UNIVERSITY OF LJUBLJANA
BIOTECHNICAL FACULTY
DEPARTMENT OF ANIMAL SCIENCE
CHAIR OF DAIRY SCIENCE

YOGURT & CHEESE

INSTRUCTIONS FOR PRACTICUM

NAME

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Domžale, 8 - 9 May, 2018

METHODS FOR FERMENTED DAIRY PRODUCTS

YOGURT, SOUR MILK, KEFIR

1. DETERMINATION OF TITRATABLE ACIDITY AND pH

ISO/TS 11869 IDF/RM 150: 2012. Fermented milks – Determination of titratable acidity – Potentiometric method

DEFINITION
amounts in milliliters of a 0.25 M NaOH required to titrate 100 ml of product to standard pink color and expressed in SH (Soxhlet Henkel)

SAMPLE PREPARATION
Agitate the yogurt sample properly and thoroughly until it is homogenous

REAGENTS
a) 0.25 M NaOH = for titration
b) 2 % phenolphthalein; 2 g in 100 ml of 95% ethanol = indicator
c) 5 % $CoSO_4*7H_2O$ = for standard color solution preparation

METHOD
Standard color: pipet 25 ml of yogurt into plastic beaker, and add 0.5 ml of 5 % $CoSO_4*7H_2O$. A standard pink color develop and can be used for the next 3 h.
Titration: pipet 25 ml of yogurt into plastic beaker, and add 1 ml of 2 % phenolphthalein. Titrate with 0.25 M NaOH , while stirring constantly, to the first permanent color change to faint standard pink color, that persists for 30 sec using phenolphthalein as indicator. Then, rinse the remaining yogurt form the pipette with already titratted yogurt and contunuie with titration to the second

Acidity is expressed in SH (Soxhlet-Henkel)

RESULT
Titratable acidity is obtained according to equation:

SH = volume of used 0.25 M NaOH (counted in mL) × 4

SLO regulation
a) Plain yogurt/sour milk/kefir: up to 55 SH
b) Fruit yogurt: not below pH 3.5 (pH measurement)

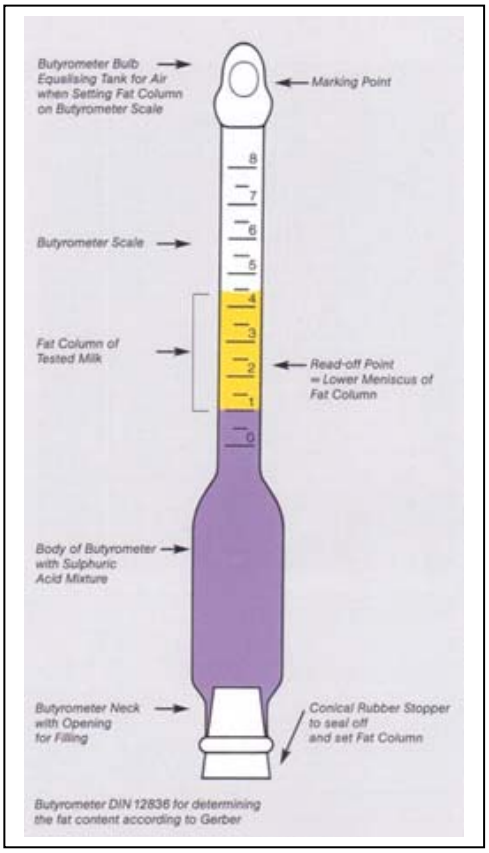
2. DETERMINATION OF FAT CONTENT – GERBER METHOD

DEFINITION

An empirical procedure which gives a value for fat content in grams of fat per 100 g or per 100 ml of milk/dairy product

PRINCIPLE

Separation of fat of the milk in butyrometer by centrifuging after dissolving the protein with sulphuric acid, the separation being aided by the addition of a small quantity of amyl alcohol. The butyrometer is graduated to give a direct reading of fat content.



