Role of Starters in Preparation of Fermented Milks

1. Fermented milks are the products obtained by souring of milk by use of starter cultures.
2. These products have evolved around the world over thousands of years and are believed to have originated in the area that is now the Middle East. These products probably resulted from the need to extend the shelf life of milk in the absence of refrigeration.
3. Today yoghurt, buttermilk, and sour cream are probably the most widely consumed fermented milk products.
4. The fermented milks are popular because of following reasons:

Preservation- Fermented milk has higher shelf-life than normal raw milk that results in a product that is hostile to undesirable bacteria.

Organoleptic Properties- A variety of flavours in fermented milk, refreshing taste, specific consistency and viscosity favourably influence the consumption of these products. Several modifications in fermented milks done to have varieties like sweet or sour, salted or spiced, beverage or gel, etc.

Ideal for probiotics- Fermented milk is an ideal medium for carrying probiotic microorganisms and also make probiotic and symbiotic foods.

Types of Fermented Milks:

(1) Numerous types of fermented milks exist around the world.

(2) Products range from yoghurt, which is probably the most widely known, especially in the Western world, to more regional products such as maziwalala) of Kenya, which is manufactured using mesophilic cultures, and dahi of India, which is largely made either in the home or by small-scale dairies.

(3) Bulgarian buttermilk has a very strong acid flavor (2–4% lactic acid), whereas yoghurt has a milder acidic and acetaldehyde flavor.

(4) Kumiss, which is traditionally made from mares' milk, is slightly alcoholic, because yeasts are used in its manufacture.

(5) Texture of products also varies from liquid (cultured buttermilk and liquid yogurt) to thick gel as for yoghurt and sour cream.

(6) Villi from Scandinavia are characterized by their ropiness, which is intentionally induced by the use of cultures that produce exopolysaccharides to provide a thick body.

Table- 1: Origin of some important fermented milk products

Product	Country of Origin	Period	Characteristics & Use
Dahi	India	6000 - 4000 BC	Coagulated sour milk taken as food item and also an intermediatory product for making country butter and ghee (clarified butter).
Chhash (Butter milk)	India	6000 - 4000 BC	Diluted dahi or the butter milk left after churning of dahi into butter, relished as beverage after/along with meal.
Laban zeer/Khad	Egypt	5000 - 3000 BC	Sour milk, traditionally coagulated in earthenware.
Leben	Iraq	3000 BC	Traditional fermented milk containing yoghurt bacteria. Partially whey is drained by hanging the curd.
Zabady	Egypt and Sudan	2000 BC	Natural type yoghurt with firm consistency and cooked flavour.
Cultured cream	Mesopotamia	1300 BC	Naturally soured cream
Shrikhand	India	400 BC	Concentrated sour milk sweetened and spiced. Semisolid mass eaten with meals as sweet dish.
Kishk	Egypt and Arab World	-	Dry fermented product made from Laban zeer and par boiled wheat. It consists of small round irregular pieces, yellowish brown in colour with hard texture. It is highly nutritious with high amino acids and vitamin content.
Kumys, Kumiss	Central Asia (Mongol, Russia)	400 BC (probably known around 2000 BC)	Traditionally mare's milk is fermented by lactobacilli and yeast which give sparkling beverage containing lactic acid, alcohol and carbon dioxide.
Mast	Iran	-	Natural type yoghurt with firm consistency and cooked flavour.
Villi	Finland	-	Traditional high viscosity fermented milk with lactic acid bacteria and mold.
Taette	Norway	-	Viscous fermented milk also known as cellar milk because it was made in cellar.
Langfil, Tattemjolk	Sweden	-	Milk fermented with slime producing culture of lactococci.
Ymer	Denmark	-	Protein fortified milk fermented by Leuconostocs and lactococci from which whey is separated.
Skyr	Iceland	870 AD	Traditionally made from ewe milk by addition of rennet and starter. It is now concentrated by membrane technology.
Prostokvasha	Soviet Union	-	Archetypal fermented milk made from ancient times by fermenting raw milk with mesophilic lactic bacteria.
Kefir	Caucasusian China	-	Milk fermented with the help of kefir grains giving foamy effervescent product with acid and alcoholic taste
Yoghurt (Kisle mliako)	Bulgaria	-	Cow or ewe's milk fermented by Str. thermophilus and Lb. bulgaricus.
Yoghurt	Turkey	800 AD	Custard like sour fermented milk
Bulgarian milk	Bulgaria	500 AD	Traditional very sour milk fermented by Lb. bulgaricus alone or added with Str. thermophilus.
Trahana	Greece	-	Traditional Balkan fermented milk made by mixing fermented ewe's milk with wheat flour and then drying.
Churpi	Nepal	-	Fermented milk is churned and the butter milk remaining is heated to form a solid curd. This may be further dried.
Airan	Central Asia, Bulgaria	1253 - 1255 AD	Cow milk soured by Lb. bulgaricus, used as refreshing beverage.
Yakult	Japan	1935 AD	Highly heat treated milk fermented by *Lb. casei* strain Shirota. Used as beverage and health supplement.

