STUDIES ON CULTIVATION OF STARTER CULTURE FOR CURD FORMATION

Dissertation submitted in partial fulfillment for the degree of

Master of Science in Applied Microbiology

Submitted By

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CERTIFICATE

It is certified that the Dissertation Report entitled ‘‘STUDIES ON CULTIVATION OF STARTER CULTURE FOR CURD PREPARATION’’ which is being submitted by Ms. **SHEETAL MISHRA** in partial fulfilment of the requirements for the award of the degree of MASTER OF SCIENCE IN MICROBIOLOGY of KIIT university is a record of candidate’s own work carried out by her under my supervision and guidance during the period of December 2017-May 2018.

Signature of Supervisor

Date:

Place:

DECLARATION

I hereby declare that the dissertation entitled "Studies on cultivation of starter culture for curd formation" submitted by me, for the degree of Master of Science to KIIT University is a record of *bona fide* work carried by me under the supervision and guidance of **Dr. Praveena Bhatt Mudliar, Senior Scientist, Microbiology and Fermentation Department, CSIR-Central Food Technological Research Institute, Karnataka, India.** I further declare that, the results of the work have not formed the basis for the award of any other degree to any candidate of any university.

Date:

Place: ***(Sheetal Mishra)***

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With all respect, I praise and think Almighty God for his grace and blessing, which enabled me to complete this endeavor in a successful manner.

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Date:

Place: **(Sheetal Mishra)**

ABSTRACT

Present work is focused on selection of starter cultures for curd preparation. Microbial cultures were isolated from dairy sources namely curd and cheese. Cultures that were Gram positive, rod shaped, catalase and oxidase negative were selected and further screened for their ability to ferment milk. Isolates that displayed lesser syneresis time, less titratable acidity in terms of lactic acid, and less reduction in pH were used for further studies. The effect of three different temperatures on the setting time and parameters like pH, titratable acidity, and microbial load during 7 day storage was studied. The effect of inoculum size on all the parameters was also investigated. Finally, the fermented product was evaluated for its rheological properties like yield stress and cross over using the rheometer. Two potential isolates namely CFRS1 and CFRS2 will be further evaluated for their functional characteristics and potential to act as starter cultures for fermentation of milk to curd.

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ABBREVIATION

℃ : Degree Celsius

h: hour

µl : Micro litre

ml: milli litre

MRS: de Man, Rogosa & Sharpe

Mins: minutes

NaOH : Sodium Hydroxide

Cfu/ml : colony forming unit/ml

LAB : Lactic acid bacteria

L.A% : Lactic acid percentage

1. **INTRODUCTION**

The lactic acid bacteria frequently termed as “the Lactics”, constitute a diverse group of microorganisms usually associated with plants. The lactics are also important commercially in the processing of meats, alcoholic beverages, vegetables etc. The lactic acid bacteria are characterized as Gram positive, aerobic to facultative anaerobic, cocci or rods which are oxidase and catalase negative and produce lactic acid as the end product of fermentation.

In late nineteenth century, microbiologists identified microflora in the gastrointestinal tract of healthy animals that differed from those found in diseased animals. As further research continued into the isolation and characterization of these microorganisms, it was revealed that ingestion of these bacteria could confer a wide range of therapeutic benefits to humans. These beneficial microflora were termed as “Probiotics”. Since then the popularity of probiotics has been increasing rapidly worldwide.

Lactobacillus is a genus of bacteria which can convert sugars into lactic acid by means of fermentation. Milk contains a sugar called lactose, a disaccharide made by the glycosidic bonding between glucose and galactose (monosaccharides). When pasteurized milk is heated to a temperature of 30-40 °C, or even at room temperature or refrigerator temperature, and a small amount of old curd or whey is added to it, the lactobacillus in that curd or whey sample starts to grow. These convert the lactose into lactic acid, which imparts the sour taste to curd. Raw milk naturally contains several species of lactobacillus.

The product “Curd” is a dairy product obtained by coagulating milk in a process called curdling. The coagulation can be caused by adding enzymes like rennet or any edible acidic substance such as lemon juice or vinegar, and then allowing it to coagulate. Also, adding lactic acid bacteria into milk and subsequent acid production also results in gel formation of milk. The increased acidity causes the milk proteins (casein) to tangle into solid masses, or *curds*. Milk that has been left to sour (raw milk alone or pasteurized milk with added lactic acid bacteria) will also naturally produce curds, and sour milk cheeses are produced this way. Producing cheese curds is one of the first steps in cheese making; the curds are pressed and drained to varying amounts for different styles of cheese and different secondary agents (molds for blue cheeses, etc.) are introduced before the desired aging

finishes the cheese. The remaining liquid, which contains only whey proteins, is the whey. In cow's milk, 80 percent of the proteins are caseins.

Bacterial cultures, known as ‘starters’, are used in the manufacture of yoghurt, kefir and other cultured milk products as well as in buttermaking and cheesemaking. The starter is added to the product and allowed to grow there under controlled conditions. In the course of the resulting fermentation, the bacteria produce substances that give the fermented product its characteristic properties such as acidity (pH), flavour, aroma and consistency. The drop in pH, which takes place when the bacteria ferment lactose to lactic acid, has a preservative effect on the product, while at the same time the nutritional value and digestibility are improved.

1.1 BACKGROUND AND CONTEXT

Present work is focused towards selection of starter cultures for curd preparation.

Curd is a traditional fermented milk product. Curd differs from yoghurt in that the latter contains defined cultures that ferment milk to form the product. In the former however, there are no defined cultures and so the product is highly dependent on the way it is prepared, the old inoculum content and also other environment factors that are prevalent during the making process. Moreover, curd has a characteristic aroma and flavor different from yogurt. It usually takes 16-17 h for the preparation of traditional curd at home. The attempt in the present study was therefore to select microbial starter cultures that could ferment milk to curd in a short period of time while having all the characteristic aroma and parameters desirable of a good fermented product.

1.2 OBJECTIVES

- To isolate the lactic acid bacterial cultures from different dairy sources
- Preliminary characterization of the cultures
- Curd preparation from skim milk broth by using selected culture
- Characterization of prepared product for desired attributes
- Rheological analysis of fermented samples

1.3 OVERVIEW OF DISSERTATION

Dairy starter cultures are carefully selected microorganisms, which are deliberately added to milk to initiate and carry out desired fermentation under controlled conditions in the production of fermented milk products. These microorganisms are intentionally added to milk in order to create a desired outcome in the final product.

The present study mainly involves isolation of lactic cultures, preparation of pure cultures from individual colonies on MRS agar plates with both pour plate and streak plate method. The colonies were identified by Gram staining. Preparation of curd was done by skim milk inoculum in normal pasteurized milk at 37^0, 42^0 and 45^0C. The microbial load of curd sample, its pH, lactic acid percentage, whey amount and rheological property of the fermented samples was determined.

REVIEW OF LITERATURE

Lactic acid bacteria

Lactic acid bacteria (LAB) are group of Gram-positive bacteria that are devoid of cytochromes and prefer anaerobic conditions, are fastidious, acid-tolerant and strictly fermentative. They are usually non-motile and non-sporulating bacteria which produce lactic acid as the end product at the end of fermentation. This bacterial group contains both rods (Lactobacilli and Carnobacteria) and cocci (Streptococci). Different species of lactic acid bacteria (such as *Streptococcus, Leuconostoc, Pediococcus, Aerococcus, Enterococcus, Vagococcus, Lactobacillus, Carnobacterium*) have adapted to grow under widely different environmental conditions. They are found in the gastrointestinal tract of various animals, dairy products, seafood products, soil and on some plant surfaces (Ring & Gatesoupe, 1998). Although, lactic acid bacteria are not dominant in the normal intestinal microbiota, several trials have been undertaken to induce an artificial dominance of LAB (Verschuere et al., 2000).

Based on their carbohydrate metabolism, LAB are divided into two distinct groups. The homo-fermentative group utilizes the Embden-Meyerhof-Parnas (glycolytic) pathway to transform a carbon source chiefly into lactic acid. Hetero-fermentative bacteria produce equimolar amounts of lactate, CO2, ethanol or acetate from glucose exploiting phosphoketolase pathway. Homo-fermentative group consist of Lactococcus, Pediococcus, Enterococcus, Streptococcus. Hetero-fermentative group include Leuconostoc, Weisella (Vasiljevik & Shah, 2008). LAB are heterogeneous group of bacteria contributing to various sectors of food, beverage, health tonics and pharmaceutical industries worldwide. All the members of the group share a common property which is their gram-positive appearance and can be differentiated on their physiology and the mode of metabolic pathway they choose; either homo-fermentative or hetero-fermentative. This group on fermentation produces various anti-microbial substances that promote health modulation and many organic compounds producing flavors and aromas in the fermented food products.

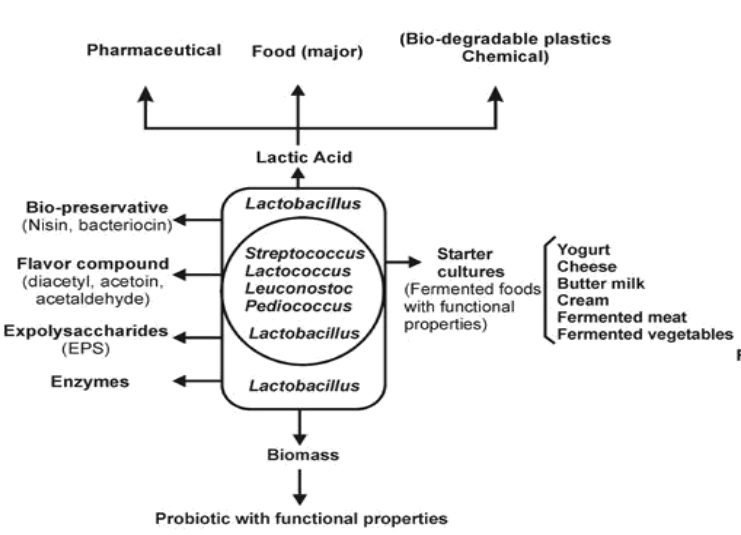
Lactic acid bacteria (LAB) are regarded as a major group of probiotics (Sharma et al., 2012; Schrezenmeir and de Vrese 2001). These lactic acid bacteria are industrially important

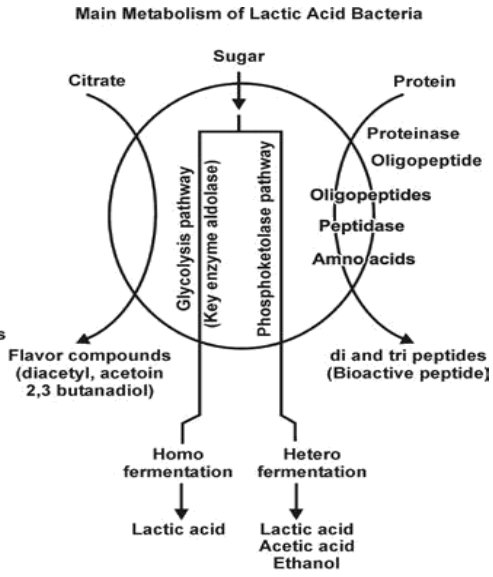
organisms recognized for their fermentative ability as their health and nutritional benefits. They are comprised of an ecologically diverse group of microorganisms united by formation of lactic acid as the primary metabolite of sugar metabolism (Carr et al. 2002). These bacteria are basically Gram-positive non-spore forming cocci, cocci-bacilli or rods, catalase-negativebacteria that are devoid of cytochromes and are of non-aerobic habit but are aero-tolerant, fastidious, acid tolerant and strictly fermentative; lactic acid is the major end-product of sugar fermentation. They are chemo-organotrophic and grow in complex media and generally are non- pathogenic to man and animals. LAB consists of several genera, which include *Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Lactosphaera, Leuconostoc, Melissococcus, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus* and *Weissella* (Ercolini et al. 2001; Holzapfel et al. 2001). Based on similarities in physiology, metabolism and nutritional needs, these genera are grouped together. A primary similarity is that all members produce lactic acid as a major or virtually sole end product of the fermentation of sugars.

