APPENDIX 1 METHODS

1. Determination of milk's density

1.1. General

Milk density is defined as relation between the mass of definite milk volume at temperature 20 ^{o}C and the mass of equal volume distilled water at temperature 4 ^{o}C.
Density, alone, could not be used as a control parameter at milk quality control. Using the density the tentative figures for the SNF and solids could be determined.

1.2. Sampling and preparation for analyses

Sampling milk or other milk derivatives and their preparation for analyses is done according corresponding Appendices.
Milk density is determined not earlier than 2 h after milking. The milk must be with temperature from 10 to 25 ^{o}C.
Before determination of density the milk must be well stirred. To avoid foam formation, it has to be carefully poured on the cylinder's walls. The cylinder must be slightly tilted.
Before taking the readings the cylinder, with the milk must be placed on an even surface, facing the light, so the readings could be easily seen.

1.3. Basic principles.

The density of the milk is determined using aerometer, also called lacto-density-meter (milk density meter) and is expressed with a number, representing milk density meter degrees, decreased 1000 times or only with milk density meter degrees.

1.4. Necessary devices and reagents

- Aerometer /lacto-density-meter, milk meter/.
- Cylinder – with inner diameter not less than 5 cm, and length, corresponding to the dimensions of the lacto-density-meter.
- Ammonium with preliminary defined relative density.

1.5. Making the determination:

Dry and clean, the lacto-density-meter is slowly dipped in the milk till division 1,030, and then is left in free-floating state. The lacto-density-meter must not touch the cylinder's walls and to be on at least 5 mm from them.
When taking the readings the eyes must be on one and the same level with the meniscus. The reading is done in the meniscus' upper end with accuracy till 0,0005, and the temperature – with accuracy till 0,5 ^{o}C.

The difference between two parallel determinations must be not more than 0,0005.

1.6. Recalculating the values according lacto-density-meter at 20 oC.

If the milk, when determining its density, has temperature, higher or lower than 20 ^{o}C, the readings from lacto-density-meter are recalculated towards 20 ^{o}C.

Density recalculation towards 20 ^{O}C is done on the following way:
for every temperature degree over 20 ^{O}C from the received by the milkmeter value are added 0,2 ^{o} for the cow and goat milk and 0,25 ^{o} for sheep and buffalo milk lacto-density-meter degreed or 0,0002, respectively 0,00025 towards density; and for every temperature degree under 20 ^{o}C from the readings of milkmeter value are deducted 0,2-0,25 lacto-density-meter degrees or 0,0002, (0,00025) from the density.

2. Determination of fat content in the milk and milk derivatives.

2.1. General

For making analyses are used pure reagents for analyses (pure reagents for analyses (p.r.a.) and distilled water or water with equivalent purity.

2.2. Sampling

Milk and milk derivatives sampling is done according *Appendices Milk sampling and preparation of samples for analyses ad Sampling and preparation of samples for verification the accuracy of the milk analyser, making corrections and recalibration.*

2.3. Basic principles.

The method uses dissolving the milk and dairy products proteins with sulphuric acid with definite concentration in butyrometer and separating the fat under the influence of amilic alcohol, heating and centrifuging in a form of dense transparent layer, the volume of which is measured in the graduated part of the butyrometer.

2.4. Necessary devices and reagents

- Butyrometers for milk, special for skimmed milk and cream;
- Rubber stopples for butyrometers;
- Stand for butyrometers;
- Special pipettes or automatic for milk, sulphuric acid and изоамилов alcohol from 1, 10 and 11 cm^3;
- Pipettes from 1 and 20 cm^3;
- Glasses from 25 till 50 cm^3;

- Centrifuge for Gerber;
- Water bath;
- Mercury thermometers up to 100 ^{o}C with value scale 1 ^{o}C;
- Sulphuric acid with density 1,82 at 20 ^{o}C for determination of fat content of the milk;
- Isoamilic alcohol for Gerber with density 0,811 to 0,812.

