Co-funded by the
Erasmus+ Programme
of the European Union

LABORATORY MANUAL

- ANALYSES OF RAW MILK QUALITY

I. Introduction

Definition. Milk is the normal mammary secretion of milking animals without either addition to it or extraction from it any substances. Raw milk is defined as "milk produced by the secretion of the mammary gland of farmed healthy animals that has not been heated to more than 40ºC or undergone any treatment that has an equivalent effect."

Requirements. Raw milk must comply with hygienic requirements and standards of the Regulation (EC) No 853/2004 of the European Parliament and of the Council, of 29 April 2004, laying down specific hygiene rules for food of animal origin.

Table 1. Selected requirements for raw milk according to Regulation (EC) No 853/2004.

<table>
<tr><td colspan="3">I. Health requirements</td></tr>
<tr><td colspan="3">1. Raw milk must come from animals (cows, buffaloes, ewes, goats, other) which are in a good general state of health that do not show any symptoms of infectious diseases communicable to humans (free from any infection of the genital tract with discharge, enteritis with diarrhoea and fever, or a recognisable inflammation of the udder, free from brucellosis and tuberculosis). The animals must not have any udder wound likely to affect the milk.
2. Raw milk must come from animals to which no unauthorized substances or products have been administered and that have not undergone illegal treatment.
3. Raw milk must come from animals in respect of which, where authorized products or substances have been administered the respective for these substances withdrawal periods have been observed.</td></tr>
<tr><td colspan="3">II. Hygiene on milk production holdings</td></tr>
<tr><td colspan="3">a. Requirements regarding premises (where milk is stored, handled or cooled), equipment and utensils – minimalisation of the risk of milk contamination
b. Hygiene during milking, collection and transport – clean udder, the animals have to be checked for any symptoms of diseases and in order to eliminate animals which are under medical treatment, application of only approved teat dips and sprays.
Immediately after milking milk must be cooled to the temperature not exceeding 8 °C in the case of daily collection, or not more than 6 °C if collection is not daily.
Transport – cold chain must be maintained so as to keep the temperature of the milk on the arrival to the destination place not higher than 10 °C.
In special circumstances if the milk meets the microbiological criteria it must not be cooled down:
- if it is processed within 2 hours after milking;
- from technological reasons related to the manufacture of certain dairy products but with the permission of the respective authority.
c. Staff hygiene requirements – suitable clean clothes, washing facilities, high degree of personal cleanliness</td></tr>
<tr><td colspan="3">III. Criteria for Raw milk</td></tr>
<tr><td>Cows' milk:</td><td>Not more than:</td><td>Frequency of testing:</td></tr>
<tr><td>Plate count of microorganisms at 30 °C in 1 mL of milk</td><td>100 000</td><td>Geometric average over a two-month period, with at least two samples per month.</td></tr>
<tr><td>Somatic cell count per 1 mL of milk</td><td>400 000</td><td>Geometric average over a three-month period, with at least one sample per month.</td></tr>
</table>

Milk of other species:	Not more than:	Frequency of testing:
Plate count of microorganisms at 30 °C in 1 mL of milk	1 500 000 (or 500 000 – milk intended for manufacture of products made without involvement of heat treatment)	Geometric average over a two-month period, with at least two samples per month.
Other criteria: milk must not contain antibiotic residues (exceeding the permitted level)		
IV. Criteria for milk immediately before processing: 1. Temperature not more than 6 °C (except certain circumstances) 2. Plate count of microorganisms at 30 °C in 1 mL of cows' raw milk less than 300 000.		

Other requirements for raw milk quality

FOR ALL MILK BATCHES:

It is forbidden to deliver milk:

- adulterated (e.g. with added water, defatted, with added neutralizing agents);
- from diseased cattle (or other animals: goats, sheep, buffalos) or during medicinal treatments;
- after treatments but before the end of the withdrawal period established for used medicine;
- not later than 3 weeks before calving and not earlier than 6 days after calving (colostrum);
- in case of reception ban established by official veterinarian.

Required milk parameters:

Appearance – colour white with cream-colored shade, without visible mechanical contaminants;

Smell – specific to milk, without foreign odour;

Temperature – not higher than 8 ºC (daily reception), 6 ºC (other reception), not-cooled (reception up to 2 hours after milking).

Acidity – measure of milk freshness:

- titratable acidity – 6.0-7.5 ºSH;
- pH – 6.6-6.8
-

- density – not lower than 1.028 g/mL;
- freezing point – not higher than -0.520 ºC;
- antimicrobial substances (antibiotics, cleaning agents etc.) – not accepted.

Milk composition. There are many factors that influence the composition of milk, such as: genetic factors (species, breed, individual factors), the physiological condition of the animal (stage of lactation, age, illness – in particular mastitis) and environmental factors (climate, feeding, method of milking). Table 2 shows mean composition of cows' milk.

