THE SCIENCE BEHIND **CHEESEMAKING**

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Introduction to Cultures and Cheesemaking

Bacteria known as "Starter Cultures" and "Adjunct Cultures" are used in cheesemaking to transform liquid milk into cheese through a natural acidification process called fermentation. Historically, these Cultures were used to preserve the constituents of milk through acidification. During fermentation, these specially selected Cultures produce natural flavour and aroma compounds resulting in a lower pH and a change in the organoleptic and rheological properties of the milk. The reduction in pH, which takes place when the bacteria ferments lactose to lactic acid, has a preservative effect on the product, while at the same time the nutritional value and digestibility are improved. More recently, selective Cultures have been used to also enhance the functional characteristics of cheese.

Lactic Acid Bacteria and Cheesemaking

Starter and Adjunct Cultures used in the cheesemaking process belong to a group of important bacteria called Lactic Acid Bacteria (LAB). LAB are gram positive bacteria that belong to the bacterial order Lactobacillales [1].

A Starter Culture is defined as a collection of bacteria that are added to milk as a primary acidifier, i.e to drive the acidification process [2]. Adjunct Cultures have been defined in literature as "Selected strains of cheese related microorganisms that are added to the cheese milk to improve development of cheese sensory quality" [3].

The bacterial order Lactobacillales are widespread throughout nature and can be found in dairy and non-dairy environments. Indeed, this group of bacteria can be found in other fermented foods ranging from dairy products such as kefir and yoghurt, to plant-based fermented foods like kimchi or sauerkraut and fermented meats such as salami and chorizo. LAB transform the macronutrients (sugars, proteins and fats) in milk into important flavour and aroma compounds via pathways such as glycolysis (fermentation of sugars), proteolysis (degradation of proteins) and lipolysis (breakdown of fat) [4].

Glycolysis

Lactose is a disaccharide present in milk that is broken down by bacterial enzymes. Interestingly, LAB derive their name based on their preference to consume lactose (the sugar in milk). They utilise this disaccharide as an energy source during the fermentation process to generate adenosine tri-phosphate (ATP) to fuel cell growth and reproduction. LAB can be classified based on their fermentation outputs, those that produce just lactic acid are homofermentative only, whereas those that produce lactic acid and other molecules are referred to as heterofermentative. *Lactococcus spp.*, *Streptococcus thermophilus* and *Lactobacillus helveticus* are all homofermentative LAB. The homofermentative LAB mentioned can be used to produce both Cheddar and Mozzarella type cheeses.

Heterofermentative LAB Cultures ferment lactose into lactic acid, volatile aroma compounds and carbon dioxide. Examples of heterofermentative Cultures include members of the *Leuconostoc spp. Lactobacillus lactis* subspecies *diacetylactis*, *Lactobacillus casei*, and *Lactobacillus plantarum*. Heterofermentative species such as *Leuconostoc spp.* are added to Dutch style cheeses, such as Edam and Gouda.

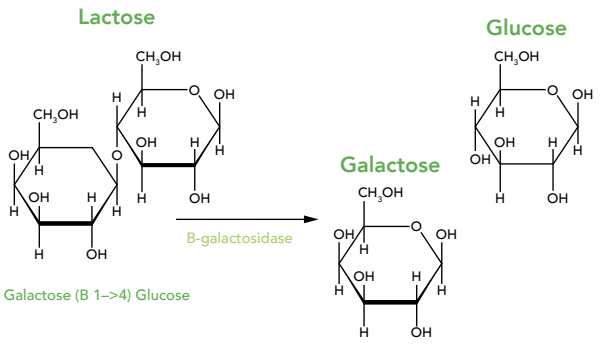

Fig. 1 Lactose catabolism into glucose and galactose. Courtesy of Thomas & M. Terry at the University of Hamburg.

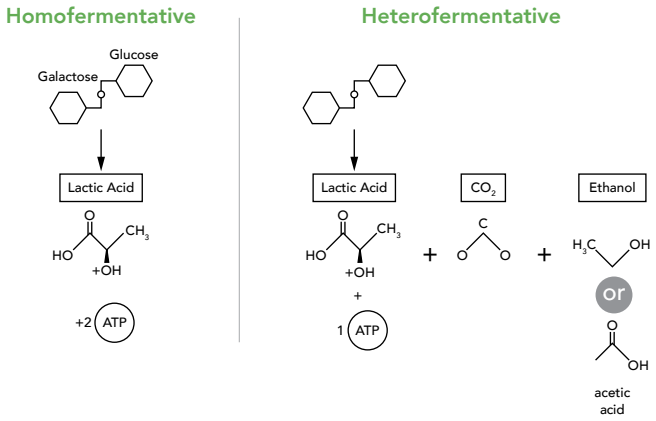

Fig. 2 The difference in these pathways can be seen in Fig.2 (Adapted from Pessione et al 2010 [5].