LAB were first isolated from milk (Carr et al. 2002) and have since been found in such foods and fermented products as meat, milk products, vegetables, beverages and bakery products (Liu 2003; O'Sullivan et al. 2002). These bacteria occur naturally in fermented food and have been detected in soil, water, manure and sewage (Holzapfel et al. 2001). LAB exist both in human (Martin et al. 2003; Schrezenmeir and de Vrese 2001) and in animals. However, some lactic acid bacteria are part of the oral flora which can cause dental caries (Sbordone and Bortolaia 2003). LAB can work as spoilage organisms in foods such as meat, fish and beverages (Liu 2003). Several *lactobacilli, lactococci* and *bifidobacteria* are held to be health-benefiting bacteria (Rolfe 2000; Tuohy et al. 2003), but little is known about the probiotic mechanisms of gut microbiota (Gibson and Fuller 2000). LAB constitute an integral part of the healthy gastrointestinal (GI) microecology and are involved in the host metabolism and *Streptococcus thermophilus*, inhibit food spoilage and pathogenic bacteria and preserve the nutritive qualities of raw food material for an extended shelf life (O'Sullivan et al. 2002; Heller 2001). The taxonomy of LAB based on comparative 16S ribosomal RNA (rRNA) sequencing analysis has revealed that some taxa generated on the basis on phenotypic features do not correspond with the phylogenetic relations. Molecular techniques, especially polymerase chain reaction (PCR) based methods, such as rep-PCR fingerprinting and restriction fragment length polymorphism

(RFLP) as well as pulse-field gel electrophoresis (PFGE), are regarded important for specific characterization and detection of LAB strains (Gevers et al., 2001; Holzapfel et al., 2001). Recently, culture-independent approaches have been applied for the detection of intestinal microbiota (Zoetendal et al., 2002). Denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) analysis of faecal 16S rDNA gene and its rRNA amplicons have shown to be powerful approaches in determining and monitoring the bacterial community.

Features of Lactic Acid Bacteria





Lactic acid bacteria in food products

LAB has a long tradition of use in the food industry, and the number and diversity of their applications has increased considerably over the years. These are the most important group of microorganisms used in the food industry for the production of various fermented products, such as yogurts, cheese, and pickled vegetables. In addition, LAB can inhibit the growth of spoilage microbes and/or pathogens in their environment by lowering the pH and/or through the production of antimicrobial peptides, called bacteriocins. Both LAB and *Bifidobacteria* are also thought to have health-promoting abilities and many are used as probiotics for the prevention, alleviation, and treatment of intestinal disorders in humans and animals. LAB is very important in the food and dairy industries because lactic acid and other organic acids produced by these bacteria act as natural preservatives as well as flavor enhancers. LAB find increasing

acceptance as probiotic which aid in stimulating immune responses, preventing infection by enteropathogenic bacteria, and treating and preventing diarrhea. Fermented foods.

Growth of spoilage and pathogenic bacteria in these foods is inhibited due to competition for nutrients and the presence of starter-derived inhibitors such as lactic acid, hydrogen peroxide and bacteriocins. Bacteriocins are heterogeneous group of antibacterial proteins that vary in spectrum of activity, mode of action, molecular weight, genetic origin and biochemical properties (Lee, 2005). Currently, artificial chemical preservatives are employed to limit the number of microorganisms capable of growing within foods, but increasing consumer awareness of potential health risks associated with some of these substances has led researchers to examine the possibility of using bacteriocins produced by LAB as biopreservatives as well as the application of bacteriocinogenic LAB in starter cultures. According to the generally poor sanitary conditions of ben saalga and other traditional fermented foods, the use of selected bacteriocinogenic LAB with antimicrobial activity against the most frequent foodborne pathogenic bacteria could be an affordable way to improve the safety of these fermented foods. For examples, a total of 14,020 lactic acid bacteria (LAB) are isolated from *Nham* and two traditional Indonesian fermented foods "*Tapai*" (fermented tapioca), and "*Tempoyak*" (fermented durian flesh). Chilli puree and fresh goat's milk are used as sources 14 for the isolation of lactic acid bacteria (LAB), and the total amount of 126 isolates are obtained (Visessanguan *et al.*, 2006).

Curds are a dairy product obtained by coagulating milk in a process called curdling. The coagulation can be caused by adding enzymes rennet or any edible acidic substance such as lemon juice or vinegar, and then allowing it to coagulate. Also, adding lactic acid bacteria into milk and subsequent acid production will also result in gel formation of milk. The increased acidity causes the milk proteins (casein) to tangle into solid masses, or *curds*. Milk that has been left to sour (raw milk alone or pasteurized milk with added lactic acid bacteria) will also naturally produce curds, and sour milk cheeses are produced this way. Producing cheese curds is one of the first steps in cheesemaking; the curds are pressed and drained to varying amounts for different styles of cheese and different secondary agents (molds for blue cheeses, etc.) are introduced before the desired aging finishes the cheese. The remaining liquid, which contains only whey proteins, is the whey. In cow's milk, 80 percent of the proteins are caseins.

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When pasteurized milk is heated to a temperature of 30-40 °C, or even at room temperature or refrigerator temperature, and a small amount of old curd or whey added to it, the lactobacillus in that curd or whey sample starts to grow. These convert the lactose into lactic acid, which imparts the sour taste to curd. Raw milk naturally contains lactobacillus.

Curd is recognized as a healthy food throughout the world due to its beneficial effects on human health. Although it contains natural compounds of high nutritional value, such as proteins, peptides, vitamins, and minerals, there is great interest in the enrichment of this dairy product to further improve its nutritional value and health benefits because it is considered as foodstuff for daily consumption (Singh et al., 2012; Williams et al., 2015). Nowadays, probiotics are the main bioactive compounds added to curd. However, efforts are being made to enrich curd with other functional ingredients originating from dairy and nondairy sources. Among them, different types of fibers, phytosterols, polyphenols, stanols, peptides, isoflavones, β-glucans from various sources, essential fatty acids, whey protein concentrate, minerals, and vitamins have been investigated (Özer and Kirmaci, 2010). It is worth mentioning that the incorporation of any functional ingredient into a dairy product such as curd will have an effect on its final cost. Consequently, the dairy industry needs to establish cost-effective strategies and processes to achieve the development of functional products at the lowest cost possible, even if these products could have the potential to mitigate some diseases, promote health, and reduce health care costs.

Traditional fermented foods are popular products since early history that have formed an integral part of the diet and it can be prepared in the household or in cottage industry using relatively simple techniques and equipment (Aidoo et al., 2006). Fermentation was evolved as a preservation technique during lean periods and prevention technique to counter spoilage of food products. It is one of the oldest and most economical methods for producing and preserving foods. In addition to preservation, fermented foods can also have the added benefits of enhancing flavor, increased digestibility, improving nutritional and pharmacological values (Jeyaram et al., 14 2009). Lactic acid bacteria perform an essential

role in the preservation and production of wholesome fermented foods. Homo-fermentative and the hetero-fermentative lactic acid bacteria are generally fastidious on artificial media but they grow readily in most food substrates and lower the pH rapidly to a point where other competing organisms are no longer able to grow. Leuconostocs and lactic Streptococci generally lower the pH to 4.0-4.5 and some of the Lactobacilli and Pediococci to about 3.5 (Steinkraus, 1983). Lactic acid bacteria (LAB) comprise large part of probiotic microflora. There are many LAB strains that have obtained ―generally regarded as safe‖ (GRAS) status and used commonly in commercial food products for human consumption. Probiotics are mono or mixed cultures of live microorganisms that might beneficially affect the host by improving the characteristics of indigenous microflora (Holzapfel et al., 1998). Lactic acid bacterial genera consist of Lactobacillus, Lactococcus, Enterococcus, Streptococcus, Pediococcus, Leuconostoc, Wesiella etc. India is traditionally rich in fermented foods. In the Indian subcontinent, making use of fermented food using local food crops and other biological resources are very common. But the nature of the products and base material vary from region to region (Sekar & Mariappan, 2007). Fermented foods like idli and dahi were described as early as 700 BC. At present there are hundreds of fermented foods with different base materials and preparation methodology. Each fermented food is associated with unique group of microflora which increases the level of proteins, vitamins, essential amino acids and fatty acids. However, fermented foods are still produced traditionally by spontaneous fermentation and only limited knowledge has been obtained regarding the microflora of these products (Jeyaram et al., 2009). Based upon the basic ingredients used, fermented foods have been divided into 7 major types (Sekar & Mariappan, 2007): (i) cereal based (with/without pulses) fermented foods, (ii) cereal/pulse and butter milk 15 based fermented food, (iii) cereal based fermented sweets and snacks, (iv) milk based fermented foods, (v) vegetable, bamboo shoot and unripe fruits based fermented foods, (vi) meat based fermented foods, and (vii) pulse based fermented food.

Various applications of Lactic acid bacteria modulating human health

- Live vaccine
- Urogenital infections
- Respiratory Infection

- Lactose intolerance
- Anti-viral
- Anti-inflammatory
- Immune modulation
- Antimicrobial
- Diarrhea & ulcers

Health benefits along with the proposed mechanism of action of lactic acid bacteria

Health Benefit by Lactic acid Bacteria	***Proposed Mechanism***
Immuno-modulation	□ Increase in IgA production. □ Non-specific defence against infection. □ Increased phagocytic activity of WBC. □ Proliferation of intra-epithelial lymphocyte.
Intestinal Tract Infections	□ Alteration of toxic binding sites. □ Stimulation of the systemic or secretory immune response. □ Adjuvant effect increasing antibody production. □ Adherence to intestinal mucosa, preventing pathogen. □ Competition for nutrients.
Lactose Intolerance	□ Bacterial β -galactosidase acts on lactose.
Anti-inflammatory and Anti-allergic.	□ Restoration of the homeostasis of the immune system □ Regulation of cytokine synthesis □ Prevention of antigen translocation into blood stream
Anti- Colon cancer	□ Mutagen binding □ Carcinogen deactivation □ Alteration of activity of colonic microbes □ Immune response □ Influence on secondary bile salt concentration
Urogenital infections	□ Adhesion to urinary and vaginal tract cells □ Competitive exclusion □ Inhibitor production (H2O2, biosurfactants)

Starter cultures

Starter cultures are those microorganisms that are used in the production of cultured dairy products such as yogurt and cheese. The natural microflora of the milk is either inefficient, uncontrollable, and unpredictable, or is destroyed altogether by the heat treatments given to the milk. A starter culture can provide particular characteristics in a more controlled and predictable fermentation. The primary function of lactic starters is the production of lactic acid from lactose. Other functions of starter cultures may include the following:

- flavour, aroma, and alcohol production
- proteolytic and lipolytic activities
- inhibition of undesirable organisms

There are two groups of lactic starter cultures:

1. simple or defined: single strain, or more than one in which the number is known
2. mixed or compound: more than one strain each providing its own specific characteristics

Thermophilic starter cultures are microaerophillic and fresh heated milk should be used to achieve a better growth of the culture since heat treatment reduces amount of oxygen in the product. The important metabolic activities of thermophilic cultures in development of fermented milk products are: a) Acid production, e.g. lactic acid b) Flavour compounds, e.g., acetaldehyde c) Ropiness and consistency, e.g., polysaccharides d) Proteolytic and lipolytic activities, e.g., peptides, amino acids, fatty acids e) Possesses therapeutic significance, such as Improvement of intestinal organisms, Produce antibacterial substances, and immunity improvement.

