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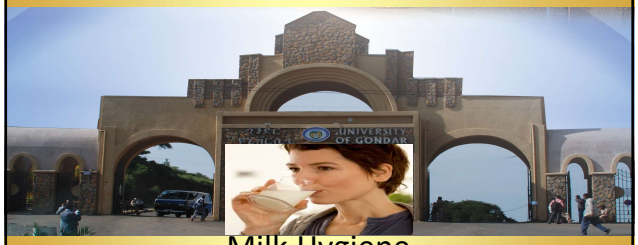

Milk Hygiene

Compiled by
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Objectives

At the end of the course, students will able to:

- Define milk
- Explain special features of milk synthesis
- Identify the Components of milk
- Identify the chemical composition

Introduction

- Milk and milk products have been used by man since prehistoric times
- **Cheese** making was discovered 7000–6000 BC **in Iraq**
- **butter** was made as far back as **2000 BC**
- The development of the **milk separator** in the **19th century** made centralized milk processing possible
- Today , up to **60%** of the milk produced in the world is converted into **dehydrated milk** products and foods containing a **large proportion of milk solids**

- In Africa, milk is produced and either
  - sold **fresh**,
  - consumed as **fermented** milk or
  - **manufactured** into products such as
    - butter, ghee and cheese.
    - Sour milk (most common product) .
  - To some extent pasturized milk

???

Define Milk

Definition

- ***The public health*** experts have defined milk as
  - fresh, clean lacteal secretion of the mammary glands of a mammal, practically free from colostrums, obtained by the complete milking of one or more healthy cows which contains not less than 8.25% milk solids-not-fat (SNF) and 3.25% milk fat."

Remark :- milk obtained within 15 days before and 5 days after calving, the period during which colostrums is produced, should be excluded.

Definition……

- ***On physical point of view***
  - Milk is an opaque, white, heterogynous fluid having various constituents in multi dispersed phases of **emulsion**, colloidal **suspension** or **solution**
- *Cow milk:- a white fluid of low viscosity and slightly sweet taste — is most commonly used as human food*

Brain storming

- List the components of healthy milk
  - 3 min

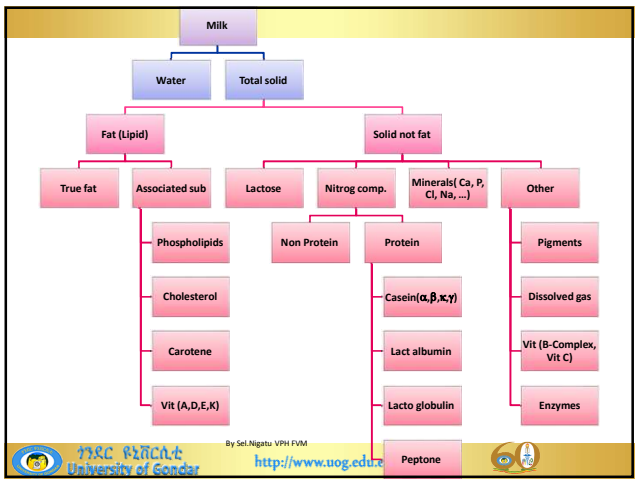

Table 1. *Composition (%) of milk of some species of mammal.*

Species	Total solids	Fat	Protein	Lactose	Ash
Human	12.4	3.8	1.0	7.0	0.2
Cow	12.7	3.7	3.4	4.8	0.7
Goat	12.3	4.5	2.9	4.1	0.8
Sheep	19.3	7.4	5.5	4.8	1.0
Horse	11.2	1.9	2.5	6.2	0.5
Donkey	11.7	1.4	2.0	7.4	0.5
Domestic rabbit	32.8	18.3	13.9	2.1	1.8
Camel	12.9	4.2	3.7	4.1	0.9

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Name of the Species	Percentage		Composition		Ash
	Water	Fat	Protein	Lactose	
Cow (foreign)	86.6	4.6	3.4	4.9	0.7
Buffalo	84.2	6.6	3.9	5.2	0.8
Ewe (sheep)	79.4	8.6	6.7	4.3	1.0
Goat	86.5	4.5	3.5	4.7	0.8
Ass	90.0	1.3	1.7	6.5	0.5
Camel	86.5	3.1	4.0	5.6	0.8
Elephant	67.8	19.6	3.1	8.8	0.7
Mare	89.1	1.6	2.7	6.1	0.5
Sow	89.6	4.8	1.3	3.4	0.9
Whale	70.1	19.6	9.5	-	1.0
Dog	75.4	9.6	11.2	3.1	0.7
Guine Pig	82.2	5.5	8.5	2.9	0.9
Cat	84.6	3.8	9.1	4.9	0.6
Liama	86.5	3.2	3.9	5.6	0.8
Human Milk	87.7	3.6	1.8	6.8	0.1

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Milk as food

- The gross energy supplied by milk can be calculated from its
  - lactose, protein and fat contents.
- Metabolically **available energy** is approximately
  - **Lactose - 4.0, kcal/g**
  - **Protein - 4.1 kcal/g**
  - **Fat - 8.9 kcal/g**
- On the basis of the above data
  - human and cow milk contain 670–720 kcal/kg

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Nutritional values of milk

Cow milk (whole)
Nutritional value per 100 g
Energy 60 kcal

Carbohydrates 5.26 g
- Sugars 5.26 g
- Lactose 5.26 g

Fat 3.25 g
- saturated 1.865 g
- monounsaturated 0.812 g
- polyunsaturated 0.195 g

Protein 3.22 g
- Tryptophan 0.075 g
- Threonine 0.143 g
- Isoleucine 0.165 g
- Leucine 0.265 g
- Lysine 0.140 g
- Methionine 0.075 g
- Cystine 0.017 g
- Phenylalanine 0.147 g
- Tyrosine 0.152 g
- Valine 0.192 g
- Arginine 0.075 g
- Histidine 0.075 g
- Alanine 0.103 g
- Aspartic acid 0.237 g
- Glutamic acid 0.648 g
- Glycine 0.075 g
- Proline 0.342 g
- Serine 0.107 g

Water 88.32 g
Vitamin A equiv. 28 µg 3%
Thiamine (Vit. B1) 0.044 mg 3%
Riboflavin (Vit. B2) 0.183 mg 12%
Vitamin B12 0.44 µg 18%
Vitamin D 40 IU 10%
Calcium 113 mg 11%
Magnesium 10 mg 3%
Potassium 143 mg 3%

Source: USDA Nutrient database (http://www.nal.usda.gov/fnic/foodcomp/search/)

Milk….