Adjunct Cultures also belong to the LAB group and may be used in combination with Starter Cultures. They have the metabolic capacity to ferment lactose via homo or hetero-fermentation pathways. They are not added to the cheesemaking process for the purpose of acidification, they are added to impart specific flavour and textural characteristics in the cheese. During maturation, Adjunct Cultures catalyse proteolysis and lipolysis which is key to the development of distinct flavours and aromas in the cheese.

Proteolysis and Cheese Maturation

Proteolysis is a major component of cheese maturation, influencing both the development of texture and flavour as ripening progresses. Proteolysis in cheese can be divided into three phases: proteolysis in milk before cheese manufacture, the enzymatically induced coagulation of the milk, and proteolysis during cheese ripening. [6]

As cheese matures, casein which is the main protein present in milk, is broken down by Adjunct Culture enzymes into peptides and amino acids, this process is called hydrolysis. The biological purpose of this process is to make peptides and amino acids available to microbial cells when carbohydrate sources become scarce [6]. As casein is hydrolysed, the body of the cheese changes from a solid rubbery mass to a smoother texture [7]. Additionally, the flavour of the cheese becomes more nuanced, through the accumulation of microbial derived taste and aroma compounds. These include amino acids such as free glutamine, which has savoury notes or sulphur containing compounds like methanethiol, dimethyl disulfide, which give a characteristic aroma to cheese [8]. Depending on the Adjunct Culture used in a recipe, a bitter or savoury flavour profile can develop in cheese as it matures. This is determined by the mix of exopeptidases and endopeptidases. Endopeptidases cleave peptide molecules in the middle at specific amino acid motifs resulting in a more savoury flavour profile. Exopeptidases on the other hand cut the peptide molecule at the end and result in longer peptides and free amino acids which give slightly different flavour characteristics.

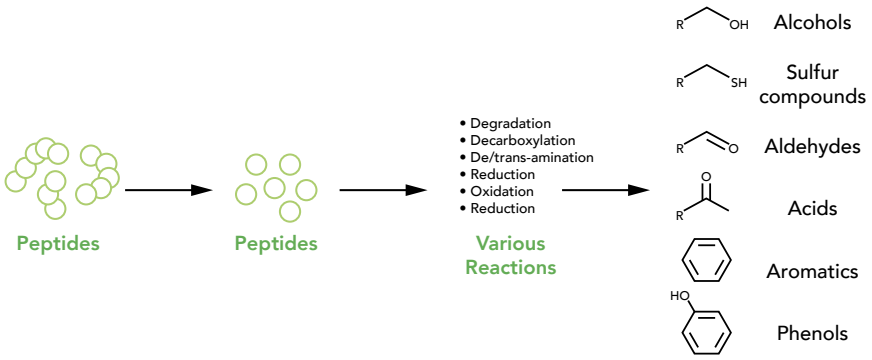

Fig. 3 TBC

Lipolysis

The enzymatic breakdown of triglycerides to fatty acids and glycerol, mono- or diglycerides (lipolysis) is essential to flavour development in many cheese varieties. Mould ripened cheese such as Blue Cheese are noted for their distinct flavour which is a result of extensive lipolysis and the release of up to 25% of the available free fatty acids (FFA) [9]. Certain FFAs are essential flavour compounds in certain cheeses, such as butyric acid in Cheddar Cheese.

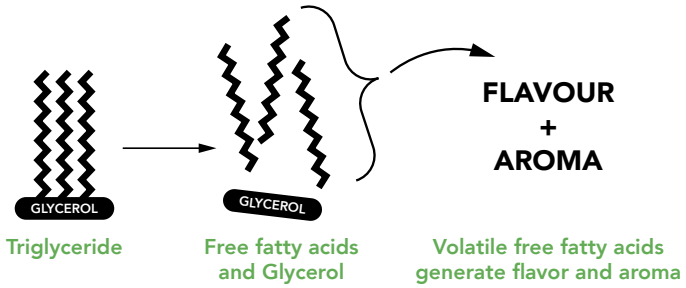

Fig. 4 TBC

Cultures in Cheesemaking

When using cultures in cheesemaking, producers have three options: Natural Whey Cultures (NWC), Bulk Cultures and Direct Vat Set (DVS).

