

A close-up photograph of a rectangular block of butter, partially unwrapped from its white paper packaging. A single pat of butter has been cut out and is lying in front of the main block. The butter is a pale yellow color with a smooth, slightly textured surface. The background is a plain, light-colored surface.

# **Microbiology of Butter**

# What is Butter?

Butter is commonly defined as a fat concentrate which is obtained by churning cream, gathering the fat into a compact mass and then working on it. It is a water-in-oil emulsion, typically salted Indian butter consisting of at least 80.2% fat, 16% water, 2.5% common salt and 1% curd.

# Types of Butter

**Creamery butter (Made in dairy plant)**

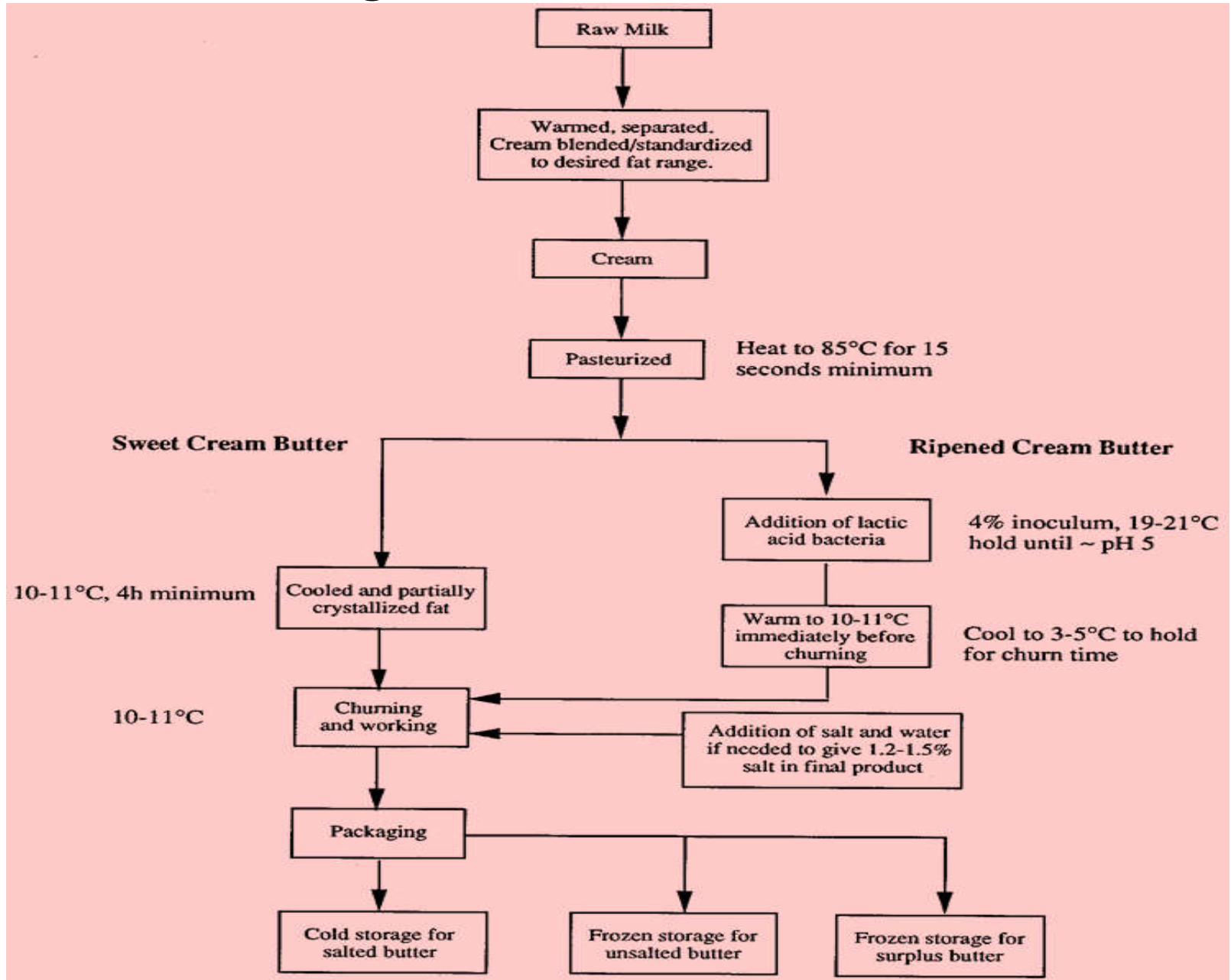
**Pasteurized cream butter  
(Made from pasteurized sweet cream)**