EQUIPMENT AND REAGENTS

- Gerber butyrometer for milk and appropriate rubber stopper;
- Volumetric pipette (10 and 11 mL);
- Gerber centrifuge;
- plastic measuring cylinder;
- ammonia, 10 %
- Sulfuric acid H_2SO_4 (density 1.825 g/L)
- Amyl alcohol (density 0.815 g/L)

SAMPLE PREPARATION

In plastic measuring cylinder, measure 90 mL of thoroughly agitated yogurt/sour milk until is homogenous, and transfer it into plastic container. Then rinse the remaining yogurt by adding 10 mL of ammonia in cylinder and pour it into plastic container with yogurt/sour milk. Mix it well.

METHOD

Measure 10 ml of H_2SO_4 into the butyrometer, using analog dispenser (dispensette). Suck the prepared yogurt/sour milk sample into the "milk pipette" (11 or 10.75 mL). Held the pipette at an angle of about 45° on the neck of the butyrometer, and allow the yogurt to flow gently down the inside of the butyrometer to form a layer on top of acid, preventing any mixing with the acid. Add 1 mL of amyl alcohol using dispenser. Seal the butyrometer with rubber stopper and without disturbing its content. Now shake an invert the butyrometer – CAUTION-butyrometer gets very warm! immediately place it in the centrifuge, and centrifuge the butyometer for 5 min. Remove the butyrometer from the centrifuge and read the result.

RESULT

The fat content in yogurt is: % fat = (B-A) × 1.1
A … is the reading at the bottom of the fat column
B … is the reading at the top of the fat column
1.1 … sample is prepared with 1/10 of ammonia

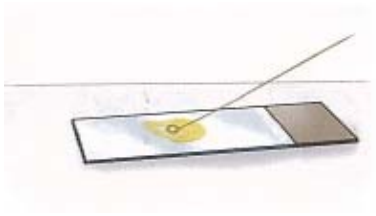
3. GRAM STAINING OF YOGURT/SOUR MILK BACTERIA

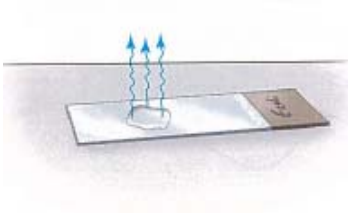
Gram staining is used to identify most bacteria by dividing them into two groups: gram-positive and gram-negative. Besides, by observing bacteria under microscope, the cell shape, arrangement and size of bacteria in a fixed smear can be determined.

EQUIPMENT
- Clean glass slides, preferably with frosted end
- Immersion oil
- Microscope
- Inoculating loop

REAGENTS
R1: Crystal violet solution
R2: Lugol
R3: decolorizer (1:1 = ethanol:acetone)
R4: Safranin solution

PROTOCOL
a) Preparing a bacterial smear from yogurt/sour milk sample: flame the loop to sterilize it, and cool it down. Transfer a loopful of yogurt to the center of the slide and spread it into a thin film (a1). Allow the smear to air dry (**a2**), and fix the dried smear by passing the slide quickly through flame three times, smear side up (**a3**). The smear is now said to be heat-fixed.