Table 2. Composition of cows' milk

Component:			Mean:	Range:
Water	87.5%			
Dry matter	12.5%, incl.:			
		Protein	3.2%	(2.6 – 4.0)
		Fat	3.6%	(2.7 – 5.5)
		Lactose	4.8%	(4.2 – 5.2)
		Ash	0.7%	(0.6 – 0,8)
		Other organic compounds	0.2%	(0.1 – 0.3)

Table of contents

II. ANALYSES

1. MICROBIOLOGICAL QUALITY

1.1. Plate count of microorganisms at 30 °C according to Polish Standard PN-93/A-86034/03-04

Principle. *Total count of microorganisms in milk is determined by the incubation of the proper dilution of a milk sample with non-selective Milk Plate Count Agar at 30 °C for 72 h under aerobic conditions and enumeration of colonies. Results are expressed as colony forming units (cfu) per 1 mL of milk.*

Apparatus/glassware

Autoclave
Incubator (30 °C)
Automated pipette (1 mL)
Vortex
Sterile Petri dishes

Chemicals/reagents

9 mL of sterile peptone water (autoclaved at 121 °C for 15 min)
Sterile medium* for enumeration of total microorganisms count (autoclaved in Schott bottles at 121 °C for 15 min)

* (composition: distilled water agar, trypton, yeast extract, glucose, antibiotic free skim milk; pH 7.0)

Procedure

1. Mix milk thoroughly in a bottle before sampling.
2. Pipette 1 mL of milk sample into the probe with 9 mL of sterile peptone water using sterile automated pipette. Mix thoroughly with vortex.
3. Follow the scheme given below to make the proper dilutions.

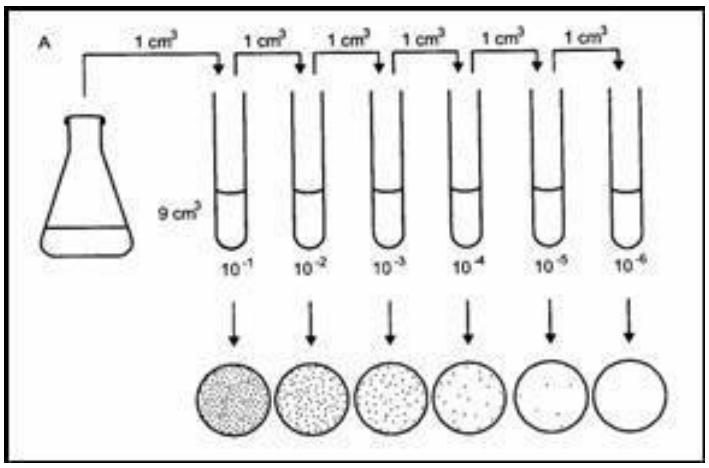

Fig. 1. Making solutions by the Lister method

4. Pipette 1 mL of each dilution into a sterile Petri dish. Each dilution should be pipetted into 2 Petri dishes.
5. Add 12-15 mL of sterile medium cooled to ~45 °C and mix gently.
6. Wait until the agar solidifies and incubate the plates (upside-down) with inoculated medium at 30 °C for 72 h under aerobic conditions.

Results. Select the plates with 10-300 colonies and enumerate microorganisms. Calculate the number of microorganisms (L) using given below equation:

$$L = \frac{C}{(N_1 + 0{,}1N_2) \cdot d}$$

where:
C– the sum of colonies from all Petri dishes chosen for enumeration,
N_1 – the number of dishes from the first dilution (e.g. 10^{-3} if dishes from 10^{-3} and 10^{-4} dilutions were enumerated),
N_2 - the number of dishes from the second dilution (e.g. 10^{-4} if dishes from 10^{-3} and 10^{-4} dilutions were enumerated),
d – index of dilution for the first dilution taken into account (e.g. 10^{-3}).

Express the results as colony forming units (cfu) per 1mL of milk (1.1 – 9.9 $\times 10^{x}$ cfu/mL).

Interpretation. The total content of microorganisms in raw milk should comply with the requirements of the Regulation (EC) No 853/2004, i.e. should be not more than $1{,}0 \cdot 10^5$ cfu/mL. If the raw milk is destined for direct consumption this number should not exceed $5{,}0 \cdot 10^4$ cfu/mL.

2. ORGANOLEPTIC ASSESSMENT

Procedure. Evaluate milk smell and appearance immediately after opening the container without mixing. Observe the milk sample for visible mechanical contaminants or fat pellets on surface.

Interpretation. The appearance of milk should be white with cream-colored or light-yellow-coloured shade without eye visible mechanical contaminants. Smell should be fresh, natural, without foreign odour. In case of doubts milk is tested for taste but after heating up to 80 ºC followed by cooling to room temperature.

3. MILK EXAMINATION FOR MASTITIS DIAGNOSIS

3.1. Whiteside test

Principle. *The test is based on the increase in number of leukocytes in mastitic milk, which react with NaOH solution to produce precipitates.*

Apparatus/ glassware

glass slide
glass rod

Chemicals/reagents

1 N NaOH

Procedure

1. Add one drop of 1 N NaOH to five drops of milk on a glass slide and stirred the mixture for 20 seconds with a glass rod.
2. Observe the consistency of milk sample on the dark background.

Results/Interpretation: Negative samples (normal milk) are entirely free of precipitate. The amount of precipitate formed is graded from slight to thick viscid mass and is considered to be indicative of the degree of irritation in the udder. The results are graded on the basis of degree of milk precipitation.