**Sweet cream butter (Made from sweet cream i.e., acidity not exceeding 0.20%)**

**Sour cream butter (Made from sweet cream i.e., acidity more than 0.20%)**

**Ripened cream butter (made from ripened cream i.e. inoculation and incubation of cream with a desirable flavour producing butter culture under optimum conditions)**

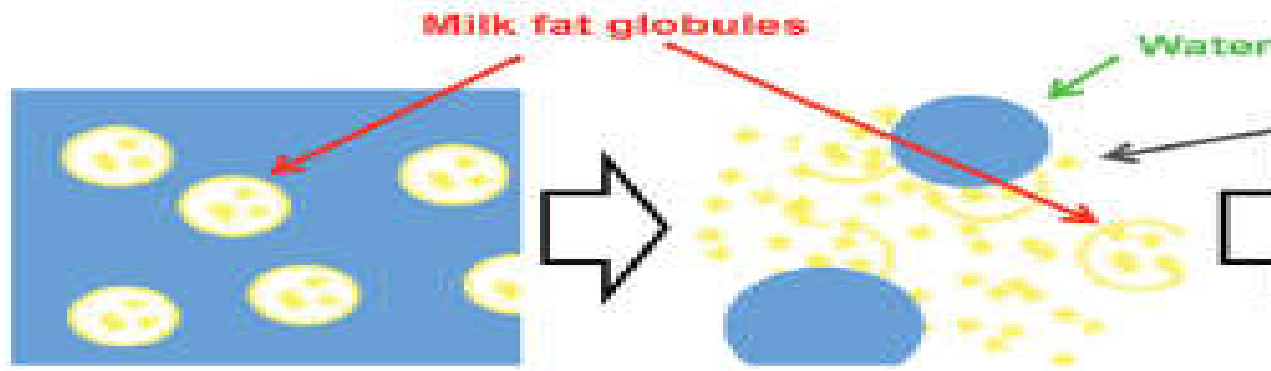
# Flow Diagram of Production of Butter



# Microenvironment of Butter

The micro-environment of butter is relatively unfavorable for the growth of microbes as compared to cream due to:

- **Compositional differences:** Fat (80%) present in greater quantities is relatively resistant to microbial decomposition. Lactose and moisture which are readily utilizable by many organisms or essential for growth is present in lower quantities. Salt sometimes present makes more unfavorable for microbial growth.
- **Structural differences:** The easy proliferation of microbes is not possible because (i) Water phase is separated by relatively resistant fat phase but however, molds and pseudomyelia forming yeasts are able to grow and penetrate through the fat phase of butter. (ii) Limited supply of nutrients in water droplets in butter while in cream, microorganisms can grow in the continuous water phase having dissolved nutrients and migrate easily from one portion to the other.



Inspite of existence of unfavorable conditions, a large number of microorganisms are being contributed by post pasteurization contamination (utensils, water, air etc.). Yeasts and molds also enter through aerial contamination.

- **Psychrophilic/psychrotrophic bacteria:** *Pseudomonas putrefaciens*, *P. fragi*, *P. fluorescens*, *P. graveolens*, *P. mephitica*, *Acinetobacter* spp., *Achromobacter*, *Enterobacter* spp.
- **Mesophilic bacteria:** Streptococci, Lactobacilli, *E. coli*, *Serratia marcescens*, *Enterobacter aerogenes*
- **Sporeformers:** *Bacillus* spp., *Clostridium* spp.
- **Yeasts and Molds:** *Torula sphaerica*, *Saccharomyces lactis*, *Torula* spp. and *Monilia* spp. (Non-lactose fermenting yeasts), *Candida pseudotropicalis*, *Geotrichum candidum*, *Cladosporium*, *Alternaria*, *Mucor* spp., *Penicillium* spp., *Aspergillus* spp., *Rhizopus* spp.