Fermented Milk Products

Fermented milks are sour milk products prepared from milk, whole, partially or fully skimmed concentrated milk or milk substituted from partially or fully skimmed dried milk, homogenized or pasteurized or sterilized and fermented by means of specific dairy starter cultures. The origin of cultured dairy product is obscure and it is difficult to be precise about the date when they

were first made. In the early part of the century, Metchnikoff claimed that owing to lactic acid and other products present in sour milks, fermented by lactic acid bacteria, the growth and toxicity of anaerobic, spore-forming bacteria in the large intestine are inhibited. Lactic acid is biologically active and capable of suppressing harmful microorganisms, especially putrefactive ones and so has a favorable effect on human vital activities. Metchnikoff's theory of longevity considerably influenced the spread of fermented milk products to many countries, particularly in Europe. He also promoted extensive studies concerning biochemical and physiological properties of fermented milks. Milk fermentation for processing of milk into fermented milk products for increasing the shelf-life and having different flavour and texture characteristics have been practiced in different parts of the world. Milk has been processed into cheese, yoghurt, acidophilus milk, kefir, dahi, kumiss and various other fermented products. In the preparation of various fermented milk products, lactic starters occupy the key position as the success or failure of such products is directly related to the types of starter used.

Curd

Dahi or curd is an Indian fermented milk product which is equally known for its palatability, refreshing taste and therapeutic importance as claimed in the ayurvedic literature. Some of its characteristics are similar to other fermented milk products such as yoghurt and acidophilus milk but it differs with regard to heat treatment of milk, starter culture, chemical composition and taste. In addition, dahi also has antibacterial properties against pathogenic and non-pathogenic organisms.

Types of Curd

Some of the fermented milks and different types of dahi consumed throughout India have been categorized as follows:

- North Zone : Dahi, Lassi
- South Zone : Dahi, Buttermilk (Mattha)
- East Zone : Payodhi or Lal dahi or Mishti dahi
- West Zone : Shrikhand, Chakka, Chhash, Dahi

Based on the acidity level (% lactic acid), dahi has been classified into categories such as sweet dahi with a maximum acidity of 0.7 per cent and sour dahi with 1.0 per cent acidity. Starter culture used in the preparation of dahi is normally dahi left over from previous day. The composition of microflora varies from one household to another and from one place to another. In general, it has been found that dahi culture is dominated by streptococci and lactobacilli. In sour dahi, however, lactobacilli predominate. For commercial manufacture by organized dairy, single starter culture (*Lactococcus lactis* subsp. *diacetylactis*) or mixed culture is used. The raw materials used are cow and/or buffalo milk, standardized milk, skim milk and reconstituted skim milk powder. The traditional method for preparation of dahi invariably involves a small scale, either in consumers' household or in the sweet makers shop in urban areas. In the household, milk is boiled, cooled to about 37°C and inoculated with 0.5 – 1 per cent of starter (previous day's dahi or butter milk) and allowed to set overnight (Figure 2.12). It is then stored under refrigeration and consumed.

Steps for preparation of dahi

Cow/buffalo milk
↓
Filtration/ clarification
↓
Standardization (fat:solid not fat ratio)
↓
Pre heating (60^0C)
↓
Homogenization and pasteurization cooling
(80℃-90℃ for 15mins)
↓
Cooling (40℃-42℃)
↓
Inoculation of starter culture
(single or mixed culture)
↓
Packaging
↓
Incubation (37℃±1℃)
↓
Cold storage (5℃)

A good quality dahi made from whole milk has a cream layer on the top, the rest being made up of a homogenous body of curd and the surface being smooth and glossy, while the cut surface should be firm and free from cracks of gas bubbles and it should have a pleasant acid taste with sweetish aroma. Composition and quality of dahi vary widely from one locality to another as it is being prepared under different domestic conditions as well as milk, with variable chemical and bacteriological quality used for the preparation. However, the chemical composition of dahi has been reported as fat ranging from 5 to 8 per cent, protein 3.3 to 3.4 per cent, ash 0.75 to 0.79 per cent and lactic acid 0.5 to 1.1 per cent. Quality of dahi can be improved with regard to increase in riboflavin and folic acid by incorporating propionic acid bacteria such as *Propionibacterium shermani* along with dahi starter culture. Regarding palatability and therapeutic importance of dahi, it has been known to create relish for food, promote the appetite, increases strength and leads to longevity.

Curd is an important part of Indian diet. In most Indian homes curd is prepared almost every day. Homemade curd is not only very simple to prepare but is also delicious. Moreover, it has no preservatives and is also economical.

Curds are obtained by coagulating milk in a process called curdling. The coagulation can be caused by adding enzymes rennet or any edible acidic substance such as lemon juice or vinegar, and then allowing it to coagulate. Also, adding lactic acid bacteria into milk and subsequent acid production will also result in gel formation of milk. The increased acidity causes the milk proteins (casein) to tangle into solid masses, or *curds*. Milk that has been left to sour (raw milk alone or pasteurized milk with added lactic acid bacteria) will also naturally produce curds, and sour milk cheeses are produced this way. Producing cheese curds is one of the first steps in cheesemaking; the curds are pressed and drained to varying amounts for different styles of cheese and different secondary agents (molds for blue cheeses, etc.) are introduced before the desired aging finishes the cheese. The remaining liquid, which contains only whey proteins, is the whey. In cow's milk, 80 percent of the proteins are caseins.

MATERIALS AND METHOD

Isolation of starter culture from curd sample

Serial dilution & pour plating plate method

- 1ml/gm of sample were serially diluted in 9ml sterile saline (0.85%).
- The samples with different dilutions were pour plate in MRS agar media by pour plate technique.
- The plates then incubated at 37℃ for 24h.
- Morphologically different colonies were selected.

Pure culture preparation - Streak plate method

- The MRS agar media was first poured on the sterile petri plates.
- Allowed for solidification.
- Open the culture and pour the cultured broth on plate.
- Streak the plates with sterile inoculating loop.
- The plates were inverted and incubated at 37°C for 24-48 hours.

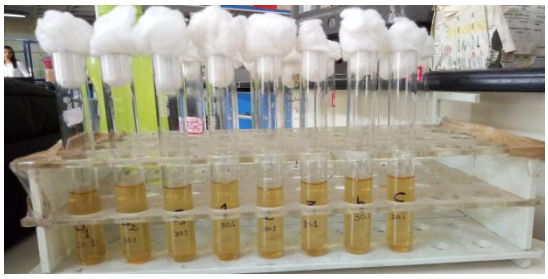
Morphology and gram staining

The pure colony of isolates were morphologically characterized for colony characteristics similar to LAB.

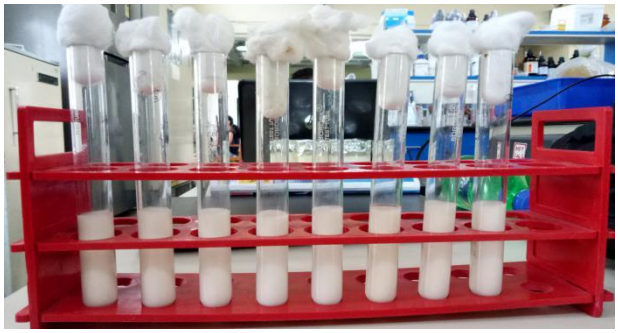
Gram staining technique

- Take drop of water on clean glass slide and take a small portion of colony using inoculating loop.
- Make a smear of colony and heat fix it.
- Flood the smear with primary stain crystal violet for 1 min.
- Pour the excess dye and wash gently with tap water.
- Flood the smear with Gram's iodine for 1 min.
- Rinse gently with tap water.
- Flood the s (12%) was prepared.

- Inoculated with cultured MRS broth in skim milk.



- MRS broth skim milk broth



- curd samples

- Allowed for curdling
- Inoculation in Milk
- Add 1gm of skim milk curd in 50ml pasteurized milk.
- Incubated at 37℃ and in every 2h check the curdling process.
- The same process was repeated in 42℃ & 45℃.

pH

- Thoroughly rinse the pH electrode with distilled water and wipe it well.
- Dip the pH electrode into the curd sample.
- Note down the value.

Lactic Acid Percentage Determination

Preparation of 0.1N NaOH , 1ltr

1N = 40gm in 1000ml

0.1N=4gm in 1000ml

Titration

- Fill the burette with 0.1N NaOH solution.
- Dilute the curd sample with distilled water in the ratio of 1:4.
- Add 3-4 drops of phenolphthalein indicator and stir well.
- Take the initial reading of the burette and final reading of the burette when the sample turn lightly pink.
- Repeat the process for each sample.

Whey amount in curd sample

- Take 10ml curd in 50ml falcon tube.
- Centrifuge at 2000rpm for 15mins.
- Then collect the supernatant and measure it in 10ml measuring cylinder.

<u>Rheological test of curd</u>

- First 5gm of curd sample was placed on the sample plate.
- The upper plate was allowed to press the curd sample completely for 10min-15min.
- Graph was obtained.
- The yield stress and cross over were detected.

RESULTS & DISCUSSION

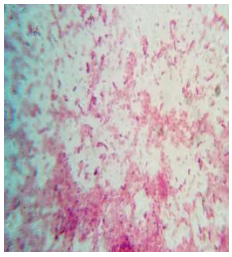
Screening and selection of microbial starter cultures for curd preparation

Experiments were carried out to isolate organisms from different sources which could potentially be used as starter cultures for preparation of curd. Some probiotic microbial cultures (7nos, CFR 1-7) which were previously isolated in the laboratory were also evaluated for their ability to form curd. A total of 9 cultures were isolated in MRS medium from different sources and characterized for their colony morphology and Gram reaction. Table 1 gives the colony morphology of the isolates obtained from dairy sources namely curd and cheese. Isolates which have been designated CFR are previous cultures which were provided by CFTRI. Only microbial isolates that were Gram positive and oxidase and catalase negative were selected for further experiments.