a1


a2

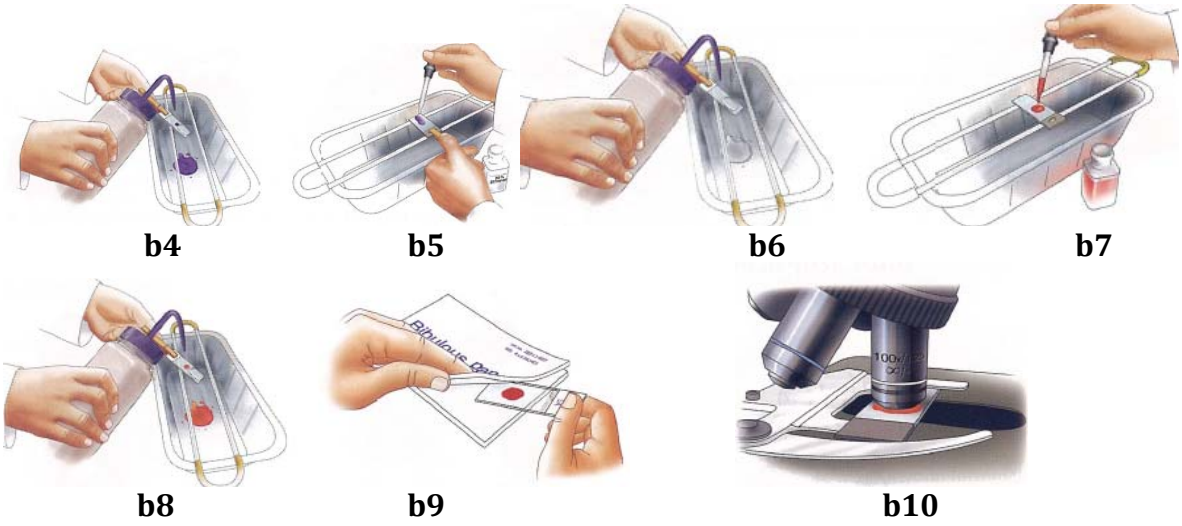

a3

b) Staining bacteria from yogurt/sour milk: cover the fixed smear with R1 (crystal violet) for 1 minute (**b1**). Rinse the slide with water to rinse excess R1 (**b2**), and cover the smear with R2 (lugol) for 1 minute (**b3**). Rinse the slide to remove excess lugol (**b4**) and decolorize with R3 until color stops running (**b5**). Rinse the slide (**b6**) and cover the smear with R4 (safranin) for 1 minute (**b7**). Rinse the slide with water to rinse excess safranin (**b8**) and dry water with paper towel (**b9**). Examine the stained smear under the microscope using the oil immersion lens (**b10**).

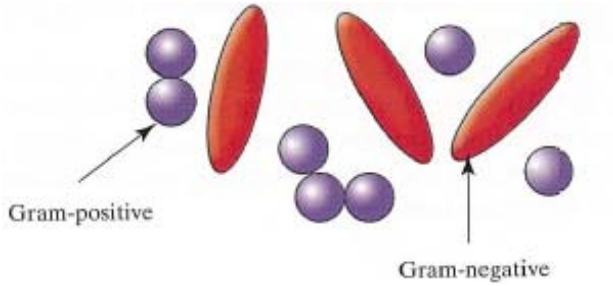

b1

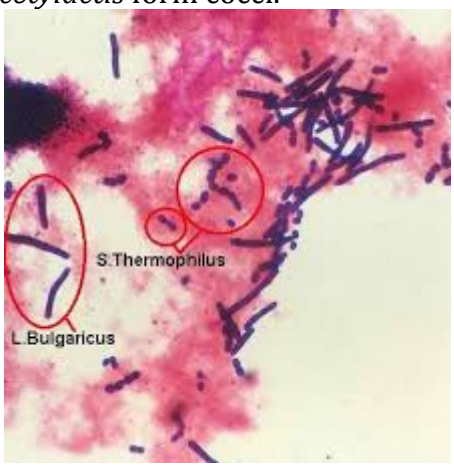

b2


b3



RESULT: all the gram-positive bacteria turn purple, and all the gram-negative bacteria turn red. Lactic acid bacteria, including yogurt and sour milk starter cultures, are gram-positive, therefore are purple to blue in color. Yogurt: *Lactobacillus delbrueckii* ssp. *bulgaricus* forms rods, and *Streptococcus thermophilus* forms cocci, usually in chains. Sour milk: *Lactococcus lactis* ssp. *lactis*, *cremoris*, *diacetylactis* form cocci.





Milk is a very complex food with over 100.000 different molecular species found. There are many factors that affect the composition of raw milk such as breed, age and physical state of the cow and seasonal variations. Therefore only an approximate milk composition of 87-88 % water and 12-13 % total solids can be given. The total solids consist of approx. 4 % fat and 9 % solids-non-fat (SNF) (proteins, lactose, minerals, vitamins, ...).

CHEESE ANALYSIS

1. TOTAL SOLIDS CONTENT

ISO 5534:2004 (IDF 4:2004) Cheese and processed cheese -- Determination of the total solids content (Reference method)

Principle

A weighed test portion of cheese is dried by heating it in a drying oven at 102 °C. The dried test portion is then weighed to determine the loss of mass.