# Sources of Microorganisms in Butter

The microbiology of butter reflects the microflora present in pasteurized cream from which it is made, water added at the time of salting butter, sanitary conditions of process equipment, manufacturing environment, and storage conditions.

**Raw material (Cream):** The main source of microorganisms in butter is cream. Raw milk may be contaminated with a wide variety of pathogenic and spoilage microorganisms. The microflora of raw milk is related to that found in and on the cow's udder, milk-handling equipment, and storage conditions. Proper handling, pasteurization, and storage conditions should result in a predominantly gram-positive microflora in milk.

**Equipments:** Butter churn is the most important source of contamination in regard to psychrotrophic organisms and yeasts and molds. The part of pasteurizer, pipelines, valves and coolers may be sources of contamination when cream is pasteurized.

**Water supplies:** Water is used to flush residual cream from holding vat into churns, for dilution of cream, as chilled wash water, for wet salting and adjusting moisture content of water. It is also used for washing butter granules during continuous butter making processes. Unhygienic water acts as source of contamination. Psychrotrophic bacteria causes butter spoilage that enter through water supplies. E.g., *Achromobacter putrescens* which causes putrid butter comes from impure water. Spoilage causing organisms (*P. fragi*, *P. fluorescens*) may also gain access into butter if well water is used for washing butter. Psychrotrophic bacteria can grow extensively in chilled water in dairy tanks, particularly when water contains some organic matter and has not been chlorinated.

**Air:** All factories do not have separate packing room or do not maintain a high standard of hygiene in butter packing. Thus, butter often gets exposed to air for long periods prior to or during packing and gets contaminated. Psychrotrophs encountered in the air of dairy plants. Mold spores remain suspended in air and contaminate walls or wooden structures in the packing room. Main sources of aerial contamination are movement of workers, ventilation, fans, dust etc.



**Personnel:** The person involved in the manufacture and handling butter may introduce microbes to butter through contaminated hands, clothing, mouth, nasal discharge etc. Unhealthy persons suffering from respiratory disorders should not be allowed to handle butter.

**Butter colour:** The colour used for incorporation in butter if kept in open containers or mixed with water in unclean containers, there are chances of contamination of butter through butter colour.

**Packaging:** Butter may be packaged either in bulk or in retail size containers. Parchment wrappers are the traditional packaging material, but plastic tubes and laminated foil packs are also common which may get contaminated by molds during transport. Bulk butter may be frozen (-30C) and stored for periods of up to a year, but good-quality butter stored at chill temperatures has expected shelf-life of 6 to 12 weeks. Bulk stored butter may be repackaged prior to sale; this process may cause some redistribution of water droplets, which may affect keeping quality and increase the risk of contamination and subsequent spoilage.

# Effect of Processing on the Microflora of Butter

## Effect of Cream Processing

- **Neutralization of Cream:** Contaminated neutralizer solution and neutral pH also affects the proliferation of microorganisms.
- **Pasteurization of Cream:** Pasteurization of cream for butter manufacture will be at or above 85°C for 15 s. This thermal treatment minimizes reactivation of lipase native to milk and many microorganisms (microbial pathogens and potential spoilage organisms) are inactivated. However, heat resistant microbes and spores of *Bacillus* and *Clostridium* will survive and cause spoilage.
- **Ripening of Cream:** There is increase in the total bacterial count since ripening involves direct addition and multiplication of added organisms. If the cultures used are contaminated, the undesirable organisms can also enter the product. However, acid production by butter cultures during ripening suppresses the growth of spoilage organisms in butter, thereby leading to extended shelf-life.

- **Cooling/Ageing of Cream:** Ageing helps in better fat recovery in butter during churning. After pasteurization, the cream is cooled to low temperature and is held at this temperature for ageing (5-10°C for 2-4 h or overnight). If the cream is properly pasteurized, there is little significance of such cooling from microbiological view point. This is due to the fact that bacterial number in pasteurized cream is relatively lower and mainly thermophilic organisms are present which are harmless in nature. If modern practices of processing are not observed, there is every possibility of microbial entry and multiplication at this stage of cream processing.

