

**EFFECT OF STABILIZERS ON TEXTURAL AND
RHEOLOGICAL PROPERTIES
OF YOGHURT**



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EFFECT OF STABILIZERS ON TEXTURAL AND RHEOLOGICAL PROPERTIES OF YOGHURT

By

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Thesis Submitted to the

National Dairy Research Institute

(Deemed University)


in partial fulfilment of the requirements for the degree of


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IN

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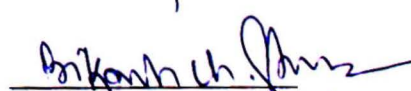

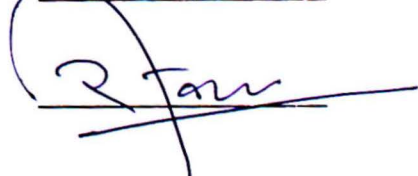
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ABSTRACT

Proper body and texture, minimum wheying off and characteristic acidic flavour are the essentials of set yoghurt of good quality. These characteristics are influenced by the composition, especially solid level, of the milk used for yoghurt production. Hydrocolloid stabilizers are permitted additives which, through their hydrophilic properties, may improve the body and texture and consistency of yoghurt. Therefore, an investigation was undertaken on the feasibility of use and comparative efficiency of hydrocolloid stabilizers to enhance consumer acceptability of set yoghurt without increasing its milk solid level. Guar, locust bean and xanthan gums were unsuitable for set yoghurt preparation as they caused casein destabilization upon their addition to milk. Gum tragacanth at 0.2% and pectin, sodium alginate and a blend of pectin and alginate at 0.1% levels produced maximum beneficial effect on rheology and syneresis of yoghurt. Estimation of syneresis and Ta-XT analysis revealed that maximum improvement in firmness (144.56 Vs 127.97 g), viscosity index (23.6 Vs 13.16 g.sec) and syneresis (4.5 Vs 6.5 ml/35ml yoghurt) was in the sample treated with tragacanth, while maximum consistency (513.13 Vs 348.96 g.sec) was achieved by using the blend of pectin and sodium alginate. The product with added tragacanth scored significantly less in organoleptic evaluation, mainly because of flavour defect. The beneficial effect of pectin and alginate on quality of set skim milk yoghurt through improvement in its rheological properties was off set by their negative impact on flavour of the product. Diacetyl was less in skim milk yoghurt compared to the product with 3% fat. Use of stabilizers resulted in further reduction in diacetyl production. In yoghurt with 3% fat, improvement in overall sensory score was noticed both with pectin and alginate probably because in this case the negative effect of stabilizers on flavour production was felt less as fat contributed to production and perception of flavour.

सारांश

सही आकार एवं रचना, अल्प ढे पृथकीकरण एवं विशिष्ट खट्टा सुवास एक अच्छे गुणवाले जमे हुए योगहर्ट की आवश्यकता है । बनावट का यह विशिष्ट गुण, खासकर योगहर्ट को बनाने के लिए प्रयोग किए गए दूध के ठोस स्तर से प्रभावित होता है । हाइड्रोकोलाइड स्टेबिलाइज़र अनुमति प्राप्त क्रिया है जो अपनी हाइड्रोफिलिक लक्षणों से योगहर्ट के आकार एवं रचना एवं गाढ़ापन में सुधार करता है । इसलिए, दुग्ध ठोस स्तर में वृद्धि किए बिना जमे योगहर्ट की उपभोक्ता स्वीकार्यता को बढ़ाने के लिए हाइड्रोकोलाइड स्टेबिलाइज़र के उपयोग की संभावना एवं तुलनात्मक क्षमता पर अनुसंधानात्मक प्रयत्न किया गया । गौर (Guar), लोकस्ट बीन (Locust bean) एवं कज़ानथान गोंद (Xanthan Gum) जमे योगहर्ट बनाने में अनुपयुक्त थे क्योंकि इनमें दूध मिलाने पर दूध की ठोसता में अस्थिरता उत्पन्न की । 0.2% ट्रेगाकान्त (tragacanth) गोंद एवं 0.1% स्तर पर पेक्टिन तथा सोडियम अलगिनेट के मिलावट ने योगहर्ट की बनावट एवं दिखावट पर अति अनुकूल प्रभाव डाला । सिनेरीसिस (Synerisis) के आकलन एवं टी ए - एक्स टी (TA-XT) विश्लेषण ने बताया कि ट्रेगाकान्त द्वारा उपचारित नमूनों ने ठोसता में अत्यधिक सुधार (144.56 प्रति 127.97 ग्रा0) लसलसाहट सूचकांक (23.6 प्रति 13.16 ग्रा0 से0) एवं सिनेरीसिस (4.5 प्रति 6.5 मि.लि./ 35 मि.लि. योगहर्ट) था जबकि पेक्टिन तथा सोडियम अलगिनेट के मिश्रण के उपयोग से अत्यधिक गाढ़ापन (513.13 प्रति 348.96 ग्रा0 से0) प्राप्त किया गया । सुवास में दोष के कारण ट्रेगाकान्त मिले उत्पाद का ऑरगेनोलेपटिक मुल्यांकन गणन काफी कम था । बनावट के लक्षणों में सुधार द्वारा जमे मलाई रहित दुग्ध योगहर्ट के गुण पर पेक्टिन तथा सोडियम अलगिनेट का अनुकूल प्रभाव उत्पाद के सुवास पर अस्वीकार्य प्रभाव के कारण प्रतिकूल साबित हुआ । 3% वसा वाले उत्पाद की तुलना में स्किम दुग्ध योगहर्ट में ड़ायसिटिल कम था । स्टेबिलाइज़र के उपयोग के परिणामस्वरूप ड़ायसिटिल उत्पादन में और कमी हुई । 3% वसा वाले योगहर्ट में, पेक्टिन तथा सोडियम अलगिनेट दोनों के साथ कुल सेंसोरी गणन में सुधार देखा गया, चूंकि इस विषय में सुवास के उत्पादन एवं ग्राह्यता के लिए वसा के योगदान की तुलना में सुवास उत्पादन पर स्टेबिलाइज़र के प्रतिकूल प्रभाव को कम महसूस किया गया ।

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Chapter- 1

INTRODUCTION

Introduction

CHAPTER 1

INTRODUCTION

Yoghurt is a semi solid fermented product made from milk by the activity of a symbiotic blend of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* culture. The starter culture utilizes the lactose in the milk for its energy requirement and as a result the production of lactic acid takes place. The gradual development of lactic acid starts to destabilize the casein micelle - denatured whey protein complex. Aggregates of casein micelles partially coalesce as the pH approaches the isoelectric point (4.6 –4.7). The α -lactalbumin and β -lactoglobulin interaction with the κ -casein probably protects the micelles against complete destabilization and as a result, the gel network consists of a regular structure which entraps within it all the other constituents of the basic mix, including the water phase. Therefore, the body and texture of yoghurt depends largely on the composition of milk and also on the additives employed in the manufacture. However, irregular behaviour of the starter organisms, faculty regulation of incubation temperature along with a number of other process variables can give rise to an end product that is deficient in overall quality.

The solid content is an important determinant in the texture and quality of yoghurt. The level of fat and solid-not-fat (SNF) required to be present in yoghurt is governed by the legal standards of the country concerned. In India, a minimum SNF of 8.5% is prescribed. The requirement of fat content in yoghurt ranges from < 0.5% to > 3.0% depending upon the type of the product. Though these are the minimum

levels required, the physical properties of yoghurt, like viscosity and consistency of the coagulum, are of great importance from the manufacturers' point of view. In general, the higher the level of solids in the yoghurt mix, the greater the consistency of the end product. The relationship between the level of solids in the milk and the consistency of yoghurt has been well established. The best yoghurt is probably made from milk containing 15-16% total solids and the composition of most commercial yoghurts falls within the range of 14 – 15% total solids. The increased protein content in the mix results in a custard like consistency following the desired fermentation. Fat content too has an important role in mouth feel and production of flavour compounds and prevention of wheying off in yoghurt. Lower solid levels lead to textural defects. To overcome this problem, stabilizers and emulsifiers are often used during the manufacture of yoghurt. The primary aim of adding stabilizer to the basic mix is to enhance and maintain the desirable characteristics of yoghurt, ie, body and texture, consistency, appearance and mouth feel. Their use is governed by legislative regulation. In India, under the PFA, a list of compounds with permitted concentrations is drafted.

These stabilizers are mostly hydrocolloids and their mode of action in yoghurt includes two basic functions: the binding of water and promotion of an increase in viscosity. The stabilizers are capable of forming a network of linkages between the milk constituents and themselves due to the presence of a negatively charged group or due to their ability to sequester calcium ions. The stabilizers bind the water as water of hydration; they may react with the milk constituents, mainly proteins, to increase their level of water of hydration. They stabilize the protein molecule to form a network that retards free movement of water.

Therefore, functions of hydrocolloids in yoghurt are gelling or thickening and stabilizing.

The list of stabilizers permitted under PFA includes carrageenan, pectin, sodium carboxy methyl cellulose, agar, guar gum, xanthan gum, tragacanth, karaya gum, gelatin and furcellaran in addition to starch. These stabilizers can be added as single compounds or as a blend. The purpose of blending these compounds together is to achieve a specific function or to overcome one of the limiting properties associated with a specific compound. The choice of a particular type of stabilizer would depend on many factors. The addition of some hydrocolloids even at low levels may destabilize the casein micelles. Although the destabilized casein micelles will eventually coagulate, the matrix has a rather limited ability to retain water and as a result, syneresis becomes evident. Therefore, such stabilizers would be unsuitable in set yoghurt though they may increase the viscosity of stirred yoghurt.

Another factor which influences the level of stabilizer to be added to the yoghurt milk is the percentage of milk solids present. At higher levels of solids, the requirement of stabilizer would be less.

It is in this context, this study on stabilizers in yoghurt is taken up. The objectives are:

- (i) To study the effect of addition of stabilizers on textural, rheological and sensory properties of yoghurt.
- (ii) To study the influence of composition of milk on the effect of stabilizers in yoghurt.

Chapter- 2

Review of Literature

CHAPTER 2

REVIEW OF LITERATURE

The types of yoghurt that are produced worldwide can be divided in to various categories based on (i) method of preparation (set and stirred yoghurt). (ii) flavours (plain, fruit or flavoured yoghurt). (iii) post-incubation processing (heat treated, frozen, dried or concentrated yoghurt). (iv) existing or proposed legal standards (yoghurt, partly skimmed yoghurt, skimmed yoghurt, sweetened/flavoured yoghurt and fruit yoghurt) (Hammer and Babel, 1957; Robinson, 2002).

2.1. Yoghurt processing

Although there is no standardized procedure for making a set/drinkable yoghurt, most processors agree on a general process. This includes pre-treatment of the milk, heat treatment, homogenization, cooling, starter culture addition and subsequent fermentation, cooling and packaging (Tamime and Robinson, 1999).