Table 3. Possible results in the Whiteside test

Grading	Description
Mixture remains opaque and free of particles	(-) Negative
Fine dispersed particles observed on close inspection at the end of test	(+/-) Trace
Mixture becomes slightly thicker, slight precipitates are observed	(+) Positive
Mixture thickens, precipitation is observed	(++) Positive
Strong mixture thickening and separation into milky whey and white particles are observed	(+++) Positive
Mixture thickens immediately and follows the glass rod, very strong precipitation is observed	(++++) Positive

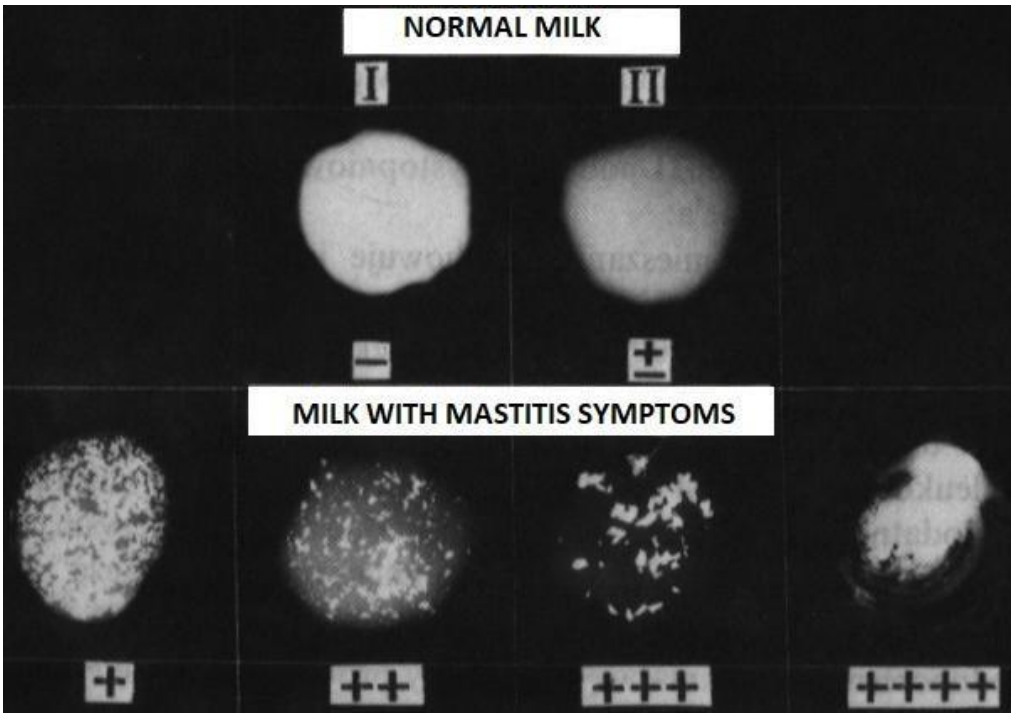

Fig.2. Possible results in the Whiteside test

Mastitis (mammary gland inflammation) changes physical, chemical (milk composition) and usually bacteriological quality of milk. It also significantly decreases the milk yield. Milk with symptoms of mastitis is not suitable for processing and cannot be purchased.

3.2. Determination of chloride concentration in milk by Mohr's method

Principle. *This method determines chloride ion concentration in milk by titration with silver nitrate in the presence of the potassium chromate as an indicator of the end point. Course of the reaction is as follows:*

$MeCl+AgNO_3=MeNO_3+AgCl\downarrow$

When all the chloride ions are precipitated, $AgNO_3$ reacts with the chromate ions to form a red-brown precipitate of silver chromate:

$2AgNO_3+K_2CrO_4=Ag_2CrO_4+2KNO_3$

Apparatus/glassware

100 mL volumetric flask
250 mL Erlenmeyer flask
10 mL, 5 mL, 1 mL pipettes
50 mL measuring cylinder

Chemicals/reagents

4% $CuSO_4$ solution (Bertrand I solution)
1 N NaOH solution
10% potassium chromate
0.1 N $AgNO_3$

Procedure:

1. Pipette 10 mL of milk into 100 ml volumetric flask, add 50 mL of distilled water, 10 mL 4% $CuSO_4$ (Bertrand I) solution and 2.2-2.3 ml 1N NaOH. Mix and wait for 10 minutes.
2. Adjust volume with distilled water to 100 mL.
3. Mix again and filter the mixture through the folded filter paper to a dry conical flask. First parts of filtrate should be returned and filtered again.
4. Transfer 50 mL of filtrate to the dry conical flask and add 0.5 mL of 10% potassium chromate solution.
5. Titrate with 0.1 N $AgNO_3$ until red-brown colour appears.

6. Calculate chloride concentration in milk from an equation:

$$X = \frac{3{,}55 \cdot a \cdot 20}{1000}[\%Cl]$$

where: a – mL 0.1 N $AgNO_3$ used for titration

1 mL 0.1 N $AgNO_3$ is equal to 3,55 mg Cl

Interpretation. Normal milk, derived from healthy cows contains on average 0.1% of chlorides expressed as Cl^- or 0.16% as NaCl. Increased level of chlorides (above 0.145%) may indicate that milk comes from animals suffering from udder inflammation (mastitis). Higher content of chlorides is also observed in milk from the beginning and the end of lactation period (colostrum). It results from the lower concentration of lactose (≤ 3.5%) in mastitic milk. Lactose and chlorides are the main components responsible for the constant osmotic pressure in milk (appr. 7.6 at 38°C). Therefore decreased concentration of the one compound (lactose) is accompanied with the rise in concentration of the other compound (chlorides).