- milk is considered as the best, ideal and complete food for all age groups.
- milk can also serve as a *potential vehicle* for transmission of some diseases
- milk can serve as *excellent source and protective medium* for certain microorganisms (including pathogens).
- it can also allow these pathogens to grow, multiply and produce certain **toxic metabolites**, thereby making itself an extremely vulnerable (potentially hazardous) commodity from the public health point of view.

Biosynthesis of Milk

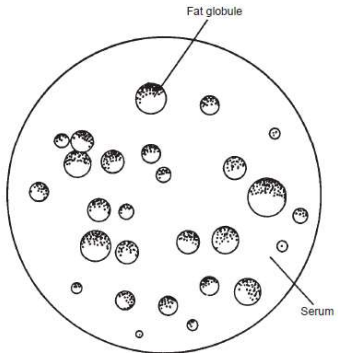
Milk is originated in the **secretary alveolus**

-> collected in ducts -> metabolites inter the secretary cell from the blood via basement membrane -> Endoplasmatic memb. -> to Golgi apparatus -> to Golgi vesicles->

The lipid phase pass to the lumen by **pinocytosis**

Agues phase milk transported to the plasma membrane and **discharged** to the lumen by **Exosytosis**

Fat globules in milk.



Chemical composition of milk

- The composition of milk is extremely complex, consisting
  - chiefly of water (> 87.35%)
  - *protein* in colloidal suspension,
  - lactose and fats in emulsion,
  - inorganic salts in solution,
  - vitamins, enzymes, gases and other substances

composition of milk….

1. Water

- This is the principal constituent of milk (80-90%) and is the **medium in which all constituents are in solution or in suspension**.
- In some countries, such as Ethiopia, where the production and distribution of milk is not properly controlled, the **adulteration of milk by the addition of water is commonly** practiced

2. Protein

- Proteins are polymers of amino acids(aa) (20)
  - R- represents the organic radical.

- Produced mainly in rER and released to alveolar lumen by exocytosis
- The three principal proteins in milk in colloidal suspension are :- **casein, lacto albumin & lacto globulin**.

- Milk proteins are grouped in to two
  - **Casein**($\alpha,\beta,\kappa,\gamma$) which accounts around 80% and
  - **whey protein** (≈ 20%)

Remark:-

Cow milk contains about 32 g/ litre protein. Of which, about 26 g/litre consists of caseins.

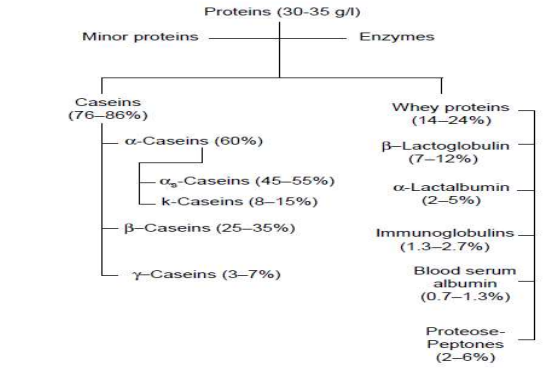
– ***Casein:-***

- is a complete protein that contains all aa,
- insoluble in water
- found only in milk.
- It exists in milk **in combination with calcium phosphate** and this combination causes precipitation
- In general caseins are high in phosphorus, low in sulphur and are not significantly affected by moderate heat.

Casein…

- are precipitated upon acidification to pH 4.6 at temperatures above 20°C
- The acids in milk (added rennin) take the calcium from calcium phosphate and thus remove the insoluble casein out of the solution as curd.
- In the normal curding or souring of milk the casein is precipitated by lactic acid produced through the action of bacteria upon lactose.

Milk-protein fractions.



- **Whey proteins**
  - Remain **in solution form** when milk is **acidified**
  - They are mainly pre formed proteins
  - Includes :-
    - α -lactalbumin,
    - ß-lactoglobulin
    - **Im g**.(IgM, IgG, IgA)
    - Enzymes, lactoferin, glucoprotein, proteus-peptone
  - ß-lactoglobulin is the principal whey protein of the cow, goat and sheep
  - **There is no ß-lactoglobulin** has been identified **in *human*, *camel* or *horse*** milk in which α -lactalbumin is the principal whey protein

- Most of the whey proteins are **denatured by heat**, i.e. they become less soluble if milk is heated.
- **Denaturation** of whey proteins and ß-lactoglobulin, in particular, is of major technological significance.
- i.e. **ß-lactoglobulin** interacts **with** ***k*** **-casein** during heating and this
  - reduces the heat stability of milk,
  - slows down **rennet** clotting during cheese manufacture and
  - gives a soft curd which tends to retain water.

- α -lactalbumin
  - represents about 20% of the protein of bovine whey (3.5% of the total milk protein) (relatively minor protein in terms of quantity).
  - It functions as part of the enzyme system involved in lactose synthesis.