2.1.1. Standardization of milk

Standardization of milk fat and SNF is an important step, not only for adherence to legal standards, but also for the overall affects on the flavour and texture attribute of the product, partially relating to the viscosity and mouthfeel. As per PFA (2008), yoghurt has been classified under five categories as yoghurt, partly skimmed yoghurt, skimmed yoghurt, sweetened/flavoured yoghurt and fruit yoghurt, with the following chemical composition:

Types of yoghurt	% fat (m/m)	% SNF (m/m)
Yoghurt	≥ 3.0%	≥ 8.5%
Partly skimmed yoghurt	0.5 % - 2.9 %	≥ 8.5%
Skimmed yoghurt	< 0.5 %	≥ 8.5%
Sweetened/flavoured yoghurt	≥ 3.0%	≥ 8.5%
Fruit yoghurt	≥ 1.5%	≥ 8.5%

The relation between the level of solid in milk and the consistency was studied by Tamime (1977), and he observed that consistency greatly improved as the milk solids increased from 12 to 20g 100g⁻¹. As a result of increasing the level of SNF in milk, the titratable acidity of milk increased due the buffering action of the additional proteins, phosphates, citrates, lactates and other miscellaneous milk constituents (Walstra and Jenness, 1984), and this function can lead to a reduced gel formation time. Higher total solid content, especially SNF improves the rheological and textural properties, which is generally practised by adding dried skim milk (Mehanna, 1988) or mixing with reconstituted milk (Balasubramanyan and Natrajan, 1988). Fat content of yoghurt also has a significant role in mouthfeel and the production of flavour compounds, especially volatile fatty acids and diacetyl in final product (Shukla and Jain, 1986; Keogh and O' Kennedy, 1998). The fortification total milk solid in the yoghurt milk can be achieved by a number of different methods such as addition of SMP, WMP, butter milk powder, whey powder, whey protein

concentrate, casein powder or concentrating by heating in open pan, by vacuum evaporation, by membrane filtration etc.

2.1.2. Additives

The preliminary treatment also includes the addition of fruit or fruit pulp (5-15%) or sweeteners (8-10%) or any permitted stabilizers, colours and flavours. Whey separation, a typical quality defect in drinkable forms of yoghurt, may be prevented by the inclusion of various stabilizers such as gelatin (Tamime and Robinson, 1999), apple pectin paste (Yukalo *et al.*, 1991).

Drinking yoghurt is normally flavoured with fruit purees or juices and consumers studied in the USA preferred strawberry and raspberry. Other flavours which have been used in drinking yoghurt are carrot and apple concentrate (Kolenikova *et al.*, 1986), pineapple (Srivastava *et al.*, 1985), lemon or orange concentrate (Arsova, 1983).

2.1.3. Heat treatment

The application of heat is a crucial step in yoghurt production and affects both the safety and quality of the product with both temperature and time being critical factors. The effect of heat treatment can be broadly summarized as (i) destruction of pathogens and other undesirable micro-organisms (ii) production of factors stimulatory/inhibitory to the yoghurt starters (iii) changes in physicochemical properties of milk constituents which are relevant in yoghurt making (Early, 1998; Tamime and Robinson, 1999; Walstra *et al.*, 1999). However, typical pasteurization does not necessarily include the destruction of bacterial spores or certain enzymes, while heating at

90-95 °C for 5 min or boiling for few minutes leads to the increase in the concentration of milk solids in basic mix and also kills all vegetative cells, enzymes and possibly some spores.

Much research has been conducted on the physical and chemical changes in milk associated with heating regimes. Flavour and viscosity changes are only a few of the many alterations. Apparent viscosity of UHT treated milk and of yoghurt prepared from this milk was studied and compared to that of vat-treated milk and yoghurt. UHT-treated milks (140 °C, 3.3 sec) had an apparent viscosity of 2.3 to 2.7 cp, while in case of vat-treated milks (63 °C and 82 °C, 30 min) it ranged from 1.9 to 2.0 cp. But in case of yoghurt the highest apparent viscosity was achieved by vat-treatments, and yoghurt from UHT-treated milk gave markedly reduced apparent viscosity (Labropoulos *et al.*, 1981). This slight increase in milk viscosity resulting from heat treatment may be attributed to the effects of the heat on the serum proteins in the milk. Often referred to as whey proteins, serum proteins consist mainly of the globular proteins β -lactoglobulin, α -lactalbumin, serum albumin, and various immunoglobulins (Walstra *et al.*, 1999). High temperatures (i.e. 70-90 °C) cause the peptide chains of these proteins to unfold, resulting in denaturation of the protein and increased viscosity (Renner and Abd El-Salam, 1991; Strange *et al.*, 1996; Walstra *et al.*, 1999). Depending on the heating temperatures, such as those used in high-temperature pasteurization, and the pH, various reactions on the side chains of these proteins may take place, preventing the peptide chain from reverting back to the original conformation. These denatured proteins are insoluble (Walstra *et al.*, 1999).

A variety of reactions involving the serum proteins may occur, but these are largely dependant upon the pH. For instance, application of heat at high temperatures (greater than 80 °C) causes β -lactoglobulin to associate with κ -casein on the outer layer of the casein micelle (Fox and McSweeney, 1998). As a result of these types of reactions, the volume of the proteins increases, resulting in a slight increase in milk viscosity (Renner and Abd El-Salam, 1991).

Heating and cooling the milk, followed by acidification to the isoelectric pH of casein, as is done in the production of yoghurt, causes the denatured serum proteins to associate and precipitate with the casein (Fox and McSweeney, 1998). Walstra *et al.* (1999) indicate that other reactions may occur that cause dimers and other small, soluble aggregates of protein to be formed. Depending upon pH and other conditions, these may continue to aggregate. These larger complexes of denatured serum proteins are insoluble. Proteose-peptone is the only serum protein to which denaturation does not apply since this protein is not globular (Renner and Abd El-Salam, 1991; Walstra *et al.*, 1999). Similarly, casein does not denature. However, very high heat treatments, such as those used for sterilization of concentrated milk, can cause the casein to irreversibly aggregate. Again, depending primarily on pH (generally below pH 6.2), the large aggregates, bonded by covalent crosslinking, coagulate and a gel may be formed (Fox and McSweeney, 1998; Walstra *et al.*, 1999). Other reactions and conditions, such as those involving calcium bridging or the depletion of κ -casein from the micelles, may play a role in the heat coagulation. Although this aggregation of casein and possible subsequent coagulation may affect the viscosity of the milk more than the denaturation of the serum

proteins, very high temperatures and other specific conditions must be met. Hence, these reactions do not typically occur in the regular processing of milk for yoghurt (Fox and McSweeney, 1998; Walstra *et al.*, 1999).

In addition to the effects on viscosity, the application of heat has many other effects on milk. Flavour changes due to Maillard reactions, formation of free sulphhydryl groups, protein interactions, and lactone and methyl ketone formation are apparent (Fox and McSweeney, 1998; Walstra *et al.*, 1999). Furthermore, colour, nutrients, and many other attributes of milk are affected (Robinson, 1994).

The gel matrix of yoghurt has been examined by scanning electron microscopy (SEM). In heated milk, chains and clusters of casein micelles are formed, which serves to immobilize the liquid phase (Kalab, 1981). Micelles of milk heated at 95 °C for 10 min or autoclaved at 121.7 °C for 15 min were observed by SEM to bear filamentous appendages, where as micelles from raw milk had relatively smooth contours with no appendages (Davies *et al.*, 1978).

This also results in protein being evenly distributed, producing a firmer and more stable yoghurt (Tamime and Robinson, 1999). However, in yoghurt made from unheated milk, this does not occur as the casein micelles fuse into larger aggregates that are not distributed evenly in the matrix. As a result, a weaker yoghurt is produced and is more susceptible to syneresis (Kalab, 1981; Tamime and Robinson, 1999). According to Tamime and Robinson (1999), the porosity of the protein matrix is impacted by the amount of casein and non-casein protein in the milk. SEM revealed that the yoghurt is more susceptible

to whey separation with larger pores in the yoghurt matrix (Kalab, 1981; Tamime and Robinson, 1999). Electron microscopy was also used to examine the effects of some stabilizers on the yoghurt gel matrix (Kalab, 1981). Ingredients such as carageenan caused aggregation of casein or the formation of fibers from the stabilizer to the casein micelles (Kalab, 1981). SEM was also used to analyze the effects of the starter cultures on the gel matrix of the yoghurt. This revealed that the lactic acid bacteria used in yoghurt production formed pockets in the gel structure as a result of bacterial action (Kalab, 1981, Tamime and Robinson, 1999). Further study of exopolysaccharide-producing starter cultures showed that filaments of the polysaccharides produced are formed. These filaments attach to the protein of the yoghurt coagulum, which possibly improves the viscosity and stability of the gel (Kalab, 1981; Modler and Kalab, 1983; Tamime and Robinson, 1999).

2.1.4. Homogenization

The next step in yoghurt processing also impacts the physical and chemical aspects of the milk. Homogenization breaks the large fat globules into smaller ones, thereby creating a stable emulsion out of an oil-in-water mixture (Early, 1998; Spreer, 1998). This is typically accomplished by directing the standardized milk through small valves at high pressure (Tamime and Robinson, 1999). This action reduces the size of the fat globules, which may be from 1 to 18 μm in diameter (average size is 3-4 μm) to less than 2 μm (Early, 1998; Tamime and Robinson, 1999). The new fat globules are stabilized by binding to some of the casein that was broken during this processing step (Fox and McSweeney, 1998; Tamime and Robinson, 1999). As a result,

homogenization serves several purposes, including preventing the separation of the cream layer, improving stability, altering the physical attributes, etc. (Tamime and Robinson, 1999; Walstra *et al.*, 1999). These effects are dependant upon the amount of fat in the milk, as well as the pressure and temperature used for homogenization and may be minimal in skim milk products (Fox and McSweeney, 1998; Tamime and Robinson, 1999). In addition, some references indicate that this processing step should occur prior to the heat treatment (Tamime and Robinson, 1999). Regardless, both the serum proteins and the fat in the milk may be affected by homogenization. Some denaturation of these proteins, primarily by β -lactoglobulin and α -lactalbumin, may occur along with the resultant effects as previously discussed with the heat treatment (Tamime and Robinson, 1999). Heating the homogenized milk at a temperature above 70 °C may induce the denatured serum proteins to interact with other denatured β -lactoglobulin to form a gel. Interactions with κ -casein both on the surface of suspended micelles and adsorbed on the fat globules are possible, as are interactions with the residual fat globule membrane. Adsorbed casein can also be displaced on the fat globule by adsorbing onto the globule surface (Tamime & Robinson, 1999). The reduction in size of the fat globules and increase in the adsorption on the casein micelle leads to an increase in total volume, resulting in an increased viscosity and the formation of a softer gel (Spreer, 1998; Tamime and Robinson, 1999). Colour is also affected, with homogenized milk appearing whiter due to the scattering and reflectance of light by a larger number of fat globules (Fox and McSweeney, 1998; Tamime and Robinson, 1999). The interaction between the casein and the fat globules, as well as other protein effects, result in an increase in hydrophobicity and water binding which limits

syneresis (Tamime and Robinson, 1999). Protein stability may also be decreased from the various protein interactions.

2.1.5. Starter cultures

Yoghurt starter bacteria consist of one or more strains of *S. thermophilus* (coccus) in conjunction with single or multiple strains of *L. bulgaricus* (rod). The pairing of coccus and rod is important because there is an associative beneficial relationship between two types. In biology, such a beneficial relationship is called symbiosis (Vedamuthu, 1991). For this reason, both the interaction of the cultures and their individual characteristics are important for flavor and texture development.