3.3. Calculation of the chloride/ lactose ratio

Principle. Chloride to lactose ratio is calculated based on the results derived from determination of chloride concentration in milk by Mohr's method (ch. 3.2) and lactose content (ch. 5.4):

$$chloride/lactose\ \ ratio = \frac{100 \cdot Cl\%}{lactose\%}$$

Interpretation. Chloride to lactose ratio for normal milk, coming from healthy animals usually do not exceed 2.1. Milk derived from animals suffering from mastitis is characterised with 5-6 (or higher) chloride/lactose number. Lower lactose and chloride concentrations at the same time may indicate that milk is adulterated (diluted with water).

4. PHYSICAL PROPERTIES

4.1. Titratable acidity

Principle. *The titratable acidity is expressed as ºSH and is determined by titration of a known amount of milk with 0.25 N NaOH using phenolphthalein as indicator. The acidity expressed in ºSH (Soxhlet-Henkel degrees) is obtained by titrating 100 mL of milk with 0.25 N NaOH, using phenolphthalein as the indicator. This method is common in Central Europe.*

Apparatus/glassware

250 mL Erlenmeyer flask

25 mL pipette

Chemicals/reagents

0.25 N NaOH

2% ethanolic solution of phenolphthalein

Procedure. Pipette 50 ml of milk into a 250 mL Erlenmeyer flask. Add 2 ml of 2% alcoholic solution of phenolphthalein and titrate with 0.25 N NaOH until a faint pink colour persists for 30 sec.

Results: Titratable acidity should be calculated using the following equation (V- mL of 0.25 N NaOH used in the analysis):

$$ºSH = V \times 2$$

Interpretation. The level of titratable acidity and pH is a measure of milk freshness. The normal range for titratable acidity of fresh herd cow's milk is 6.0 – 7.5 ºSH (V - mL of 0.25 N NaOH used per 100 mL of milk). The acidity of 8-9 ºSH denotes slight acidification of milk, milk characterized with the acidity equal to 10-12 ºSH precipitates during heating, under the 24-28 ºSH milk precipitates at room temperature.

4.2. pH measurement

Principle. *The pH (called also active acidity or actual acid degree) means the amount of the hydrogen ions (negative logarithm of the hydrogen ion concentration) of milk and is measured by the electrometric way using a pH-meter.*

Apparatus/ glassware

pH-meter

~100 mL beaker

Procedure

1. Pour approximately 50 mL of milk into a small beaker.
2. Rinse the electrode of pH-meter with distilled water and place it into the milk sample together with the temperature compensation probe.
3. Turn on the pH-meter and wait for a moment for a stabilization of pH value.
4. Read the pH value from display.
5. Rinse electrode of pH-meter with distilled water and place it into the KOH storage solution.

Interpretation. According to the requirements the pH of milk at 20° C should vary within a relatively narrow range of 6.6 to 6.8. There are many components in milk which provide a buffering action. The major groups of substances which determine milk buffering capacity are caseins and phosphates. Acidification of milk lowers the pH value (pH < 6.5), whereas neutral or alcaline pH is measured in case of mastitis.

4.3. Milk density

Principle. *The density is read directly from the scale of lactodensimeter (specially graduated hydrometer).*

Apparatus/ glassware

lactodensimeter

250 mL cylinder

Procedure

1. Pour slowly (to avoid aeration) the milk into a clean and dry cylinder.
2. Immerse gently the lactodensimeter into the milk.
3. Read the measured density from the upper scale and the milk temperature from the thermometer (bottom scale). Unit: Lactodensimeter degree, (°Ld); 32°Ld = 1,032 g/ml)
4. Read the milk density at 20 °C from the correction table (given below).

Interpretation. According to the requirements of Polish Standard the density of milk at 20 °C should be not lower than 1.028 g/cm^3. The density of milk is given by the densities of its

components according to their mass content (SNF content contributes to higher milk density, whereas water and fat have negative impact on this parameter). Other factors which influence milk density are temperature (the table which allow to recalculate results obtained at different temperatures is given below) and inclusion of air. The density of raw milk may be used for quality control as by its measurement, deviations of milk composition due to addition of water (or skimming) may be revealed. 10% of added water results in approximately 0.03 (g/cm^3) decrease in milk density. However, measurement of milk density cannot alone constitute the absolute proof of watering as skimming and watering at the same time may give the "normal" milk density.