- **Minor protein constituents**
  - About 50 enzymes have been detected in bovine milk.
    - may play either beneficial or harmful roles
- **Catalase.**
  - catalyses the decomposition of hydrogen peroxide (H_2O_2) to H_2O and O_2.
  - Its activity is higher in mastitic milk and colostrum than in normal milk and
  - increases with increase in bacterial numbers.

- **Lactoperoxidase.**
  - The enzyme catalyses oxidation of thiocyanate to products that inhibit certain bacteria.
  - It is **relatively heat stable**; it is not inactivated by pasteurization (72°C x 15 seconds) but is destroyed when milk is heated above 80°C.
  - The **absence** of lactoperoxidase in milk indicates that the milk has been **heated to at least 80°C**

- **Phosphatase.**
  - Milk contains an acid and alkaline phosphatase.
  - Alkaline phosphatase has a pH optimum near 9 and is **inactivated by heating** milk to 72°C for 15 seconds. Its absence indicates that milk has been properly pasteurised.
- **Other milk enzymes.**
  - lipases
  - proteases,
  - amylases, xanthine oxidase, carbonic anhydrase and lysozyme.

?

Milk Fat

- **Objectives**
  - At the end of this session students can list down the **components** of milk fat
  - Chemical and physical properties of milk fat

Biosynthesis of milk fat

- Is initiated in the ergastoplasm of alveolar cells of the udder
- In **ruminants** large amount of acetate produced in the rumen is absorbed to the blood stream and serve as major **source of "C"** atom for the synthesis of f.a.
- In non ruminants glucose serve as major source of "C" atom for the synthesis of f.a.

- Acetate -> Acetyle CoA
- Glucose -> Acetyle CoA

Glycerol phosphate

Triglycerides

Finally the milk fat is released by **pinocytosis** in the form of **fat globules** together with the fat globular membrane

Milk Fat

– exists in the milk in the form of small globules (oil-in-water type emulsion), which average approximately 2 to 5 microns in size.

Size	Diameter in micron	%
Small	< 2	70-90%
Intermediate	3-5	10-30%
Large	8-10	0.001%

- The **size** of the fat globule depends on
  - The breed
    - Jersey and Guernsey -> produce largest globule
    - Holstein and Ayrshire -> produce smaller size
  - Lactation period
    - As lactation period progresses , the size becomes smaller

- **The surface of fat globules is coated with**
  - **Fat globule membrane** (that contains **phospholipids, and proteins** in complex form and stabilizes the fat emulsion)
- **The emulsion may, however, be broken by**
  - **agitation,**
  - **churning,**
  - **homogonization,**
  - **heating and**
  - **freezing**

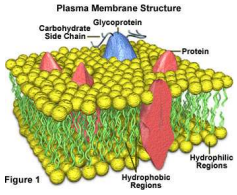


Figure 1

- The milk fat *globular membrane*
  - prevents the fat globules from coalescing and **separating** from one another
  - Protects the fat from action of lipase (prevents from **rancidity**)
  - From practical point of view the size of the milk fat globule affects
    - Time required for Separation of the milk
    - Churning of the cream
    - Transportation of milk or cream
    - Cheese making

milk fat….

- About 98% of milk fat is a mixture of triacyl glycerides

Lipid	%
Carotenoids + vitamin A	trace
Cholesterol esters	0.02
Triglycerides	98.3
Diglycerides	0.3
Monoglycerides	0.03
Free fatty acids	0.1
Cholesterol	0.20–0.40
Phospholipids	0.20–1.0

- Milk Fats are partly solid at room temperature
- Chemically, milk fat is composed of a number glyceride-esters of fatty acids
  - Fatty acids are esterified with glycerol as follows:
  - Glycerol + fatty acids ———> triglyceride (fat) + water
  - H–C–OH + HOOC–R2 ———> H–C–OOCR2 + $3H_2O$

- When the fat is hydrolyzed the free fatty acids are liberated and as a result the fat becomes rancid
- Chemically milk fat is composed of about 25 different fatty acids combined with glycerol
- Fatty acids vary in
  - " C" chain length from 4 carbon atoms, as in *butyric acid*; to 20 carbon atoms, as in *arachidic acid*
  - Saturation. Saturated fatty acids are relatively stable

(It is the double bonds in the carbon chain that make the fatty acid unsaturated)

Principal fatty acids found in milk triglycerides

Fatty acid	Molecular formula	Average amount in milk fat (%)	Chain length (No. of carbons)	Melting point (°C)
Butyric	$CH_3(CH_2)_2COOH$	3.7	4	-8
Caproic	$CH_3(CH_2)_4COOH$	2.0	6	-2
Caprylic	$CH_2(CH_2)_6COOH$	1.6	8	16
Capric	$CH_3(CH_2)_8COOH$	2.6	10	31.5
Lauric	$CH_3(CH_2)_{10}COOH$	3.3	12	44
Myristic	$CH_3(CH_2)_{12}COOH$	8.7	14	58
Palmitic	$CH_3(CH_2)_{14}COOH$	27.0	16	64
Stearic	$CH_3(CH_2)_{16}COOH$	10.0	18	70
Oleic	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	35.0	18	13
Linoleic	$CH_3(CH_2)_4(CH=CH.CH_2)_2(CH_2)_6COOH$	4.5	18	-6
Linolenic	$CH_3.CH_2(CH=CH.CH_2)_3(CH_2)_6COOH$	0.6	18	-13
Arichidic	$CH_3(CH_2)_{18}COOH$	1.0	20	77

VOLATILE

- Commonly the f.a. of milk fat are grouped in to two:-

(1) Volatile f.a. (17%)

- That gives the **characteristic flavor** for butter and cream
- That includes Butyric, Caproic, Caprylic, Capric & Lauric . Of them **Butyric** a. is the most variable and is largely responsible for **rancied** flavor when decomposed (hydrolyzed) and released (become free f.a.).