Apparatus

- Analytical balance, capable of weighing to the nearest 1 mg, with a readability of 0,1 mg
- Forced ventilation drying oven, electrically heated, with ventilation port(s) fully open, capable of being maintained at 102 °C±2 °C throughout its working space
- Aluminium foil
- Grinding or grating devices, easy to clean, appropriate for preparing the test sample
- petri dish

Preparation of test samples

Prior to analysis, remove the rind, the smear or the mouldy surface layer of the cheese in such a way as to obtain a test sample representative of the cheese as it is usually consumed. Grind or grate the test sample by using an appropriate grinding or grating device. Mix the ground mass quickly. Clean the device after preparing each sample. Care shall be taken to avoid moisture loss. Store the test sample in an airtight container (petri dish) until commencing the analysis, which shall be carried out as soon as possible after grinding. First, weigh the aluminium foil and then weigh between 2-3 g of grated cheese onto foil. Fold the foil over cheese and flatten the cheese sample. Uncover the foil and put it into the oven. Dry the cheese until the constant weight.

Result

$$\% \text{ dm} = \frac{(c - a)}{(b - a)} * 100$$

% dm = dry matter

a - aluminium foil weight
b - aluminium foil and cheese weight before drying
c - aluminium foil and cheese weight after drying

% of water: 100 - % dm

To determine the cheese texture we express it as water in non-fat matter:

$$\% \text{ water in non-fat matter} = \frac{\% \text{ water}}{100 - \% \text{ f}} * 100$$

% f = fat

Cheese classification based on texture:
Very hard cheeses: contain less than 51 % of water in non-fat matter or less than 35 % of absolute water
Hard cheeses: contain between 49-56 % of water in non-fat matter or between 35-40 % of absolute water
Semi hard cheeses: contain between 54-63 % of water in non-fat matter or 40-50 % of absolute water
Soft cheeses: contain more than 66 % of water in non-fat matter or at least 50 % of absolute water

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2. FAT CONTENT

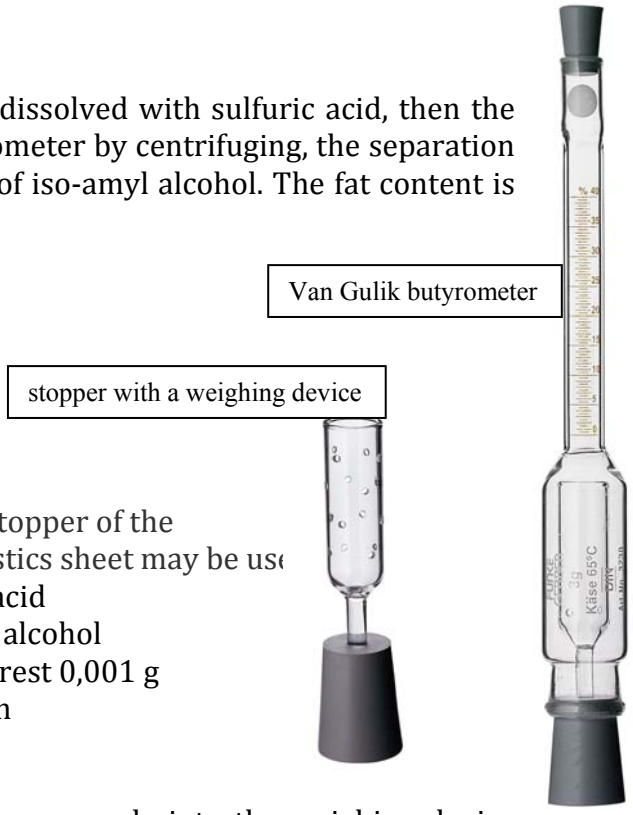
I**SO 3433:2008 (IDF 222:2008)** Cheese -- Determination of fat content -- Van Gulik method

This method is applicable to all types of cheese.

Terms and definitions
Van Gulik method is an empirical procedure which, when applied to a cheese, gives a value for fat content, expressed in grams per 100 g of cheese (numerically equivalent to a percentage mass fraction).

Principle
The protein layer surrounding the fat globules is dissolved with sulfuric acid, then the fat of the cheese is separated in a Van Gulik butyrometer by centrifuging, the separation being assisted by the addition of a small quantity of iso-amyl alcohol. The fat content is then read directly from the butyrometer scale.

