APPENDIX 4 SAMPLING AND PREPARATION OF SAMPLES FOR VERIFICATION THE ACCURACY OF THE MILK ANALYSER, MAKING CORRECTIONS AND RECALIBRATION.

1. Necessary consumables and devices

- Distilled water;
- Minimum 3 milk samples with known content of fat, SNF, protein, density, lactose, solids;
- Heating water bath;
- Cooling water bath or chamber;

2. General

Milk sampling and storage of samples of raw, thermally treated milk and its derivatives (cream, whey, butter-milk etc.) aiming verification the accuracy of the analyser, making corrections and recalibration is accomplished following the recommendations below:

- Sample to be taken from homogeneous batches, observing all the requirement;
- The sample's volume to be enough for making minimum 5 measurements for each sample or not less than 0.5 l;
- The samples to correspond to the standard physic-chemical and microbiological requirements, to be pure, without admixtures, without added cleaning or other unusual substances and without falsifications;
- Do not use samples with total acidity of milk more than 17^{o}T;
- Vessels, where the samples will be handled have to be clean, dry, glass, metal or other suitable material, to be tightly closed with rubber or other stopples. The stopples not to absorb water and fat and not to influence the analyzed sample content;
- Till the start of the analyses the samples are stored in conditions, assuring preservation of their content and quantities (advisable low temperature – 1-3 ^{o}C).

For longer storage of the samples a preservative is added as was already described in p.9.1.1, and then the sample has to be well stirred.

3. Representative Samples

The samples have to be representative for the corresponding milk type. Changes in the analyzed parameters in the samples have, if possible, to cover the whole measuring range – i.e. used samples to be with low, middle and high content of the analyzed components.

Exemplary recommended values:

Cow milk

Parameter	Low value	High value
% fat content	2,00	6,00

% Solids-Non-Fat content	8,00	9,00

The Lactose percentage content (4,0-5,5; average-4, 7), Protein (2,00-4,00; average-3, 3), salts (0,7-0,8) is proportional to the SNF content. When preparing samples these values vary within limited bounds.

Sheep milk

Parameter	Low value	High value
% fat content	5,50	10,00
% Solids-Non-Fat content	9,00	11,50

The Lactose percentage content (average - 4,6), Protein (average - 5,8), salts (average - 1,0) is proportional to the SNF content. When preparing samples these values vary within limited bounds.

Buffalo milk

Parameter	Low value	High value
% fat content	5,50	10,50
% Solids-Non-Fat content	9,00	11,00

The Lactose percentage content (average - 4,7), Protein (average - 4,3), salts (average - 0,8) is proportional to the SNF content. When preparing samples these values vary within limited bounds.

Goat milk

Parameter	Low value	High value
% fat content	2,00	6,00
% Solids-Non-Fat content	8,00	9,00

The Lactose percentage content (average - 4,6), Protein (average - 3,7), salts (average - 0,8) is proportional to the SNF content. When preparing samples these values vary within limited bounds.

Cream

Parameter	Low value	High value
% fat content	8,00	20,00
% Solids-Non-Fat content	2,50	5,00

The cream samples are diluted with distilled water. Degree of dilution is 2-3 times, in dependence of the initial fat content in the cream.

Whey

Parameter	Low value	High value
% fat content	0,20	0,80
% Solids-Non-Fat content	5,00	7,50

The content of fat and SNF in the whey depends on the kind of the dairy product as a result of which the whey is received.

4. Samples preparation

Milk – raw or thermally treated

For raw milk sample with average content of the analyzed components is advisable to be used milk, collected from at least 10 animals from the most commonly met breed in the region where the analyser will be functioning.

Low fat and high fat samples are prepared on the following way:

Available fresh or thermally treated milk is poured in a separating funnel, which is place in a refrigerator for at least 12 hours at temperature 4 - 6 °C in order to stratify. For a bigger stratification a longer time is required.
The layer at the bottom is poured in a vessel. It is well mixed by pouring it from vessel to vessel and is warmed up to 40 °C in a water bath.
The upper layer is poured in another vessel.
Using the certified methods the density and the concentration of the analyzed components - fat, protein, SNF, lactose, salts are determined.

The analyser's accuracy depends only on the correctness of the chemical analyses of the components in the samples and the normal acidity during calibration!

It is recommended the first cow milk sample with low fat content to be with the following parameters:

1.8-2% FAT; 8.7-9% SNF; 3,3-3,5 % Protein; 4,8-4,9% Lactose; 0,75 Salts; 1030-1033 kg/m^3 Density.
The second cow milk sample with high fat content to be with the following parameters:
5-5,5% FAT; 8.4-8,79% SNF; 3,1-3,2% Protein; 4,6-4,7% Lactose; 0,7 Salts; 1028-1029 kg/m^3 Density.
Samples with medial values of the separate parameters are received by mixing the two boundary values in a definite proportion.
Preserve the samples, using above described method for their longer storage.
When using samples, stored shortly, preliminary pour the sample from one vessel to another in order to distribute the milk components evenly paying attention not to form foam in the sample.
When the samples are stored for a longer period it is recommended to warm it up to 35-45 °C, and the vessel to be shaken carefully. In case that there is a cream stuck on the vessel's surfaces – remove it. The sample is poured from vessel to vessel several times and is cooled down (advisable to 20 °C /.

If there is separated liquefied fat or white particles with irregular form on the vessel's walls this sample could not be used.