Curd and cheese samples collected from Mysuru region was used for isolation of starter culture. The sample was serially diluted and plated on MRS agar plate and observed for colonies after incubation period. Total of 9 morphologically different cultures were isolated by streak plate method and used for further studies

Table 1: Colony characteristic and gram staining results of the isolates

SAMPLE	MORPHOLOGY	Oxidase	Catalase
Curd 1	Gram positive short rod	-ve	-ve
Curd 2	Gram positive curve small rod	-ve	-ve
Curd 3	Gram positive rod	-ve	-ve
Curd 4	Gram positive short rod	-ve	-ve
Curd 5	Gram positive short, thick rod	-ve	-ve
Curd 6	Gram positive thick rod	-ve	-ve
CFRS1	Gram positive thick rod	-ve	-ve
CFRS2	Gram positive short rod	-ve	-ve
CFRS3	Gram positive thick rod	-ve	-ve
CFRS4	Gram positive short rod	-ve	-ve
CFRS5	Gram positive curve , small rod	-ve	-ve
CFRS6	Gram positive small rod	-ve	-ve
CFRS7	Gram positive thick rod	-ve	-ve
CS8	Gram positive small rod	-ve	-ve
CS9	Gram positive small rod	-ve	-ve
CS10	Gram positive thick rod	-ve	-ve



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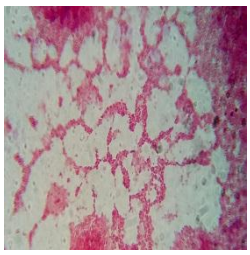

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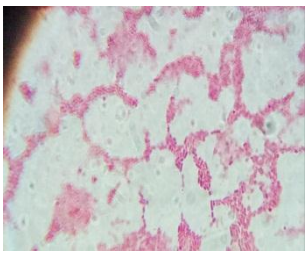

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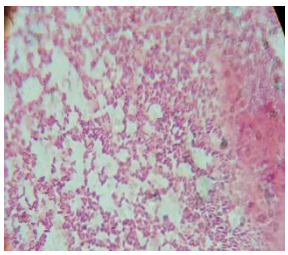

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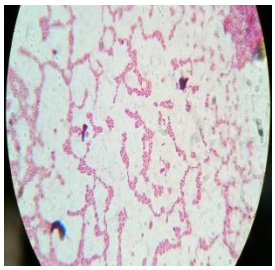
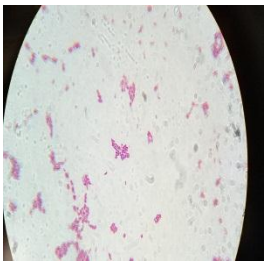
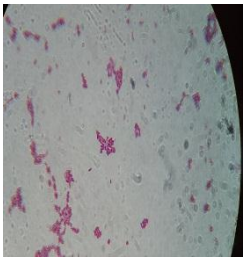


C5

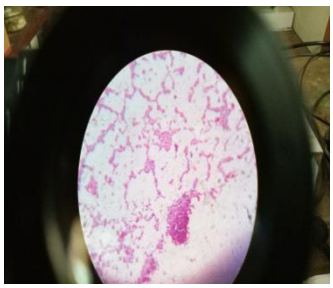
C6



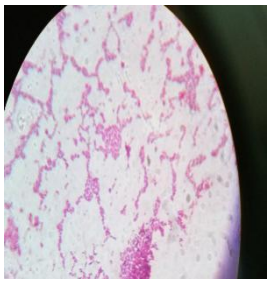
CFRS1



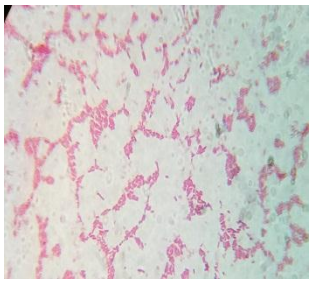
CFRS2



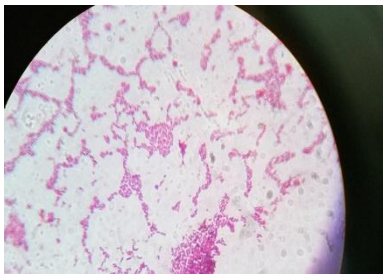
CFRS3



CFRS4

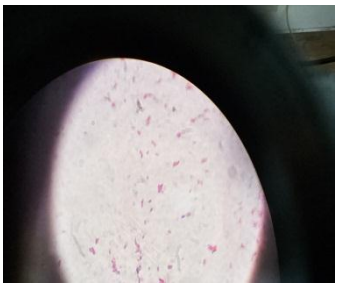


CFRS5

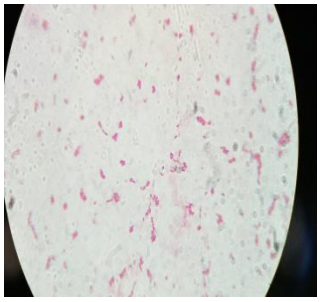


CFRS6

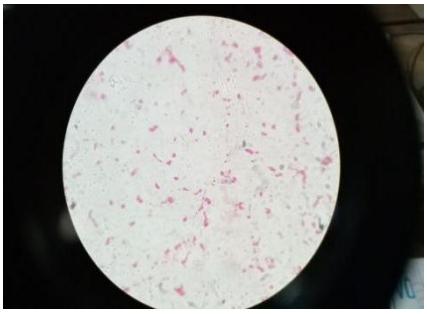
FRS7



CS8



CS9



CS10

Selection of starter culture for curd preparation

The selected organisms were evaluated for their ability to ferment milk to form curd of desirable attributes. The experiments were carried out initially using skim milk and later using commercial samples of milk (pasteurized). The primary criteria for the selection of the suitable culture was measured as the "Syneresis" i.e formation of gel with separation of liquid. Therefore curd setting time and amount of whey separation were considered for selection of cultures. The titratable acidity in terms of lactic acid and change in pH was also evaluated. Effect of parameters such as inoculum size and temperature were studied during the screening process.

Studies on Syneresis during fermentation of milk using isolated cultures

Table 2 gives the results of syneresis observed for individual cultures which were inoculated to pasteurized milk. The amount of whey separation along with decrease in pH at the end of set time and total titratable acidity, texture and aroma was analysed. The skim milk (12%) was prepared and inoculated with culture which was overnight grown in MRS broth at 37℃. In skim milk, it was observed that the fermentation time was 5h for all the cultures to form a gel. As can be seen, the pH of the set curd ranges from 4.54 - 5.34. The fermented milk inoculated with isolate CSc had the lowest pH of 4.54 and was more acidic than other samples while isolate CFRS3 was the least acidic. The difference in pH may be due to lactic acid formation by the cultures.

Table 2 Synresis time and other parameters of milk inoculated with isolated cultures

Sample Name	Set Time	pH	Whey	Texture	L.A%
C1	5h	5.25	0	Smooth	0.22%
C2		5.13	0.1ml		0.19%
C3		5.03	0		0.23%
CFRS1		5.18	0		0.18%
CFRS2		5.10	0.2ml		0.21%
CFRS3		5.34	0.01ml		0.18%
CSb		5.06	0		0.19%
CSc		4.54	0		0.21%

The mother inoculum from the curd formed using skim milk was then inoculated into pasteurized milk. The results are presented in Table 3. It was observed that, the whey

amount was negligible for some of the isolates (C1, 2, 3 & 5, and CFR S1, S2, S3, S6). The other 8 isolates showed longer curdling time of 15h and greater whey separation (0.9ml -1.5ml) with unpleasant aroma. Based on these results only isolates C1, 2, 3 & 5, and CFR S1, S2, S3, S6 were used for further studies.

Table 3: Curdling ability of the isolates

Sample name	Syneresis time	Amount of liquid separation	Sensory
C 1	12 h	0.1ml	pleasant
C 2	13 h	0	pleasant
C 3	12 h	0.2ml	pleasant
C 4	15 h	1.5ml	unpleasant
C 5	12h	0.01ml	pleasant
C 6	15h	1ml	unpleasant
CFRS1	12h	0.3ml	pleasant
CFRS2	12h	0.01ml	pleasant
CFRS3	13h	0	pleasant

4.2 *Studies on effect of temperature on fermentation of milk using isolated cultures*

Experiments were conducted to optimize the temperature required for setting of curd using individual cultures. Table 4 presents the results of the experiment. Results show that the pH & whey amount were highest at 45℃in almost all the samples. The LA% however, was highest in 37℃ incubated sample the curd formed at different temperatures and using different cultures were stored at 4°C and studied for changes in the parameters including the microbial count in the fermented product. Table 4,Table5 ,Table 6 presents a comparative analysis of 3rd day, 5th day & 7th day storage of the curd samples at 37°C, 42°C and 45°C. During fermentation, the formation of lactic acid made the pH decrease. Incubation temperature & number of storing days of sample affected the pH, whey amount & lactic acid content (%). The 3rd day, pH value of curd sample incubated at 37℃, 42℃ & 45℃ were different and ranged from 4.3 - 5.1, 4.2 - 5.4 & 4.2 - 5.7 respectively. LA (%) of curd sample incubated at 37℃, 42℃ & 45℃ was in the range of 0.08-0.14, 0.07-0.13 & 0.05-0.13 respectively. The

pH, whey amount and lactic acid content increased with increase in storage time. Results indicate that milk fermented with CFR S1, S2 and C3 and stored at 4°C, showed less whey separation, reduction in pH and lactic acid content at all the incubation temperatures tested.

Table 4 – showing the 0th day analysis of curd

Sample name	pH of 37℃	pH of 42 ℃	pH of 45℃	L.A% of 37℃	L.A % of 42℃	L.A% of 45℃	Whey amount in 37℃	Whey amount in 42℃	Whey amount in 45℃
C1	5.2	4.9	5.7	0.11	0.10	0.13	0.8ml	0.1ml	1ml
C2	5.3	4.8	5.6	0.13	0.10	0.10	0.06ml	0.09ml	1.3ml
C3	4.9	4.5	5	0.12	0.09	0.13	0.01ml	0.01ml	0.1ml
CFRS1	4.9	5.1	4.5	0.15	0.10	0.07	0.01ml	0.02ml	0.09ml
CFRS2	4.6	5.4	5.8	0.16	0.14	0.14	0.09ml	0.6ml	0.5ml
CFRS3	5	5.1	4.4	0.10	0.08	0.06	0.09ml	0.4ml	0.6ml
CSb	5.3	4.4	5.4	0.09	0.10	0.07	0.7ml	0.5ml	0.3ml
CSc	4.5	4.2	5.3	0.15	0.13	0.10	0.09ml	0.6ml	0.2ml

Table 5 – showing the 3rd day analysis of curd

Sample name	**pH of 37℃**	**pH of 42 ℃**	**pH of 45℃**	**L.A% of 37℃**	**L.A % of 42℃**	**L.A% of 45℃**	**Whey amount in 37℃**	**Whey amount in 42℃**	**Whey amount in 45℃**
C1	5.1	4.9	5.6	0.09	0.10	0.12	0.9ml	0.1ml	1ml
C2	5.1	4.8	5.5	0.11	0.09	0.09	0.08ml	1ml	1.5ml
C3	4.6	4.5	4.9	0.11	0.08	0.11	0.01ml	0.01ml	0.1ml
CFRS1	4.8	5.1	4.3	0.14	0.09	0.06	0.02ml	0.02ml	1ml
CFRS2	4.4	5.4	5.7	0.14	0.13	0.13	0.1ml	0.7ml	0.5ml
CFRS3	4.8	5.1	4.2	0.09	0.07	0.05	0.1ml	0.5ml	0.7ml
CSb	5.1	4.4	5.3	0.08	0.09	0.05	0.8ml	0.5ml	0.4ml
CSc	4.3	4.2	5.1	0.14	0.12	0.09	0.1ml	0.7ml	0.2ml

Table 6- showing results of 5th day analysis

Sample name	pH of 37℃	pH of 42 ℃	pH of 45℃	L.A% of 37℃	L.A % of 42℃	L.A% of 45℃	Whey amount in 37℃	Whey amount in 42℃	Whey amount in 45℃
C1	5	4.8	5.4	0.11	0.12	0.14	0.9 ml	0.2 ml	1 ml
C2	4.9	4.6	5.4	0.13	0.10	0.10	0.09 ml	1 ml	1.5 ml
C3	4.5	4.4	4.7	0.14	0.09	0.11	0.02 ml	0.02 ml	0.1 ml
CFRS1	4.7	5	4.3	0.16	0.10	0.07	0.04 ml	0.03 ml	1.1 ml
CFRS2	4.3	5.3	5.6	0.15	0.15	0.16	0.1 ml	0.8 ml	0.6 ml
CFRS3	4.7	5	4.2	0.11	0.09	0.07	0.1 ml	0.7 ml	0.8 ml
CSb	5	4.3	5.3	0.09	0.10	0.06	0.9 ml	0.8 ml	0.4 ml
CSc	4.2	4.1	5.2	0.16	0.13	0.11	0.2 ml	0.8 ml	0.2 ml