Table 4. Milk density in different temperatures

°Ld	Temperature of milk during measurement, °C															
	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
	Milk density at 20 °C															
25	1,0233	1,0235	1,0236	1,0237	1,0239	1,0240	1,0242	1,0244	1,0246	1,0248	1,0250	1,0252	1,0254	1,0255	1,0258	1,0260
26	1,0242	1,0244	1,0245	1,0247	1,0249	1,0250	1,0252	1,0254	1,0256	1,0258	1,0260	1,0262	1,0264	1,0266	1,0268	1,0270
27	1,0251	1,0253	1,0254	1,0256	1,0257	1,0259	1,0261	1,0263	1,0265	1,0268	1,0270	1,0272	1,0275	1,0277	1,0279	1,0282
28	1,0260	1,0261	1,0263	1,0265	1,0266	1,0263	1,0270	1,0273	1,0275	1,0278	1,0280	1,0282	1,0285	1,0287	1,0290	1,0292
29	1,0269	1,0271	1,0273	1,0275	1,0276	1,0278	1,0280	1,0283	1,0285	1,0288	1,0290	1,0292	1,0295	1,0297	1,0300	1,0302
30	1,0279	1,0281	1,0283	1,0285	1,0286	1,0288	1,0290	1,0293	1,0295	1,0298	1,0300	1,0302	1,0305	1,0307	1,0310	1,0312
31	1,0288	1,0290	1,0292	1,0294	1,0296	1,0298	1,0301	1,0303	1,0305	1,0308	1,0310	1,0312	1,0315	1,0317	1,0320	1,0322
32	1,0298	1,0300	1,0302	1,0304	1,0306	1,0307	1,0310	1,0312	1,0315	1,0318	1,0320	1,0323	1,0325	1,0328	1,0330	1,0333
33	1,0307	1,0308	1,0311	1,0313	1,0315	1,0317	1,0320	1,0322	1,0325	1,0328	1,0330	1,0333	1,0335	1,0338	1,0341	1,0343
34	1,0317	1,0319	1,0321	1,0323	1,0325	1,0327	1,0330	1,0332	1,0335	1,0338	1,0340	1,0343	1,0344	1,0348	1,0351	1,0353
35	1,0326	1,0328	1,0331	1,0333	1,0335	1,0337	1,0340	1,0342	1,0345	1,0347	1,0350	1,0353	1,0355	1,0358	1,0361	1,0363
36	1,0335	1,0338	1,0340	1,0343	1,0345	1,0347	1,0349	1,0352	1,0356	1,0357	1,0360	1,0362	1,0365	1,0367	1,0370	1,0373

5. CHEMICAL COMPOSITION

5.1. Fat content by the Gerber method

Principle. *The Gerber Method was developed and patented by Dr. Niklaus Gerber of Switzerland in 1891. In this method the fat is separated from fat-containing milk through the addition of sulphuric acid, which digest all milk components except oil. The separation is facilitated by using amyl alcohol and centrifugation. The fat content is read directly on a special calibrated glassware, called butyrometer. Measurements should be carried out in duplicate.*

Apparatus/glassware

special Gerber butyrometer - scale 0-6%; 0-7% or 0-10%,
caoutchouc stoppers
pipette - 11 ml
special Gerber centrifuge, ~1200 rpm,
water bath - 65°C

Reagents

sulphuric acid, H_2SO_4 - density 1.85 g/mL 90-91%, clear, colourless.

amyl alcohol, $C_5H_{12}O$ - density 0.81 g/mL

Procedure

1. Pour successively into the butyrometer: 10 mL sulphuric acid, 11 mL milk and 1 mL amyl alcohol.
2. Close the butyrometer with the caoutchouc stopper and shake until the milk is dissolved. Turn the butyrometer upside-down 5 times. Put the butyrometer into water bath and hold at 65 -70 °C for 5 minutes.
3. Spin in the centrifuge for 5 minutes and put again into water bath at 65 -70 °C for 5 minutes. Adjust the fat column by using the stopper, so that it will be in the graduated part of the butyrometer. The fat percentage can then be read directly from the scale.

Remarks

1. It is important to wear acid resistant gloves, protection glasses or a protection shield.
2. Samples containing sugar must not be analysed by this method. Sugar can react very violently with concentrated sulphuric acid and cause an explosion.
3. If the expected fat content is close to 1%, or if a higher precision of the fat content is required, it is recommended to use the Röse Gottlieb (extraction) method.

5.2. Calculations of dry matter (total milk solids) and solids non-fat content

Principle. *The content of total milk solids is calculated on the basis of density and fat content according to the Fleischmann formula:*

$$\%TMS(DM) = 1{,}2 \cdot t + \frac{2{,}665 \cdot 100 \cdot (d - 1)}{d}$$

where:

t – fat content determined by means of Gerber method (%);

d – milk density at 20°C.

The content of solids non-fat content is obtained by subtraction of fat content from dry matter content:

$$SNF = dm - t$$

Interpretation. According to the requirements of Polish Standard raw cow's milk must contain not less than 8.0% of solids non-fat.

5.3. Total ash content – mineralization method (dry ashing)

Principle. *The ash content in milk is analysed through the mineralization of milk in muffle furnace at 550 °C.*

Apparatus/ glassware

muffle furnace
porcelain or quartz dishes/crucibles
water bath - boiling
electrical heat plate
oven - 130 °C
desiccator
analytical weighing balance with accuracy of ± 0.0001

Reagents

concentrated acetic acid

Procedure

1. Weigh 20 g (± 0.001) of milk sample into an incinerated and pre-weighed quartz or porcelain dish
2. Add few drops of concentrated acetic acid (to precipitate proteins and facilitate the complete evaporation)
3. Place a dish into an oven and dry at ~100 °C and reduce the milk to obtain the thick (syrupy) consistency
4. Place a dish on an electric heating plate or oven and evaporate until no more fumes are evolved.
5. Place a dish into a muffle furnace (porcelain dishes must be placed into the cold furnace) and incinerate at ~550 °C until it is free of black carbon particles and turn into white or light grey in colour (6 hours).