Classification of Fermented Milks:

There are several types of fermented milks and are classified based on several parameters:

(1) Type of cultures used

(2) Type of fermentation

(3) Temperature at which prepared

(4) Type of flavour and body-texture characteristics

(5) Type of raw materials used

(6) Form of product (liquid, gel etc)

Classification of Fermented Milks

A. Acid Fermentation

A.1 Mesophilic Products

A.1.1 Normal flavour and consistency

Cultured Butter Milk, Dahi

A.1.2 With slimy consistency

Nordic fermented milks, e.g. Piima, Villi, Langfil

A.2 Thermophilic Products

Yoghurt & Similar products

A.3 Products with intestinal bacteria

Acidophilus milk, Bifidus milk, Yakult

B. Acid+Alcohol Fermentation

Kefir, Koumiss, etc.

C. Concentrated type fermented milks

Cellar milk, Shrikhand, Laben, Skyr

D. Milk-plant fermented products

Kishk, Curd-rice, etc.

Microorganisms used to manufacture ferment milk:

1. Microorganisms used to manufacture fermented milk primarily include those that can ferment lactose to lactic acid and may be either of the mesophilic or thermophilic type.
2. In addition to lactic acid producers, other types of organisms impart desired flavor or therapeutic properties to fermented products. Examples include organisms that produce diacetyl or acetaldehyde for flavor or small amounts of alcohol in products such as kefir.
3. Organisms such as *Bifidobacterium* spp. and *Lactobacillus acidophilus* are added for therapeutic purposes.
4. Leuconostocs are used in products such as cultured buttermilk to produce diacetyl via citrate fermentation.

Functions and Applications of Microorganisms in Fermented Milks

Culture	Function	Application
Lactobacillus delbrueckii subsp. *bulgaricus*	Acid and flavor	Bulgarian buttermilk, yogurt, kefir
Lactobacillus acidophilus	Acid	Acidophilus milk
Lactobacillus kefir	Acid	Kefir
Streptococcus thermophilus	Acid	Yogurt
Lactococcus lactis subsp. *lactis* (biovar. *diacetylactis*)	Acid and flavor	Sour cream, cultured buttermilk
Lactococcus lactis subsp. *lactis* & *Lactococcus lactis* subsp. *cremoris*	Acid	Cultured buttermilk, sour cream
Leuconostoc lactis & *Leuconostoc mesenteroides* subsp. *dextranicum*	Flavor	Cultured buttermilk, sour cream, ripened cream butter
Bifidobacterium longum *Bifidobacterium bifidum* *Bifidobacterium breve*	Acid and flavor	Yogurt

Yoghurt:

1. Most popular fermented milk product in the world derived from the Turkish word 'Jugurt' which is generally manufactured from pasteurized milk.
2. In India, Yoghurt has been defined by PFA as coagulated product obtained from pasteurized or boiled milk or concentrated milk, pasteurized skimmed milk and or pasteurized cream or a mixture of two or more of these products by lactic acid fermentation through the action of *L. bulgaricus* and *S. thermophilus*.
3. This is a traditional food and beverage in the Balkans and the Middle East. But its popularity has spreaded to Europe and many other parts of the world.
4. Its fat content ranges from 0 to over 4% depending on region and legislation. High-temperature pasteurization of the yogurt mix is employed to obtain a smooth and firm body. Nonfat dry milk or stabilizers may also be added to increase the water-holding capacity and therefore improve its body.
5. Several different types of yoghurt are commercially available including plain, flavored, liquid, carbonated, and low lactose.

6. **It may also contain cultures of *Bifidobacterium bifidus* and *Lactobacillus acidophilus* and other cultures or suitable lactic acid producing harmless bacteria.**
7. **It may contain milk powder, skimmed milk powder, unfermented buttermilk, concentrated whey, whey powder, whey protein, whey protein concentrate, water soluble milk proteins, edible casein, and caseinates manufactured from pasteurized products.**
8. **It may also contain sugar, corn syrup or glucose syrup in sweetened yoghurt and fruits in fruits yoghurt.**
9. **It shall have smooth surface and thick consistency without separation of whey.**
10. **It shall be free from vegetable oil/fat, animal body fat, mineral oil and any other substance foreign to milk.**

Product	Milk Fat	Milk solids not fat	Milk Protein	Sugar
1	2	3	4	5
(i) Yoghurt	-	Not less than 8.5 - percent m/m	Not less than 3.2 - percent m/m	-
(ii) Partly skimmed Yoghurt	Not less than 3.0 percent m/m	Not less than 8.5 - percent m/m	Not less than 3.2 - percent m/m	-
(iii) Skimmed Yoghurt	Not less than 0.5 percent m/m and not more than 3.0 percent m/m	Not less than 8.5 - percent m/m	Not less than 3.2 - percent m/m	
(iv) Sweetened / Flavoured Yoghurt	Not less than 3.0 percent m/m	Not less than 8.5 - percent m/m	Not less than 3.2 - percent m/m	Not less than 6.0 percent m/m
(v) Fruit Yoghurt	Not less than 1.5 percent m/m	Not less than 8.5 - percent m/m	Not less than 3.2 - percent m/m	Not less than 6.0 percent m/m

Microbiological Requirements as per PFA

Sr. No	Requirements	Yoghurt/Dahi
1.	Total Plate Count	Not more than 1,00,000/gm
2.	Coliform Count	Not more than 10/gm
3.	*E. coli*	Absent in 1 gm
4.	Salmonella	Absent in 25 gm
5.	*Shigella*	Absent in 25 gm
6	*Staphylococcus aureus*	Not more than 100/gm
7	*Yeast and Mold count*	Not more than 100/gm
8	*Anaerobic spore count*	Absent in 1 gm
9	*Listeria monocytogenes*	Absent in 1 gm

Microbiological Requirements for Yoghurt

Essential (Starter culture)

Lactobacillus delbrueckii subsp bulgaricus

Streptococcus thermophilus

Non-essential: These are not essential for acid production but sometimes used for other purposes. They can be added with specific label on the pack.