Table 7- showing the 7th day analysis

Isolate	**pH of 37℃**	**pH of 42 ℃**	**pH of 45℃**	**L.A% of 37℃**	**L.A % of 42℃**	**L.A% of 45℃**	**Whey amount in 37℃**	**Whey amount in 42℃**	**Whey amount in 45℃**
C1	4.9	4.6	5.2	0.13	0.12	0.17	0.1ml	0.4ml	1.2ml
C2	4.7	4.4	5.3	0.15	0.10	0.12	0.09ml	1.2ml	1.6ml
C3	4.4	4.3	4.5	0.16	0.09	0.14	0.03ml	0.04ml	0.2ml
CFRS1	4.6	4.9	4.1	0.18	0.10	0.10	0.06ml	0.05ml	1.3ml
CFRS2	4.2	5	5.4	0.16	0.15	0.18	0.1ml	0.9ml	0.7ml
CFRS3	4.6	4.9	4.1	0.13	0.09	0.10	0.1ml	0.9ml	0.8ml
CSb	4.8	4.1	5.1	0.11	0.10	0.09	0.1ml	0.1ml	0.5ml
CSc	4	4	5	0.17	0.13	0.13	0.3ml	0.1ml	0.2ml

The effect of storage on the microbial load of the fermented product was studied for the samples prepared at different temperatures. Table 7 gives the results of the study. Isolate CSb inoculated samples at 37°C was found to have more microbial count as compared to all the other products after 7 days of storage. At 42°C, curd inoculated with isolates C3, C2, CFRS3 and CSb had the highest counts while at 45°C, isolates C1, C2 and C3 had the highest microbial counts.

Table 8 showing Microbial load of the fermented milk incubated at different temperatures and stored at 4C (after 7 days of storage)

Sample name	Incubated at 37℃ (cfu/ml)	Incubated at 42℃ (cfu/ml)	Incubated at 45℃ (cfu/ml)
C1	$109x10^5$	$88x10^5$	$1x10^6$
C2	$14x10^5$	$12x10^6$	$7x10^6$
C3	$88x10^5$	$13x10^6$	$28x10^6$
CFRS1	$78x10^5$	$89x10^5$	$46x10^5$
CFRS2	$61x10^5$	$65x10^5$	$48x10^5$
CFRS3	$65x10^5$	$12x10^6$	$88x10^5$
CSb	$1x10^7$	$117x10^5$	$93x10^5$
CSc	$84x10^5$	$13x10^6$	$114x10^5$

4.3 *Studies on effect of inoculum size on fermentation of milk using isolated cultures*

Table 9- Analysis of set time, pH, aroma & L.A%, texture in 1% & 2% inoculum.

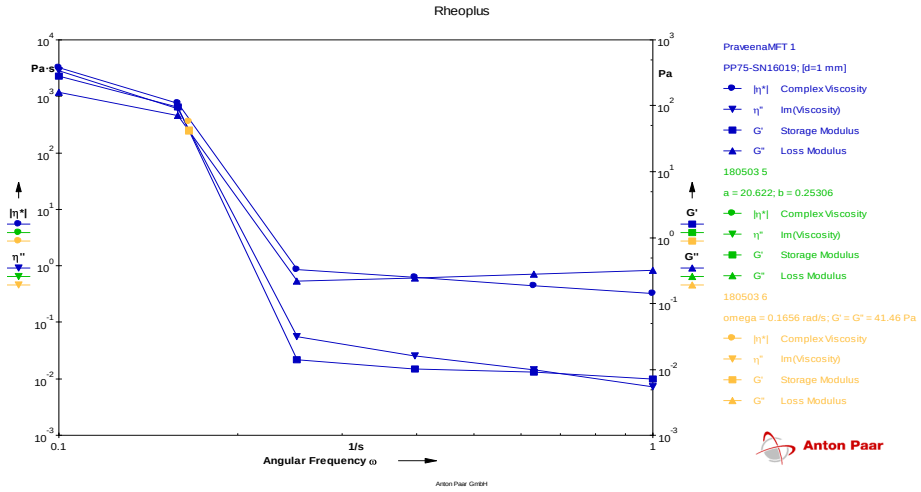
Isolate	Inoculum % (w/v)	Set Time	pH	Aroma	Lactic acid percentage	Texture
C1	1%	14h	4.8	Pleasant	0.17	Smooth
	2%	12h	4.8		0.22	
C2	1%	14h	4.5		0.14	
	2%	11h	4.3		0.18	
C3	1%	13h	4.3		0.09	
	2%	12h	4.2		0.15	
CFRS1	1%	15h	4.9		0.13	
	2%	13h	5		0.20	
CFRS2	1%	14h	4		0.17	
	2%	12h	4.2		0.25	
CFRS3	1%	14h	4.7		0.20	
	2%	11h	4.4		0.27	
CSb	1%	15h	4.5		0.16	
	2%	12h	4.8		0.20	
CSc	1%	13h	4.7		0.14	
	2%	12h	4.7		0.20	

Studies were carried out to investigate the effect of varying inoculum size on the setting parameters of curd. The curd previously formed after fermentation of skim milk was used as the seed inoculum and measurement was made in grams on a wet basis (1% & 2% inoculum). The milk inoculated with 1% curd took longer time than milk inoculated with 2%. Table 7 shows the effect of inoculum size on the production of lactic acid, curd set time, pH value and curd formation, aroma and texture during milk fermentation. It was observed that, the higher the inoculum size, the higher was the lactic acid content. The LA% of 1% inoculum was in the range of 0.09%-0.20% & at 2% inoculum level, LA % was in the range of 0.15%- 0.27%. 1% inoculated curd sample took 13 h-15h & the 2% inoculated milk samples took around 10h-11h. The pH of 1% inoculum was in the range of 4-4.8 and 2% inoculum 4.2-5. The texture of all the curd sample were smooth & had pleasant aroma.

Rheological Analysis of Samples

The milk fermented by individual cultures and Curd formed after the incubation period were analyzed for various rheological tests including shear test and cross over.

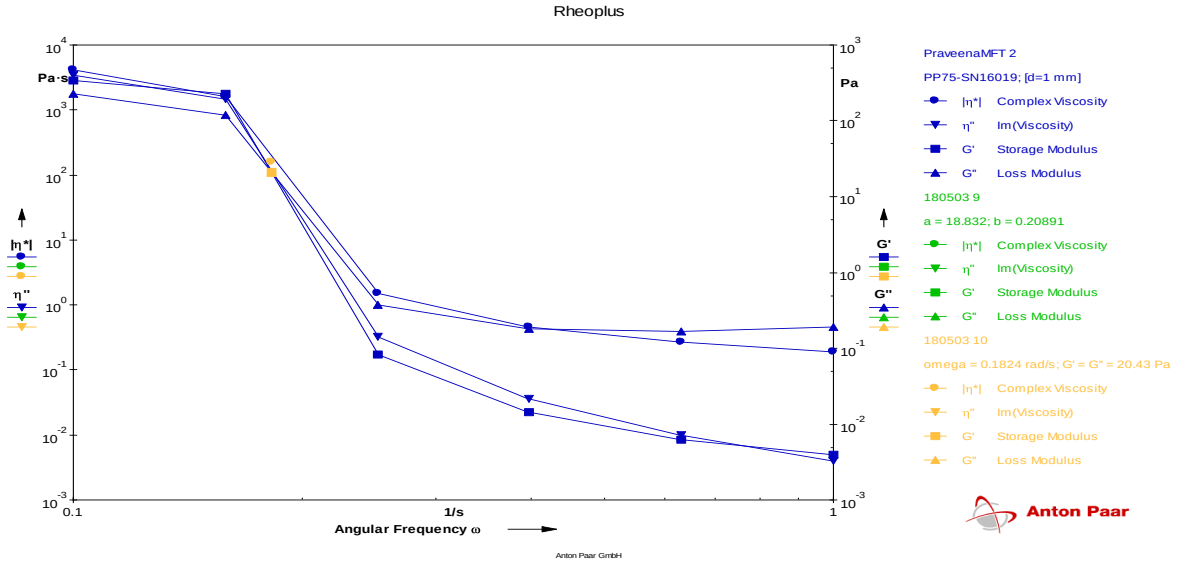
C1



Meas. Pts.	Angular Frequency	Storage Modulus	Loss Modulus	Damping Factor
	[rad/s]	[Pa]	[Pa]	[1]
1	0.1	2.85E+02	1.60E+02	0.56
2	0.158	9.62E+01	7.20E+01	0.749
3	0.251	1.38E-02	2.16E-01	15.6
4	0.398	1.01E-02	2.46E-01	24.4
5	0.631	9.06E-03	2.81E-01	31
6	1	7.13E-03	3.22E-01	45.2

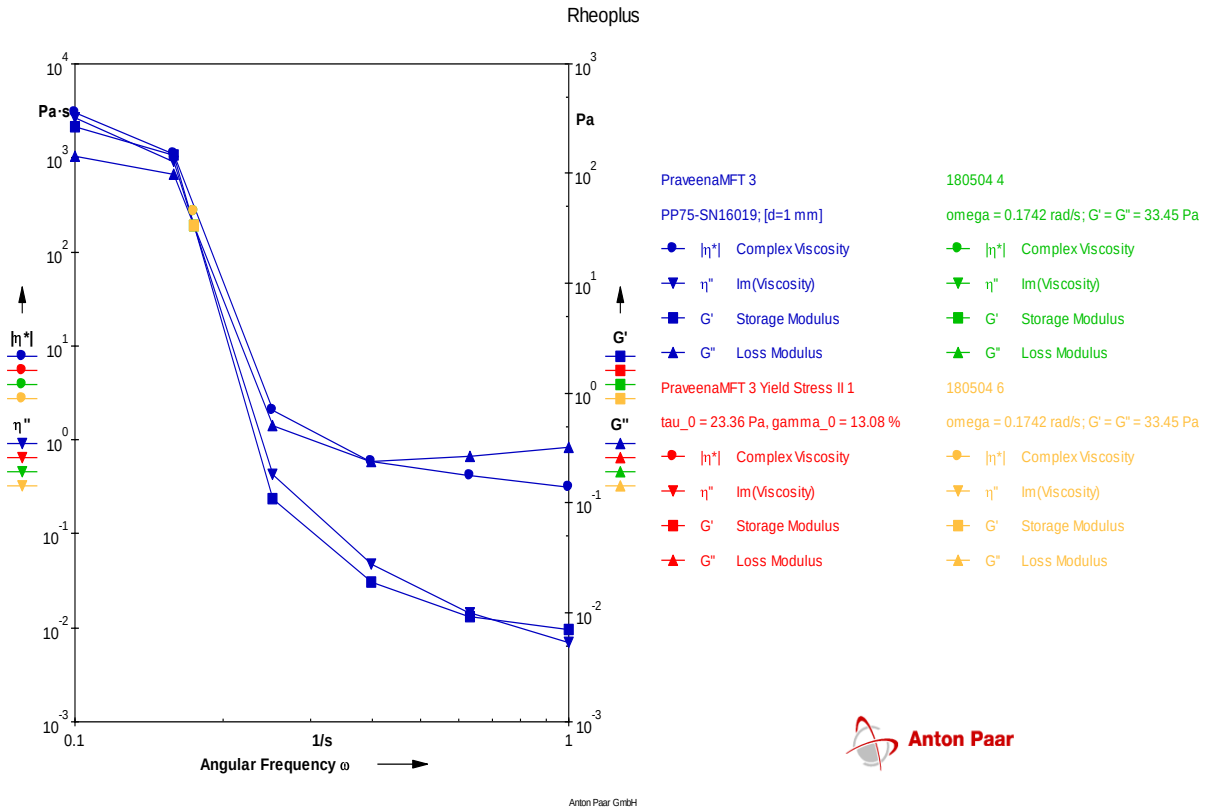
Shear Stress	Shear Rate	Complex Viscosity	Deflection Angle	Torque	Status
[Pa]	[1/s]	[Pa·s]	[mrad]	[µNm]	[]
1.18E+00	3.62E-04	3.27E+03	9.66E-02	9.79E+01	M- ,WE-
4.03E+01	5.31E-02	7.58E+02	8.94E+00	3.33E+03	
4.91E+01	5.70E+01	8.63E-01	6.05E+03	4.07E+03	WA+,WMa
5.50E+01	8.90E+01	6.19E-01	5.96E+03	4.55E+03	WA+,WMa
6.29E+01	1.41E+02	4.46E-01	5.97E+03	5.21E+03	WA+,WMa
7.24E+01	2.25E+02	3.22E-01	5.99E+03	5.99E+03	WA+,WMa