Reagents
- Sulfuric acid
- Iso-amyl alcohol



Apparatus
- Van Gulik butyrometers
- Weighing device which can be fitted to the large stopper of the butyrometer. Alternatively, a dish, a capsule or plastics sheet may be use
- Pipette or automatic measure, to deliver sulfuric acid
- Pipette or automatic measure, to deliver iso-amyl alcohol
- Analytical balance, capable of weighing to the nearest 0,001 g
- Centrifuge, in which the butyrometers can be spun

Sampling - Cheese portion
Weigh, to the nearest 0,005 g, 3,000 g of the cheese sample into the weighing device fitted to a suitable stopper, or into a capsule, or on plastics sheet.

Determination

If a stopper with a weighing device is used, close the neck of the butyrometer with this stopper, including weighing device and test portion, and add sulfuric acid to the small opening until the acid level reaches a height of about two-thirds of the body of the butyrometer and the weighing device is completely surrounded with sulfuric acid. Place the butyrometer with its neck (large opening) downwards in the water bath maintained at 65 °C for 5 min. Remove the butyrometer from the water bath and mix it well for 10 s; repeat the operation until the protein (cheese) is completely dissolved. Remove the butyrometer from the water bath and add, after thorough shaking of the butyrometer, 1 ml of iso-amyl alcohol to the small opening. Immediately shake the butyrometer for at least 3 s, and add sulfuric acid to the small opening until the level reaches the 35 % graduation mark. Close immediately with the small stopper and invert the butyrometer. As soon as the fat has ascended into the body, shake the butyrometer thoroughly for 10 s. Invert again so that the acid drains out of the stem. Repeat the shaking and inversion twice and centrifuge the butyrometer at a relative centrifugal acceleration of 350) for 10 min. Remove the butyrometer from the centrifuge and carefully adjust the large stopper to bring the bottom of the fat column, with the minimum movement of the column, to a graduation mark, preferably a main graduation mark. This should preferably be done by slightly withdrawing the stopper and not by forcing it further into the neck.

Method of calculation

The fat content of the cheese, expressed in grams per 100 g of cheese, is equal to B - A where A is the reading obtained at the bottom of the fat column; B is the reading obtained at the top of the fat column.

Absolute % fat content: direct reading from the butyrometer

% fat in dry matter: $= \frac{\%\ f}{\%\ dm} * 100$

On the basis of fat content, expressed on dry matter, cheeses are classified as follows:

High-fat cheese: at least 55% of fat content in dry matter

Full fat cheese: at least 50% of fat content in dry matter

Fat cheese: at least 45% of fat content in dry matter

¾ of fat cheese: at least 35% of fat content in dry matter

Half fat cheese: at least 25% of fat content in dry matter

¼ of fat cheese: at least 15% of fat content in dry matter

Low (or no) fat cheese: less than 15% of fat content in dry matter

3. CHLORIDE CONTENT

ISO 5943:2006 Cheese and processed cheese products -- Determination of chloride content -- Potentiometric titration method

Is applicable to all cheeses and processed cheese products containing more than 0,2 (m/m) of chloride ion. The principle is based on the suspension of a test portion in water, acidification with nitric acid and subsequent potentiometric titration of chloride ion with standard volumetric silver nitrate solution.

Analysis

2 to 3 g of grated cheese is weighed into a Erlenmeyer flask. Addition of 25 mL of 0.1 M $AgNO_3$ is followed by addition of 25 mL of conc. HNO_3. The mixture is heated to boiling point and during boiling, 10 ml of $KMnO_4$ is added. When slowly boiling solution decolorize, another portion of $KMnO_4$ is added until the dark brown color of solution remains. The excess of $KMnO_4$ is removed by the addition of point of the knife of glucose. Sample is diluted with addition of 100 mL of water and 5 mL of $NH_4Fe(SO_4)_2$ is added as a titration indicator. The excess of $AgNO_3$ is titrated with 0.1 M NH_4SCN mL until the red-brown color.