(2) Non Volatile f.a. (≈ 83%)

- Includes Oleic, Stearic, Myristic & Palmitic acid
- From these groups **Oleic acid** the most variable one and **relatively low in melting point**. Therefore it is the main f.a. that influences the hardness and softness of the milk fat

- In General **cow milk fat** consists of
  - 95-99% triglycerides
  - 1.26- 1.59% diglycerides
  - 0.016-0.038% monoglycerides
- Based on molecular wt.
  - 52.9% high m. wt.
  - 18.9% medium m. wt
  - 28.2% low m. wt
- Based on saturation
  - 65% saturated
  - 32% mono unsaturated
  - 3% poly unsaturated
- Based on "C" atom length
  - 7% short chain f.a. (C4-C8)
  - 15-20% medium chain f.a. (C10-C14)
  - 73-78% long chain f.a. (>C16)

- The melting point and hardness of the fatty acid is affected by
  - the length of the carbon chain and
  - the degree of unsaturation.
- As chain length increases, melting point increases.
- As the degree of unsaturation increases, the melting point decreases.
- Fats composed of *short-chain* or *unsaturated* fatty acids have low melting points and are **liquid** at room temperature, i.e. oils(ex. Plant origin)
- Fats high in *long-chain* and *saturated* fatty acids have high melting points and are **solid** at room temperature. (ex. Animal origin)

- What is the melting point of butter fat from animal?
- Is it solid or liquid at room temperature?

- Butterfat dose not have defined melting point b/c it is a mixture of fatty acids with different melting points.
- some of the fat is liquid and some solid at temperatures between16 and 25°C

- Fat is the most variable in the constituents of milk. The amount varies with different animals and even in the same animal from time to time
- Variations in the fat content are influenced by factors such as
  - Breed : Holstein produce more milk but less %in fat
  - Age and health of cow: Fat content decreases with age and poor health.
  - Stage of lactation: Fat content decreases during the first 2 or 3 months of lactation.
  - Season of the year: Fat content is lowest in late spring (rainy season or early summer(dry)
  - Feeding habit: The fat content of milk varies depending on the type of fodder that the cows use

– Variation during milking:

A) The “fore milk” or “first milk” drawn is lowest or poorest in fat content.

B) The “middle portion” is average in fat content.

C) The “last milk” drawn is highest or best in fat content.

?

Physical and chemical properties of the milk

Objectives of the study

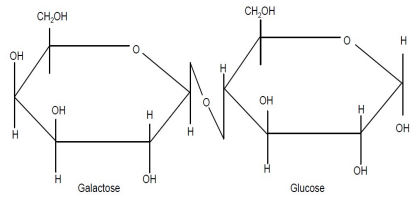
- At the end of this session students will
  - Explain the milk C/H properties
  - Describe the general physical and chemical properties of cow milk
  - List factors that determine the components of the milk

What are the C/H of milk???

1 min

Carbohydrates of the milk

- Lactose is the major C/H
  - It is a disaccharide (glucose + galactose)



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Milk C/H

- Present in a true solution form
- The average lactose content of milk
  - 4.7 - 4.9% (in a form of α and β)
- β-lactose is more **water** soluble than ' **α** '
- but in general lactose is less soluble in water than sucrose and is also less sweet (has only 16-33% sweetening power than glucose)

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Milk C/H

- Therefore adding sucrose to milk forces lactose out of solution and crystallizes.
- This causes sandiness of lactose in such products as ice cream.
- Heating milk above 100°C causes lactose to combine irreversibly with the milk proteins (amyle group). (= milyard reaction)
  - This reduces the nutritional value and flavor of the milk and also turns it **to brown**

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– bacteria that have the enzyme ß-galactosidase (lactase) break down to glucose and galactose which then to lactic acid. -> souring of milk.

– Under controlled condition they can be fermented to propionic acid necessary for Swiss-cheese production (with large holes)

NB

– Mastitis reduces lactose secretion.

Physical Properties of Milk

- Chemists consider that matter exists in three forms and milk has all the three states of dispersion

Forms	Size	examples
Coarse dispersion (emulsion)	> 1 x 10^{-4} mm	Milk fat
Colloidal dispersion	10^{-4} - 10^{-6} mm	Milk protein
Molecular dispersion	< 10^{-6} mm	Minerals. lactose

- color, specific gravity, freezing point and boiling point.
  - These are influenced by the composition of milk.
  - They are also a great help in the processing and testing of milk for adulteration.

Color

– The milk pigments influencing the color of milk are:2 type

- Fat soluble pigment - Carotene (Vitamin A) of the fat in the milk that gives a **golden** yellowish color and it is not synthesized by the cow but from feed.
- The **yellow** color of carotene is confined to the fat phase and therefore, becomes **prominent in cream** layer and more so in butter.

Color cont……

- Water soluble pigment- Riboflavin (B2) & and xanthophylls of the **whey** gives a **bluish** tint (greenish yellow pigment) color.
- The characteristics **white** opalescent color of milk is due to scattering of light by the **colloidal particles** which it contains.

Color cont……

- The color of milk :-
  - Bufallo Milk -> Creamy White
  - Cow's Milk -> Yellowish creamy white
  - Skim Milk -> Bluish
  - Whey -> Greenish yellow (This is due to pigment Riboflavin).

Color cont……

- The intensity of **yellow** color of cow milk depends on various factors such as
  - breed, feeds, size of fat globules, fat percentage of milk.
  - The greater intake of **green feed**, the deeper the color of cow milk.
  - The larger the **fat** globules and the higher the fat percentage, the greater the intensity of the **yellow** color.
  - Milk with **low fat** % show a **bluish** tint

pH and acidity

- Freshly drawn milk is **amphoteric** to litmus i.e. it turns red litmus to blue and blue litmus red.
- The pH of cow milk is 6.4 - 6.6 (**slightly acidic**)
  - due to the presence of
    - casein,
    - acid phosphates and
    - citrates and
    - to a lesser degree to albumin, globulin and carbondioxide.