Sample C2]



Meas. Pts.	Angular Frequency	Storage Modulus	Loss Modulus	Damping Factor	Shear Stress	Shear Rate	Complex Viscosity	Deflection Angle	Torque	Status
	[rad/s]	[Pa]	[Pa]	[1]	[Pa]	[1/s]	[Pa·s]	[mrad]	[µNm]	[]
1	0.1	3.40E+02	2.28E+02	0.67	1.20E+00	2.92E-04	4.10E+03	7.79E-02	9.89E+01	M- ,WE-
2	0.158	2.30E+02	1.19E+02	0.517	4.06E+01	2.48E-02	1.64E+03	4.18E+00	3.36E+03	
3	0.251	8.25E-02	3.71E-01	4.5	5.49E+01	3.63E+01	1.51E+00	3.86E+03	4.54E+03	WMa
4	0.398	1.43E-02	1.82E-01	12.8	4.11E+01	8.95E+01	4.59E-01	5.99E+03	3.40E+03	WA+,WMa
5	0.631	6.17E-03	1.69E-01	27.3	3.78E+01	1.42E+02	2.67E-01	5.98E+03	3.13E+03	WA+,WMa
6	1	3.96E-03	1.91E-01	48.2	4.29E+01	2.25E+02	1.91E-01	6.00E+03	3.55E+03	WA+,WMa

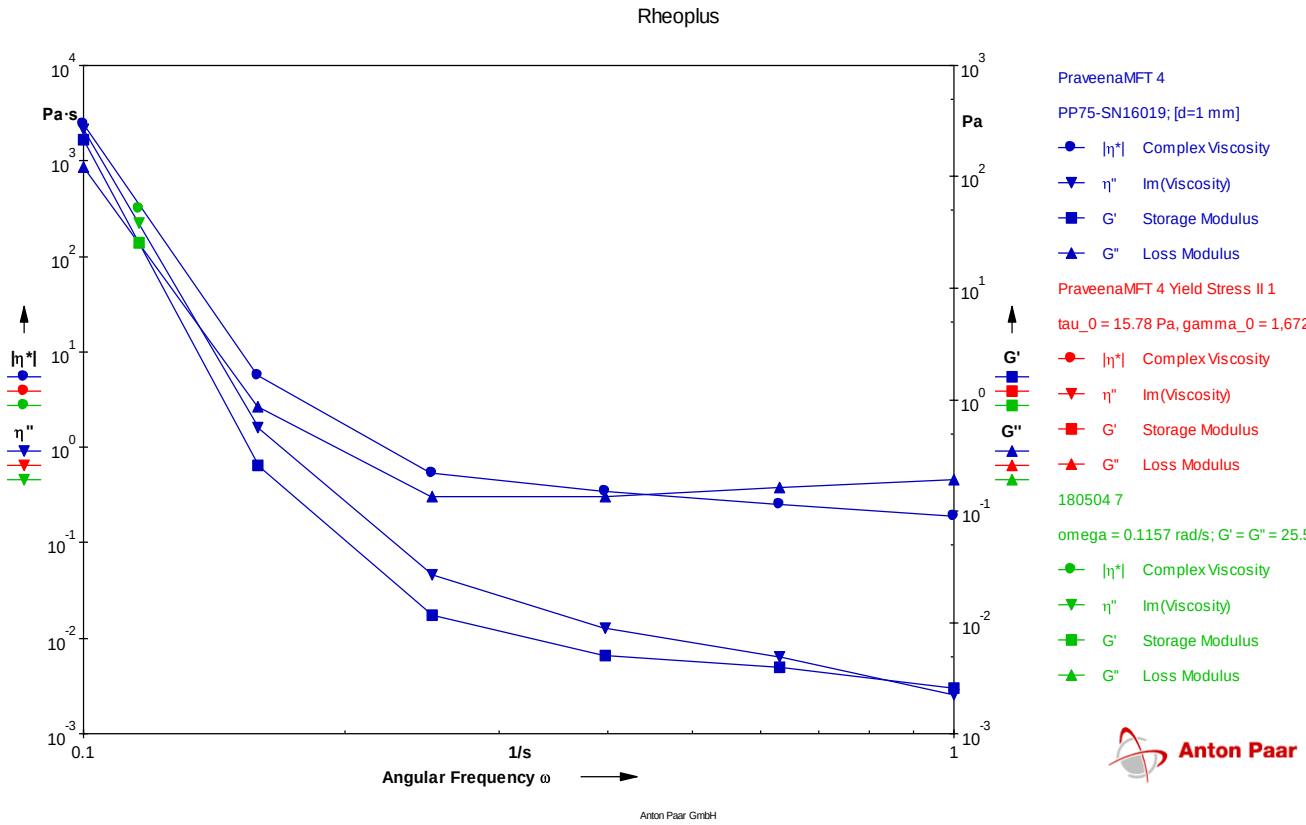
SAMPLE C3



Meas. Pts.	Angular Frequency	Storage Modulus	Loss Modulus	Damping Factor
	[rad/s]	[Pa]	[Pa]	[1]
1	0.1	2.65E+02	1.45E+02	0.545
2	0.158	1.47E+02	9.90E+01	0.675
3	0.251	1.10E-01	5.02E-01	4.58
4	0.398	1.88E-02	2.36E-01	12.5
5	0.631	9.13E-03	2.67E-01	29.2
6	1	6.99E-03	3.17E-01	45.3

Shear Stress	Shear Rate	Complex Viscosity	Deflection Angle	Torque	Status
[Pa]	[1/s]	[Pa·s]	[mrad]	[µNm]	[]
1.18E+00	3.91E-04	3.02E+03	1.04E-01	9.77E+01	M- ,WE-
4.04E+01	3.62E-02	1.12E+03	6.10E+00	3.34E+03	
5.82E+01	2.84E+01	2.04E+00	3.02E+03	4.81E+03	WMa
5.32E+01	8.96E+01	5.94E-01	6.01E+03	4.40E+03	WA+,WMa
5.98E+01	1.41E+02	4.23E-01	5.98E+03	4.95E+03	WA+,WMa
7.12E+01	2.25E+02	3.17E-01	5.99E+03	5.89E+03	WA+,WMa

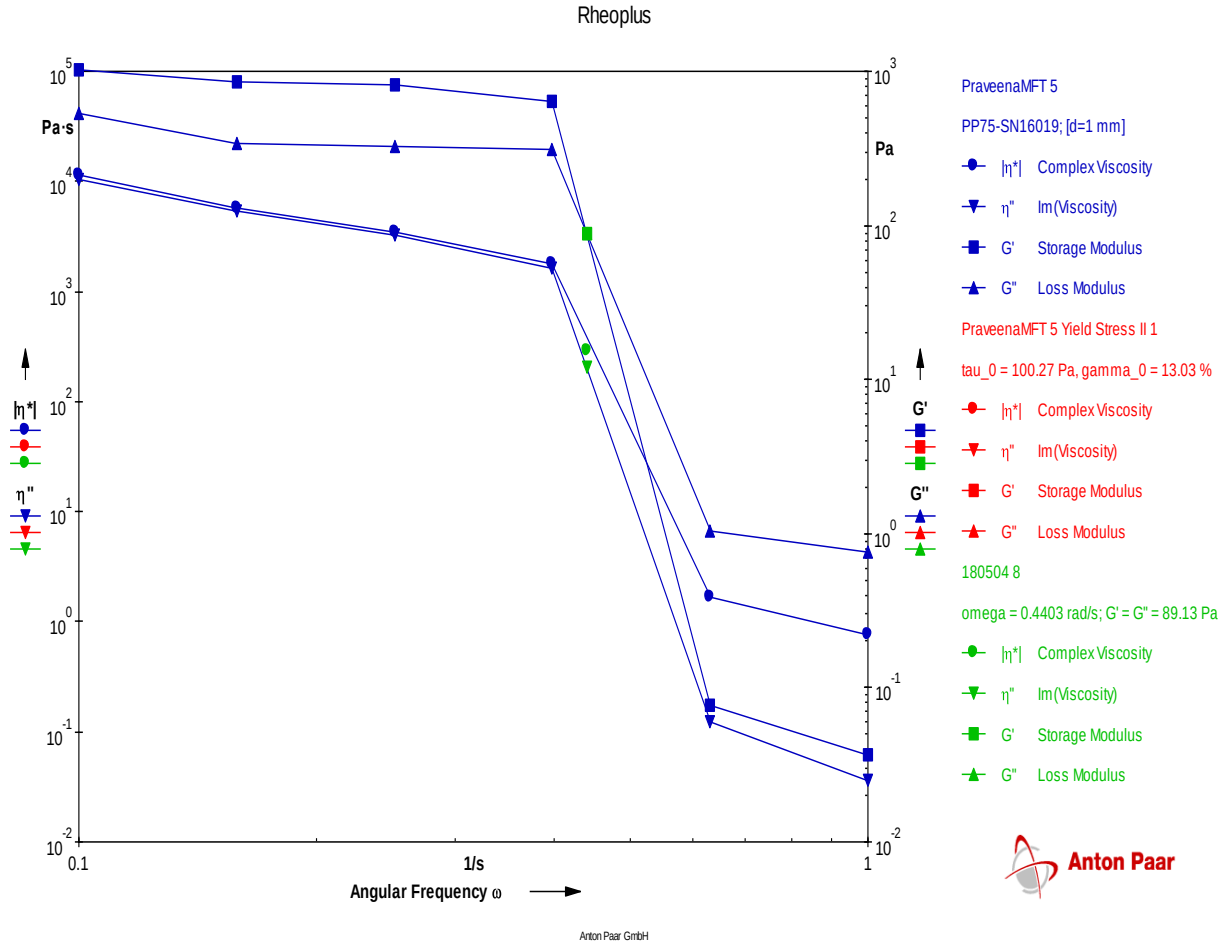
SAMPLE CFRS1



Meas. Pts.	Angular Frequency	Storage Modulus	Loss Modulus	Damping Factor	Shear Stress
	[rad/s]	[Pa]	[Pa]	[1]	[Pa]
1	0.1	2.14E+02	1.22E+02	0.568	1.18E+00
2	0.158	2.56E-01	8.72E-01	3.4	3.03E+01
3	0.251	1.15E-02	1.35E-01	11.8	3.06E+01
4	0.398	5.01E-03	1.36E-01	27.2	3.05E+01
5	0.631	3.97E-03	1.61E-01	40.6	3.61E+01
6	1	2.56E-03	1.93E-01	75.2	4.33E+01