L. bulgaricus is a lactic acid bacteria usually seen as medium to long, slender rods sometimes bent or curved, in pairs and in chains. As a homofermentative thermophile, *L. bulgaricus* is known to be relatively tolerant to heat, with an optimum growth temperature of approximately 45-50 °C and acid production in milk occurs optimally around 43-46 °C (Rasmussen, 1981; Vedamuthu, 1991; Rybka and Kailasapathy, 1995). As a result of this high temperature resistancy, this yoghurt starter microorganism is commonly used in high temperature fermentations (Rasmussen, 1981). The association of *L. bulgaricus* with other lactobacilli is under debate (Tamime and Deeth, 1980). Certain characteristics, however, serve to distinguish these bacteria from other members in the family Lactobacillaceae, such as *L. helveticus*, which is also found in some yoghurt and cheeses (Rasmussen, 1981). As described by Rasmussen (1981), these differences include the production of granules by *L. bulgaricus*, as seen under a microscope, an attribute

which *L. helveticus* lacks. A second distinguishing feature is the maximum titratable acidity production of approximately 1.7 % for *L. bulgaricus*. This is considerably lower than *L. helveticus*, which maintains a maximum titratable acidity for acid production in milk of about 2.7 %. A third characteristic of *L. bulgaricus* as compared to *L. helveticus* is the inability of *L. bulgaricus* to ferment maltose (Rasmussen, 1981). *L. bulgaricus* is considered to be a relatively slow-growing organism, due to a lack of strong tolerance for oxygen. As a result of this, until the oxygen levels are reduced during fermentation, this microorganism will not grow rapidly (Marth and Steele, 1998). Lactose degrading enzyme system in *L. bulgaricus* consists of β -galactosidase as well as β -D-phosphogalactoside galactohydrolase and has galactose accumulates in milk fermentations (Vedamuthu, 1991).

The other starter microorganism involved in yoghurt production, *S. thermophilus*, is spherical-shaped, typically found in pairs or long chains, and is noted for the ability to withstand higher temperatures (up to 120 °F or 49 °C), contributing to the classification of homofermentative thermophile and was described first by Orla-Jensen in 1916 (Tamime and Deeth, 1980; Rasmussen, 1981; Vedamuthu, 1991). The thermal resistance is demonstrated in the evidence of *S. thermophilus* survival during heating at 60 °C for 30 minutes and characterized by the use of these bacteria in various high temperature fermentations (Rasmussen, 1981; Tinson *et al.*, 1982; Wilkins *et al.* 1986). Despite being able to survive at higher temperatures, *S. thermophilus* fails to grow at 10 °C, separating this microorganism from other streptococci that grow at lower temperatures (Tamime and Deeth, 1980; Marranzini, 1987). Other characteristics of this member of the

Streptococceae include the inability of *S. thermophilus* to grow in high salt concentrations and the inability to produce ammonia from arginine (Marth and Steele, 1998). These attributes are important for identification, since *S. thermophilus* does not possess a necessary antigen for serological identification and, therefore, physiological techniques must be utilized (Tamime and Deeth, 1980; Marranzini, *et al.*, 1987). Other characteristics of this member of the streptococceae family include a sensitivity to phosphates and, as is the case with *L. bulgaricus*, production of capsules and exopolysaccharides by some strains. Much research has been conducted on the capsule material in terms of the effects on viscosity and texture. Some characteristics of coccus strains make them vulnerable in dairy fermentations. First is sensitivity to the penicillin and other antibiotics residues in milk and second is they are also somewhat retarded by high sucrose concentration (Vedamuthu, 1991).

S. thermophilus and *L. bulgaricus* exist in a complex cooperative relationship in yoghurt, in which one bacterium produces stimulatory agents for the other (Tamime and Deeth, 1980; Wilkins *et al.*, 1986; Vedamuthu, 1991). *L. bulgaricus* has been shown to produce certain amino acids, such as valine, leucine, and histidine, which are essential for *S. thermophilus* to grow. These amino acids are the result of proteolysis of casein by *L. bulgaricus* (Marranzini *et al.*, 1987; Abu-Tarboush, 1996). Numerous additional studies have been performed to determine which amino acids stimulate *S. thermophilus* under varying conditions. However, according to Abu-Tarboush (1996), *S. thermophilus* is stimulated by valine, histidine, methionine, glutamic acid, and leucine released by *L. bulgaricus*. In reciprocation, *S.*

thermophilus stimulates *L. bulgaricus* by the production of various compounds under anaerobic conditions (Tamime and Deeth, 1980; Abu-Tarboush, 1996). As listed in a study by Abu-Tarboush (1996), several of these substances include peptides, purines, pyrimidines, oxaloacetic acid, and fumaric acids, though Tamime and Deeth (1980) cited previous research that contends that peptides, purines, and pyrimidines may not necessarily stimulate growth of *L. bulgaricus*. In addition to these compounds, *S. thermophilus* produces formic acids and carbon dioxide, and reduces the oxygen-reduction potential, which serves to increase the acid produced by *L. bulgaricus* (Marranzini, *et al.* 1989). As both *L. bulgaricus* and *S. thermophilus* partially convert the lactose in the milk to lactic acid, this is an important aspect of the carbohydrate fermentation during yoghurt production, as is the production of acetaldehyde by these microorganisms (Labell, 1989). As a result of both lactic acid and acetaldehyde generation from this cooperative growth, the characteristic flavor of yoghurt is produced (Richter and Mull, 1975; Rasmussen, 1981; Wilkins *et al.*, 1986a; Wilkins *et al.*, 1986b; Marranzini *et al.*, 1989; Schmidt *et al.*, 1989).

Both *L. bulgaricus* and *S. thermophilus*, as well as other lactic acid bacteria, have ropy strains that produce exopolysaccharides, either as a capsule or secreted as slime (Vedamuthu, 1991; Wood and Holzapfel, 1995; Marth and Steele, 1998; Tamime and Robinson, 1999,). However, Hassan *et al* (1996a, 1996b) reported that several strains of encapsulated lactic acid bacteria could not be characterized as ropy and, therefore, differentiated between encapsulated ropy, encapsulated nonropy, and unencapsulated nonropy strains. In other research, no distinction is necessarily made between the versions of polysaccharide

secretions. Regardless, these strains have been studied for their possible benefit of the polysaccharide production to the viscosity, mouthfeel, and other rheological properties. Water-binding capacity of yoghurts made with these bacteria related to the syneresis or wheying off defect in set and stirred yoghurt have also been examined (Tamime and Robinson, 1999).

A study conducted by Schellhaas (1983) indicated that the apparent viscosity of skim milk gels made by ropy cultures was more compared to that made by non-ropy cultures (Tamime and Robinson, 1999). Further experimentation by Schellhaas and Morris (1985) maintained that, at higher shear rates, gels produced by ropy cultures exhibit less of a decrease in viscosity. Electron microscopy revealed that this might be due to the crosslinking of the exopolysaccharide with both the cell surface and the protein of the gel (Schellhaas and Morris, 1985). However, as a result of this bonding, gel firmness is not necessarily improved by the presence of these exopolysaccharides. Rawson and Marshall (1997) compared the effects of ropy and nonropy yoghurt cultures on the viscosity and gel texture of stirred yoghurt. Results indicated that apparent viscosity was increased by using both ropy strains of *L. bulgaricus* and *S. thermophilus* as compared to yoghurt made with nonropy cultures. However, after destructive shear rate-thinning and subsequent recovery time, viscosity was not recovered as well for yoghurt made with the two ropy cultures. Rheological data from this study also suggest that although ropiness may improve cohesiveness, firmness (defined as the force necessary to attain a given deformation) of the yoghurt matrix may not be affected. These results correlate to previous research (Schellhaas and Morris, 1985), where the

increase in viscosity was attributed to a shorter relaxation time after shearing. This suggests that the polysaccharide-protein association might have more of an impact on the viscosity than the protein-protein interactions of nonropy yoghurt (Rawson and Marshall, 1997; Tamime and Robinson, 1999). Other researchers determined that these bonds are stretched under low shear rates but are broken with higher shearing (Rawson and Marshall, 1997). According to Rawson and Marshall (1997), the reduction in firmness may result from the excess slime produced prohibiting the polysaccharide-protein bonds from being formed, resulting in a weaker structure of the gel (Rawson and Marshall, 1997). The researchers concluded that the relaxation time of the bonds do not affect the gel firmness. The polysaccharide-protein interactions from ropy bacteria do, however, contribute to the overall viscosity, cohesiveness, and adhesiveness, which may have an influence on the overall mouthfeel of the yoghurt (Rawson and Marshall, 1997).

Hassan *et al.* (1996a; 1996b) examined the rheological and textural properties of yoghurt made with strains differentiated as encapsulated ropy (in which an exopolysaccharide capsule is formed), encapsulated nonropy (secretes extracellular slime), and unencapsulated nonropy. Yoghurts made with ropy cultures exhibited increased viscosity and shear stress values; however, differences attributed to the type of polysaccharide secretion (capsule or slime) were apparent. Results from these experiments showed that the presence of bacterial capsules may enhance some rheological properties, such as viscosity, but may weaken the gel structure. This caused lower shear stress values compared to slime-producers, which produce a more stretchable gel structure (Hassan *et al.*, 1996a). The size of the capsule also affected the gel structure.

Larger capsules created structures with large pores, which further served to enhance viscosity (Hassan *et al.*, 1996a). Overall, encapsulated nonropy cultures produced yoghurt that were more stable for shearing, were more viscous, and had a lower yield stress (Hassan *et al.*, 1996a; Marth and Steele, 1998). The type of extracellular polysaccharide produced by the yoghurt bacteria was shown to have effects on the texture and syneresis, as well. Yoghurt made with encapsulated nonropy cultures had the lowest firmness and curd tension, but exhibited less syneresis than unencapsulated cultures (Hassan *et al.*, 1996b). The lower firmness in the yoghurt made by slime producing cultures may have been due to the polysaccharide interfering with the casein structure (Hassan *et al.*, 1996b). Hassan *et al.* (1996b) also demonstrated that, in addition to the type of polysaccharide produced, yoghurt texture was affected by pH, whether a single strain or a mixed culture was used, and size of the capsular material produced.

Production of exopolysaccharides by starter cultures influences the flavor intensity of yoghurt. High polysaccharide-producing cultures may impart a higher intensity due to the carbohydrate masking the flavor (Tamime and Robinson, 1999). Mouthfeel and other attributes may be affected as well.

2.1.6. Stabilizers

Stabilizers are a group of water-soluble or water-dispersible biopolymers used in small amounts (typically 0.2%) in ice cream, sorbets, water ices, fermented dairy foods and other foods (Clarke, 2004). Stabilizers have been used in food products for a variety of purposes, including thickening, aiding stability, and improving

mouthfeel (Harris, 1990; Imeson, 1997; Phillips and Williams, 2000). According to Glicksman (1982), stabilizers can be categorized according to the manufacturing process (Tamime and Robinson, 1999). Hydrocolloids can either be of natural, modified, or synthetic origin. Selection of the stabilizer or stabilizer combination to be used in a food system greatly depends on several variables. Functional properties of the stabilizer, intended use and outcome, interactions with other ingredients, and legal aspects are only a few considerations. In yoghurt, stabilizers are added for two main reasons as thickening or gelling agents and to stabilize the yoghurt matrix. Their mode of action in yoghurt includes two basic functions: first is binding of water and second is forming network of linkages between milk constituents and themselves (Early, 1998; Tamime and Robinson, 1999; Phillips and Williams, 2000). In this capacity, the hydrocolloids, which are generally added to the milk prior to fermentation, can improve the viscosity, maintain the yoghurt structure, inhibit syneresis, alter the mouthfeel, and, in the case of yoghurt with added fruit, help keep the fruit in suspension (Early, 1998). Gelatin, carboxy methyl cellulose, and high methoxy pectin all may be used to achieve these results. The synergistic effects can be achieved from the combination of two stabilizers, for example, greater increase in viscosity than would be expected from either component individually, or gelation, when the separate component do not gel (Clarke, 2004). In yoghurt, stabilizers permitted by PFA (2008) are carrageenan, pectins, sodium CMC, agar, guar gum, xanthan gum, tragacanth gum, karaya gum, gelatine and furcellaran @ 0.5% (max) while gelatin can be added @ 1% (max).