2.5. Making the determination:

Preparation of samples for analyses.
The milk is mixed well in order to become homogenous mixture (if necessary it is slowly heated up to 35-40 ^{o}C) and is carefully shaken and tempered to 20±2 ^{o}C. The samples from whey and buttermilk are preliminary filtered through double layer gauze and is then tempered to 20±2 ^{o}C. Cream samples are placed in water-bath at temperature 35 till 40 ^{o}C, stirred till homogenous sample is received and cooled down to 20±2 ^{o}C.

2.6. Making measurement

With butyrometer for milk
For milk, whey and buttermilk.
With automatic or special for acids pipette are measured 10 cm^3 sulphuric acid with d=1,820 кg/m^3 at 20 ^{o}C in the milk butyrometer. Carefully on the butyrometer's walls are piled up 11 cm^3 from the prepared sample. The pipette is held till its full drainage.
For cream
From the prepared sample is measured 10 g with error up to 0,001 g and 50 cm^3 water are added. Mixture is well stirred and heated up to 30-35 ^{o}C, then is again stirred and cooled down to 20±2^{o}C, and the following steps are as with the milk sample using sulphuric acid with d=1,789 till 1,790 кg/m^3.
With butyrometer for cream
For cream
5 g from the sample are measured with butyrometer with error up to 0,0001 g and then 5 cm^3 water are added, 10 cm^3 sulphuric acid with d=1,780 to 1,790 кg/m^3 at 20 ^{o}C and 1 cm^3 изоамилов alcohol. The butyrometer is closed with rubber stapple and is shaken till the proteins are fully dissolved.

2.7. Calculating the results

By using milk butyrometer
Milk, whey, buttermilk.
Using the butyrometer's graded scale the grams fat in 100 g product are read directly. When the milk is curdled, the result is increased with 0,1 g for every degree.
By using cream butyrometer.

Cream

Using the butyrometer's graded scale the fat content in the products is directly read in percentages.

2.8. Measurement accuracy

By using milk butyrometer
The difference between two parallel determinations could not exceed:
For skimmed milk, whey and buttermilk - 0,05 g for 100 g product;
For cream - 0,5 g for 100 g product;
For milk - 0,1 g for 100 g product;
By using cream butyrometer
The difference between two parallel determinations could not exceed 0,5 g for 100 g cream.

3. Determination of water content and solids in the milk and milk derivatives.

3.1. General

The solids represent the fat content, proteins, carbohydrates and salts.
Sampling is done according *Appendices Milk sampling and preparation of samples for analyses ad Sampling and preparation of samples for verification the accuracy of the milk analyser, making corrections and recalibration.*

3.2. Basic principles.

Water content is determined by weight when drying at temperature (102±2) ^{o}C of the weighted product till constant mass, expressed in grams for 100 g product.
The solids/dry substance is the mass of the dry remainder, received after dehydration of determined quantity product at temperature (102±2) ^{o}C till constant mass and is expressed in grams for 100 grams of the product.

3.3. Necessary devices and reagents

- Assay balance with loading bounds 200 g and error 0,0002 g.
- Mercury thermometers from 0 to 100 ^{O}C and from 0 to 150 ^{o}C with value of scale division 1 ^{o}C;
- Pipettes from 5 to 10 cm^3, class II;
- Glass banks with grind stopples with volume 100-200 cm^3;
- Drying-oven with thermal regulator for keeping the temperature (102±2) ^{o}C;
- Exicator with silicagel or another hygroscope material;
- Weight plates;
- Peg for the weight plates;
- Glass pods with rounded ends;
- Quartz, sea or river sands.

3.4. Making the determination:

Sample preparation for analyses.
The milk (whey, cream, and buttermilk) is well shaken. If needed, the sample is heated slowly up to 38-40^{o}C, it is well mixed and cooled down to 20^{o}C. Mixing and pouring are done at least three times in dry and clean vessel.