Shear Rate	Complex Viscosity	Deflection Angle	Torque	Status
[1/s]	[Pa·s]	[mrad]	[µNm]	[]
4.78E-04	2.46E+03	1.28E-01	9.75E+01	M- ,WE-
5.28E+00	5.73E+00	8.89E+02	2.51E+03	WMa
5.67E+01	5.40E-01	6.02E+03	2.53E+03	WA+,WMa
8.93E+01	3.42E-01	5.98E+03	2.53E+03	WA+,WMa
1.42E+02	2.55E-01	5.99E+03	2.99E+03	WA+,WMa
2.25E+02	1.93E-01	6.00E+03	3.58E+03	WA+,WMa

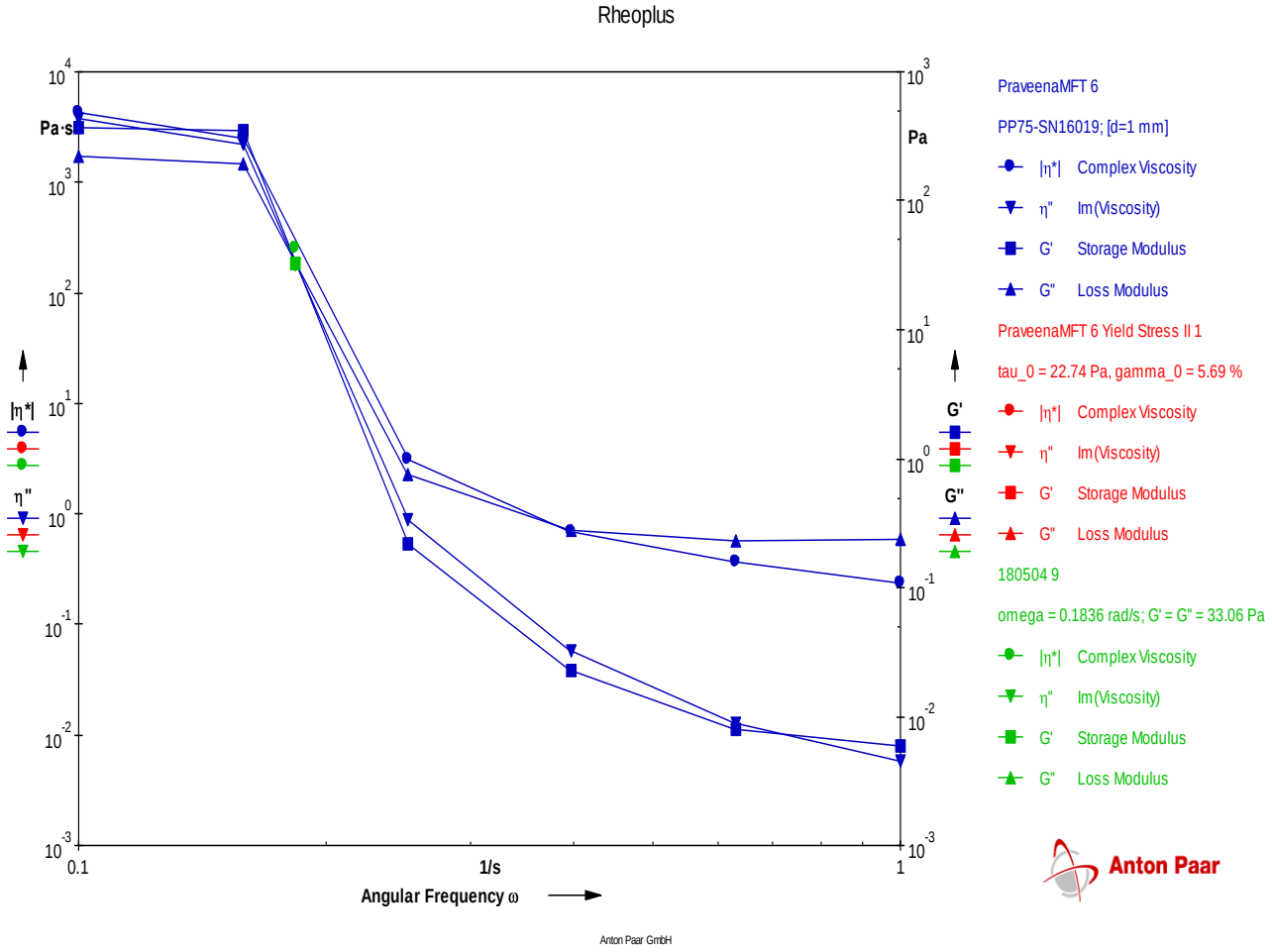
SAMPLE CFRS2



Meas. Pts.	Angular Frequency	Storage Modulus	Loss Modulus	Damping Factor	Shear Stress
	[rad/s]	[Pa]	[Pa]	[1]	[Pa]
1	0.1	1.03E+03	5.37E+02	0.522	1.20E+00
2	0.158	8.52E+02	3.40E+02	0.399	4.07E+01
3	0.251	8.14E+02	3.24E+02	0.399	8.04E+01
4	0.398	6.41E+02	3.09E+02	0.482	1.20E+02
5	0.631	7.67E-02	1.04E+00	13.6	1.36E+02
6	1	3.64E-02	7.67E-01	21.1	1.73E+02

Shear Rate	Complex Viscosity	Deflection Angle	Torque	Status
[1/s]	[Pa·s]	[mrad]	[µNm]	[]
1.03E-04	1.16E+04	2.76E-02	9.94E+01	M- ,WE-
7.03E-03	5.79E+03	1.18E+00	3.37E+03	
2.31E-02	3.49E+03	2.45E+00	6.65E+03	
6.72E-02	1.79E+03	4.50E+00	9.93E+03	
8.16E+01	1.66E+00	3.45E+03	1.12E+04	WMa
2.26E+02	7.68E-01	6.02E+03	1.43E+04	WA+,WMa

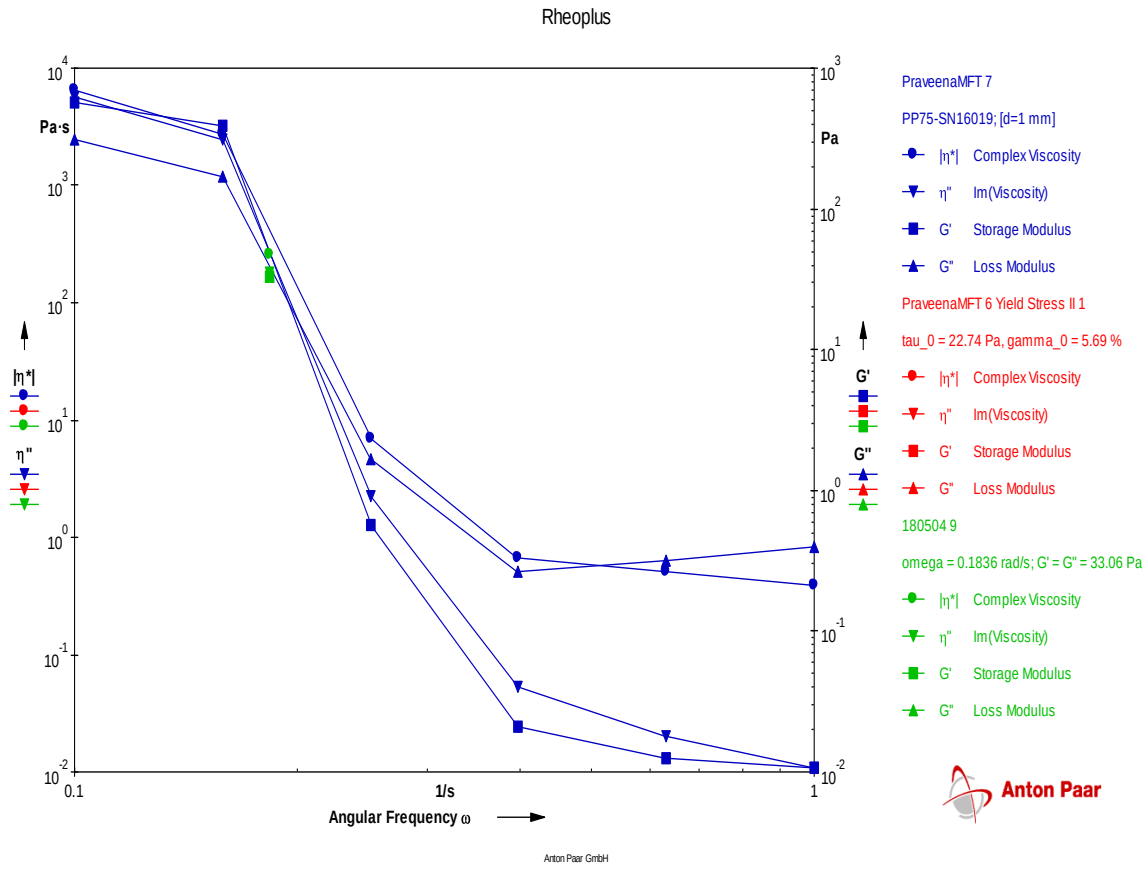
SAMPLE CFRS3



Meas. Pts.	Angular Frequency	Storage Modulus	Loss Modulus	Damping Factor	Shear Stress
	[rad/s]	[Pa]	[Pa]	[1]	[Pa]
1	0.1	3.72E+02	2.24E+02	0.601	1.19E+00
2	0.158	3.48E+02	1.95E+02	0.561	4.07E+01
3	0.251	2.21E-01	7.55E-01	3.42	5.85E+01
4	0.398	2.29E-02	2.77E-01	12.1	6.33E+01
5	0.631	8.02E-03	2.31E-01	28.8	5.18E+01
6	1	5.89E-03	2.40E-01	40.7	5.39E+01