2.1.6.1. Carrageenan

Carrageenans are complex polysaccharides of esters of galactose and 3,6-anhydrogalactose, found in red seaweeds (*Rhodophyceae*), such as *Chondrus crispus* (Irish Moss), *Kappaphycus alvarezii* and *Eucheuma denticulatum*. Carrageenan can have several structures, usually classified as one of three types that have different properties: *kappa* (κ), *iota* (ι) (both of which come from Indonesia and the Philippines) and *lambda* (λ) (from the Pacific coast of North America, France, Denmark, Norway, Ireland and Great Britain). Carrageenans are polyelectrolytes in aqueous solution and form gels after heating and cooling in the presence of cations such as K^+ or Ca^{2+} . Carrageenans can also form gels with both milk proteins and locust bean gum. κ -Carrageenan has a specific function in ice cream and added at about 0.02%, it reduces phase separation of milk proteins and polysaccharides (Clarke, 2004).

2.1.6.2. Pectin

Pectin is extracted from citrus peel and apple pomace. It is a polysaccharide consisting of linear chains of galacturonic acid and galacturonic acid methyl ester units. Pectin is classified according to its degree of esterification. High methoxy (>50% esterified) and low methoxy (<50% esterified) pectins possess different properties. For example, low methoxy pectin requires calcium to gel whereas high methoxy pectin gels at low pH and in the presence of high concentrations of sugar. Pectin is the setting agent used in jam making as fruits contain some pectin. Some fruits, such as apples and gooseberries, usually contain enough natural pectin to form a gel, whereas pectin must usually be added to set the jam for other fruits, such as strawberries and

cherries (Clarke, 2004).

2.1.6.3. Sodium carboxymethyl cellulose

Sodium carboxymethyl cellulose is derived from purified cellulose from cotton and wood pulp. It is a sodium salt polymer of anhydro glucose residues. The long, negatively charged molecules produce a stable thickener that can also reduce casein precipitation. However, its perception as a 'chemical' has resulted in fairly low usage (Clarke, 2004).

2.1.6.4. Agar

Agar is the extract of *Gelidium* and other species of red algae. Chemically, agar is believed to be composed of three of 3, 6-anhydro-L-galactose and D-galactopyranose residues in varying proportions. It is insoluble in cold water and soluble in boiling water and it forms a firm, resilient gel when cooled to 32-39 °C which does not melt below 85 °C. Major food uses of agar are as gelling agents in the bakery industry, in confectionary, in meat and fish products and in dairy products (Klose and Glickman, 1972).

2.1.6.5. Guar gum

Guar gum is extracted from the seeds of *Cyamopsis tetragonolobus*, an annual crop grown in the Indian subcontinent. Guar has a similar structure to LBG. It has a backbone of mannose units, about half of which have galactose side branches. Guar has a higher molecular weight than LBG and the side groups are more evenly spaced. The larger proportion of galactose units makes guar coldwater soluble. The regions of backbone that are free of side chains are smaller than in

LBG. Hydrogen bonding between them is therefore not strong enough to form permanent cross-links, but does result in 'hyper-entanglements' (Clarke, 2004). In practice, it is found that cultured dairy foods stabilized with guar gum has a slightly shorter body and a lighter viscosity than cultured dairy foods containing locust bean gum (Bassett, 1983).

2.1.6.6. Xanthan gum

It is a polysaccharide gum (D-Glucose + D-Mannose + D-Glucuronic acid) produced on commercial basis from the fermentation of corn sugar by the bacterium *Xanthomonas campestris*. This bacterium is an organism which has been isolated from cabbage plant and has long been known to produce colonies that are viscous and gummy in nature. It is non-pathogenic and non-toxic. It may be used in amounts sufficient for the purpose intended. Too high a usage level causes a rubbery consistency (Bassett, 1983).

2.1.6.7. Gum karaya and gum tragacanth

When the bark of some trees and shrubs is injured, the plants exude a sticky material that hardens to seal the wound and gives protection from infection and desiccation. Such exudates are commonly found on plants that grow in semiarid climates.

Gum Karaya is one such exudates obtained from tree. It is a polysaccharide containing L-rhamnose, D-galactose and D-galacturonic acid units. It does not dissolve in water to give a clear solution but absorbs water readily to form viscous solution at low concentrations. It is used in salad dressings, icepops, sherbets, cheese spreads, meat products, whipped cream products etc.

Gum Tragacanth is an exudate obtained from shrubs. It is a complex polysaccharide containing D-galacturonic acid, D-galactose and L-arabinose, with associated calcium, magnesium and potassium cations. It also contains small amounts of cellulose, proteins and starch. Gum tragacanth swells in cold water to give extremely viscous colloidal solutions, probably the most viscous of all the plant gums. Due to these properties it has been extensively used in low pH salad dressings, confectionaries, ice-creams and bakery products (Klose and Glickman, 1972).

2.1.6.8. Gelatin

Gelatin is a natural stabilizer derived from collagen (Harris, 1990; Imeson, 1997), usually from pigskin, cattle hides, or cattle bones (called ossein). The conversion from this animal protein into gelatin is accomplished by either an acid or alkali hydrolysis pre-treatment. It is not suitable for vegetarians, and has now generally been replaced by other stabilizers. It comprises mostly of amino acids held together with peptide bonds in a left-hand helix with no covalent crosslinking. Proline, hydroxyproline, and glycine are predominant, though nearly all amino acids are found. The exception is tryptophan, though cysteine is only present in minute quantities (Imeson, 1997). The gelatin is melted upon the application of heat due to the relatively low melting point (27 °C to 34 °C). It forms three-dimensional gel structure, properties of which are important in terms of applications in food products.

2.1.6.9. Locust bean gum

Locust bean gum, also known as LBG, carob gum or St. Johns Bread, is extracted from the seeds of the Mediterranean *Ceratonia*

siliqua tree. LBG is a polysaccharide consisting of a mannose backbone with galactose side branches on about a quarter of the mannose units. The side branches occur in blocks, giving LBG 'smooth' regions of free mannose backbone and 'hairy' regions of galactose side groups. LBG is only partially soluble in cold water and it must be heated >85 °C to hydrate it fully. In solution, strong hydrogen bonds can form between the large smooth backbone regions. This leads to gel formation under certain conditions. LBG is the best stabilizer for many ice cream applications and its ability to gel is crucial to some aspects of its use. However, it is also expensive, and subject to large fluctuations in availability and price (Clarke, 2004).

2.1.6.10. Starch

Starch is made up of amylose and/or amylopectin molecules arranged radially. Ordinary starch is common thickener in many cooked foods such as gravies and sauces in acid production such as cultured dairy foods, and it breaks down and loses its natural ability to swell in water and increase the viscosity of the food. Starch chemists have modified starch by cross-linking with phosphates and other edible products so that it doesn't break down in acid products. These modified starches are being successfully used in cultured dairy foods to thicken the body, prolonged shelf life, prevent wheying off to make a better looking all around product (Bassett, 1983).

2.1.6.11. Sodium alginate

Sodium alginate is a polysaccharide of guluronic acid and mannuronic acid, which is extracted from brown seaweeds such as *Macrocystis pyrifera* (found off the Pacific coast of North America,

Australia and New Zealand) and *Laminaria digitata* (from Ireland, Norway, France and Scotland). It consists of a negatively charged polymer chain with ionic bonds to positively charged sodium ions (Na^+). In aqueous solution, the sodium ions dissociate from the polymer so it becomes charged. Calcium ions (Ca^{2+}) or other doubly charged cations can bind to negative charges on two different polymer molecules. These intermolecular interactions lead to the formation of a gel. In ice cream, alginates are blended with phosphate, citrate or tartrate ions to prevent premature gelation due to the calcium from the milk solids. The major advantage of alginate is its resistance to acid conditions, particularly when heated, whereas other stabilizers would lose their functionality (Clarke, 2004).

2.1.7. Incubation

During the manufacture of yoghurt, the heat treated milk is cooled to the incubation temperature of the starter culture (*S. thermophilus* and *L. bulgaricus*) and, in general, the milk is fermented at 40-45 °C, which is optimum growth condition for the mixed culture. In industry, generally two methods of incubation are followed for yoghurt preparation. The actual incubation stage can be take place either in the retailed container for the production of set yoghurt or the milk is incubated in bulk tank for manufacture of stirred yoghurt. Thus the only real differences between set and stirred yoghurt are the rheological properties of the coagulum. In the former type the milk is left undisturbed during the incubation period and the resultant gel is in the form of a continuous semi-solid mass, while in stirred yoghurts breaking of gel structure takes place at the end of incubation period, prior to cooling and further processing (Hammer

1957; Tamime and Robinson, 1999).

Short incubation method: In this method yoghurt is incubated at higher temperature i.e. 40-45 °C for the period of as short as 2½ hours. Culture is added at higher concentration i.e. ≥3 %.

Long incubation method: For this method incubation conditions are 30 °C for around 16-18 hours (over night) or until the desired acidity is reached.

Hrabova and Hylmar (1987) studied the effect of incubation conditions (42 °C for ≥3 hours vs. 30 °C for 17 hours) on rheological properties (viscosity and whey separation) of fruit yoghurt. The influence of coagulum temperature (incubation temp.) during stirring (range – 25 to 42 °C) was also examined, results showed that in order to maintain a good creamy consistency, the temperature must not exceed 37 °C and it was concluded that the low temperature incubation (30 °C for 16-18 hours) had marked favourable effect on the quality of fruit yoghurt, particularly its rheological properties. Same observations were noted by Cho-Ah-Ying *et al.*, (1990). They prepared yoghurt by fermenting milk at 38 °C and 43 °C and concluded that the temperature of incubation had significantly affected only one sensory characteristic (e.g. texture), and overall the yoghurts made at 38 °C had scored higher.

2.1.7.1. Gel formation

The gel formation during incubation of yoghurt is basically due to destabilization of casein complex. The milk casein micelles are composed of different protein fractions and in yoghurt gel they associated with one another via Ca-phosphate bridges. During

fermentation of milk, the micellar or colloidal Ca^{2+} content increases in the serum as the pH is lowered due to the solubilization of micellar Ca-phosphate (Pouliot *et al.*, 1989). Yoghurt gel is the acid gel formed by the acid fermentation of milk. It could be argued, however, that the partially yoghurt gel is enzyme induced gel because proteinases originating from the yoghurt starter culture may have role in the matrix formation of denatured protein (Tamime and Marshall, 1997).

According to Heertje *et al.* (1985) the gel formation is the result of both biological and physical action on the milk, such as the fortification, homogenization and heat treatment of milk base and the catabolism of lactose in the milk by the starter culture for its energy requirement and as a result, the production of lactic acid and other compounds.

2.1.8. Stirring

In case of set yoghurt the coagulum is not subjected to stirring or any mechanical damage and the yoghurt containers are cooled directly. For the organoleptic quality of stirred/drinkable yoghurt the most sensitive production stage is stirring of coagulum after its fermentation. (Silfverberg, 2003).