3.5. Making the measurement

The weight plate with 20-30 g washed out and tempered sand and glass rod is dried at 102±2 ^{o}C for 1 h, and then is taken out, covered with the cap, tempered with exicator (up to 30 min) and the mass is weighted with accuracy up to 0,0005 g. In the weight plate, using pipette, at about 10 cm^3 milk are poured, covered and weighted. With the help of the glass rod milk is well mixed with the sand and without a cap is heated on a water-bath till a homogenous mass is formed. Then the weight plate is put in a drying-oven at temperature 102±2^{o}C, it is dried out for 3 h, it is taken out of the oven, covered with the cap, tempered in exicator (up to 30 min) and the mass is weighted. Weight-glass is placed in the drying-oven again and is dried 1 h, then is taken out, tempered and weighted. This procedure is repeated till the difference between two consequent measurements becomes not more than 0,004g. In case that at the second or following drying procedure mass increases, then for the calculation is taken the previous measurement.

3.6. Calculating the results

Water content in grams for 100 g product (milk or milk derivatives), is calculated by the formula:

$$X = \frac{M_2 - M_3}{M_2 - M_1} * 100$$

Where
M1 - the mass of the plate with the sand and the glass rod, g;
M2 - the mass of the plate with the sand, the glass rod, and the sample before drying, g;
M3 - the mass of the plate with the sand, the glass rod, and the sample after drying, g;

The dry substance (Y) is calculated using the formula:

$$Y = 100 - X,$$

Where:

X is the calculated water content.

3.7. Measurement accuracy.

The difference between tow consecutive measurements of one and the same sample could not be more than 0,2 g for 100 g product.

4. Determination of casein content in the milk.

4.1. General

The methods are based on the Volker's method.
For making the analyses are used pure reactive for analyses (p.r.a.) and distilled water or water with equal purity.

4.2. Sampling

According corresponding *Appendices*.

4.3. Basic principles.

Added to the milk formalin liberates acidic residuum from the protein's end groups, which are titrated with soda caustic solution. The soda caustic quantity is proportional to the casein in the milk content.

4.4. Necessary devices and reagents

- Glass 250 cm^3.
- Pipettes Foll - 25,5 cm^3.
- Pipettes Mor from 1 cm^3, with division 0,1 cm^3.
- Soda caustic p.r.a. - 0,143 n solution.
- Formalin 40% p.r.a - freshly neutralized.
- Phenolphthalein - 2 % solution in 70 % ethyl alcohol.
- Potassium oxalate p.r.a. 28 % water solution.
- Cobalt sulphate p.r.a. 5 % water solution.

4.5. Making the determination:

For cow milk
Reference sample preparation.
20 cm^3 from the measured milk are poured in a glass vessel together with 1 cm^3 3 % water solution of cobalt sulphate. The sample is shaken and a slight rose color of the solution is received, which serves as a standard in the research.

4.6. Making the measurement

20 cm^3 from the milk are measured in a glass and a titrated with 0,1 N soda caustic, using phenolphthalein as an indicator, till the color of the standard sample is reached. The volume of the used soda caustic is not taking into consideration.
4 cm^3 38-40 % formalin are added towards the neutralized sample and the rose color disappears as a result of the liberated carboxylic groups. It is well

stirred and titrated with 0,1 N soda caustic, till slight rose color is recovered. At the second titration the volume of the used soda caustic is measured.

For sheep milk
Casein content in sheep milk is determined on the same way. The only difference is that instead of 4 cm^3 38-40 % formalin in the milk are added 6 cm^3, and the standard/reference sample is prepared with 1 cm^3 4 % solution of cobalt sulphate.