L. acidophilus

Bifidobacterium bifidus

Lactococcus lactis

L. lactis subsp diacetylactis

Pediococcus

Contaminants: Should not be present in the final product.

Yeast and molds

Coliforms

Other bacteria

Important characteristics of Yoghurt cultures

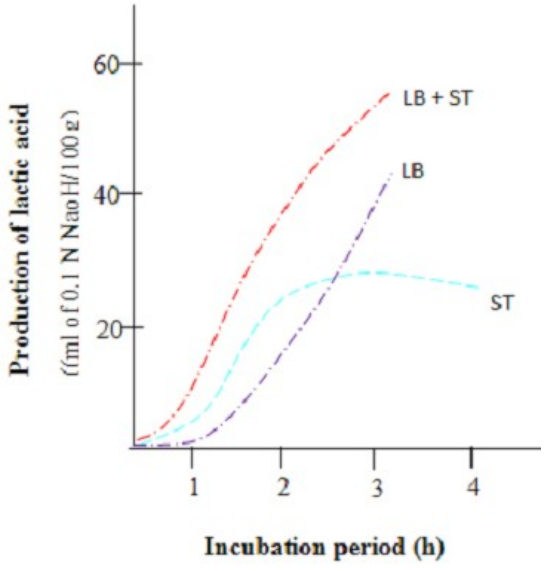
	S .thermophilus (ST)	*L. bulgaricus* (LB)
1	Gram positive spherical or ovoid cells occurring in pairs and chains (0.7 to 0.9 micro diameter)	Gram positive, short to filamentous rods with rounded ends, 0.8-1.0 μx 4-6 μ size.
2	In milk it produces 0.7-0.8% Lactic acid of L(+) form.	Can produce up to 1.7%, D (-) form of lactic acid in milk
3.	Non motile	Non-motile
4	Some strains can produce capsule or slime in milk.	Presence of metamorphic granules can be easily demonstrated in old cultures.
5	No group specific antigen and classified in viridans group	A typical milk bacterium of thermobacterium group.
6	Growth temperature: Minimum- 20° C optimum- 37- 42° C Maximum- 45-50° C	Growth temperature: Minimum- 22° C optimum- 45° C Maximum- 50-55° C
7	Do not hydrolyse arginine (NH_3 from arginine-absent)	Do not hydrolyse arginine
8	Ferment only a small number of sugars e.g. Fructose, sucrose, glucose and lactose.	Able to ferment glucose, galactose, fructose, lactose.
9.	It is highly sensitive to many antibiotics and sanitizing agents.	Sensitive to phosphate salts in milk.
10	Weak proteolytic activity	Shows mild proteolytic activity and is only weakly lipolytic.
11	On solid media it produce very small 'pin point' colonies.	On agar medium they produce rough (R) and smooth (S) colonies of various shapes like star, spindle, round, triangular etc. It is claimed that strains forming rough colonies are the most active producers of antibiotics.
12	In milk it produces volatile acids such as formic, acetic, propionic, butyric, caproic, isovaleric acids, acetoin, small amounts of acetaldehyde and acetone.	Produce acetaldehyde, acetone, butanone and traces of acetoin. Some fatty acids are also produced.

Qualities of an ideal yoghurt starter

1. Purity, i.e. free from contaminants
2. Vigorous growth
3. Production of the right consistency
4. Production of good flavour without off flavour
5. Stability i.e. its balance should be easily maintained
6. No tendency to induce syneresis.
7. Should not develop excessive acidity on cold storage.
8. Should have a reasonable tolerance to sugar.
9. Should be resistant to penicillin and other antibiotics.
10. Its maintenance should be easy.
11. It should be phage resistant.

Mechanism of symbiosis between cultures

1. *L. bulgaricus* has got cell wall bound proteases. It has been established that numerous amino acids liberated from casein by proteases produced by *L. bulgaricus* stimulate the growth of *S. thermophilus*. *S. thermophilus* produces formic acid which stimulates the growth of *L. bulgaricus*. Apart from formic acid, Carbon dioxide, creating anaerobic conditions and pyruvic acid produced by *S. thermophilus* stimulate growth of *L. bulgaricus*.
2. As a result of symbiosis, several benefits have been observed: Higher rate of acid production: As compared to either *S. thermophilus* or *L. bulgaricus* alone, the combination of culture, produces higher amount of acid at all times (



Higher amount of flavour produced: The major flavour producer is *L. bulgaricus*.

Resistance to sugar: More than 11% sugar slows down growth of yoghurt cultures individually, but they produce acid at faster rate even with 15% sugar in combination.

Higher cell numbers: Overall, the cell count increases, but depends on the stage of incubation.

The stimulatory effect by *L. bulgaricus* results in a shortened generation time of streptococci and increased numbers. As a result, the streptococci grow faster in the early part of incubation, outnumbering the lactobacilli by 3 or 4 to 1 after the first hour. During this stage it drives excess O2 and produce formate, CO2 and lactic acid for stimulatory growth of lactobacilli. Hence in the later stage, proportion of lactobacilli increases and streptococci decreases due to inhibitory effect of lactic acid. The ratio of rod: cocci become 1:1 after 4 hours.