What factors affect the pH of the milk???

Brainstorm 1 min

pH……

- In General:-
  - pH in cow milk is **not affected by the feed** , rather by breed, stage of lactation, composition and health
  - **Colostrum** has high natural acidity due to its high protein content
  - In **early lactation** the value is above average but the value falls to about normal in second month and then remain fairly stable until the last month of lactation, and then decline at the last month.
  - The **higher the solid not fat** content of milk, the higher the natural acidity and vice versa
  - **Heating** of milk increases acidity due to conversion of **colloidal casein in to soluble casein** and
  - formation of acids by **degradation of lactose.**
  - **Mastaitic** milk has an **alkaline pH** b/c of exudates from the cell that mixed with milk

Titratable vs developed acidity

- When the milk is kept for some time, the **bacteria** will multiply and utilizes lactose and converts in to **lactic acid**, there by increasing the acidity and decreasing the pH value. This acidity is known as **developed or real acidity**.
- The sum of natural acidity and developed acidity is known as **titratable acidity.**
- In chemistry, **Titratable acid** generally refers to any acid that can lose proton(s) in an acid-base reaction

- There are two fundamentally different methods of expressing acidity:
  - (a) titratable acidity expressed as percent lactic acid,
    - measures total acidity but does not measure the strength of the acids.
  - (b) hydrogen ion concentration or pH.
    - The pH indicates the strength of the acid condition.

- The true neutral point is pH 7.0;
  - pH < 7.0 => an acid
  - pH >7.O => an alkaline.
- One pH unit means a tenfold difference in strength;
  - for example, a pH 5.5 indicates an acidity that is ten times as great as pH 6.5.

pH.....

- Milk acidity is estimated by titrating 10 ml of milk against N/9 NaOH solution (or 0.1N) in the presence of phenolphthalein indicator
  - and expressed as % of lactic acid.
  - In titration the standard alkali required to shift the pH of milk to 8.4 pH, which change the colour of phenolphthalein to pink is taken as cut off line

- Titratable acidity (as lactic acid per 100 ml of milk

$$\frac{9 V_1 N}{V_2}$$

- **Where:-**
  - **V1** = volume in ml of standard NaOH used for titration
  - **V2** = Volume in ml of milk taken for the test
  - **N** = Normality of standard hydroxide solution

- Milk having titratable acidity more than 0.17% is not suitable to prepare heat treated products b/c milk coagulates on heating.

Specific gravity of milk

What is sp.gr. ????

Specific gravity of milk

- The wt of a given volume of milk compared with the wt of the same volume of water at the same temp.
- The **specific gravity** of a substance (when referred to water at 4°C) is numerically equal to the **density** of that substance.

Sp gr

- The specific gravity of milk is usually expressed at 15.5°C
- The average specific gravities of
  - Cow Milk - 1.032

    sp.gr.= (Lc / 1000) + 1
- The specific gravity of milk is **influenced** by
  - the **proportion of its constituents** each of which has different specific gravity:-

Sp gr

– Water	- 1.000
– Fat	- 0.930
– Protein	- 1.346
– Lactose	- 1.666
– Salts	- 4.12
– SNF	- 1.616
– Skim Milk	- 1.035 – 1.037

Sp gr

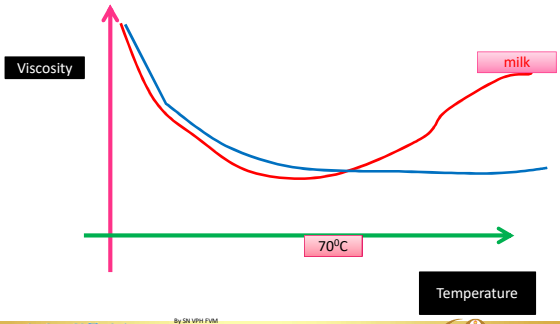
- The specific gravity of milk is decreased by
  - Addition of water
  - Addition of cream (fat)
  - Increased temperature.
- The specific gravity of milk is increased by
  - Addition of separated milk (skim milk)
  - Removal of fat
  - Reduction of temperature.

FLAVOURS

- Affected by food, chemicals ,heat ,lactation period (salty at the end) and microorganisms:-
  - Malty Flavour - *Streptococcus Lactis* var *maltigenes*.
  - Unclean flavour - *E-Coli, Aerobactes aerogenes*
  - Potato Flavour - *Pseudomonas graveolens*
  - Medicinal Flavour - *Aerobacter aerogenes*
  - Fishy Flavour - *Pseudomonas Fluorescens*
  - Phenol Flavour - *Bacillus Circulans*
  - Amyl alcohol Flavour - *Micrococcus caseolyticus*
  - Putrid - *Pseudomonas putrefaciens*
  - Fruity - *Pseudomonas fragi*

Viscosity

- Resistance to flow
- Expressed in centipoise
- In genera viscosity decrease with increment of temp.
  - Water has at 20 ^{o}C =1.005 centipoise and milk **1.5 X** greater
  - But at 100 ^{o}C = 0.280 centipoise
- Though in milk similarly heating to pasteurization temp, and prolonged agitation decrease the viscosity due to breakdown of fat clusters ,
  - boiling increases the viscosity due to denaturation of proteins



Viscosity

- Butter milk
  - that is churned from pasteurized milk is less viscous than from row milk, though the fat % is the same
- This has impact on consumers view

Freezing point(F.Pt)