2.1.9. Cooling

Yoghurt production is a biological process and cooling one of popular methods used to control the metabolic activity of the starter culture and its enzymes. Cooling of the coagulum commences directly when the product reaches the desired acidity or pH (4.6/0.9 % LA). Since the yoghurt organisms show limited growth activity around $<10^{\circ}\text{C}$, the primary objective of cooling is to drop the temperature of the

coagulum from 30-45 °C to <10 °C (best at around 5 °C) as quickly as possible so as to control acidity of the final product. In stirred yoghurt, the coagulum is first stirred in incubation tank before cooling commences, while in case of set yoghurt the coagulum in separate retail containers are cooled without disturbing much. The process of cooling yoghurt may be carried out in one or two phases.

2.1.9.1. One-phase cooling

The coagulum is cooled directly from the incubation temperature to <10 °C prior to the addition flavouring materials and packaging. The product at 20 °C is less viscous compared to <10 °C; as a consequences, the product can be transferred from one section of the processing equipment to another with minimal structural damage. One phase cooling is not widely used in industries (Tamime and Robinson, 1999).

2.1.9.2. Two-phase cooling

This process involves two stages of cooling. In the first stage, coagulum is cooled from 30-45 °C to about 20 °C within 5-6 hours prior to addition of the flavoring materials and filling. In the second stage, cooling takes place in the refrigerated cold store below 10 °C. To achieve maximum effect on yoghurt quality, the second stage of cooling <10 °C must be carried out as slowly as possible over a 12h period (Silfverberg, 2003).

Silfverberg (2003) has described a multistage cooling process for yoghurt in which packed product is subjected to an intermediate stock cooling in a chilling tunnel before reaching the refrigerated cold store. This process entails stock cooling from 42 °C to 30 °C, dysgental stage

from 30 °C to 20 °C, lact-less phase to 14-15 °C, holding phase at 2-4 °C.

The two-phase cooling is widely used in the industry for the production of acceptable viscous yoghurt (Tamime and Robinson, 1999).

2.1.10. Storage

In order to minimize some of biological and biochemical reactions such as fat oxidation, changes in colour of added fruit in acidic medium, physical change in appearance of the exposed surface due to slightly dehydration etc. The storage of yoghurt below 10 °C is essential, and with the provision, the keeping quality of the product could be increased up to three weeks from the date of production (Tamime and Robinson, 1999).

According to Bylund (1995), the shelf life of the stirred yoghurt can be extended up to several months at ambient temperature by altering the post fermentation processing parameters i.e. pasteurization, UHT treatment etc. During the first 24-28 hours of cold storage physical characteristics of coagulum improved mainly due to the hydration of and/or stabilization of the casein micelles, and hence it may be desirable to delay the sale/distribution of yoghurt and to store it at <10 °C (Tamime and Robinson, 1999).

2.2. Influence of stabilizers on quality of yoghurt

Main criteria of quality assessment of yoghurt are mouthfeel, flavour, syneresis, acidity/pH and rheological properties of yoghurt gel. Research has shown that these attributes are significantly affected by the type of stabilizers and their levels used in product preparation.

2.2.1. Texture and rheology

Impact on body and texture of fermented milk products is the prime consideration while developing products. Information available on the application of stabilizers is concerned with their role in controlling syneresis, gelation and consistency of cultured dairy products. With the natural solid content of milk, the gel form is weak and fragile. The mouthfeel is also poor and watery. The addition of 2-5% milk solids as milk powder to the milk base before incubation can remedy this situation. However, the gel shows syneresis. To inhibit this, the milk powder may be totally or partially replaced by some stabilizers like starch, gelatin, isolated milk protein or combinations thereof.

Incorporation of 0.3-0.6 % gelatin could reduce the whey separation, while above 0.7 % gave excessive increase in consistency of the coagulum imparting to yoghurt an atypical jelly like structure but it did not affect the acidification process even when added @ 1.0 % concentration (Ledder and Thomasow, 1975). Pectin was also used in yoghurt and it improved gel strength by 20-30 % when added @ 0.06-0.07 % (Gupta and Prasad, 2000). In another study of pectin, by Zmarlicki *et al.*, (1977), the addition of ≥ 0.15 % pectin caused progressive deterioration of gel structure and consistency, while smaller addition increased gel strength by 20-30 %. Addition > 0.1 % pectin partially increased syneresis and least syneresis was obtained at 0.06-0.07 %. In another study, Jawalekar *et al.*, (1993), also tried gelatin along with other stabilizers, and observed that in the yoghurt prepared from cow/buffalo milks, viscosity and curd tension were highest when gelatin was used followed by Na-alginate and starch. Similar observation

was made by Shukla and Jain (1986) who reported on the basis of Instron parameters that addition of gelatin (0.2-0.3 %) improved the textural characteristics of yoghurt followed by Na-alginate. For Na-alginate the optimum level (0.2 %) in yoghurt was suggested by Jiang *et al.*, (1995) and yoghurt with 0.2 % pectin had best flavour, with a score that was only slightly lower than that of control. Gelatin (0.2, 0.4 and 0.6 %) was tried with other stabilizers such as CMC (0.06, 0.08 and 0.1 %) and starch (0.6, 0.8 and 1.0 %) to study the physico-chemical properties of fat free yoghurt and results indicated that acidity, pH and acetaldehyde content of yoghurt were not clearly affected by any of the applied stabilizers while curd tension and Organoleptic properties were highest for gelatin, followed by starch and CMC (Harby and El-Sabie, 2002).

Changes in certain rheological characteristics of yoghurt due to addition of potato starch have been studied in concentrations of 0.5, 1.0, 1.5 and 2.0 %. Higher than 1.0 % resulted in increased curd tension, while viscosity of set yoghurt increased as the level of starch in milk increased from 0 to 1.5 %. But at 2.0 % level, yoghurt had a slightly decreased in viscosity (Chawala and Balachandran, 1993). In fruit yoghurt also starch was found to give excellent body, texture and flavour (Winterton and Micklejohn, 1978; Jomrichova, 1985). Volker (1971) has suggested to add starches @ 1.8-3.0 % and gelatin @ 0.3-0.8 %. It was also proved that starch and gelatin gave best result in combination compared to starch alone (Thomasow and Hoffmann, 1978; Katz, 1991; McGlinchey, 1997). In another study of (Jogdand *et al.*, 1991) reported that there was no significant improvement in viscosity, consistency and

overall acceptability of yoghurt when the level of starch, gelatin or alginate was increased above 1.5 %.

According to Powell (1969) some stabilizers such as Na-CMC, guar gum, locust bean gum can even at low level destabilize the casein micelles and give rise to a coarse coagulum with an open texture leading to syneresis becomes. And this problem can be minimized by blending with carrageenan and alginate (Dexter, 1976). Gonc *et al.*, (1994) made yoghurt by adding 0.04-0.06 % CMC and results showed that viscosity and consistency of yoghurt increased and syneresis decreased.

2.2.2. Acidity development

The application of additives in indigenous milk product dahi was reported by Baisya and Bose (1974). According to them, dahi sample prepared with or without addition of pectin and gelatin did not show any appreciable difference in their final acidity. Subsequent studies on yoghurt also did not show any significant effect on the biochemical performance of the starters with added gelatin (1-5ml / 100ml milk), glycerol monostearate (0.1-0.6 g / 100ml milk) or their mixtures. Studies on the effect of various additives revealed enhanced acidity in yoghurt prepared with added additives.

Addition of stabilizers lowers the acid development in yoghurt during storage. Higher acid development was recorded at the time of curd setting in yoghurt containing 0.5-1.0 % gum acacia. Maximum acid development was observed in yoghurt with 1.0 % tapioca starch (Gupta and Prasad, 2000). Similarly Athar *et al.*, (2000), who conducted trials using pectin, guar gum, CMC, carrageenan, Na-alginate, corn starch and

gelatin in yoghurt to study the acid development during storage. Maximum increase was recorded in control as compared to other treated samples. Less acidity develop sample treated with corn starch, gelatin and pectin. Thus the acidity in yoghurt especially during storage varied with type and level of stabilizers used and they don't have appreciable effect on acidity development during incubation (Mehanna and Mehanna, 1989).

2.2.3. Flavour development

Stabilizers do not significantly affect the natural flavour of yoghurt. Main compounds responsible for the yoghurt flavour are acetaldehyde, diacetyl and volatile fatty acids. Stabilizers and additives, although improve the quality of yoghurt, cause the decrease in the production of diacetyl and volatile fatty acids content (Shukla and Jain, 1986). Similar trend of results has been reported by Volker (1971) that the use of additive in excess tended to mask the flavour of the product. Stabilizers like gelatin, CMC, gum acacia, sodium alginate and pectin had no adverse influence on flavour producing ability of yoghurt starters, whereas addition of fruit pectins, agar, carrageenans, alginic acid, guar gum, gum Arabica, carrob's gum, kernel flour might reduce the flavour of yoghurt even in small amount (Tamime and Robinson, 1999). Guar gum was added in acidified milk at different levels (0.1-05%) and partition coefficients of acetaldehyde, ethanol and diacetyl were determined by headspace analysis using gas chromatography. Guar gum did not affect the partition coefficients of the flavour compounds under any experimental conditions (Lo *et al.*, 1996).

Thus, review shows that some stabilizers are having beneficial effect on characteristics of set yoghurt. They don't affect the activity of the starter, production of flavour compounds and acidity development during incubation but most of stabilizers lower the acidity during storage. Thus, there is possibility of using stabilizers in the preparation of set yoghurt without addition of SMP or WMP. Hence the present study has been taken to identify suitable stabilizers for the preparation of yoghurt and study their effect on textural and rheological properties of yoghurt.

Chapter- 3

Materials & Methods

CHAPTER 3

MATERIALS AND METHODS

Raw materials used and the methodology employed in the preparation and analysis of set yoghurt are described in this chapter.

3.1. Milk

Fresh, raw cow milk, skim milk and cream were obtained from the Experimental Dairy, NDRI, Bangalore for trials. The milk was standardized to the required level of fat and SNF using appropriate amounts of cream/skim milk.

3.2. Starter Cultures

Separate strains of *S. thermophilus* and *L. bulgaricus*, provided by Dairy bacteriology department, NDRI, Bangalore were used for the production of yoghurt.

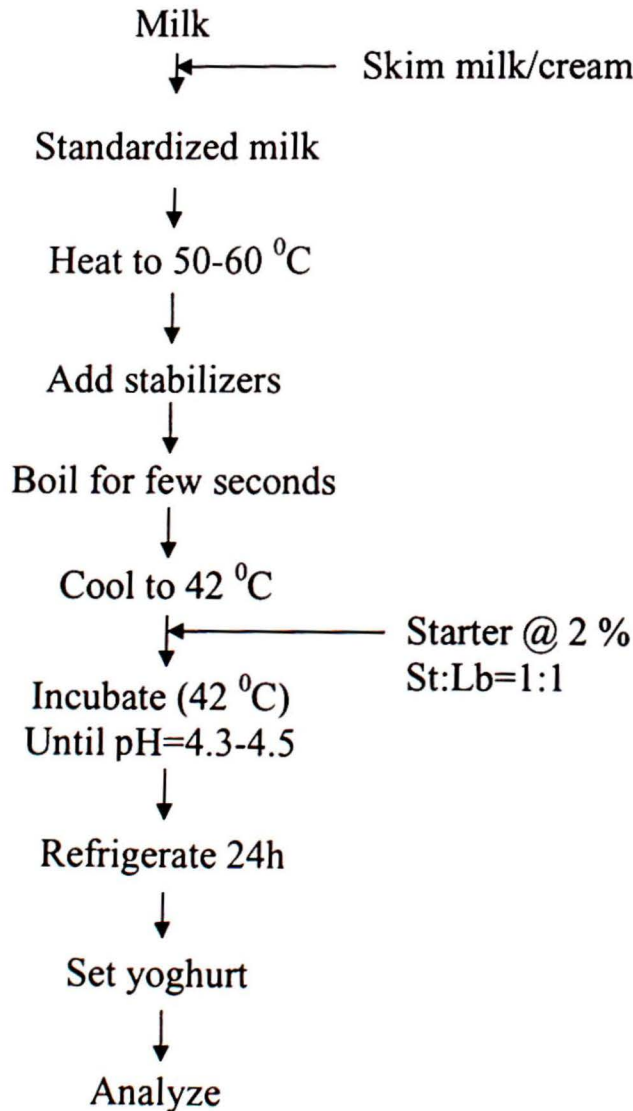
3.3. Stabilizers

Carrageenan (HiMedia Laboratories Pvt. Ltd.), high methoxy pectin (Loba Chemie Pvt. Ltd.), CMC sodium salt (s.d. Fine-chem Limited), guar gum (HiMedia Laboratories Pvt. Ltd.), xanthan gum (Loba Chemie Pvt. Ltd.), gumtragacanth (Loba Chemie Pvt. Ltd.), locust bean gum (HiMedia Laboratories Pvt. Ltd.) and sodium alginate (HiMedia Laboratories Pvt. Ltd.) were used for trials.