Enumeration of yoghurt bacteria

1. **Counting each microbe by cultivating separately on selective media.**

L. bulgaricus **on MRS medium or reinforced clostridia agar at pH 5.5.**

S. thermophilus **on LPA or M17 medium or PCMA medium at pH 7.0.**

2. **Use of single medium to allow the growth of both bacteria.**
3. **Certain media can give differential count of both yoghurt starters.**

- **PPY-Eriochrome agar (Tryptose, proteose-peptone yeast extract, Erichrome black T-dye, agar, glucose, lactose,Tween-80). After 24 h of incubation, *S. thermophilus* appeared as circular colonies, 1-3 mm in diameter, convex, opaque, and white-violet, often with a darker centre while *L. bulgaricus* appeared as transparent, diffuse colonies (4-6 mm diameter) of undefined shape, irregular edge.**
- **LAB medium containing lactose, typtone, meat extract, yeast autolysate, tomato juice, Tween 80, K2HPO4 and agar using double pour plate technique. Differentiation is made by colony type under a low power microscope. *S. thermophilus* gives smooth, round or lenticular colonies and *L. bulgaricus* gives irregular, or rough colonies in the depth of the medium.**
- **A medium in which β-glycerophosphate is incorporated. This inhibits the growth of *L. bulgaricus* but not that of *S. thermophilus*.**

Types of Yoghurt

1. Legal / Standards/ Chemical composition: FAO/WHO (1973)

Full fat > 3% fat

Medium fat 0.5 to 3% fat

Low fat < 0.5%

Balkan yoghurt – 4.5 to 10% fat

2. Method of production

Set yoghurt

Stirred yoghurt

Fluid yoghurt – diluted or stirred yoghurt (yoghurt with <11% T.S.)

3. Flavours

Natural or plain yoghurt – traditional type with sharp, acidic taste

Fruit yoghurt- addition of fruits & sweetening agents to plain yoghurt

Flavoured yoghurt – synthetic flavouring & colouring agents are added.

4. Post –incubation processing

Pasteurized yoghurt

Frozen yoghurt

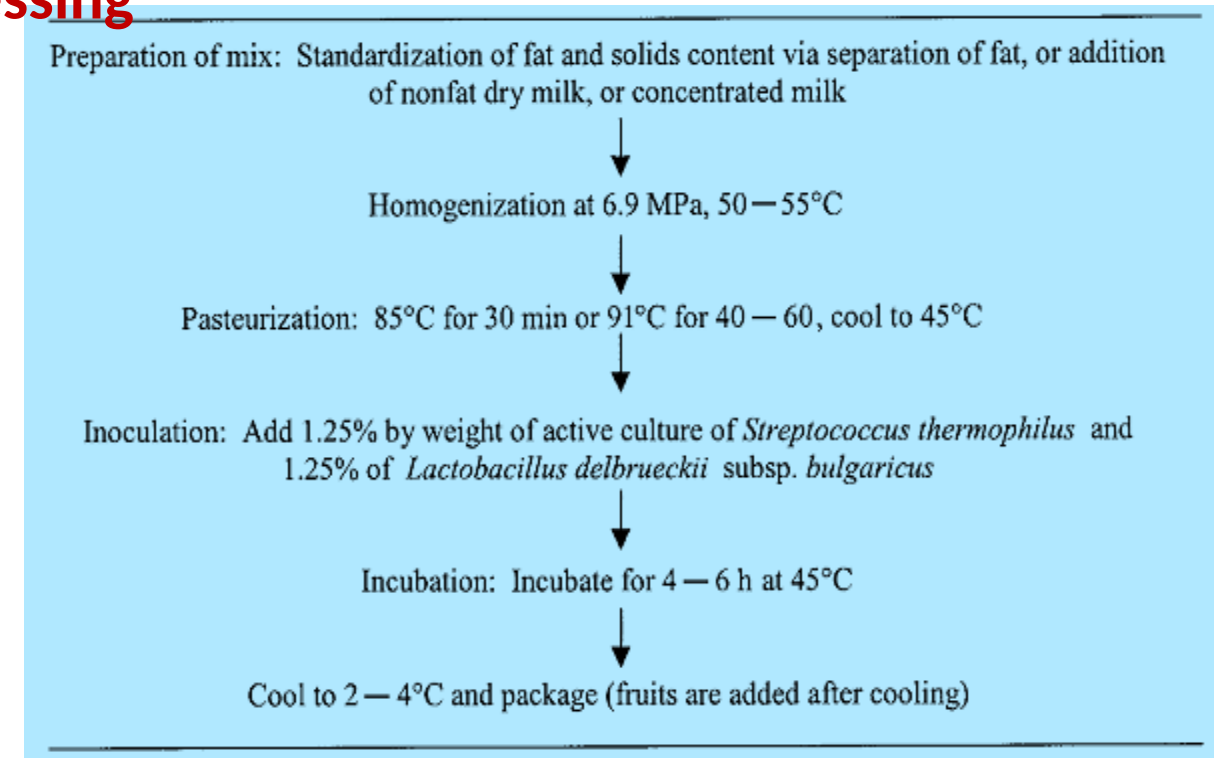
Dietetic yoghurts- may include less calorie yoghurt, low lactose yoghurt or vitamin/protein fortified yoghurt.

