color is produced.
- **PRECIPITATION BY HEAVY METALS**: To 2ml solution of protein and 2-3 drops of lead acetate solution. Casein is precipitate as white precipitate.
- **PRECIPITATION BY ALKALOIDAL REAGENTS**: To 3ml solution of protein, add 2 drops of 20% solution of sulphosalicylic acid, CASEIN is precipitated as white precipitate.
- **HEAT COAGULATION TEST**: Fill the protein solution up to 2/3rd of the test tube. Boil upper portion of test tube by slightly tilting the test tube. Add 2-3 drops of 1% acetic acid and again boil vigorously for 2-3 min. No appearance of turbidity in test tube confirm the caseine.

- **CONFIRMATIVE TEST (NUMANN'S TEST)**: To 5ml protein solution add 0.5ml of 40% NaOH. Heat for 1 min and cool spontaneously. Add 1.5ml of concentrated nitric acid (till PH acidic). To this solution add 1 ml of ammonium molybdate solution (saturated) and heat. A canary yellow precipitate is formed which confirms casein.

FIG. 4: TEST SAMPLES

References:

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