Natural Whey Cultures
Traditionally whey from a previous batch of cheesemaking is added to the cheese milk after pasteurisation to start the cheesemaking process. This type of Culture is called a Natural Whey Culture (NWC). NWC are still used by some artisanal cheesemakers and even in the manufacture of Parmigiano Reggiano PDO [10]. This type of Culture is often referred to as an undefined or mixed Culture strains because the species and strains present in the mixture are not known. This type of Culture system is cost-effective to operate, can offer more Phage robustness but can also be more variable due to the undefined nature of the strains present in the NWC.

In the manufacture of Parmigiano Reggiano PDO, non acid cooked whey is recovered before acidification has occurred and is incubated overnight to develop the mixed strain NWC. This NWC is then ready to be inoculated into pasteurised cheese milk mixture for the next day's cheesemaking. This process is commonly referred to as "back slopping", a process similar to this is used for the manufacturing of sourdough bread, sauerkraut and kombucha.

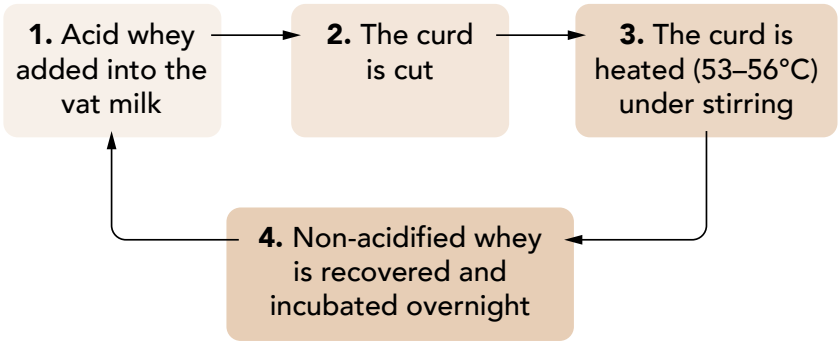

Fig. 5 Figure from Bertani et al [10].

Bulk Cultures
The second type of Culture system is known as a Bulk Culture System. In this system pure Cultures of Starter and Adjunct bacteria are grown up in a sterilised liquid media to a high cell density. These are then added to the cheese milk after pasteurisation as it is being filled into the vat. Most manufacturers of Cheddar Cheese switched to using defined Bulk Cultures over 50 years ago [11]. Defined strains of Bulk Starter Cultures have been selected based on their cheese manufacturing stability and acidification properties. The pH of the Bulk Starter Cultures typically drops the pH of the cheese milk by 0.05-0.1 pH units due to the presence of lactic acid in the Bulk Culture mixture.

The Bulk Culturing Process:

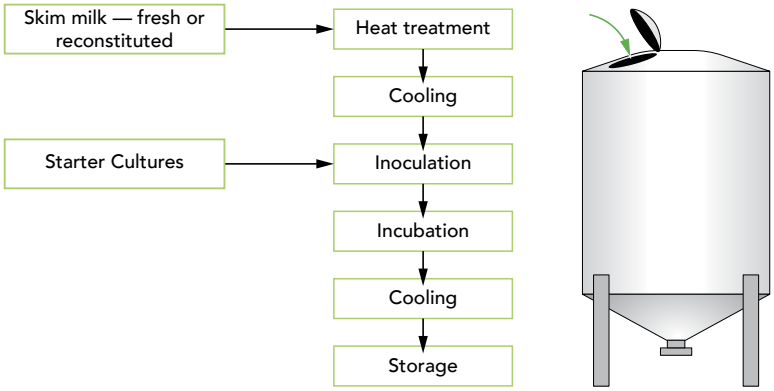

Fig. 6 Figure adapted from The Tetra Pak Dairy Processing handbook (12).

Sterilisation

The environment in which these Cultures are cultivated is tightly controlled to eliminate the risk of contamination by unwanted bacteria, yeast, mould, and bacteriophage. Bulk Cultures are grown in liquid media that has been sterilized prior to Culture inoculation. This heat treatment is a crucial step in achieving a safe, consistent, and reliable Bulk Culture.

Inoculation and Incubation

After sterilisation, media tanks are cooled prior to being inoculated with the specific Starter or Adjunct Culture of choice. The incubation temperature varies depending on the culture being grown. Cultures are grown to target higher cell density than Direct Vat Set (DVS).

Cooling

The Bulk Culture liquid is cooled and positively released once determined to be contaminant-free. The activity (rate of acidification) is determined, and this informs the dosage rate in each recipe where this batch of Culture is being utilised and is controlled by flow meters to verify volumes added to each vat.