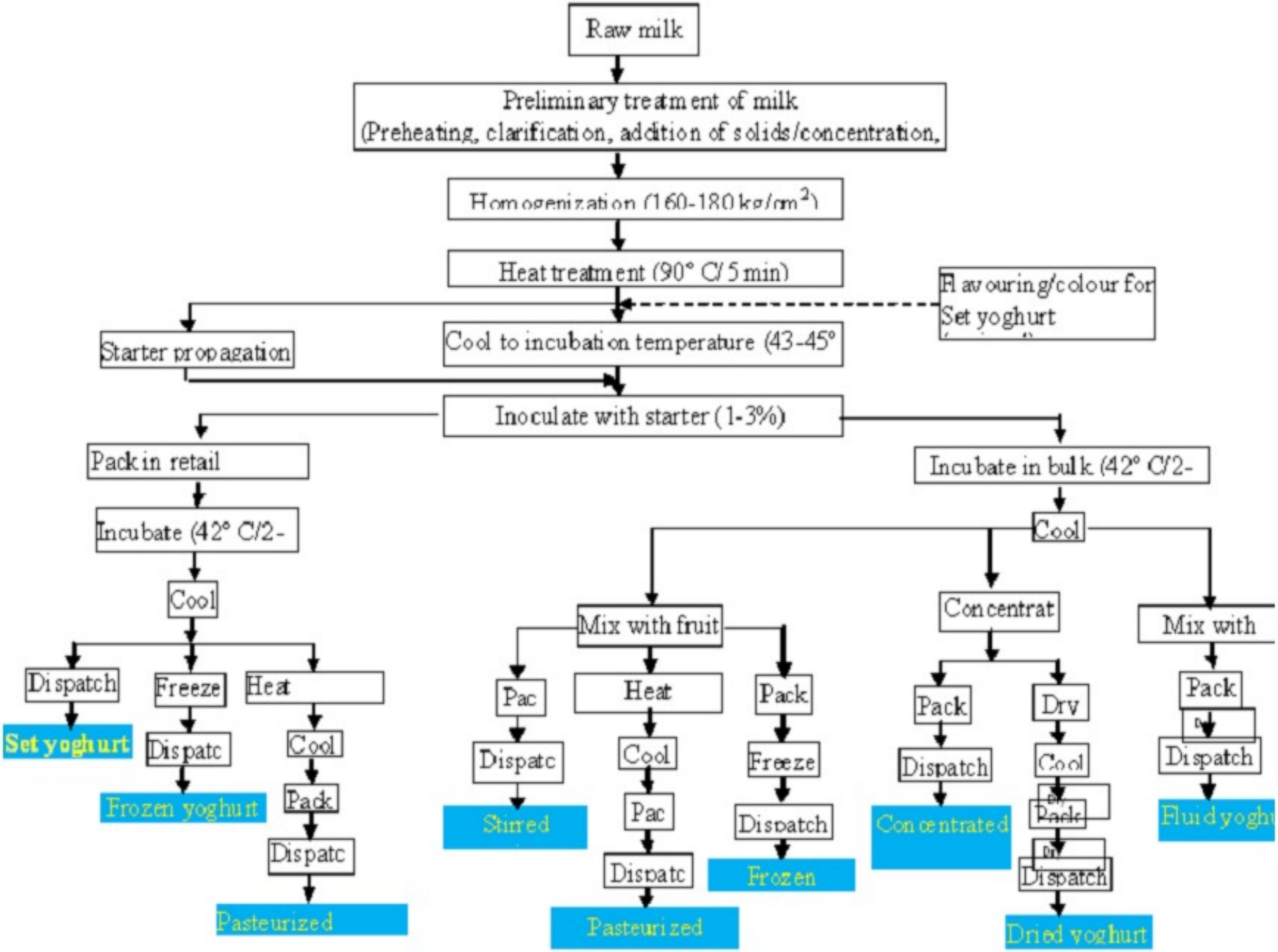
Concentrated (24% T.S.)

Dried yoghurts (90-94% T.S.).

Production of Yoghurt: The manufacture of yoghurt involves:

1. Selection of raw materials
2. Standardization
3. Homogenization
4. Heat processing
5. Inoculation
6. Incubation
7. Cooling
8. Post incubation processing
9. Packaging




Raw milk
Preliminary treatment of milk
(Preheating, clarification, addition of solids/concentration,
Homogenization (160-180 kg/cm2)
Heat treatment (90° C/5 min)
Flavouring/colour for
Set yoghurt
Starter propagation
Cool to incubation temperature (43-45°
Inoculate with starter (1-3%)
Pack in retail
Incubate (42° C/2-
Cool
Dispatch
Set yoghurt
Freeze
Dispatc
Frozen yoghurt
Heat
Cool
Pack
Dispatc
Pasteurized
Incubate in bulk (42° C/2-
Cool
Mix with fruit
Pac
Dispatc
Stirred
Heat
Cool
Pac
Dispatc
Pasteurized
Pack
Freeze
Dispatch
Frozen
Concentrat
Pack
Dispatch
Concentrated
Dry
Cool
Pack
Dispatch
Dried yoghurt
Mix with
Pack
Dispatch
Fluid yoghu

Selection of raw materials: Raw milk is the major raw material used in manufacture of yoghurt. It should be fresh, have normal composition, free from mastitis and other diseases, free from antibiotics and other inhibitors, free from off-flavours, has low bacterial count and free from pre-enzymatic activities.