3.4. Yoghurt production

3.4.1. Preparation of yoghurt milk

The flow diagram given below shows the process used for making the set yoghurt for this study. Milk adjusted to 3% fat and >8.5% SNF and skim milk with >8.5% SNF and were dispensed equally in different beakers to test for the influence of different stabilizers and their combinations.



3.4.2. Addition of stabilizers

The stabilizers and their levels used in this study were selected after noting results of texture analysis and syneresis during preliminary studies. The milk samples were warmed up to 50-60 °C before the stabilizers were added. Required quantity of stabilizers were mixed thoroughly in a small volume of warm milk separately under constant rapid stirring, using high speed magnetic stirrer and then added to sample milk to set the yoghurt mix.

3.4.3. Preparation of Yoghurt

The method for preparation of yoghurt described by Athar *et al.* (2000) was used with slight modification. The yoghurt mix was boiled for few seconds in SS pan and then the milk was immediately cooled to 42 °C, inoculated with 2 % yoghurt culture and filled in 100ml plastic cups. Then containers were incubated at 42 °C in incubator till body was set and desirable pH was obtained (4.2-4.5). At this stage, the acidity was 0.7-0.9 % lactic acid. The set yoghurt was cooled to 4 °C in order to stop further fermentation and stored for 10 days for further studies. Yoghurt was refrigerated for at least 24h before analysis. Control batch was also prepared without addition of stabilizers.

3.5. Chemicals and Glassware

All the chemicals utilized for the preparation of different reagents were of “Analytical Grade” (AR) and all glassware were properly washed, cleaned and dried.

3.6. Instruments

3.6.1. pH meter

pH meter (Cyberscan 2500, Eutech Instruments) was used. The electrode assembly was calibrated against standard buffer of pH 9.2 and 4.0.

3.6.2. Spectrophotometer

Elico, scanning minispec, SL-177, INDIA was used for absorbance measurements.

3.6.3 Texture analyser

TA-XT plus, Stable Micro System, England was used for measuring the rheological properties of set yoghurt.

3.7. Rheological analysis

The texture analyser was used to evaluate the firmness, consistency and viscosity index of set yoghurt. The following steps were undertaken to run the texture analyser:-

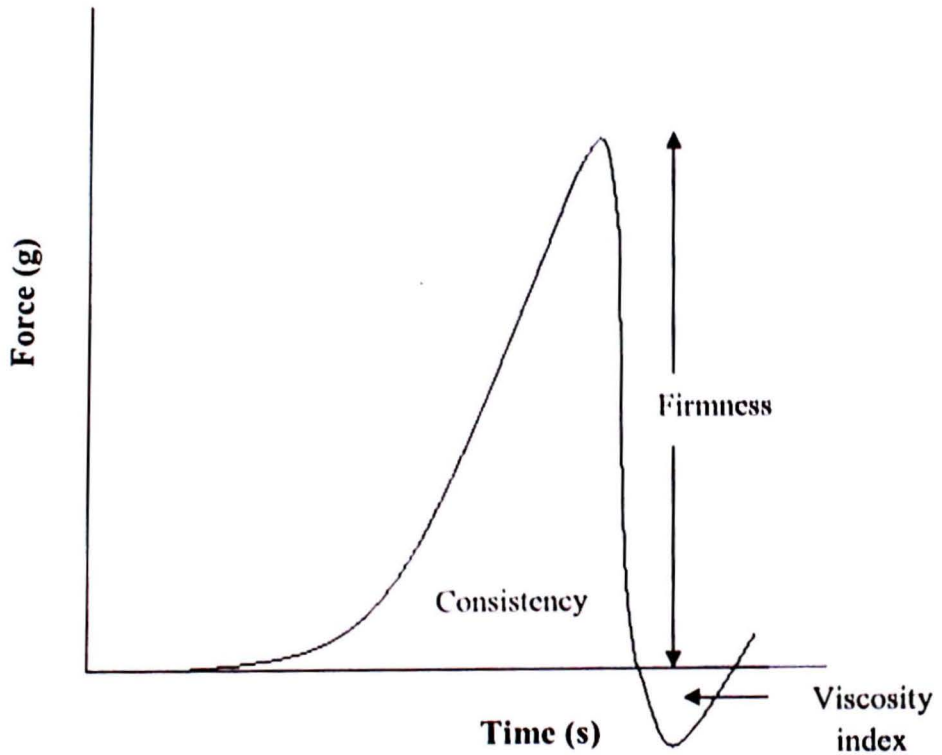
1. Computer was linked to the texture analyser and texture exponent 32 programme was marked.
2. The p/25 aluminium cylindrical probe was fixed to the machine.
3. Under TA setting command, library option was opened.
4. Under library, "return to start" was selected which meant the probe would return to original position after penetrating the

sample to the required distance. The following project settings were loaded:-

Test mode	: Compression
Pre-test-speed	: 1.00 mm/sec
Test-speed	: 2.00 mm/sec
Post-test speed	: 10.00 mm/sec
Target mode	: Distance
Distance	: 10.00 mm
Trigger type	: Auto (Force)
Trigger force	: 2.0 g
Tare mode	: Auto
Advanced options	: On
Break mode	: Off

5. The probe height was calibrated to 50mm.
6. Yoghurt (8 ± 2 °C) in 100ml plastic cup was kept on the platform and probe was positioned centrally and close over the sample surface.
7. When test was run, the probe travelled to a distance of 10mm into the product after 2g of force was sensed by the probe and returned to original position, generating a force-time curve as shown below.
8. The maximum force on positive peak of the curve was noted and expressed as firmness (i.e. the force required to penetrate the probe to the specified distance in to the sample) in terms of grams.
9. Consistency (i.e. work done during the travel of the probe) was determined by calculating the area under the positive peak of the curve in terms of grams seconds.

10. Area under negative peak was taken as viscosity index (i.e. pulling force exerted by the sample during withdrawal of the probe from the product) and expressed as grams seconds.



Force-time curve

3.8. Physico chemical analysis

3.8.1. pH and titratable acidity

Yoghurt pH was measured directly using a pH meter. The electrode assembly was calibrated against standard buffer of 9.2 and 4.0 pH. The titratable acidity (TA) of 24h stored set yoghurt was determined according to the procedure given in I.S.I (1960). Ten grams of the

sample mixed with equal volume of distilled water was titrated against N/9 NaOH using 1.0ml of phenolphthaleine (0.5 %) as indicator. The acidity was expressed as per cent lactic acid (by weight)

3.8.2. Whey separation

Whey separation was measured using centrifugation method (Mangashetti *et al.*, 2003). Thirty five ml of yoghurt mix of each sample were poured into 50ml plastic graduated centrifugation tube and allowed to set within the tubes only. Set yoghurt tubes were stored under refrigeration for 24h. Immediately after this storage, two ventricle cross cuts from top to bottom of coagulum were made using spatula and tubes were centrifuged (International Equipment Company) at 2500rpm for 5min. Volume of supernatant liquid separated on the top of coagulum was visually measured.

3.8.3. Diacetyl content

3.8.3.1. Reagents

The reagents used for the estimation of diacetyl are listed below:-

- 1) Hydroxylamine prepared by dissolving 17.5g of $\text{NH}_2\text{OH}\cdot\text{HCl}$ in 500ml distilled water.
- 2) Acetone-phosphate reagent prepared by dissolving 29g of K_2HPO_4 in distilled water and adding 40ml of pure acetone, and making up the final volume to 200ml with distilled water.

- 3) Alkaline tartrate prepared by mixing aqueous 50% w/v solution of sodium-potassium tartrate and concentrated NH_4OH in the ratio of 22:3.
- 4) Ferrous sulphate reagent prepared by dissolving 5g of FeSO_4 in 100ml 1% H_2SO_4 .

3.8.3.2. Method

Diacetyl content was measured using the method described by Walsh and Cogan (1974). Twenty grams of yoghurt was steam-distilled in a distillation apparatus and the first 10ml of distillate collected. To 5ml of the distillate was added 1.5ml of the hydroxylamine reagent. The mixture was heated to 75°C for 20min in a thermostatically controlled water bath and while still warm, 0.5ml of the acetone-phosphate reagent was added, mixed and cooled to room temperature. 1.5ml of alkaline tartrate reagent was added, mixed and immediately 0.1ml of the ferrous sulphate reagent was added and mixed. The volumes were adjusted to 10ml using 33% K_2HPO_4 and after 15min absorbance was read at 530nm in a spectrophotometer. Standard curve was obtained by distilling sterilized non fat skim milk samples containing different levels (i.e. 1, 3, 5, 7, 9ppm) of diacetyl.

3.8.4. Sensory evaluation

The sensory evaluation of set yoghurt with and without stabilizers with respect to overall sensory acceptance was carried out after 24h of storage under refrigeration, by a panel of judges, on the basis of a 9 point hedonic scale wherein a score of 1 represented “disliked extremely” and a score of 9 represented “liked extremely”. The samples for evaluation were coded appropriately during sensory evaluation.

Chapter- 4

Results & Discussion

CHAPTER 4

RESULTS AND DISCUSSION

The results of the study made on the application of different stabilizers in set yoghurt without added milk solids are presented in this chapter.

4.1. Preliminary selection of stabilizers

Gum tragacanth, CMC sodium salt, carrageenan, Na-alginate, high methoxy pectin, guar gum, xanthan gum and locust bean gum were incorporated into skim milk (<0.5 % fat and 8.5 % SNF) at different levels upto the maximum permitted level of 0.5% and yoghurt was prepared. The appearance of the set yoghurt and the extent of whey separation in the products were noted to draw inference on the applicability of a particular gum.

Guar gum, locust bean gum and xanthan gum caused casein destabilization upon their addition to the milk and that eventually resulted in distinct curd settling at the bottom and whey separation at the top of the yoghurt container, as shown in the Figure 1. Though xanthan is known to be a thickening agent, and is widely used in foods, it has been reported that xanthan would give rubbery consistency to yoghurt curd (Bassett, 1983). These three gums, even at low concentration, were found unsuitable and therefore were not taken up for further studies. Dexter (1976) had suggested that the problem of syneresis would be minimized by blending these gums with carrageenan and alginates.