Whey and buttermilk

The samples are poured several times from vessel to vessel and if needed gradual heating with stirring with cooling down is done.

Cream

The sample is slowly warmed up to 35 – 40 °C in water bath. The fat is dispersed wholly by carefully shaking and if necessary, by stirring and pouring it from vessel to vessel till its full homogenization.
From homogenized cream is prepared sample for analyses by diluting it with distilled water in degree, sufficient for the components of the diluted cream to be reached in the measuring range of the analyser.

5. Advisable scheme for independently determination the content of different parameters in milk and its derivatives

When is not possible to use the help of authorized laboratories and above mentioned milk analysers we recommend you to follow the scheme:

5.1. For cow milk (whole milk, low fat, skimmed milk) and UHT milk

Determination of fat content – Gerber's method, described in *Appendix Methods.*
Density determination – using aerometer, described in *Appendix Methods.*
SNF determination – by formula – p. 3.2.3.1.B
Determination of Lactose content – by formula – p.3.2.3.2.A
Determination of salts content – by formula – p. 3.2.3.3.A
Total protein content determination – by formula – p. 3.2.3.4.A

Example: Determination of the quality parameters for two samples cow milk (low fat and high fat), obtained and prepared according p. 2.3.1 and 2.4.1.

First – determine the fat content in the samples, using the Gerber's method (p.3.2.)
Suppose that for the first sample the result is 2,0 %F, for the second – 5,9 %F.
Second – determine the milk density, using aerometer (p.3.1.)
Suppose that the results are 1,0316 for the first sample and 1,0274 for the second
Third – Calculate the SNF content using the formula (p.3.2.3.1.B)

$$SNF = \frac{0{,}075 * 2{,}0 + 100 - 100/1{,}0316}{0{,}378} = 8{,}50\%$$

$$SNF = \frac{0{,}075 * 5{,}9 + 100 - 100/1{,}0274}{0{,}378} = 8{,}23\%$$

Fourth – determine the lactose content by the formula (p.3.2.3.2.A)
Lact. = SNF * 0,55 = 8.50 * 0.55 = 4.67 %
Lact. = SNF * 0,55 = 8.23 * 0.55 = 4.53 %
Fifth – determine the salts content by formula (p.3.2.3.3.A /
Salts = SNF * 0,083 = 8.50 * 0.083 = 0.71 %
Salts = SNF * 0,083 = 8.23 * 0.083 = 0.68 %
Sixth – determine the total protein content by formula (p.3.2.3.4.A)
Proteins = SNF * 0,367 = 8.50 * 0.367 = 3.12 %
Proteins = SNF * 0,367 = 8.23 * 0.367 = 3.02 %

So, when calibrating the milk analyser we'll use samples with the following parameters:

	I Ist sample (low fat)	II nd sample (high fat)
milk fat	2,00	5,90
SNF	8,50	8,23
density	1,0316	1,0274
lactose	4,67	4,53
salts	0,71	0,68
proteins	3,12	3,02

5.2. For sheep milk

Determination of fat content – Gerber's method, described in Methods p. 3.4.
Density determination – using aerometer, described in Methods p. 3.3.
SNF determination – by formula – p. 3.2.3.1.B
Determination of Lactose content – by formula – p. 3.2.3.2.A
Determination of solids/salts content – by formula – p. 3.2.3.3.A
Total protein content determination – by formula – p. 3.2.3.4.A

Example: Determination of the quality parameters for two samples sheep milk (low fat and high fat), obtained and prepared according p. 2.3.1 and 2.4.1.
First – determine the fat content in the samples, using the Gerber's method (p.3.2.)
Suppose that for the first sample the result is 5,6 %M, for the second – 9,8 %M.

Second – determine the milk density, using aerometer (p.3.1.)
Suppose that the results are 1,0352 for the first sample and 1,0300 for the second
Third – Calculate the SNF content using the formula (p.3.2.3.1.B)

$$SNF = \frac{0{,}075 * 5{,}6 + 100 - 100/1{,}0352}{0{,}378} = 10{,}11\%$$

$$SNF = \frac{0{,}075 * 9{,}8 + 100 - 100/1{,}0300}{0{,}378} = 9{,}65\%$$

Fourth – determine the lactose content by the formula (p.3.2.3.2.A)

Lact. = SNF * 0,45 = 10.11 * 0.45 = 4.55 %
Lact. = SNF * 0,45 = 9.65 * 0.45 = 4.34 %
Fifth – determine the salts content by formula (p.3.2.3.3.A /
Salts = SNF * 0,075 = 10.11 * 0.075 = 0.76 %
Salts. = SNF * 0,075 = 9.65 * 0.075 = 0.72 %
Sixth – determine the total protein content by formula (p.3.2.3.4.A)
Proteins = SNF * 0,475 = 10.11 * 0.475 = 4.80 %
Proteins = SNF * 0,475 = 9.65 * 0,475 = 4.58 %
So, when calibrating the milk analyser we'll use samples with the following parameters:

	I Ist sample (low fat)	II nd sample (high fat)
milk fat	5,60	9,80
SNF	10,11	9,65
density	1,0352	1,0300
lactose	4,55	4, 34
salts	0,76	0,72
proteins	4,80	4,58

5.3. For wheat, buttermilk and cream

Determination of fat content – Gerber's method, described in Methods p. 3.4.
Density determination – using aerometer, described in Methods p. 3.3.
SNF determination – using drying - p. 3.3. and formula – p. 3.2.3.1.A