Standardization and pre- treatments:

Buffalo milk should be preferred for set types of product as it gives firm curd while cow milk may be good for stirred product, which will be smoother and uniform.

The manufacturer has to standardize the milk to meet legal requirements for fat and SNF (Solids-not-fat). Technologically, good quality set yoghurt is obtained from the milk having 13 -15% total milk solids.

Fat contributes to integrated pleasant flavour and richness to the product. About 3% fat is sufficient to have good quality product, while SNF can be increased to 10-12%, preferably by concentration or by supplementation with skim milk powder.

The ingredients added during this standardization process should be carefully selected as poor quality ingredients can not make good quality product. If SMP, Whey powder, Butter or any other materials used for standardization has high microbial count, especially spore counts, it will increase load in fermented milk and the product will show defects early.

As such stabilizer is not needed in the product, but to increase the smoothness and decrease whey separation in stirred product, it can be added at the rate of 0.1-0.2%, while heating the milk. This should not add to the total microbial load of the product.

Pre-treatments like filtration/straining removes dirt, dust and extraneous matters and reduce microbial load to a great extent. Forewarming will lead to activation of microbes, but before they multiply, the milk should be heat processed so that the microbial count does not increase.

Homogenization: The milk may be homogenized at 100 Kg/ cm^2 at 60-70 C. This is an optional step done in between the final heat processing of milk. This process is useful as it gives uniform mixing of all raw materials, reduce the problem of fat separation in curd, improves gel stability and improves digestibility. Homogenizer and the line connecting to it becomes an additional source of contamination. If it is not properly cleaned, it may add to the total microflora of the milk. The process of homogenization also breaks the clumps of microflora in milk and hence, if such milk is tested by plating methods, it apparently shows more cfu/ml, while actually the number of microflora might not have increased.

Heat Processing:

The milk intended for fermented product manufacture must be heated in the range of 80-95 C for 5-30 minutes. Generally it is practiced at 80C for 30 min, or 85C for 20 min or 90C for 10 min or 95 C for 5 min.

The heat treatment is made legal from public health point of view, as heat processing should destroy the pathogens likely to come in milk and make the milk safe for human consumption.

It supports good growth of the culture as it destroys other competing microflora giving free ground for the starters to proliferate.

It inactivates natural inhibitory substances in milk, drives out oxygen, produce some growth stimulating agents for starters.

Higher heat treatment denatures whey proteins which help in improving the gel stability.

Heat treatment is the only severe treatment applied during the entire process of fermented milk manufacture, which can destroy microorganism. So, heat treatment is considered as critical control point (CCP) in HACCP program for yoghurt.

Cooling:

Just after heating, the milk is cooled to incubation temperature, which is around 42-45C.

Cooling is essential before inoculation, because if the culture is added in hot milk, obviously it will die.

Care should be taken that milk after cooling should be inoculated as early as possible. If the time gap before inoculation is more, the survivors of pasteurization will grow.

The microbial count just after cooling should be minimum.

If set-product is to be prepared, the colour and flavour in appropriate concentration is mixed at this stage. In case of stirred product, flavouring is done while stirring. The milk is now ready for inoculation.

Inoculation:

The milk is inoculated with active yoghurt cultures, *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* at the rate of 2% (v/v) of milk. Usually, both the cultures are added in equal proportion (1% each).

Filling in retail packs for set yoghurt:

If set yoghurt is required, the inoculated milk is required to be packed in retail containers before incubation. The packing material must efficiently be treated, sanitized to minimize contamination. Risk of mold and bacterial spores decreases half -life.

Incubation:

After adding culture in the milk, it is uniformly mixed without aeration.

It is then incubated in bulk or in the same tank, if stirred product is to be made. If set-product is required, the milk is filled in retail containers before incubation.

Incubation is purely a biological process during which the culture grows and brings necessary transformations in milk to get a desirable fermented product. Incubation temperature should be kept 42 C.

The period of incubation varies between 3-6 h, depending upon the rate of acid production by the culture in the milk. However, the best end point to stop fermentation is just after the milk sets.

Setting takes place at about 0.6% acidity and the remaining acidity required in the product can develop while cooling.

During incubation, the milk is very sensitive to mechanical disturbances and other changes. So, it should not be disturbed.

Cooling:

As soon as the curd sets or desired acidity in the product is achieved, it must be cooled.

Cooling is done to reduce the rate of multiplication of starter cultures and stop their growth at the end of cooling. This is essential to avoid over acidification in the product.

The final acidity desired in the product and total count of starter cells excepted in the product will depend on the rate of cooling and how much times it takes to reduce the temperature below 5 C.

The rate of cooling affects the quality of the product and decided according to the %lactic acid expected in the final product.

Rapid cooling may lead to more contraction of gel and separate more whey, while too slow cooling may sour the product. In yoghurt, two stage cooling is preferred, i.e. in first stage cooling from 42 C-20 C and in second stage from 20 C-5 C in cold store.

In the stirred products, cooling and stirring are done simultaneously.

It is advisable to stir the product at lower temperature to reduce the problems of wheying-off. In most cases, the product is stirred at about 20 C and also blended with colour, flavour, fruits, nuts and other additives and then packed in retail containers. The product is to be stored at less than 5 C, until its consumption.

Packaging:

The packaging for set product is done in retail containers before incubation.

However, for stirred product, the packing is done during cooling. The packaging materials may be polythene pouches, polystyrene cups, bottles or cartons.