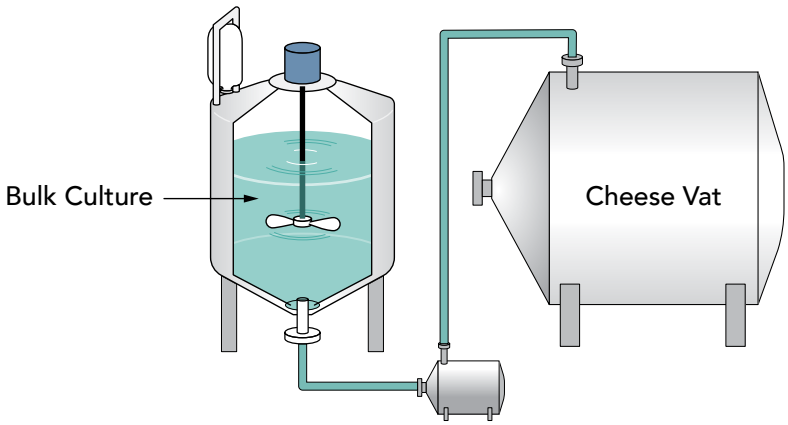

Fig. 7 Bulk Culture being added to the vat

Direct Vat Set

Direct Vat Set (DVS) Cultures were introduced by microbial Culture manufactures in the 1980's. These Cultures come in the form of frozen or freeze dried concentrated defined Culture mixes. These types of Cultures are produced by industrial Culture manufacturers and supplied to Cheesemakers ready to use [11]. They offer flexibility and ease of use as there is no equipment required to grow them, as in the case of Bulk Culture use. These Cultures are inoculated directly into the cheese milk after pasteurisation. It is important to note that cheesemaking recipes need to be adjusted to increase the ripening time (time between milk fill and the addition of rennet). This time is needed for the Cultures to hydrate and activate the Cultures.

The DVS Process

As mentioned above DVS Cultures are supplied in either frozen or freeze-dried format. The inoculation of both these types of Culture occurs as the cheesemilk is filling into the vat after pasteurisation [13].

The Cheesemaking Process

The Starter Cultures and any Adjunct Cultures are added to the milk post pasteurisation and typically held at 32°C for 30 minutes to ripen, for a DVS type recipe this maybe extended. The cheese ripening step allows the bacteria to grow, proliferate and begin fermentation, which lowers the pH and develops the flavour of the cheese.

The curd is allowed to ferment until it reaches an optimal firmness. The gel is then cut within the vat with cheese knives into small pieces of curd and heated to 38°C. The heating step helps expel more whey from the curds (this process is called syneresis). The curd and whey mixture is then pitched to a Cheddaring belt system when the pH of the whey reaches a specific target in the vat. In the belt system whey is drained from the curd, the curds then form a continuous mass as it moves through the Cheddaring belts. This step is called cheddaring. Cheddaring facilitates further syneresis, allowing the fermentation to continue until a pH of 5.1 to 5.5 is reached within the curd mass [14].

When sufficient acidity has developed (~pH5.4), the curd is milled (cut) into small pieces and dry salted. Dry-salting causes a rapid increase in the salt- in -moisture content of the curd and Starter Culture activity stops immediately. Different cheese varieties will have different target pH's that will determine the processing parameters. For instance, Mozzarella has a lower final pH than Cheddar. The final pH of the cheese is an important characteristic to consider when designing a recipe as it will determine the flavour and functionality of the final product.

After salting, the curd pieces are pressed into the form of a uniform block before ripening at 6–8°C for 2 months to > 24 months for Cheddar, the maturation period will vary depending on the degree of maturity required. For Mozzarella, the dry salted curd is stretched into a hot molten mass to create the typically fibrous texture prior to being moulded, cooled and brine salted. The cheese will require up to 14 days to ripen, at this point the cheese is functionally ready to be sold to customers.