6. Remove a dish carefully, cool in desiccator and weigh (with the accuracy of four decimal places).
7. Process of ashing, cooling and weighing should be repeated till no further loss in weight is indicated.
8. Calculate the ash content in milk from an equation:

$$\%ash = \frac{100 \cdot (c - a)}{b - a}$$

where: a – mass of an empty dish (g)

b- mass of a dish with milk (g)

c – mass of a dish with ash (g)

Interpretation. Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food. Cows' milk contains on average 0.7% of ash (range of: 0.66-0.77%). Milk contains on average:

Table 5. The content of basic minerals in milk (mg/ 100 mL of milk)

Cations		Anions	
Potassium (K)	138	Phosphorus (P)	96
Calcium (Ca)	125	Chlorine (Cl)	103
Sodium (Na)	58	Sulphur (S)	30
Magnesium (Mg)	12	CO_2	20

The ash content and concentration of mineral compounds cannot been used as synonyms as minerals are present in milk in different form than in the ash (some of them is present in the form of oxides). The prevalence of alkali elements in milk ash is advantageous in terms of human nutrition and distinguish (favours) milk and dairy products among the other foodstuffs such as meat or cereal products.

5.4. Lactose content – polarimetric method

Principle. *This method of lactose quantification is based on the measurement of the specific rotation of the polarized light due to the asymmetric carbon of lactose. The measurement is done after discarding fat and precipitation of proteins from milk.*

Apparatus/ glassware

Polarimeter

Pipette - 10 mL

Cylinder – 100 mL

Volumetric flask – 100 mL

Conical flask

Reagents

Carrez solution I - potassium hexacyanoferrate(II) trihydrate [$K_4Fe(CN)_6$-$3H_2O$]:15 g in 85 mL of distilled water

Carrez solution II - zinc sulfate heptahydrate ($ZnSO_4 \times 7H_2O$): 30 g/ 100 mL ammonia, 25% (w/w)

Procedure

1. Transfer 30 mL of milk to a 100 mL volumetric flask
2. Add 15 mL of Carrez solution I and 15 mL of Carrez solution II, mix and add two or three drops of ammonia solution and mix again
3. Adjust the volume with distilled water
4. Wait for 10 minutes
5. Filter the mixture through the folded filter paper to a dry conical flask
6. Wait for 30 minutes and adjust the temperature to 20 °C
7. Fill a clean polarimetric tube with filtrate in such manner to avoid air bubbles, place the glass window on the top followed by the gasket and screw the top.
8. Place the filled tube into the polarimeter and adjust the angle according to the proper directions. Record the value "α" . The measurement should be repeated twice.
9. Calculate the lactose content from the given below calculation:

$$C[g/100\,mL] = \frac{\alpha \cdot 100 \cdot 100}{52.5 \cdot l \cdot 30}$$

where: α: observed reading
30 - volume of milk used
l - the length of a polarimeter tube in dm (l = 2)
52.5: rotation of an aqueous solution of lactose at equilibrium.

The accuracy of this method is 10%.

Interpretation. The mean content of lactose (as monohydrate) in normal fresh milk is 4.8%. This value is lower at the beginning and at the end of lactation period. During the mammary gland inflammation some decrease of the lactose concentration in milk is observed (to 3% or less) as a result of the inhibition of its synthesis. It is usually accompanied by the increase of the content of chlorides.

6. THE TECHNOLOGICAL QUALITY OF MILK INTENDED FOR PRODUCTION OF CONCENTRATED MILK PRODUCTS OR CHEESE

6.1. The alcohol number

Principle. *The amount of 96% ethanol which causes the precipitation of 10 ml of milk is called "alcohol number". This method belongs to the group of indirect methods of evaluation of milk heat stability. The higher the alcohol number the higher the heat stability of the milk.*

Apparatus/glassware

100 mL conical flask

10 mL pipette

Chemicals/reagents

96% ethyl alcohol (in burette)

Procedure

1. Pipette 10 ml of milk into a 100 mL Erlenmeyer flask.
2. Titrate the milk sample with 96% ethanol until it precipitates. The amount of 96% ethanol which causes the precipitation of 10 ml of milk is called "alcohol number".

Interpretation. The alcohol number higher than 6 means that the milk is of high ethanol stability and can be qualified for the production of milk concentrates. The main reasons of low ethanol stability is when the milk is getting sour or its content is abnormal (e.g. too high content of whey proteins, Ca, Mg phosphates, citrates). In that case the balance of the colloid system changes and the proteins precipitate.

6.2. Rennet-induced coagulation test

Principle. *This test is conducted to eliminate the milk samples which incorrectly coagulate after addition of rennet enzyme. The test relies on the measuring the coagulation time after addition of rennet to milk (in proper proportions and temperature) and on the estimation of the quality of coagulum.*

Apparatus/glassware

50 mL beaker

25 mL pipette

1 mL pipette

water bath, 35°C

glass rod

thermometer

stop-watch

Chemicals/reagents

rennet solution (1 g of powder/100 g)

Procedure

1. Transfer 25 ml of milk into a 50 mL beaker and warm to 35°C in water bath.
2. Add 0,25 mL of rennet solution and start to measure the coagulation time
3. Mix the milk vigorously for few seconds and left it to coagulate.