All such packaging materials serve as additional source of contamination and their sterility should be ensured before packaging.

Packaging materials should also prevent the contamination during storage and distribution.

Yoghurt packaging machines are based on one of the following principles.

1. Volumetric level filling- When fluid yoghurt is poured in to glass bottles.
2. Volumetric piston filling- As applied to the packaging of stirred yoghurt in the plastic container.

Storage:

All packaged retail units are packed in larger carton and stored in cold stores.

The yoghurt must be stored at <5 C to ensure inhibition of growth starters and non-starter microorganism.

If there are temperature fluctuations or temperature increases during storage, the growth of culture as well other microorganisms may take place and it will make the product sour or produce other defects. Hence, maintenance of temperature during storage is very important.

The distribution of the finished product should always be through cold-chain.

A good quality yoghurt has shelf-life of 2-3 weeks days at 5 C.

Post production processing of yoghurt into various types

Pasteurized yoghurt: If yoghurt is heated after incubation, it is called as pasteurized yoghurt/heated yoghurt/thermized yoghurt/UHT yoghurt.

Pasteurization of yoghurt can be done to improve the shelf-life. It can increase shelf-life between 4-6 weeks at refrigeration temperature.

The heat treatment given to yoghurt is less because yoghurt has low pH, short time of heat treatment is required to achieve in habitation of starter and contaminants. The range of temperature required is 60-70 C for 1-20 min.

The disadvantages of heat treatment is loss of flavour, increased whey syneresis, loss of vitamins, loss of enzymes (lactase), destroy live starter flora which may reduce therapeutic value of the product, violates legal FAO/WHO definition of yoghurt stating that yoghurt should have starter flora in abundance.

Liquid/drinking yoghurt: Yoghurt is cooled to about 20-25C and then stirred with addition of pasteurized water. The quantity of water depends upon the final total solids desired in the product. Liquid yoghurt generally is defined as the one having less than 9% total solids. This product resembles to cultured buttermilk. It can spiced and taken as a refreshing beverage.

Carbonated yoghurt: Carbon dioxide is injected in stirred and diluted yoghurt to make carbonated yoghurt. The carbonation of finished yoghurt beverage improves its thirst quenching quality and enhance its refreshing character. It may have higher shelf -life but show more whey separation.

Frozen yoghurt: The yoghurt base produced in conventional way and the mix can be prepared by adding several other ingredients including milk, cream, skim milk powder, etc. The mix is then frozen like ice-cream and air is incorporated to have over run. Yoghurt can be hard frozen, or can be served as soft with or without additional flavourings.

Dietetic yoghurt: Various types of dietetic yoghurts can be prepared by supplementations of required ingredients and standardization. These may low fat yoghurt, low calorie yoghurt, high calcium yoghurt, etc. Most important form of dietetic yoghurts is the probiotic yoghurt, which is prepared by incorporation of probiotic culture in yoghurt.

Lactose hydrolyzed yoghurt: This product is designed for lactose intolerant people. The milk is first treated with lactase to partially hydrolyze lactose and then finally heat treated and inoculated with yoghurt cultures. The product will have faster growth of starters requiring less incubation time. Yoghurt will be milder and sweeter.

Types and cultures of yoghurt

Name	Cultures used
Acido-yoghurt	*L.bulgaricus* (LB) + *S. thermophilus* (ST) +0.25% of intestinal strain of *L. acidophilus*
Acidophilus yoghurt	(LB+ST) + acidophilus (final product contains 30 million/ml of *L. acidophilus*)
Acidophilus bifidus yoghurt	LB+ST + L.acidophilus + B.bifidus
Bioyoghurt	ST + *L. acidophilus* (or S. lactis var taette + L. acidophilus)
Bifighurt	ST + *B. bifidus*
Biograde	ST + *L. acidophilus* + *B. bifidus*

Biochemical changes during yoghurt production

Acid production: The main biochemical change during yoghurt production is fermentation of lactose to produce lactic acid as the major end product through EMP. The acid produced brings down the pH of the milk which leads to coagulation of milk proteins forming the gel. The percentage of lactic acid is affected by temperature of incubation, starter inoculums rate, ratio of rod: cocci, age of yoghurt and level of lactic acid produced. *S. thermophilus* produces L(+) form of lactic acid while *L. bulgaricus* produces D(-). During storage, the level of D (-) lactic acid increases due to activity of *L. bulgaricus*. In later part of incubation, *L. bulgaricus* grows faster.

Flavour production: The flavour compounds are carbonyl compounds-acetaldehyde, acetone, acetoin and diacetyl. Acetaldehyde is a major flavour compound. The normal con. is around 13-17 mg/kg, which gives normal yoghurt flavour. *L. bulgaricus* is the main contributor to acetaldehyde.

Acetaldehyde is produced from various substrates. It is produced from lactose via pyruvate and acetyl CoA. It can also be produce from milk protein via threonine with the help of enzyme aldolase. Flavour also comes through thermal degradation compounds of fat, protein, lactose, volatile fatty acids, amino acids.

Proteolysis: Yoghurt cultures are weakly proteolytic. Proteolysis in yoghurt brings changes in physical structure, contribution to flavour production e.g. peptides, amino acids directly act as flavour compounds or act as precursor for reaction that produces flavour compounds. Yoghurt bacteria increases soluble NPN by 50%. *L. bulgaricus* is more proteolytic and has ability to hydrolyze casein. Beta and kappa casein more degraded than α-casein by *S. thermophilus*.