Solutions

Titrant c($AgNO_3$) = 0.1 mol/L

Method of calculation

$$\% \text{ NaCl} = \frac{0{,}585 * (a - b)}{c}$$

a 25 mL of added $AgNO_3$
b the usage of 0.1 M NH_4SCN for titration (in mL)
c mass of cheese sample (in g)

1 ml 0,1 M $AgNO_3$ = 0,00585 g NaCl

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4. CLOTTING ACTIVITY

ISO 11815:2007 (IDF 157:2007) Milk -- Determination of total milk-clotting activity of bovine rennets

Bovine rennets (calf and adult) contain, in various amounts, both chymosin and bovine pepsin as main milk-clotting enzymes. Each of these enzymes has its own characteristics as far as milk-clotting activity and cheese-making properties are concerned. The most obvious difference between these enzymes is the stronger pH dependence of the milk-clotting activity of pepsin. For economic reasons, therefore, it is very important to know the total milk-clotting activity of a certain rennet type and to have that characterized

relative to an internationally recognized reference standard with known composition and milk-clotting activity.

Definition
Milk clotting activity is defined as the volume of milk clotted by one volume of rennet at 35 °C in 40 minutes.
Protocol
0.1 g of powdered rennet is dissolved with 100 mL of dH_2O (in 100 mL measuring flask). Prior analysis the titratable acidity of milk is determined (see below!) that should be between 6.5 – 7.5 SH. 100 mL of milk is heated to 35 °C and during mixing, 5 mL of prepared rennet solution is added. When 2.5 mL of rennet we start to measure the coagulation time.

Calculation of milk clotting activity

$$L = \frac{M * 1000 * 2400}{l * t}$$

M – amount of milk in ml (100 mL)
l – amount of added rennet (5 mL)
t – coagulation time in s

As milk clotting activity depends on titratable acidity of milk, we calculate the milk clotting activity to 7.0 SH.

Expression of milk clotting activity:
1 : 100.000 means that 1 unit of rennet is capable to rennet 100.000 units of milk at 35 °C in 40 minutes.

MILK FRESHNESS - determination of acidity

- titratable acidity: from **6,0** to **7,5 SH**
- pH measure: from **6,6** to **6,8**

Titratable acidity – Soxhlet Henkel (SH) method

Objective
The **titratable acidity** according to Soxhlet-Henkel (SH) is the volume of NaOH solution (0,25 M) which is used up in the titration of **100 mL of a milk**. The titration is performed to a certain standard colour shade using phenolphthalein as indicator that changes color from colorless to faint pink.
The acidity in milk indicates the consumption of NaOH necessary to shift the pH-value from 6,6±0,1 (corresponding to fresh milk) to a pH-value of 8,2-8,4 (phenolphthalein).

Method
<u>Production of the standard color</u>

50 ml of the milk are pipetted into a titration flask and mixed with exactly 1 ml of cobalt sulphate solution ($CoSO_4$). The standard faint pink is developed and it holds for maximum 3 hours.

Determination of the titratable acidity:
50 ml of the milk are pipetted into a titration flask and 2 ml of phenolphthalein (2%) are added. Then you titrate, while continuously swirling, with 0,25 M NaOH until you reach the standard colour shade. You should not need more than one minute for the titration.

Result: acidity=$V_{0,25M\ NaOH} \times 2$
Fresh milk: titratable acidity up to 7,2 SH (according to regulation)