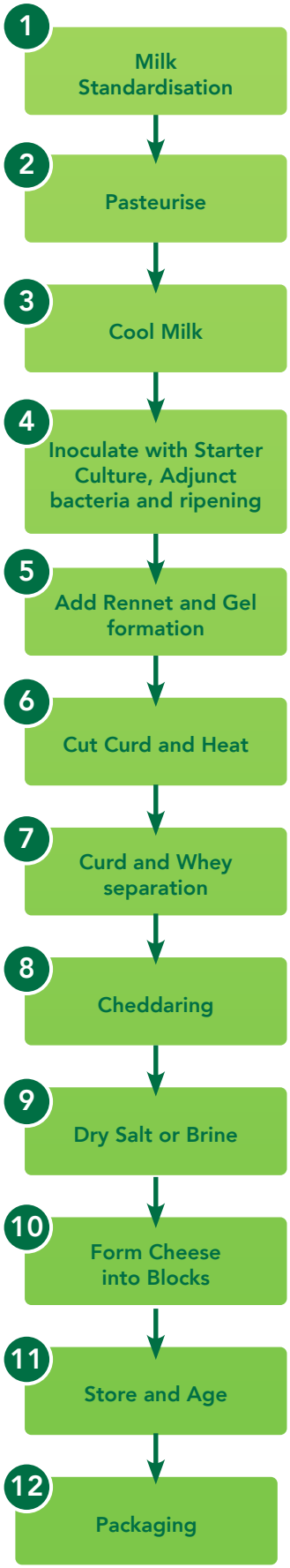

Fig. 8 Steps in the cheese-making process

Bacteriophage

Bacteriophage, commonly referred to as 'Phage' in the dairy industry, are a class of viruses that selectively replicate within bacterial strains. (Fig.8) A strain is a unique member of a bacterial species, it can carry out the same metabolic function as other strains within the same species but may differ from other members of the same species by a change in its cell wall or in its genetic sequence. Phages selectively target individual strains of bacteria. Once a Phage affects a strain it will replicate inside the strain until its numbers have reached typically 1000 Phage units. It then ruptures the cell to infect all other cells of the same strain. Phages are omnipresent in the natural environment and have no effect on humans. As a result of this, it is widely accepted that Phage are present in the cheese plant environment, and they are a normal part of the dairy plant ecosystem [15]. Phage may interfere with the production of lactic acid and will impact the quality the culture and subsequently the cheese.

Dairy producers have adopted multiple mitigation strategies to control phage in the dairy production environment. To minimize the risk of such issues occurring, it is common practice in most cheese plants to regularly rotate Starter Cultures, implement strict hygiene practices, and adjust plant designs [16].
More recently, some producers have implemented Phage testing surveillance and screening strategies to monitor the Phage numbers and types present in the production environment. Information generated from these surveillance and screening strategies aids the management of, and selection of appropriate Starter Cultures and when to rotate them Starter Culture rotation.

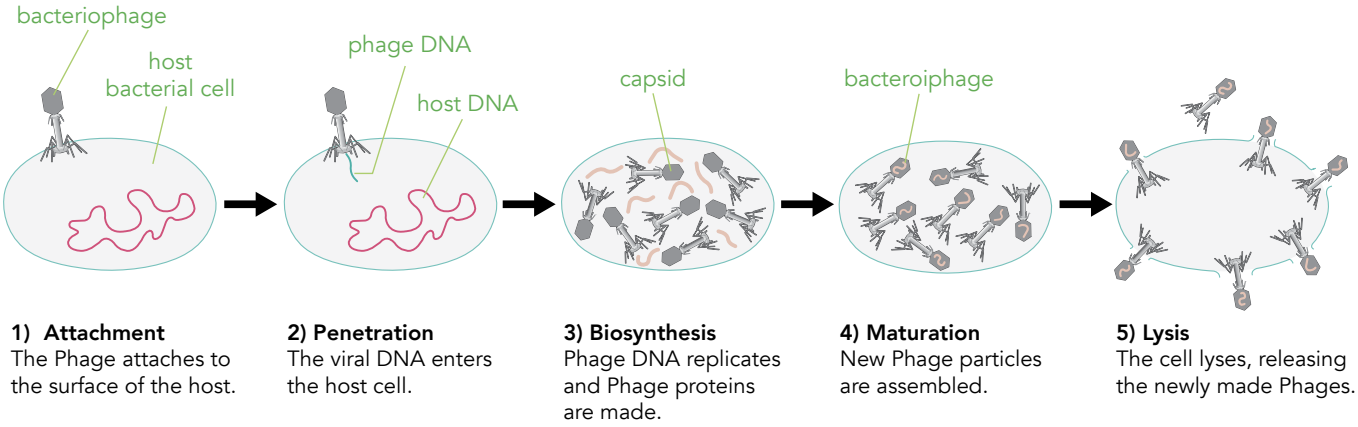

Fig. 8 A virulent phage shows only the lytic cycle pictured here. In the lytic cycle, the phage replicates and lyses the host cell.

Conclusion

Cultures are an integral element of the cheese making process, and over time the importance of developing and selecting specific Cultures has enabled cheesemakers to produce cheeses with unique flavours and functionalities.

These characteristics are controlled primarily by the selection of specific blends of Starter and Adjunct Cultures.

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