4.7. Calculations

The quantity of the 0,1 N soda caustic in cm^3, used in the second titration, multiplied by the coefficient 0,7335 is equal to the casein content in the milk in percentages.
The following tables could be used for quicker readings of casein's percentage on the base of used cm^3 0,1 N soda caustic:

Table I

Calculation of casein content in the cow milk on the base of used cubic centimeters 0,1 N soda caustic:

0,1 n NaOH cm^3	Casein%	0,1 n NaOH cm^3	Casein %	0,1 n NaOH cm^3	Casein %
3,00	2,20	3,35	2,46	3,70	2,71
3,05	2,24	3,40	2,49	3,75	2,75
3,10	2,27	3,45	2,53	3,80	2,79
3,15	2,31	3,50	2,56	3,85	2,82
3,20	2,35	3,55	2,6	3,90	2,86
3,25	2,38	3,60	2,64	3,95	2,90
3,30	2,42	3,65	2,68	4,00	2,93

Table II

Calculation of casein content in the sheep milk on the base of used cubic centimeters 0,1 N soda caustic:

0,1 n NaOH cm^3	Casein%	0,1 n NaOH cm^3	Casein %	0,1 n NaOH cm^3	Casein %
5,40	3,96	6,10	4,47	6,80	4,99
5,45	4,00	6,15	4,51	6,85	5,02
5,50	4,03	6,20	4,55	6,90	5,06
5,55	4,07	6,25	4,58	6,96	5,10
5,60	4,10	6,30	4,62	7,00	5,13
5,65	4,14	6,35	4,66	7,05	5,17
5,70	4,18	6,40	4,69	7,10	5,21
5,75	4,22	6,45	4,73	7,15	5,24
5,80	4,25	6,50	4,77	7,20	5,28
5,85	4,29	6,55	4,80	7,25	5,32
5,90	4,33	6,60	4,84	7,30	5,35
5,95	4,36	6,65	4,88	7,35	5,39
6,00	4,40	6,70	4,91	7,40	5,43
6,05	4,44	6,75	4,95	7,45	5,46

4.8. Measurement accuracy.

Two parallel samples are measured and the difference between them could not exceed 0,1 %.

The accuracy of the method require the work to be done at place with good natural illumination, titration to be done evenly, without interruptions, colorless formalin to be used, preliminarily neutralized with soda caustic and phenolphthalein indicator.

Formalin titration is easy method, but it is not enough precise. More accurate results for casein content are obtained using Kjeldhal's method, but it requires special appliances.

5. Determination of salts in the milk

5.1. General

For the mineral substances in the milk conclusions can me made on the ashes content.

5.2. Sampling

According *Appendices Milk sampling and preparation of samples for analyses ad Sampling and preparation of samples for verification the accuracy of the milk analyser, making corrections and recalibration.*

5.3. Basic principles.

Milk is dried, carbonized and turned to ashes till constant mass. The ashes received are calculated in percentages.

5.4. Necessary devices and reagents

- Assay balance;
- Crucibles;
- Water-bath or infrared lamp;
- Hot plate or burner;
- Drying-oven with thermal regulator;
- Muffle furnace;
- Exicator;
- Quantity filter.

5.5. Making the determination:

In preliminary tempered and weighted crucible of the assay balance at about 10 g milk is weighted with accuracy up to 0,0005 g. The crucible with the sample is placed in a water-bath or infrared lamp till the evaporation of milk to dry state. Then it is carbonized with the burner or on a hot plate, paying attention not to be splashed out. The crucible is placed in a muffle oven and turns to ashes slowly, without the sample to be kindled, at temperature 500-550 ^{o}C till white or grey-white ashes. It is tempered in an exicator and is weighted till the appointed accuracy. Heating up in the oven is repeated till a constant mass is received.

5.6. Calculations

Ashes content is calculated using the formula

$$ashes = \frac{(C - A)}{(B - A)} * 100$$

Where:
A – the mass of empty, tempered crucible, g
B – the mass of the crucible together with the milk, g
C – the mass of the crucible with the received ashes, g

5.7. Measurement accuracy

The difference between tow parallel determinations could not be more than 0,02 %.