Lipolysis: Lipolysis in yoghurt occurs due to bacterial lipases and esterases. Occurs very slightly, affects flavour, increases FFA during storage and *L. bulgaricus* produces more volatile fatty acids than *S. thermophilus*.

Nutritional value of yoghurt

Milk contains well-balanced macronutrients including carbohydrate, fat and protein and micronutrients including calcium, phosphorus, magnesium and zinc.

Milk proteins have high nutritive value due to the favourable balance of essential amino acids.

Constituent	Milk		Yoghurt		
	Whole	Skim	Full fat	Low fat	Low fat/fruit
Water (g)	87.8	91.1	81.9	84.9	77.0
Energy value (kcal)	66	33	79	56	90
Protein (g)	3.2	3.3	5.7	5.1	4.1
Fat (g)	3.9	0.1	3.0	0.8	0.7
Carbohydrate (g)	4.8	5.0	7.8	7.5	17.9
Calcium (mg)	115	120	200	190	150
Phosphorus (mg)	92	95	170	160	120
Sodium (mg)	55	55	80	83	64
Potassium (mg)	140	150	280	250	210
Zinc (mg)	0.4	0.4	0.7	0.6	0.5

While manufacture of yoghurt, quality and quantity of nutrients are affected by two ways.

Changes due to modification/fortification: Changes due to modification or fortification of supplementation of any ingredient can easily be calculated.

Changes due to fermentation: Depend upon fermentation conditions and the type of cultures.

- During fermentation; lactose, protein, fat and some vitamins decrease while lactic acid, peptides, amino acids, volatile flavour compounds, vitamins, enzymes and bacterial proteins increase.
- Fermentation improves the digestibility of the proteins. It helps in formation of softer curd and its digestibility is facilitated by presence of lactic acid.
- Vitamin content may increase or decrease depending upon the strain of the culture. Yoghurt cultures synthesize vitamin B2, B12, Folic acid, Niacin with improvement in nutritive value.

Therapeutic value of yoghurt

Used as therapeutic agents in gastro-intestinal disorders: Yoghurt flora is known to inhibit several food spoilage organisms and intestinal pathogens due to presence of several inhibitory substance and low pH. This helps in prevention of infections and control of several gastro-intestinal tract illness including diarrhea and constipation.

Can be digested by lactose intolerant people: Yoghurt has lower lactose due to fermentation and it also provides lactase from autolyzed bacteria in gut and hence it helps in digestion of lactose.

Hypocholesterolemic activity: Yoghurt flora can reduce cholesterol in the body thus; reducing the risk of heart diseases.

Anticarcinogenic activities: Yoghurt, especially having probiotic cultures possess some tumor inhibiting properties. They also reduce the activity of enzymes that convert pro-carcinogen to carcinogen in gut which reduces the possibility of colon cancer.

Immunostimulatory properties: Increase in specific and non-specific immune functions occurs by consumption of fermented milks.

Quality Testing of yoghurt

Physical tests: Physical appearance, free whey, presence of gas slits, package conditions, etc.

Organoleptic tests: Colour and appearance, body and texture, flavour and taste.

Chemical tests: Titratable acidity, pH, presence of flavour compounds etc.

Microbiological tests: Microscopic examination, bacterial count, Coliform count, Yeast and mold count, etc

Tests for specific properties: If any specific property or function is claimed from the product, a test to justify is required to be done.

Shelf-life of good quality yoghurt is 2-3 weeks at refrigeration temperature. It is required to be stored in cold conditions, transported and distributed in cold chain, otherwise several defects develop and the shelf-life is reduced.

Defects of yoghurt

- Defects in yoghurt can be classified as Appearance defects, Body & texture defects, Flavor defects, Acidification defects and Storage related defects.

Sr. No.	Defect	Causes	Remedies
1	Whey Separation/ settled (separation of clear liquid at the top of the product)	Over acidification, mechanical damage to curd, high incubation temperature, low total solids, aeration, improper heat treatment of milk, etc.	Homogenization, increase total solids (protein content >3.5%), adequate heat treatment of milk, low incubation temperature, use of slime producing cultures, use of stabilizers, etc.
2	Sour/high acid	Over incubation, slow cooling, contamination with wild LAB, improper storage, high rate of inoculum	Start early cooling, maintain low temperature, use least over-acidifying cultures.
3	Insipid/dull	Under incubation, slow cultures, too early cooling after incubation, page attack.	Incubate till pH<4.6, ensure starter activity and purity, optimum temperature of incubation, higher rate of inoculation.
4	Bitter taste/sweet curdling	Contamination by aerobic spore formers, proteolytic action	Hygenic care, sufficient heat treatment of milk
5	Gassiness/bulging	Growth of contaminants like yeasts, coliforms, unhygienic conditions, un-refrigerated storage	Prevent contamination, observe hygienic conditions, maintain cold chain
6	Yeasty flavour	Contamination with yeast and molds.	Use strict hygienic practices to avoid contamination.
7	Ropiness/ slimy ness	Slime producing contaminants, too low temperature of incubation, too high concentration of stabilizers	Use appropriate incubation temperature, prevent contamination with slime producers, low level of stabilizers.
8	Rancid/oxidised/ stale	Improper storage, contamination with fat degraders, exposure to light	Prevent contamination with lipolytic organisms, prevent light exposure, low temperature storage.