**Effect of Churning:** Vigorous agitation of cream at 10°C (batch method) leads to breaking of bacterial clumps and increasing the total bacterial count. Contamination of butter churn from extraneous sources may further add microbial load in butter. The bacterial load of buttermilk is typically greater than that of cream or butter. However, continuous process yields low count and coliform-free butter.

## **Effect of Moisture Distribution (Working, Printing,**

**Reworking):** The diameter of water droplets in conventionally made butter is  $<1$  to  $>30\text{ }\mu\text{m}$ . The number of water droplets greater than  $30\text{ }\mu\text{m}$  in diameter is inversely proportional to the time of working during conventional (batch churn) butter manufacture. A consequence of uneven distribution of droplets containing microorganisms is a high degree of non-homogeneity regarding microbial distribution in butter. Inadequate working of the butter in batch churns results in poor dispersion of large water droplets and promotes microbial spoilage.

**Effect of Salting:** Salt added to butter inhibits microbial growth. However, salt must be distributed evenly in the moisture phase of butter effectively to inhibit microbial growth in water droplets. Insufficient working results in a non-homogeneous distribution of salt in the water droplets. The microbiological quality of water used for washing or for brines is critical to production of a safe and stable product.

***Listeria* survive in a saturated brine solution held at 4C for 132 days. Thus, brines used to salt butter must be free of *Listeria*. Water is frequently contaminated with pseudomonads, and consequently care must be taken to insure water and brines used are free of these bacteria. The most common form of spoilage in butter occurs with *Pseudomonas* sp. Addition of salt to butter lowers the freezing point so that psychrotrophic microorganisms present may be able to grow at less than 0C. Distribution of salt in the moisture phase of butter has less impact on growth of yeasts and molds on the surface of butter as compared to bacteria. Humid conditions appear to have a greater impact on mold growth. Bacterial spoilage may occur in areas of butter with low salt in large droplets of moisture (poor working).**

**Effect of Packaging:** In batch operations, butter is loaded directly from the churn into hoppers and wheeled to packaging machines. Handling butter this way exposes it to air, workers, plant environment, and ambient temperatures that may accelerate spoilage. Control of the microbiological quality of air in the packaging room is therefore important. HEPA (High Efficiency Particulate Arrestor) quality air with the filtration after temperature modification is desired. Practices that result in standing water on the floor or residual and spilled product facilitate growth of environmental contaminants. Aerosolization of contaminants often produce unacceptable levels of microbiological contamination in the air. Personnel hygiene is critical during butter manufacture, because contaminants from hands, mouth, nasal passages, and clothing may be transmitted to butter during packaging. Direct packaging into consumer-size containers is preferable over bulk packaging, because such butter must be reworked and repackaged before sale. Such reworking increases the risk of contamination and subsequent spoilage of butter. Polyethylene is the preferred material based on its physical properties (low density, high impact, cost effectiveness, absence of copper, and near sterile condition).

**Effect of Storage:** At room temperature, butter becomes soft and moisture droplets aggregate and increase in size, leading to faster growth of microorganisms. At low temperature storage, psychrotrophs multiply. In sweet cream butter, micrococci predominate during cold storage and in ripened cream butter, yeasts usually predominate during cold storage.

# Spoilage Defects in Butter

## Fresh Butter Defects

**Cream borne Defects:** Feed, weed, barny, cowy, metallic, cheesy, malty, musty, yeasty, rancid and cooked. Pasteurization, ripening of cream and washing of butter can decrease the defects in the butter.

**Flat or insipid Flavour:** Causes can be dilution of cream with water or excess washing of butter granules. Avoiding dilution of cream or excess washing of butter granules can control this defect.