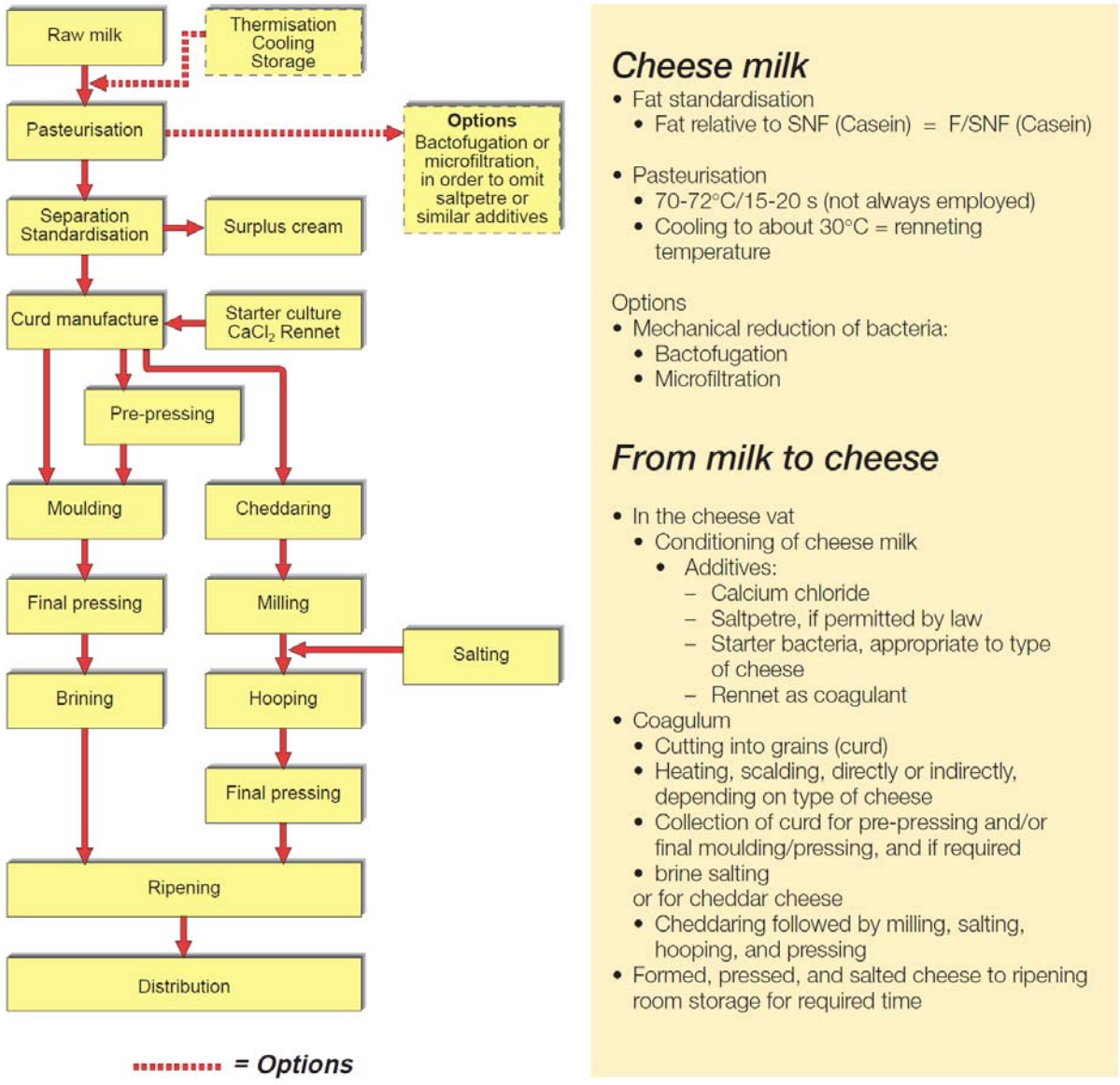
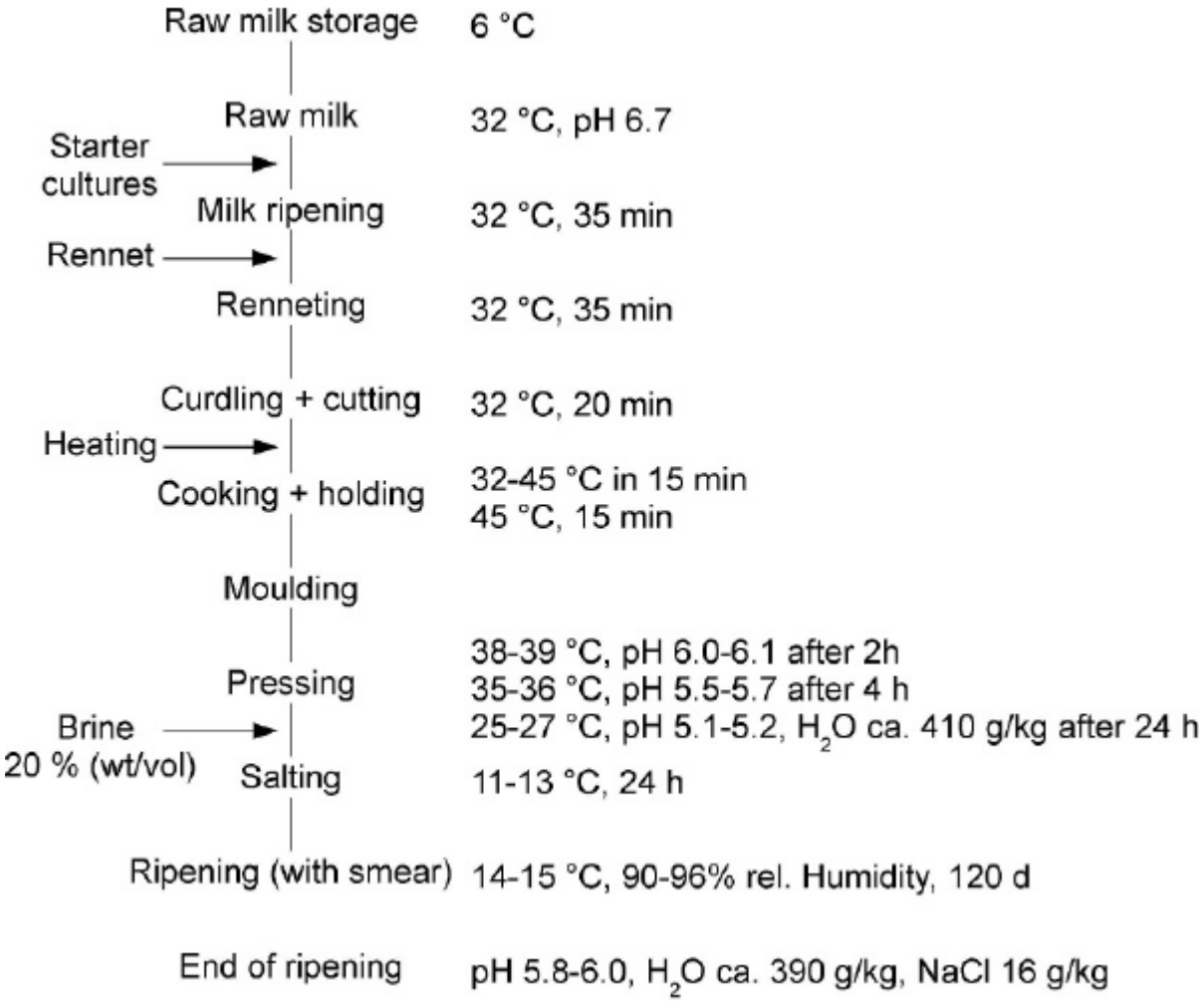
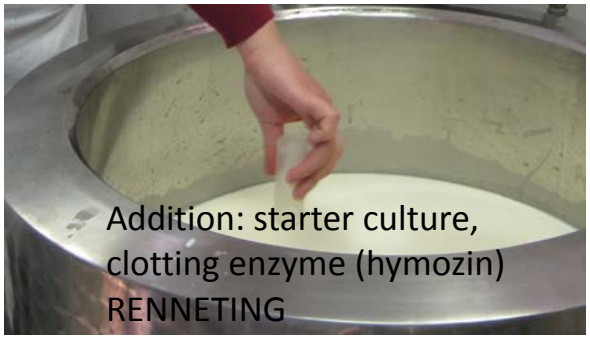


Fig. 14.1 *Process flow in production of hard and semi-hard cheese.*


Raw milk storage
6 °C
Raw milk
32 °C, pH 6.7
Starter cultures
Milk ripening
32 °C, 35 min
Rennet
Renneting
32 °C, 35 min
Curdling + cutting
32 °C, 20 min
Heating
Cooking + holding
32-45 °C in 15 min
45 °C, 15 min
Moulding
Pressing
38-39 °C, pH 6.0-6.1 after 2h
35-36 °C, pH 5.5-5.7 after 4 h
Brine
20 % (wt/vol)
Salting
25-27 °C, pH 5.1-5.2, H2O ca. 410 g/kg after 24 h
11-13 °C, 24 h
Ripening (with smear)
14-15 °C, 90-96% rel. Humidity, 120 d
End of ripening
pH 5.8-6.0, H2O ca. 390 g/kg, NaCl 16 g/kg


Milk in cheese vat


T: 32-34 °C


Addition: starter culture,
clotting enzyme (hymozin)
RENNETING


Coagulated milk;
firmness of coagulum


Cutting of coagulum


Cheese grain making


Scalding of cheese grain


Firm cheese grain


moulding


Pressing (intensive fermentation)