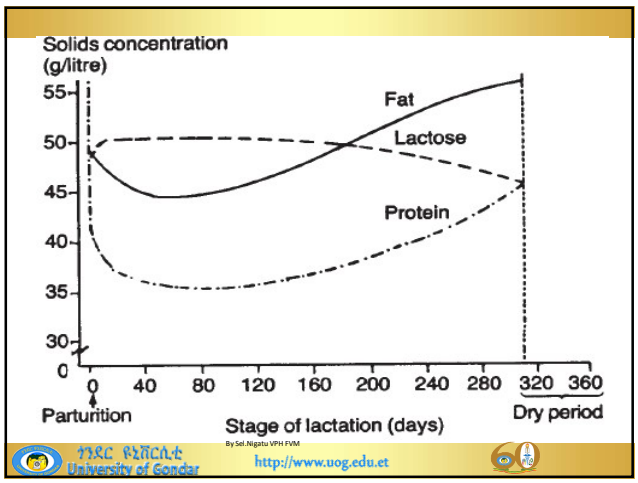
- Water freezes at 0 ^{o}C
- Milk freezes at –ve 0.55 ^{o}C
- Mainly affected by Soluble sub, lactose & minerals ,but little by fat & proteins.
- Added water increase F.Pt
  - Eg 1% added water increase f pt to *0.006 ^{o}C*
- Added salt decrease F.Pt.

Boiling pt

- Milk is slightly heavier than water and needs higher temp to boil (100.1- 100.17 ^{o}C)
- Boiling -> ↑ acidity , and therefore permits rapid coagulation
- Boiling in air -> formation of thin film on the surface due to coagulation of casein and albumin

Factors influencing the composition of milk

- **Species**
- **Breed**
  - High milk yielder have lower fat%
- **Individuality**
- **Age**
  - with age yield decrease, but fat increases till 3rd month
- **Health**
- **Stage of lactation**
  - Lactose is quite constant
  - Protein, fat and SNF increases to the end



Factors ….

- **Season**
- **Interval between milking**
  - Longer interval, more milk , less fat
  - Evening have more fat than morning
- **Completeness of milking**
  - Milk at the end of milking time have more fat than the first drops
- **Feed**
- **Oestrus cycle**
  - Fat% decrease in heat period
- **Exercise**
  - Exercise before milking decrease milk yeld but increase fat %
- **Milker**
- **Excitement**
- **Treatment**

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Milk preservation and Pasteurizations

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Objectives of the lesson

- At the end of this session students can
  - describe the General principles of food preservation in relation to growth characteristics of bacteria.
  - list down the different environmental factors Influencing Growth of mic org.
  - Explain the various methods of milk pasteurization and preservation

What do we mean by Growth of Micro Organism ?

Growth of Microbes

- Increase in ***number*** of cells, not cell size
- One cell becomes colony of millions of cells

- Bacteria exhibit a wide diversity in metabolic activities, but all have similar cellular structure and reproduction mechanisms.

Bacterial Growth Curves

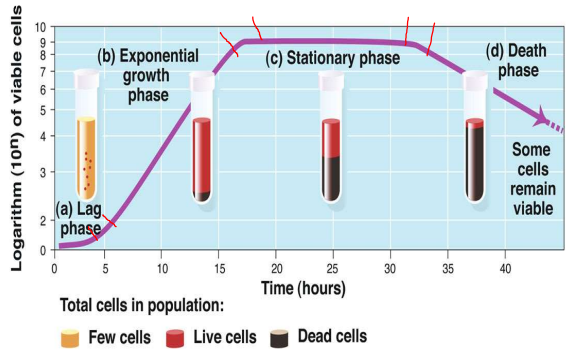
What are the phases involved ?

2 min brain storming

4 Major Phases

Bacterial growth curve

- The lag phase—preparing for division
- The logarithmic phase—reproduction relationship to disease
- The stationary phase—equalization
- The decline phase—cell death



1. Lag Phase

- Bacteria are first introduced into an environment or media
- Bacteria are "checking out" their surroundings
- cells are very active metabolically
- making new enzymes in response to new medium
- # of cells not changed or changes very little(in *Let lag phase* or *positive acceleration phase*)
- Needs one hour to several days

2. Log Phase (Exponential phase)

- Rapid cell growth (exponential growth)
- population **doubles** every generation
- microbes are sensitive to adverse conditions:-
  - antibiotics
  - anti-microbial agents
- At the end follows negative acceleration phase –in which the rate of multiplication is decreasing

3. Stationary Phase

- nutrients becoming limiting or waste products becoming toxic.
- Death rate = rate of reproduction
- cells begin to encounter environmental stress
  - lack of nutrients
  - lack of water
  - not enough space
  - metabolic wastes
  - oxygen
  - pH

Endospores would form now

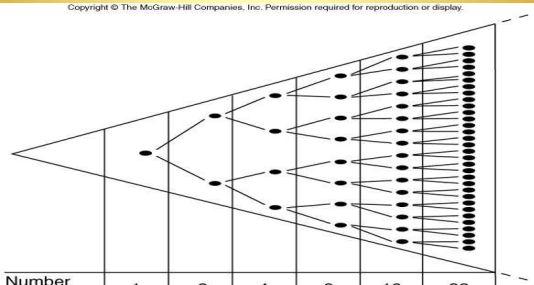
4. Death Phase (phase of decline)

- The initial part is *accelerated death phase*
- Death rate > rate of reproduction
- Due to limiting factors in the environment
- The last part of this phase is *survival phase* in which no cell division occurs and cells survive on endogenous nutrition

- Vegetative cells of the organism are *least resistant* to lethal agents at the *logarithmic* phase, but more *resistant at late lag* phase and *stationary* phase

Generation Time

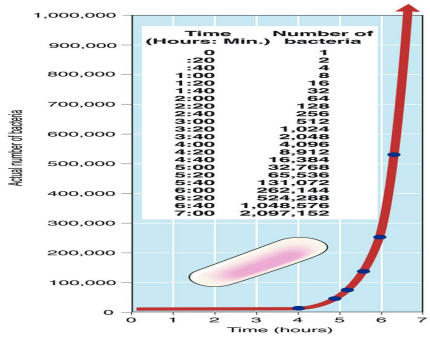
- Time required for cell to divide/for population to double
- Average for bacteria is 1-3 hours
- *E. coli* generation time = 20 min
  - 20 generations (7 hours), 1 cell becomes one million cells!