CMC at 0.5% level resulted in whey separation at the top of the curd and was found unsuitable at high levels. However, at very low levels (<0.1 %), firmness of the curd had improved. CMC would react with casein near its isoelectric point producing soluble complex (Imerson, 1997). As a result, CMC finds use in yoghurt based drinks and in that case the stabilizer is added after fermentation. As in the present study, Imerson (1997) too had noted protein agglomeration and whey separation when it was added prior to fermentation. However, this stabilizer was also included for further studies, especially to find out whether it could be added at low concentration to improve yoghurt firmness.

Sodium alginate and carrageenan, when added at 0.5 % formed gels in milk even before incubation. While marked difference in syneresis was not noticed in the case of sodium alginate, carrageenan appeared to increase the syneresis. Both these gums were selected for further studies at low levels only.

Gum tragacanth decreased the whey separation in yoghurt at all the levels tried. But tragacanth was difficult to be incorporated into milk and gave an inferior appearance to the product at high levels. This gum was taken up for further studies.

Pectin gave yoghurt curd a gelly like structure at higher levels, and also increased syneresis. Same results were reported also by Fox *et al.*, (1990) who concluded that, at extremely high dosage, pectin causes flocculation owing to excluded volume effects. But some improvement in syneresis was noted at low levels. The lower levels might be suited for set yoghurt.

These observations indicated that though addition of stabilizers at 0.5 % is permitted under PFA, none of them, with the probable exception of tragacanth, could be used alone at such concentration for improving the appearance and texture of set yoghurt.

4.1.1. Effect on rheology and syneresis

4.1.1.1. Single stabilizers

4.1.1.1.1. Gum tragacanth

The results presented in Table 1 show the beneficial effect of tragacanth in improving the textural and rheological properties of set yoghurt when it is used at an appropriate level. Firmness of the product improved at all the levels tried, but the optimum was at around 0.2-0.3 % of addition. Similar trend was noticed in the case of consistency also. Consistency increased from 380.11 in the control to 494.85 g.sec at 0.1%

Table 1 – Effect of gum tragacanth on rheology and syneresis of set yoghurt

Parameters	Control	Gum tragacanth %				
		0.1	0.2	0.3	0.4	0.5
Firmness (g)	120.47 ± 0.9	135.65 ± 3.93	141.50 ± 3.12	140.93 ± 0.67	126.72 ± 3.8	123.53 ± 2.9
Consistency (g.sec)	380.11 ± 1.55	494.85 ± 14.08	489.24 ± 31	471.87 ± 36.25	416.52 ± 42.62	382.06 ± 7.89
Viscosity index (g.sec)	15.28 ± 0.58	21.26 ± 0.81	24.10 ± 0.76	21.91 ± 0.20	18.73 ± 0.82	17.37 ± 1.31
Syneresis (ml/35ml yoghurt)	6.5 ± 0.25	4.5 ± 0.25	4.0 ± 0.25	4.5 ± 0.25	5.0 ± 0.25	6.0 ± 0.25

Values are average of four trials

addition of tragacanth. Further increase in the levels progressively reduced the consistency, and at 0.5 % addition the consistency of the product was similar to that of the control. Viscosity index also was maximum when the addition of stabilizer was at 0.2 % level (24.1 Vs 15.28 g.sec in control). Use of tragacanth had reduced the syneresis at all the levels tried, but the maximum beneficial effect was noticed at 0.2% level. Considering all these parameters the optimum level of this stabilizer in set yoghurt could be set at 0.2 %. However, it had to be ascertained whether such addition of the stabilizer would affect the colour and flavour of the product, and consequently the consumer acceptability.

4.1.1.1.2. CMC sodium salt

Based on the preliminary observations that CMC at higher level caused casein destabilization and wheying off in milk, suitability of CMC for set yoghurt was tried at low level of 0.1 % only. Firmness of the curd was markedly higher than in the control (206.5 Vs 118.8 g). Slight increase in consistency (390.8 Vs 360.8 g.sec) was also noted (Table 2). However, viscosity index was markedly less in the treated sample than in the control (12.0 Vs 3.7 g.sec). Syneresis in the product increased nearly 3 fold by the use of this stabilizer even at 0.1 % level (Figure 2). These observations revealed that CMC alone, even at the low level of 0.1% is not suited for set yoghurt, though CMC has been used with advantage in other types of yoghurt. Gonc *et al.*, (1994) had used CMC at very low concentration (0.04-0.06 %) in drinkable flavoured yoghurt to improve viscosity and consistency and to reduce syneresis.

Figure 1 - Curd settling

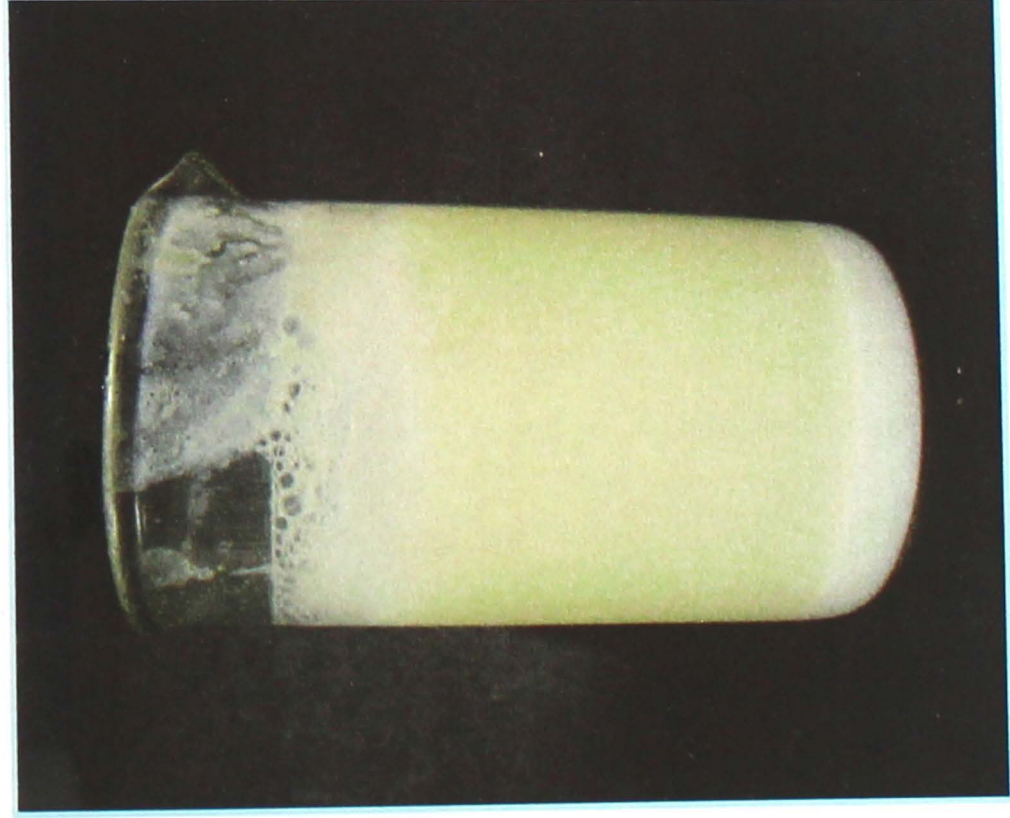
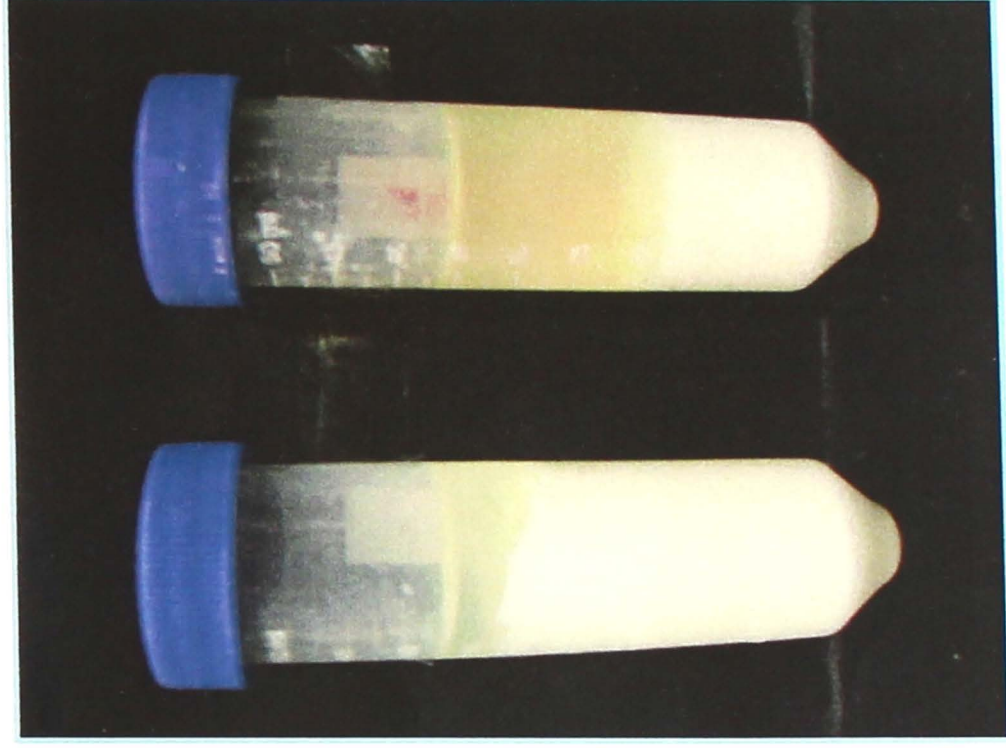


Figure 2 - Syneresis test



4.1.1.1.3. Carrageenan

In the preliminary trials, carrageenan had increased the wheying off in yoghurt considerably especially at higher levels. Further studies revealed that even at a level of 0.1 % there was 2- fold increase in the syneresis (Table 2). Firmness of treated sample (94.38 Vs 118.75 g.sec)

Table 2 - Effect of CMC and Carrageenan on rheology and syneresis of set yoghurt

Parameters	Control	CMC (0.1%)	Control	Carrageenan (0.1%)
Firmness (g)	118.75 ± 4.5	206.50 ± 27.58	135.52 ± 14.32	94.38 ± 7.24
Consistency (g.sec)	360.80 ± 49.22	376.42 ± 17.6	407.16 ± 45.63	330.98 ± 17.41
Viscosity index (g.sec)	12.00 ± 4.38	3.70 ± 2.36	13.78 ± 1.56	13.46 ± 1.10
Syneresis (ml/35ml yoghurt)	6.50 ± 0.75	18.50 ± 1.5	5.5 ± 0.86	11.00 ± 1.47

Values are average of four trials

was found less as compare to its control. Consistency decreased from 407.2 g.sec in the control to 331.0 g.sec in the carrageenan added sample. Therefore, this stabilizer was judged unsuitable for set yoghurt when it is used alone.

4.1.1.1.4. Sodium alginate

The results of the rheological analysis of yoghurt added with different levels of sodium alginate are presented in Table 3. Firmness of the product increased marginally as the concentration of the stabilizer was raised to 0.2 %, but decreased thereafter. Best consistency was

Table 3 – Effect of Sodium alginate on rheology and syneresis of set yoghurt

Parameters	Control	Sodium alginate and pectin %		
		0.1	0.2	0.3
Firmness (g)	119.32 ± 1.16	132.13 ± 1.21	125.86 ± 4.39	108.37 ± 2.30
Consistency (g.sec)	362.93 + 7.27	502.11 ± 8.7	469.76 ± 13.5	393.61 ± 8.67
Viscosity index (g.sec)	13.35 ± 0.57	20.08 ± 0.68	17.55 ± 0.46	12.32 ± 1.04
Syneresis (ml/35ml yoghurt)	6.0 ± 0.25	5.0 ± 0.25	5.0 ± 0.25	8.0 ± 0.25

Values are average of four trials

obtained when the stabilizer added at 0.1% level. The same was the case with viscosity. A perusal of the data presented in the Table 3 shows that alginate could be used with advantages at an optimum level of 0.1 %. Sodium alginate was not suitable at higher concentration because, on its addition, a gel was formed in milk even before incubation and the final product was gummy. Shukla and Jain (1986) had observed the beneficial effect on rheology of yoghurt when alginate was added at 0.2 % level. Jiang *et al.*, (1995) tried both sodium alginate and pectin at low concentration (0-0.2 %) and noticed that alginate gave better sensory score than pectin. Yoghurt added with alginate had smooth shiny appearance.