Interpretation. The milk at the given conditions should coagulate after 4 to 10 minutes and the curd should be firm. The renneting time is affected by many factors: e.g. the higher enzyme concentration, temperature, the protein (casein) concentration and Ca content will accelerate the curd formation. Milk with elevated acidity will flocullate at an earlier stage. Milk which was subjected to heat treatment at high temperature will be characterized with increased clotting time, a weaker curd and high degree of syneresis.

6.3. Fermentation tests

Principle. *The principle of this method is to find what kind of microbiota dominates in raw milk. After few hours of incubation at 37-38 °C lactic acid bacteria (LAB) causes milk coagulation. The quality of the obtained coagulum, its consistency and appearance are affected by the kind of microorganisms present in milk. Used conditions i.e. 37-38 °C rather favour the growth of harmful bacteria, e.g. E. coli and putrefactive bacteria than the growth of LAB. The fermentation test with added rennet additionally gives the information about the quality of rennet curd.*

Apparatus/glassware

~50 mL sterile probes

20-40 mL sterile pipettes

1 mL pipette

incubator, 37-38 °C

Chemicals/reagents

rennet solution (0,5 g of powder/100 g)

Procedure.

1. Transfer 20-40 ml of milk into a sterile probe for the fermentation test
2. Transfer 20-40 ml of milk into a sterile probe, add 1 mL of rennet solution and mix for the fermentation-rennet test
3. Place the probes into the incubator at 37-38 °C for 12 hours (fermentation-rennet test) or 24 hours (fermentation test)
4. Observe the quality of curd according to given below classification.

Interpretation. The correct evaluation of curd is performed after 24 of hours by the following classifications [Table 6]:

Type of milk	Sub-type	Description	Interpretation
L (liquid milk, without symptoms of coagulation)	L_1	Milk completely smooth, with sweet taste or purely acidic	Milk of L type may be exceptionally clean, contains very little microorganisms or has the outstanding characteristics of bacteriocides. This type of milk is rather rare. If it does not show any other defects it is suitable for cheese processing.
	L_2	A slight amount of whey under the cream layer , the lack of signs of clot	
	L_3	The initiating curling can be seen	
J (jelly – smooth curd without cracks, proper odour rand taste)	J_1	Even curd, without wheying-off	Milk of J type is suitable for processing
	J_2	Curd with a few cracks or grooves produced by gas	
	J_3	Curd with cracks and grooves produced by gas, with possible slight whey separation	
type W (wheyey – curd retracted to a lesser or greater extent, usually along the wall of the tube, but not torn, greeny whey not acidic separation)	W_1	curd slightly retracted with small amount of whey	Milk of W type may be used for processing but with the signs stronger than in W_3 type cannot be used for cheese making
	W_2	curd retracted like a pencil, greeny whey slightly acidic	
	W_3	curd heavily retracted, partly torn with not clear whey	
type G (granular – granular curd in the form of small flakes, whey clouded or yellow)	G_1	thin granular curd but uniform	Milk of G type may be used for processing if the characteristic for this type symptoms are not intensive (poorly G_1)
	G_2	thick granular curd with visible whey separation	
	G_3	thick granular and torn curd	
type S (swelly - torn, loose curd with a large number of gas bubbles in a curd and cream)	S_1	swelled curd, gas bubbles present in a curd and cream	Milk of S type is completely defective and not suitable for processing.
	S_2	curd and cream layer strongly swelled	
	S_3	curd intensively swelled, spongy or torn	

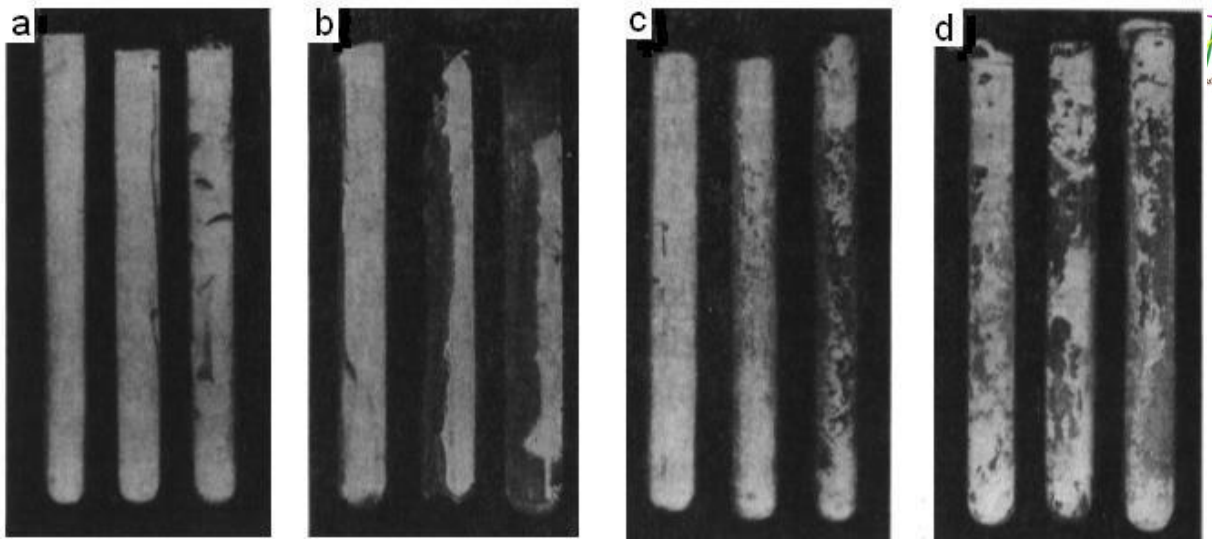

Fig.3. Possible results in fermentation test: a) curd of jelly type; b) curd of wheyey type; c) curd of granular type; d) curd of swelly type