Number of cells	1	2	4	8	16	32
Number of generations		1	2	3	4	5
Exponential value		2^1 (2×1)	2^2 (2×2)	2^3 (2×2×2)	2^4 (2×2×2×2)	2^5 (2×2×2×2×2)

(a)

E.Coli have Rapid generation times



What are the general principles of food preservation??

General principles of food preservation

1. To prevent or delay decomposition by micro org
   - Keeping out mic org (aseptic)
   - Removal of mic org(filtration)
   - Hindering the growth and activity of mic org (Temp, drying…)
   - Killing of mic org
2. Prevent or delay self –decomposition
   - By distraction or inactivation of enzymes
3. Prevent mechanical and insect…… destruction

Possible ways of food preservation from micro organism

- Reduce amount of contaminants
  - The fewer the organism , the longer the lag phase
- Create unfavorable environment
  - pH, O2, …….
- Distraction of mic org.
  - Heat, radiation

What factors affect the heat resistance of micro organisms?

Pair discussion – for 2 min

Discussion in group of 4 pn – 3 min

Factors affecting heat resistance

- Temp-Time combination
- Initial concentration of cells(spores)
- Nutrients in the medium
  - The better the medium, the more resistant the mic org.
- Incubation temp of the agent
  - Resistance increases when the mic org is in its optimum incubation temp environment
- Growth phase
  - Greatest resistance at late lag and stationary phases.

Factors..

- The nature of the mic organism :-
  - more resistant to heat are
    - Cocci than roads
    - Thermophilic mic org than other
    - Bacteria that clamp together than not
    - Bacteria with capsul
    - Bacteria with high content of lipid than less
      - *Gm-ve than +ve*

Environmental Factors Influencing Growth

- Temperature
- O_2
- pH
- Moisture (freely available H2O) (a_w)
  - Less a_w => prolonged lag phase
  - Most mic org grows well when a_w approaches to 1.00
  - In general bacteria requires more moisture than yeast and yeast more than molds
- Others: radiation, atmospheric pressure, light, available food

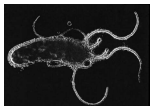
Oxygen Requirements

- Obligate aerobes – require O_2
- Facultative anaerobes – can use O_2 but also grow without it
- Obligate anaerobes – die in the presence of O_2

pH

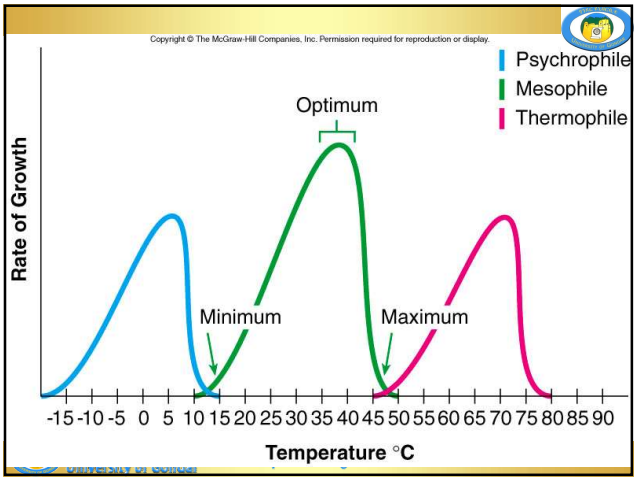
- Most bacteria grow between pH 6.5 and 7.5
- Acid (below pH 4) good preservative for cheeses
- Acidophiles can live at low pH

  Eg. Helicobacter pylori lives in stomach under mucus layer



Temperature Optima

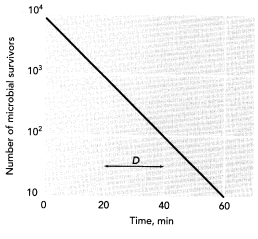
- Psychrophiles: cold-loving (7°C)
- Mesophiles: moderate temperature-loving (37°C)
- Thermophiles: heat-loving (45°C)
- Thermoduric :- can survive heat treatment like pasteurization
- Psychrophylic (Psychrotropic) : can grow at refrigeration temp but optimum is 27°C
- Each has a minimum, optimum, and maximum growth temperature



Thermal processing principles

- Heat resistance of mic org and their spores are usually expressed in terms of Thermal death time (TDT) and Thermal death points (TDP)
- **Thermal Death Time (TDT)** is the length of *time* required to kill all bacterial in a liquid culture at a given temperature and specific condition.
- **Thermal Death Point (TDP)-** is the lowest *temperature* at which all the microbes in a liquid culture will be killed in 10 minutes.

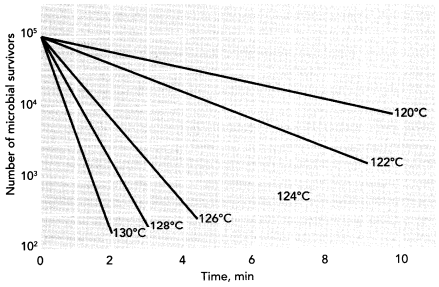
- **Decimal Reduction Time (DRT) (D- value)** is the length of time required to kill 90 % of a bacterial population at a given temperature.



