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COMPARATIVE STUDY ON MILK CASEIN ASSAY METHODS

STUDIU COMPARATIV ASUPRA METODELOR DE DETERMINARE A CAZEINEI DIN LAPTE

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Casein, the main milk protein was determined by different assay methods: the gravimetric method, the method based on the neutralization of the NaOH excess used for the casein precipitate solving and the method based on the titration of the acetic acid used for the casein precipitation. The last method is the simplest one, with the fewer steps, and also with the lowest error degree. The results of the experiment revealed that the percentage of casein from the whole milk protein represents between 72.6–81.3% in experiment 1, between 73.6–81.3% in experiment 2 and between 74.3–81% in experiment 3.
***Key words**: casein, milk, isoelectric point*

Introduction

Milk contains three different forms of globular proteins: caseins, lactalbumins and lactoglobulins. Globular proteins fail to interact with themselves and form colloid suspensions more easily than fibrous proteins.

Casein is a mixture of several similar proteins, called alpha, beta, kappa, and gamma caseins. Casein is a phosphoprotein and the difference between the forms of casein is the number of phosphate groups contained in the protein.

About 80% of the total protein in milk is casein; the difference is represented of the whey proteins and in small amounts of various enzymes (e.g. lipoprotein lipase, alkaline phosphatase, lactoperoxidase). Casein, the dominant protein in cow's milk, can be fractioned by electrophoresis into four major components: alpha-, beta-, gamma-, and kappa-casein (Clayden, 2001). Caseins are phosphoproteins, with molecular weight > 20,000 which are precipitated at pH 4.6 (for cow's milk) or by action of the enzyme chymosin (rennin) (Jensen, 1995; Fox, 1992).

Whey protein, which is more heterogeneous than casein, consists predominantly of beta-lactoglobulin and alpha-lactoalbumin. Other whey proteins present in smaller amounts are serum albumin, immunoglobulins (e.g. IgA, IgG, IgM), protease peptones, lactoferrin, and transferrin. Casein forms with albumin colloidal dispersions.

The goal of the study was to compare the different separation methods and to establish the most favorable assay method for casein.

Casein was determined using several methods: the gravimetric method, the retitration method, and the method based on the titration of the acetic acid. The methods are based on the same principle, namely the precipitation of casein at the isoelectric point, pH_I. It is very important not to exceed the pH_I value, because the casein is resolved.

Material and Methods

Casein from milk samples was separated and determined using several methods: the gravimetric method, the method based on the retitration of the NaOH excess used for the solubilization of the precipitated casein, and the method based on the titration of the acetic acid used for the casein precipitation. All the methods are based on the same principles, namely the precipitation of casein at the isoelectric point, pH_I (Caprita, 2001).

The gravimetric method consists in the casein precipitation with 2N CH_3COOH at $pH_I = 4.7$. The precipitate is then filtered, dried and weighed.

The retitration method implies the casein precipitation followed by its solubilization. The calculation of the casein content is based on the 0.1N NaOH volume used for the complete solubilization of casein.

The third method is based on the titration of the acid acetic used for the casein precipitation. After the neutralization of the milk free acidity, casein is precipitated with 0.1N CH_3COOH. The clear filtrate is titrated with 0.1N NaOH.

The proportion of casein from the total protein content was expressed as percentage. The total protein was determined with the Sörensen method.

Results and Discussions

The experimental data are presented in Tables 1, 2 and 3.

The gravimetric method is indicated for skim milk. The fat is separated relatively difficult, and has a negative effect on the results. The method implies considerable quantities of solvent.

The retitration method is laborious, with many difficult steps, which represent potential sources of errors. In order to avoid the loss of a part of the casein, which might remain adherent to the filter paper, we tried the precipitate decantation together with the filter paper. So, the error due to the incomplete retaken of the precipitate is reduced, but the titration of the NaOH excess is more difficult.

The method is based on the titration of the acid acetic used for the casein precipitation has the advantage of using the same milk sample used for the determination of the titratable acidity with 0.1N NaOH.

Table 1

The casein, protein and the casein proportion from total protein in experiment I

Sample	Protein, g/dL	Casein, g/dL	% Casein from protein
1	3.51	2.65	75.5
2	3.26	2.63	80.7
3	3.65	2.65	72.6
4	3.51	2.60	74.1
5	3.22	2.33	72.4
6	3.51	2.48	70.6
7	3.31	2.68	81.0
8	3.26	2.47	75.8
9	3.51	2.59	73.8
10	2.97	2.18	73.4
11	3.36	2.70	80.3
12	3.80	3.00	78.9
13	3.12	2.44	78.2
14	3.41	2.76	81.0
15	3.46	2.77	80.0
16	4.48	3.62	80.9
X±DS	3.45±0.33	2.65±0.31	76.82±3.67

Table 2

The casein, protein and the casein proportion from total protein in experiment II

Sample	Protein, g/dL	Casein, g/dL	% Casein from protein
1	3.56	2.80	78.7
2	3.32	2.44	73.5
3	3.37	2.57	76.2
4	3.37	2.51	74.5
5	3.37	2.74	81.3
6	3.95	3.20	81.0
7	3.51	2.80	79.8
8	3.17	2.42	76.3
9	3.51	2.80	79.8
10	2.83	2.23	78.8
11	3.42	2.66	77.8
12	3.90	2.98	76.4
13	3.12	2.48	79.4
14	3.32	2.64	79.5
15	3.37	2.66	78.9
16	4.49	3.52	78.4
X±DS	3.47±0.38	2.71±0.31	78.14±2.21

Table 3

The casein, protein and the casein proportion from total protein in experiment III

Sample	Protein g/dL	Casein g/dL	% Casein from protein
1	3.34	2.64	79.2
2	3.29	2.65	80.8
3	2.96	2.31	78.3
4	3.24	2.57	79.6
5	2.96	2.33	78.9
6	2.87	2.23	77.7
7	3.20	2.57	80.4
8	3.19	2.47	77.5
9	3.38	2.66	78.7
10	2.40	1.92	80.2
11	3.06	2.27	74.3
12	3.57	2.79	78.2
13	2.96	2.31	78.0
14	3.29	2.63	80.1
15	2.63	2.13	81.0
16	4.32	3.43	79.5
X±DS	3.16±0.42	2.49±0.34	78.9±1.64

As regarding the experimental data in the three experiments, we observed differences between the total protein and casein values of the milk samples. No significant differences were observed between the milk samples drawn from the same cow.

Conclusions

The study of the different methods for the casein determination allowed the following to be inferred:

- The most laborious method and with the greatest time and reagent consume is the gravimetric method. This technique needs a precise analytical balance, a desiccator, a ventilating room for the work with solvents, and a pH-meter for precipitating the casein at the isoelectric point. The method is not very precise.
- The retitration method of the NaOH excess used for the solubilization of the precipitated casein has the advantage to reduce the determination steps, and consequently the error sources. So, the drying and the weighing of the filter paper are not necessary. A source of error might be the washing of the precipitate from the filter paper into a beaker.

- The method based on the titration of the acetic acid used for the casein precipitation is the simplest one, with the fewer steps, and also with the lowest error degree.
- From the experimental data we observed that the proportion of casein from the total protein is between 72.6–81.3% in experiment 1, between 73.6–81.3% in experiment 2 and between 74.3–81% in experiment 3.
- The casein content in the milk from the same cow at two weeks interval remains almost constant.

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