Classification of milk in fermentation test with rennet addition [Table 7]:

Type	Description	Classification
I	Curd in the form of smooth, straight or slightly cranky, cylindrical rod (like pencil), medium hard, firm, dense, elastic, completely smooth in the cross-section or with a few holes, clear whey with characteristic regular smell and acidic taste	Normal milk of clear lactic fermentation, suitable for cheese manufacturing
II	Curd twisted, uneven, weakly dense, with many holes in the cross-section, whey not clear with taste and smell not completely clear	Milk of II type may be conditionally used for processing if the characteristic for this type symptoms are not intensive
III	Curd heavily twisted, spongy, too hard or pulped, torn, high number of holes or visible blowings in the cross-section, whey unclean with defective taste and smell	Completely defective, not suitable for cheese manufacturing

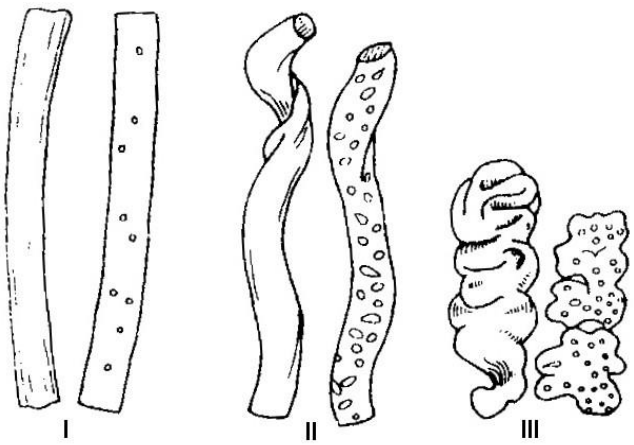

Fig. 4. Types of curds in fermentation test with added rennet

References (including figures):

1. Bylund G. 2003. Dairy Processing Handbook. Tetra Pak Processing Systems AB, Lund, Sweden. Available at: https://dairyprocessinghandbook.com/
2. Jurczak M. 2005. Mleko - produkcja, badanie, przerób. SGGW, Warszawa [in Polish].
3. Polish Standard PN-A-86002: 2002. Raw milk for sale. Requirements and tests [in Polish].
4. Regulation (EC) No 853/2004 of the European Parliament and of the Council, of 29 April 2004, laying down specific hygiene rules for food of animal origin.
5. Walstra P., Geurts T. J., Noomen A., Jellema A., van Boekel M. A. J. S. 1999. Dairy Technology. Marcel Dekker Inc., New York.
6. Zmarlicki S. (red.) 1978. Ćwiczenia z analizy mleka i produktów mlecznych: opracowanie zbiorowe. SGGW, Warszawa [in Polish].

Checking questions (choose the correct answer):

Q1. According to the proper standard (Regulation (EC) No 853/2004) plate count of microorganisms assessed at 30 ° C in fresh, raw milk should:

A. Not exceed 100 000 cfu in 1 L of milk;
B. Be tested at least two times per month;
C. Not exceed 400 000 cfu in 1 mL of milk.

Q2. Negative result in the Whiteside test indicates that:

A. Milk is fresh;
B. Milk is not suitable for processing and cannot be consumed;
C. Milk comes from healthy cows without mammary gland inflammation (mastitis).

Q3. The proper analysis to check if milk is suitable for the production of milk concentrates is:

A. Rennet-coagulation test;
B. Determination of the alcohol number;
C. Fermentation test.

LABORATORY MANUAL

- ANALYSES OF RAW MILK QUALITY

Dr hab. inż. Dorota Najgebauer-Lejko, prof. URK
Department of Animal Product Technology
University of Agriculture in Krakow
e-mail: dorota.najgebauer-lejko@urk.edu.pl

This work was co-funded by the Erasmus+ Programme of the European Union

Innovation of the structure and content of study programs profiling food study fields with a view to digitizing teaching

Táto publikácia bola spolufinancovaná programom Európskej Únie Erasmus+

Inovácia štruktúry a obsahového zamerania študijných programov profilujúcich potravinárske študijné odbory s ohľadom na digitalizáciu výučby

FOODINOVO | 2020-1-SK01-KA203-078333

Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Education and Culture Executive Agency (EACEA). Neither the European Union nor EACEA can be held responsible for them.

Financované Európskou úniou. Vyjadrené názory a postoje sú názormi a vyhláseniami autora(-ov) a nemusia nevyhnutne odrážať názory a stanoviská Európskej únie alebo Európskej výkonnej agentúry pre vzdelávanie a kultúru (EACEA). Európska únia ani EACEA za ne nepreberajú žiadnu zodpovednosť.

FOODINOVO | 2020-1-SK01-KA203-078333