22 Microbial population versus time on semi-log coordinates.

- **D-value (Survivor Curves):-** is the time required for a one log cycle reduction in microbial population

D-Value (Survivor Curves) at Different Temp.



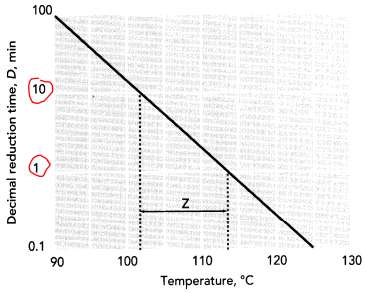
2.3 Decimal reduction or survivor curves at different elevated temperatures.

- **Z – Value** :- is the interval of Temperature in ^oF (Zc in ^oC) required to reduce the TDT by **ten fold**
  - It provides the relative resistance of an organism to different destructive temp.

Thermal Resistance Curve

- This linear relationship is the thermal resistance curve for a given microbial population.

Thermal Resistance Curve



24 Figure Thermal resistance curve of microbial population.

- **F-Value :-** is the **time in minute** required to destroy spoors or vegetative cells of a particular organism at 250 ^{0}F **(121^{o}C)**

Decreasing order of resistance

Most Resistant

- Prions
- Endospores of bacteria
- Mycobacteria
- Cysts of protozoa
- Vegetative protozoa
- Gram-negative bacteria
- Fungi, including most fungal spore forms
- Viruses without envelopes
- Gram-positive bacteria
- Viruses with lipid envelopes

Least Resistant

Major source of milk contamination

- Dairy animals
  - Hygiene
  - Health status
- Human handlers
  - Milk man hygiene
  - Health
- Environment
  - Water in the farm (used for cleaning purpose)
  - Containers , equipments
  - Temperature ……
- Additives in the milk
  - Sugar
  - Powder…
- Medicaments used for animal treatment
  - Not keeping the withdrawal period

Regulatory demands

From 2006 new EU hygiene regulations

- Temperature and age
  - ≤ 6ºC in milk cooling tank
  - ≤ 10ºC at reception
  - ≤ 6ºC until 36 hrs before processing
  - ≤ 4ºC until 48 hours before processing
- Microbiological
  - ≤100.000 TPC in milk cooling tank
  - ≤300.000 TPC before processing - fresh milk

Contamination Preventive measures

Improved farm hygiene and milking practices

Improved milk handling and processing technology

Educated consumers and higher consumer awareness on food safety

Preventive……

- Clean milk production (on the farm level)
  - Animal health
  - Animal hygiene
  - Health of the working personnel
  - Hygiene of the environment (working environment, equipments …)
- Pasteurization
- Proper storage (cold chain maintenance)
- Attention on the expire date and on the use before date

Pasteurization

- **Two objectives**
  - ***Public health aspect***
    - (by destroying pathogens)
  - ***Keeping quality aspect***
    - (by destroying spoilage microorganisms and undesirable enzymes)
    - Can extend the shelf life up to 16 days

• The extent of microorganism inactivation depends on the combination of temperature and holding time

- "*Shelf-life*" can be defined as the length of time that a food can be held under recommended or practical storage conditions and still maintain its "freshness" or acceptable quality

- Brainstorm on objectives of pasteurization??
  - 1 min

Pasteurization

- Initially 61.7°C for 30 min. targeting *Mycobacterium tuberculosis*
- Later to 62.8°C for 30 min. targeting *Coxiella burnetti* which is more heat tolerant than *M. tuberculosis*

Type of pasteurization

- Based on temp and time combination

1) Batch Pasteurization (Low Temperature Long Time pasteurization) **(LTLT)** (Holding Time)

- 62.8°C for 30 min. followed by cooling to 5 °C or below

2) HTST that requires

- 72°C for 15 sec

3) **Ultra heat treatment** 135ºC – 150ºC with out holding time (for **UHT** –milk)

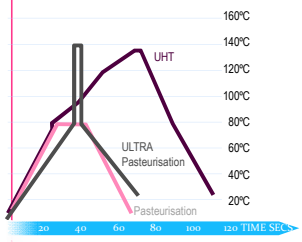
"Ultra-Pasteurized" means that the milk is heated to a minimum of 137.7°C for a minimum of 2 seconds.

The average shelf-life of Ultra-Pasteurized milk products can be as much as 90 days

4) **In pouch** (in the bottle) pasteurization – after packing

5) pasteurization before packing

- **Ultra Pasteurization**
  - **Direct steam injection from 80°C to 140ºC for 1-4 seconds**
  - **Flash cooling from 140ºC to 80ºC**
- **Pasteurization**
  - **Indirect heat treatment from chilled temperature to 80ºC back to chilled temperature**
- **UHT**
  - **Indirect heat treatment from chilled temperature to 137.7ºC and cooled to room temperature**



- **Milk**:
  - 63° C for not less than 30 min.,
  - 72° C for not less than 16 sec., or equivalent destruction of pathogens and the enzyme phosphatase
  - ***Milk is deemed pasteurized if it tests negative for alkaline phosphatase.***

- **Frozen dairy dessert mix** (ice cream or ice milk, egg nog):
  - at least 69° C for not less than 30 min;
  - at least 80° C for not less than 25 sec; or **other time temperature combinations which is approved** (e.g. 83° C/16 sec).
- **Milk based products**- with 10% milk fat or higher, or added sugar (cream, chocolate milk, etc)
  - 66 ºC / 30 min, or 75° C / 16 sec

Chemical method of milk preservation

- Activation of the lactoperoxidase
-

Lactoperoxidase

SCN^- + H_2O_2 ---------- $OSCN^-$ + H_2O

(Thiocyanate ion) + (Hydrogen Peroxide)---------- (Antibacterial compound)