## Storage Defects:

The storage defects of butter are mainly dependent upon the conditions of storage mainly in cold butter storage. Sometimes storage at ambient conditions can also complicate the situation and categorized into 3 groups:





Type of spoilage	Symptoms	Control Measures
<b>Chemical deterioration:</b> Presence of salt, moisture, lactic acid, sunlight, copper may induce certain chemical reactions in butter leading to defects.	<ul style="list-style-type: none"> <li>•Rancidity is caused due to oxidation of fat on exposure to copper or sunlight.</li> <li>•Fishiness in butter is due to the production of trimethylamine as a result of decomposition of lecithin</li> </ul>	Avoiding exposure of butter to sunlight
<b>Enzymatic deterioration:</b> Certain enzymes (lipases, proteinases, and phospholipases) cause deterioration of butter during storage.	<p>Psychrotrophic bacteria especially pseudomonads produce heat stable lipases. Lipases cause fat degradation in butter during storage even at -10C.</p> <p>Psychrotrophic bacteria (<i>P. putrefaciens</i>) produce proteinases which are usually heat stable (bitter, sour, fruity and unclean flavor when butter is stored in refrigerators)</p>	<p>Discouraging prolonged low temperature storage. Proper pasteurization of cream will destroy the milk lipase. Hygienic measures can avoid post-pasteurization entry of psychrotrophs.</p>

Type of spoilage	Symptoms	Control Measures
<b>Microbial deterioration:</b> Growth of microorganisms in butter causes a variety of colour and flavour defects. The defects mainly caused by psychrotrophic bacteria, yeasts and molds.	<p><b>Colour defects:</b> Surface of butter gets discoloured, the colour changes depend on the type of organisms involved.</p> <ul style="list-style-type: none"> <li>• <b>Bacterial discolouration:</b> Black discolouration caused by <i>P. nigrifaciens</i>.</li> <li>• <b>Fungal discolouration:</b> Ambient storage conditions, moisture content and acidity of butter encourages moldy butter.  Black: <i>Cladosporium herbarum</i>  Brown: <i>Aspergillus</i> sp.  Green: <i>Penicillium</i> sp.  Orange and yellow: <i>Geotrichum candidum</i>  Reddish pink: <i>Fusarium</i>  Black-<i>Torula</i> sp.  Pink-<i>Rhodotorula</i> spp.</li> </ul>	<p>Control of water supply and proper plant sanitation. Elimination of excess moisture from butter surface during packaging.</p> <p>Control of air-borne contamination. Treatment of packaging materials like UV exposure, Sorbic acid, Calcium propionate, Sodium benzoate, Dihydroacetic acid, Proper sanitization of butter churn, clean handlers</p>

**Flavour defects:**

**Rancid flavour:** Butter gets rancid due to microbial, enzymatic or chemical degradation of fat constituents.

- **Hydrolytic rancidity:** Action of lipase on fat to produce free fatty acids. Short chain fatty acids give rancid flavour (Majorly butyric acid).

- **Oxidative rancidity (tallowy flavour):** Oxidation of unsaturated fatty acids, catalyzed by light and heavy metals (iron and copper). The other factors favoring the defect are high temperature, low pH, salt, long storage etc. During this type of rancidity, free radicals are formed from fatty acids which get converted to peroxides and then to hydroperoxides. The breakdown products give off-odour.

Proper pasteurization of cream, proper moisture and salt distribution in butter.

Addition of various antioxidants, avoiding exposure of butter to sunlight or metal ions

## Type of spoilage

## Symptoms

## Control Measures

- **Ketonic rancidity:** Growth of molds causes ketonic rancidity in butter where fatty acids are converted to various ketones which are responsible for off-flavours.

Avoiding mold growth

**Putrefactive Taint:** Breakdown of proteins by various putrefactive organisms (*Pseudomonas putrefaciens*, coliforms, *Flavobacterium maloloris*). The chemical closely related to isovaleric acid has been found to be responsible for this off-flavour. The causative organisms enter butter through unchlorinated butter supplies and through cream vats, butter churns etc.

Proper pasteurization, proper chlorination of water, uniform distribution of salt, ripening of cream as use of *S. lactis* subsp. *diacetylactis* inhibits multiplication of *Pseudomonas*.

**Cheesy Taints:** Development of cheese like flavour in butter stored at 10C is due to the growth of Gram- negative bacteria.

## Type of spoilage

## Symptoms

## Control Measures

**Musty Taint:** This defect develops in cold stored butter due to *Achromobacter* sp.

**Apple like aroma:** *Flavobacterium lactis* growing at 5C has been found to be associated with butter.

**Skunk like flavour:** *Pseudomonas mephitica* which is usually observed in unsalted butter held at cold storage.

**Fishy Taint:** Fishiness is developed due to the decomposition of lecithin to trimethylamine by microbial activity (*P. ichthyosmia*).