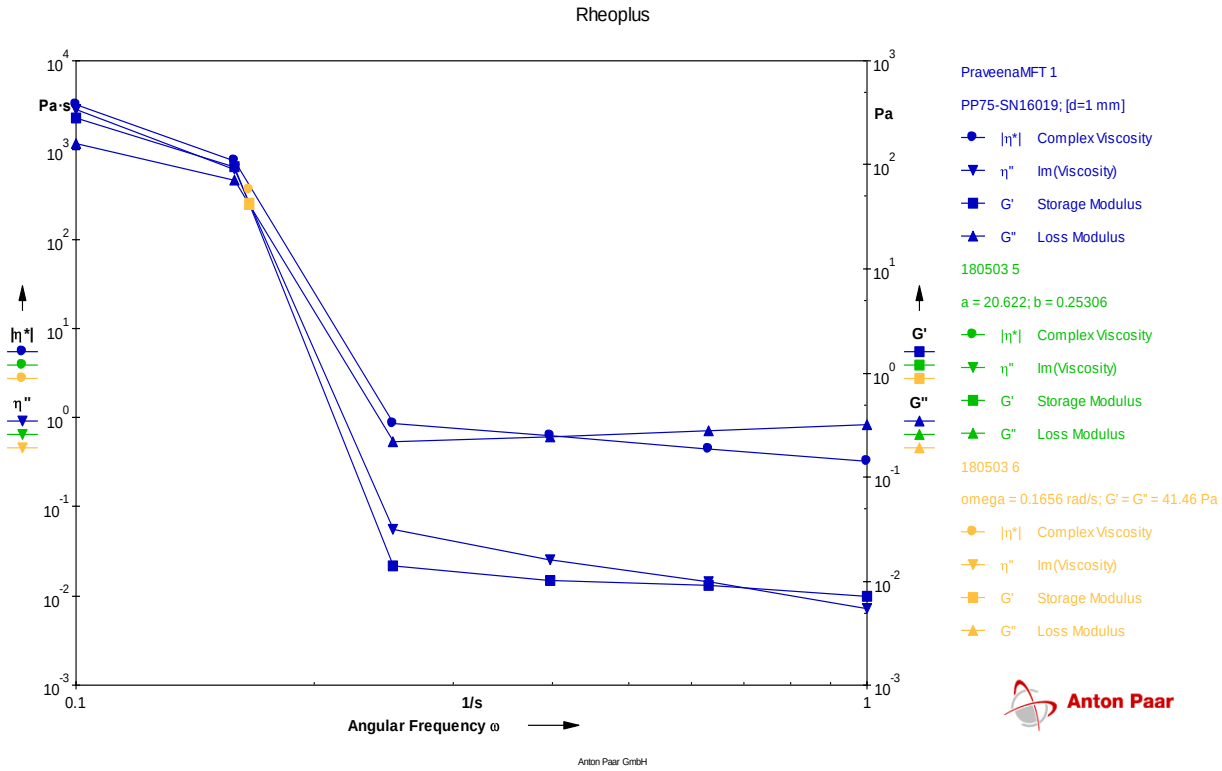
Shear Rate	Complex Viscosity	Deflection Angle	Torque	Status
[1/s]	[Pa·s]	[mrad]	[µNm]	[]
2.74E-04	4.34E+03	7.32E-02	9.85E+01	M- ,WE-
1.62E-02	2.52E+03	2.72E+00	3.36E+03	
1.87E+01	3.13E+00	1.99E+03	4.84E+03	WMa
9.06E+01	6.99E-01	6.07E+03	5.23E+03	WA+,WMa
1.41E+02	3.67E-01	5.98E+03	4.29E+03	WA+,WMa
2.25E+02	2.40E-01	6.00E+03	4.46E+03	WA+,WMa

SAMPLE CFRS4



Meas. Pts.	Angular Frequency	Storage Modulus	Loss Modulus	Damping Factor	Shear Stress	Shear Rate	Complex Viscosity	Deflection Angle	Torque	Status
	[rad/s]	[Pa]	[Pa]	[1]	[Pa]	[1/s]	[Pa·s]	[mrad]	[µNm]	[]
1	0.1	5.67E+02	3.11E+02	0.548	1.20E+00	1.85E-04	6.46E+03	4.93E-02	9.89E+01	M- ,WE-
2	0.158	3.94E+02	1.72E+02	0.436	4.07E+01	1.50E-02	2.71E+03	2.53E+00	3.36E+03	
3	0.251	5.63E-01	1.65E+00	2.94	6.52E+01	9.38E+00	6.96E+00	9.96E+02	5.40E+03	WMa
4	0.398	2.09E-02	2.68E-01	12.8	6.06E+01	8.98E+01	6.74E-01	6.02E+03	5.01E+03	WA+,WMa
5	0.631	1.25E-02	3.19E-01	25.4	7.14E+01	1.41E+02	5.06E-01	5.97E+03	5.91E+03	WA+,WMa
6	1	1.07E-02	3.94E-01	36.7	8.86E+01	2.24E+02	3.94E-01	5.99E+03	7.33E+03	WA+,WMa

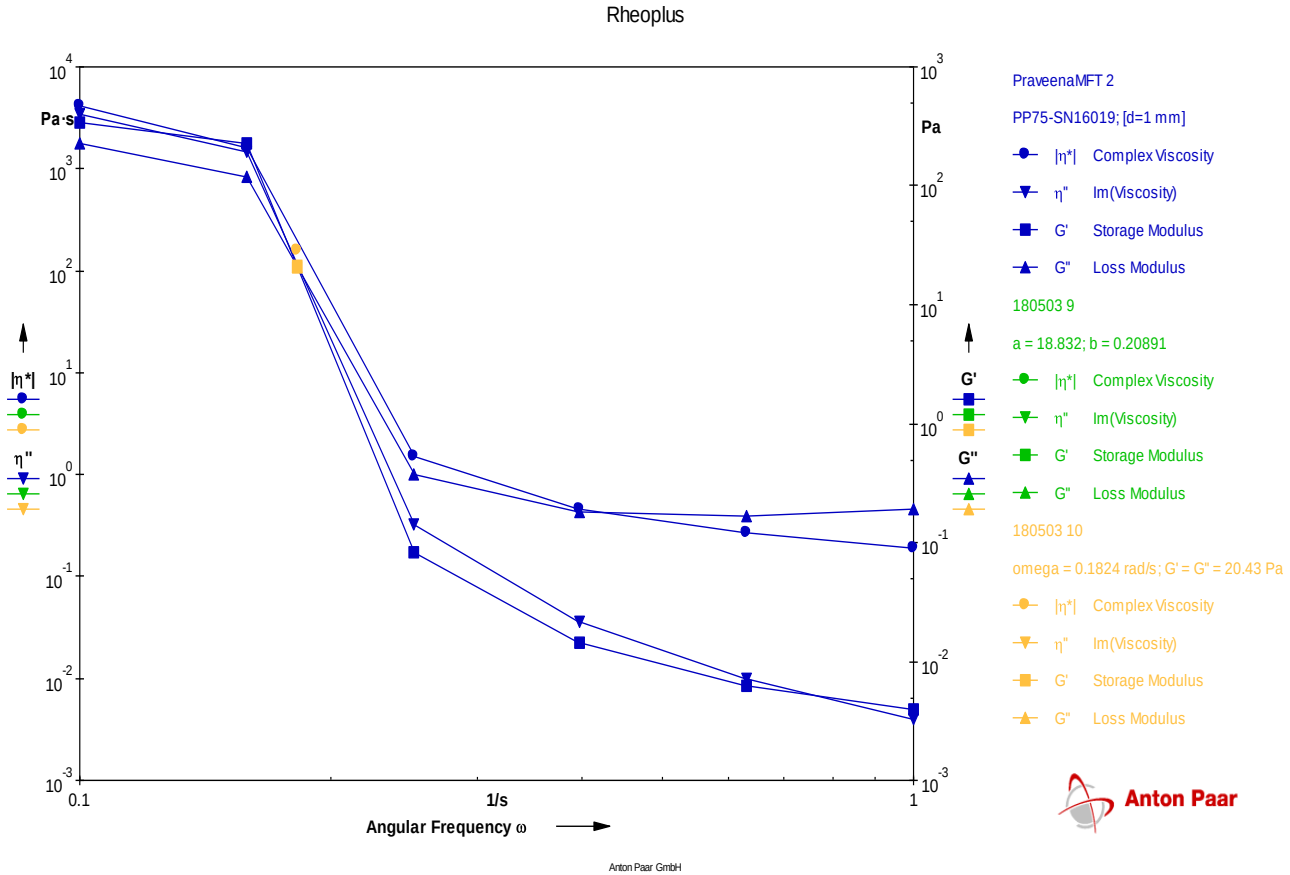
Sample CSb



Meas. Pts.	Angular Frequency	Storage Modulus	Loss Modulus	Damping Factor
	[rad/s]	[Pa]	[Pa]	[1]
1	0.1	2.85E+02	1.60E+02	0.56
2	0.158	9.62E+01	7.20E+01	0.749
3	0.251	1.38E-02	2.16E-01	15.6
4	0.398	1.01E-02	2.46E-01	24.4
5	0.631	9.06E-03	2.81E-01	31
6	1	7.13E-03	3.22E-01	45.2

Shear Stress	Shear Rate	Complex Viscosity	Deflection Angle	Torque	Status
[Pa]	[1/s]	[Pa·s]	[mrad]	[µNm]	[]
1.18E+00	3.62E-04	3.27E+03	9.66E-02	9.79E+01	M- ,WE-
4.03E+01	5.31E-02	7.58E+02	8.94E+00	3.33E+03	
4.91E+01	5.70E+01	8.63E-01	6.05E+03	4.07E+03	WA+,WMa
5.50E+01	8.90E+01	6.19E-01	5.96E+03	4.55E+03	WA+,WMa
6.29E+01	1.41E+02	4.46E-01	5.97E+03	5.21E+03	WA+,WMa
7.24E+01	2.25E+02	3.22E-01	5.99E+03	5.99E+03	WA+,WMa

Sample **CSc**



Meas. Pts.	Angular Frequency	Storage Modulus	Loss Modulus	Damping Factor	Shear Stress	Shear Rate	Complex Viscosity	Deflection Angle	Torque	Status
	[rad/s]	[Pa]	[Pa]	[1]	[Pa]	[1/s]	[Pa·s]	[mrad]	[µNm]	[]
1	0.1	3.40E+02	2.28E+02	0.67	1.20E+00	2.92E-04	4.10E+03	7.79E-02	9.89E+01	M- ,WE-
2	0.158	2.30E+02	1.19E+02	0.517	4.06E+01	2.48E-02	1.64E+03	4.18E+00	3.36E+03	
3	0.251	8.25E-02	3.71E-01	4.5	5.49E+01	3.63E+01	1.51E+00	3.86E+03	4.54E+03	WMa
4	0.398	1.43E-02	1.82E-01	12.8	4.11E+01	8.95E+01	4.59E-01	5.99E+03	3.40E+03	WA+,WMa
5	0.631	6.17E-03	1.69E-01	27.3	3.78E+01	1.42E+02	2.67E-01	5.98E+03	3.13E+03	WA+,WMa
6	1	3.96E-03	1.91E-01	48.2	4.29E+01	2.25E+02	1.91E-01	6.00E+03	3.55E+03	WA+,WMa

SUMMARY

LAB was isolated from different dairy sources by serial dilution & pour plate method. Isolation of pure culture was done by streak plate method. Primary characterization of isolates was done by Gram staining and oxidase and catalase negative isolates were further used for the study. Curd was prepared from pasteurized milk by adding the lactic culture which was incubated at 37℃ for 24h. The cultured broth was centrifuged at 6000rpm for 10mins. The pellets were added to pasteurized milk for curd formation. Effect of temperature on the fermented product was studied at three different temperatures ie; 37℃, 42℃ & 45℃.

The pH analysis, lactic acid percentage, microbial load, whey amount, texture, aroma & rheological properties were studied. The 3rd day, 5th day, 7th stored samples were analysed for pH, lactic acid percentage, whey amount and microbial load. Rheological properties like yield stress and cross over were studied in curd samples.

CONCLUSION

The present study was based on the selection of lactic acid starter cultures for curd formation. Effect of temperature on the fermented product was studied at three different temperatures i.e., 37℃, 42℃ & 45℃.The sample pH , LA% , microbial load , whey amount, texture & aroma were studied. . The pH value decreased with increased in time period, L.A% & whey amount also increased with increased time period. More the inoculum percentage shorter was the curd set time. The L.A% was seen highest in 2% inoculated sample than the 1% inoculated sample. As curd set at 37℃ had overall good acceptability, the rheological properties like yield stress and cross over were analysed for the same. Rheological studies showed good bonding ability between protein molecules in the samples fermented with cultures CFRS1 and CFRS2. Thus further optimization studies are required to select a best starter culture with commercial value.

FUTURE WORK

- The potential isolates (CFRS1 and CFRS2) that produce less whey, have good microbial load during storage and display pH & Lactic acid percentage which donot lead to sourness and takes shorter time to set can be used for commercial level curd preparation.
- However, future work regarding complete chracterization of the isolates, their synergistic effect on the sensorial attributes as well as health benifitting characters need to be studied in detail.
- The mixed cultures can be evaluated for curd preparation
- These starter cultures can also be investigated for preparation of other fermented dairy products such as cheese.

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