Proper pasteurization of cream.

Control of microbial contamination and growth in butter.

# Pathogens: Growth and Survival in Butter

Few outbreaks of foodborne disease associated with commercial butter during production, handling and package.

***Staphylococcus aureus*:** Outbreaks of staphylococcal food poisoning have been associated with butter. An outbreak in USA (1970) was linked to whipped butter and to the butter from which the whipped butter was made. The presence of staphylococcal enterotoxin A was demonstrated in both butters. It was seen that the enterotoxin formed in the cream was carried over into the finished product. A combination of poor hygiene, low salt concentration (or inadequate salt dispersal), and temperature abuse could allow growth of *S. aureus* in stored butter.

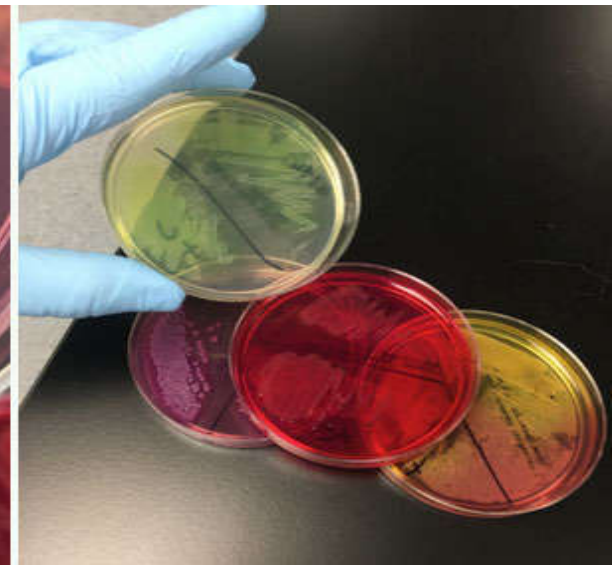
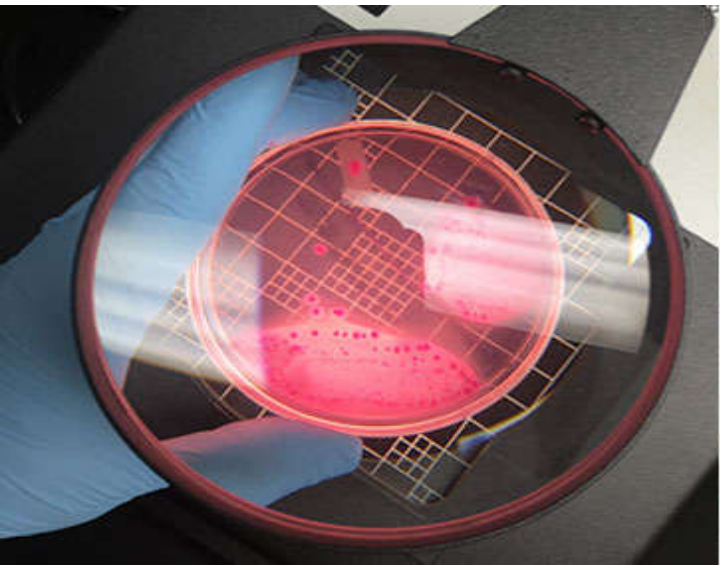
***Campylobacter*:** In 1995, an outbreak of *C. jejuni* enteritis in USA affected 30 people due to consumption of garlic butter. *C. jejuni* could survive in butter without garlic for 13 days at 5C.





# Regulatory Microbiological Standards

Products	Coliform Count	Yeast and Mold Count	SPC
Cream	10/g	<20/ml	$50 \times 10^3/\text{g}$
Butter	Absent/0.1 g	20/g	$25 \times 10^3/\text{g}$



# Regulatory Microbiological Standards

Products	<i>E. coli</i>	<i>Salmonella/</i> <i>Shigella</i>	<i>L.</i> <i>monocytogenes</i>
Cream	Absent/0.1 g	Absent/25 g	Absent/25 g
Butter	Absent/0.1 g	Absent/25 g	Absent/g