4.1.1.1.5. Pectin

Pectin at concentrations of 0.4 % and above caused in marked deterioration of the rheological properties of yoghurt. Marginal

improvements in firmness and consistency were noticed upto 0.2 % (Table 4). Slight reduction in syneresis was also noticed at these levels.

Table 4 - Effect of pectin on rheology and syneresis of set yoghurt

Parameters	Control	Pectin %			
		0.1	0.2	0.3	0.4
Firmness (g)	120.47 ± 0.9	130.32 ± 4.5	124.10 ± 14.09	115.33 ± 5.53	80.69 ± 1.31
Consistency (g.sec)	380.11 ± 1.55	402.48 ± 2.61	392.89 ± 1.81	377.45 ± 18.73	228.48 ± 16.0
Viscosity index (g.sec)	15.28 ± 0.58	16.44 ± 0.52	15.05 ± 5.53	11.94 ± 0.55	3.38 ± 0.63
Syneresis (ml/35ml yoghurt)	6.5 ± 0.25	5.5 ± 0.25	5.5 ± 0.25	8.0 ± 0.63	12.0 ± 0.73

Values are average of four trials

However, higher levels increased the syneresis. Gupta and Prasad (2000) had observed that pectin at low concentration of 0.06-0.07 % increased gel strength and reduced syneresis in yoghurt. In the present study also slight improvement in rheological properties was observed at the lowest level (0.1 %). Compared to the effect of tragacanth and alginate, the improvement in quality by addition of pectin was nominal. The results of this study are also supported by Zmarlicki *et al.*, (1977) who tried pectin at different levels in yoghurt prepared from concentrated milk and concluded that addition of >0.1 % pectin causes progressive deterioration of gel strength and consistency and increases syneresis too.

4.1.1.2. Combinations of stabilizers

Though a number of gums are permitted to be used in yoghurt, the observation reported above indicate that none of them could markedly improve the rheological properties or reduce wheying off in set yoghurt when they were used alone. PFA permits the use the above stabilizers in combination. The object of blending these compounds together is to achieve a specific function or to overcome some of the limiting properties such as wheying off, associated with a specific compound. According to Tamime and Robinson (1999) blending carrageenan and alginate with other gums which otherwise improve the rheological properties, may reduce their adverse effect on syneresis. The following three different combinations were tried in yoghurt: (i) Tragacanth and carrageenan (ii) CMC and carrageenan (iii) Alginate and pectin. Gum tragacanth and CMC are thickening agents while carrageenan and alginate have gelling and stabilizing properties (Tamime and Robinson, 1999).

4.1.1.2.1. Gum tragacanth and carrageenan

The effect on rheology and syneresis of the set yoghurt when tragacanth and carrageenan were added in combination (0.05 % each) is shown in the results presented in Table 5. Combination at higher levels had imparted an unacceptable gel like consistency to the yoghurt. Even at the 0.1% level of the stabilizer combination, firmness and consistency of the product had decreased and syneresis had increased. The beneficial effect of tragacanth noticed earlier when it was used alone was lost when it was combined with carrageenan. It is concluded that this combination is not suited for set yoghurt.



4.1.1.2.2. CMC and carrageenan

The effect of the combination of CMC and carrageenan at 0.1% concentration (0.05% + 0.5%) is presented in Table 5. A similar effect as that of the combination of tragacanth and carrageenan was noticed in this case also. Firmness and consistency decreased and syneresis increased as a result of the addition of this combination of stabilizers and hence this combination also was adjudged unsuitable for set yoghurt.

Table 5 - Effect of combinations of tragacanth or CMC with carrageenan on rheology and syneresis of set yoghurt

Parameters	Control	Gum tragacanth (0.05%) + Carrageenan (0.05%)	Control	CMC (0.05%) + Carrageenan (0.05%)
Firmness (g)	117.00 ± 17.25	91.02 ± 12.81	135.52 ± 14.32	114.90 ± 12.80
Consistency (g.sec)	316.12 ± 58.94	276.00 ± 71.83	407.16 ± 45.63	358.62 ± 53.44
Viscosity index (g.sec)	12.42 ± 2.3	12.10 ± 3.13	13.78 ± 1.56	12.73 ± 1.28
Syneresis (ml/35ml yoghurt)	5.00 ± 0.25	7.00 ± 0.40	5.50 ± 0.86	13.50 ± 0.40

Values are average of four trials

4.1.1.2.3. Sodium alginate and pectin

In Table 6, the effect of the use of the 1:1 blends of alginate and pectin during yoghurt production is presented. Yoghurt was found to be gummy and had gel like structure when the concentration of this stabilizer blend was 0.4% or higher. However, at lower concentrations, the rheological properties had improved. A 0.1% concentration of the

Table 6 - Effect of combination of Sodium alginate and pectin on rheology and syneresis of set yoghurt

Parameters	Control	Sodium alginate (0.05%) and pectin(0.05%)		
		0.1	0.2	0.3
Firmness (g)	119.32 ± 1.16	132.13 ± 1.21	125.86 ± 4.39	108.37 ± 2.30
Consistency (g.sec)	362.93 + 7.27	502.11 ± 8.7	469.76 ± 13.5	393.61 ± 8.67
Viscosity index (g.sec)	13.35 ± 0.57	20.08 ± 0.68	17.55 ± 0.46	12.32 ± 1.04
Syneresis (ml/35ml yoghurt)	6.0 ± 0.25	5.0 ± 0.25	5.0 ± 0.25	8.0 ± 0.25

Values are average of four trials

blend in the product was found to increase firmness, consistency and viscosity. Firmness increased from 119.3 g to 132.10 g at 0.1% level. Further increase in the concentration of the stabilizer blend progressively decreased firmness. Similarly, consistency was the highest at 0.1% level of the blend. Viscosity too showed same trend as firmness and consistency. Syneresis was the lowest at 0.1% and 0.2% levels of the stabilizer. Based on the above observations it is suggested that a 0.1% concentration of the blend is most suited for the product. Yoghurt had uniform shiny appearance when the stabilizer concentration was at this level.

The improvement in rheological properties of yoghurt due to the use of 1:1 blend of sodium alginate and pectin at 0.1% level was similar to the effect of addition of 0.1% tragacanth noticed earlier. However,

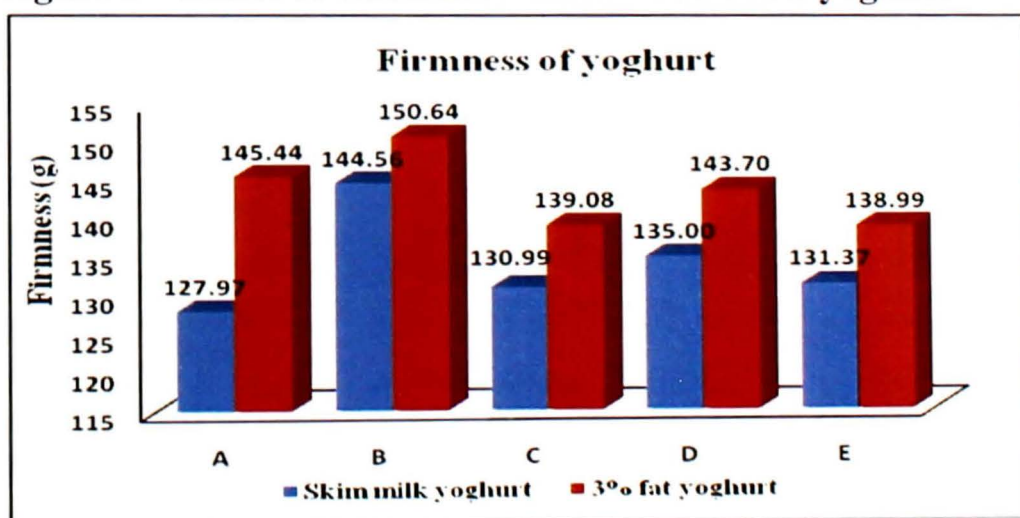
this blend had the added advantage that unlike tragacanth, it could be incorporated to yoghurt mix easily.

4.2. Overall assessment of stabilizers

The preliminary observations on the effect of the stabilizers on rheology and syneresis of yoghurt presented above indicated that gum tragacanth, sodium alginate or pectin alone or combination of pectin and alginate could improve the properties of the set yoghurt. They were to be used at a level much lower than the permitted concentration of 0.5%. However, the overall effect of these gums on sensory acceptance of the product had to be evaluated. Also, it had to be ascertained whether the observations made on the skim milk yoghurt hold good in yoghurt with the recommended 3% fat.

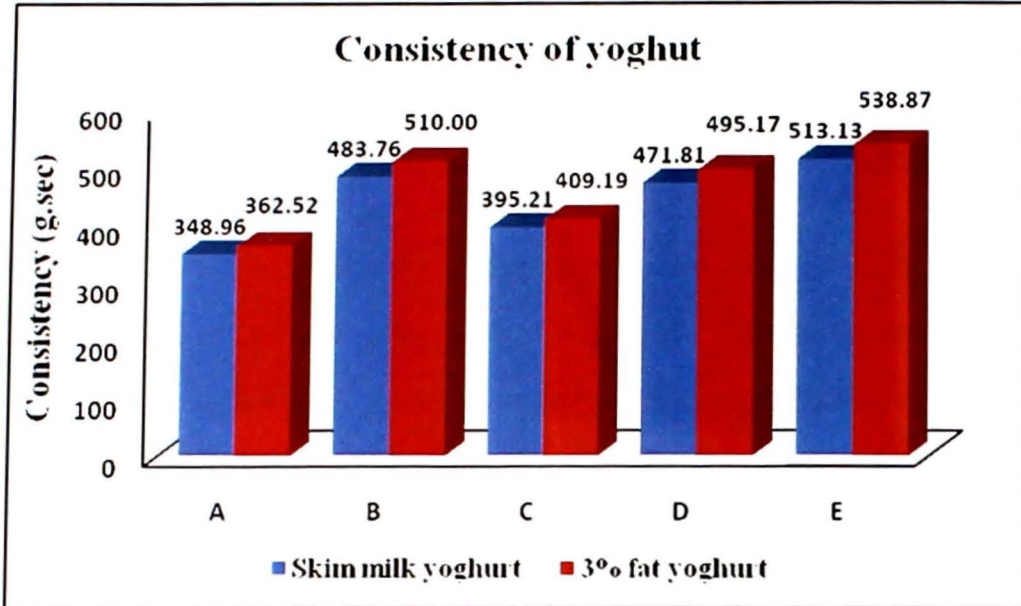
The comparative effects of the stabilizers on firmness, consistency and viscosity of skim milk yoghurt and 3% fat yoghurt are presented in Figures 3, 4 and 5 respectively. It is seen from the figures that firmness

Figure 3 – Effect of stabilizers on firmness of set yoghurt



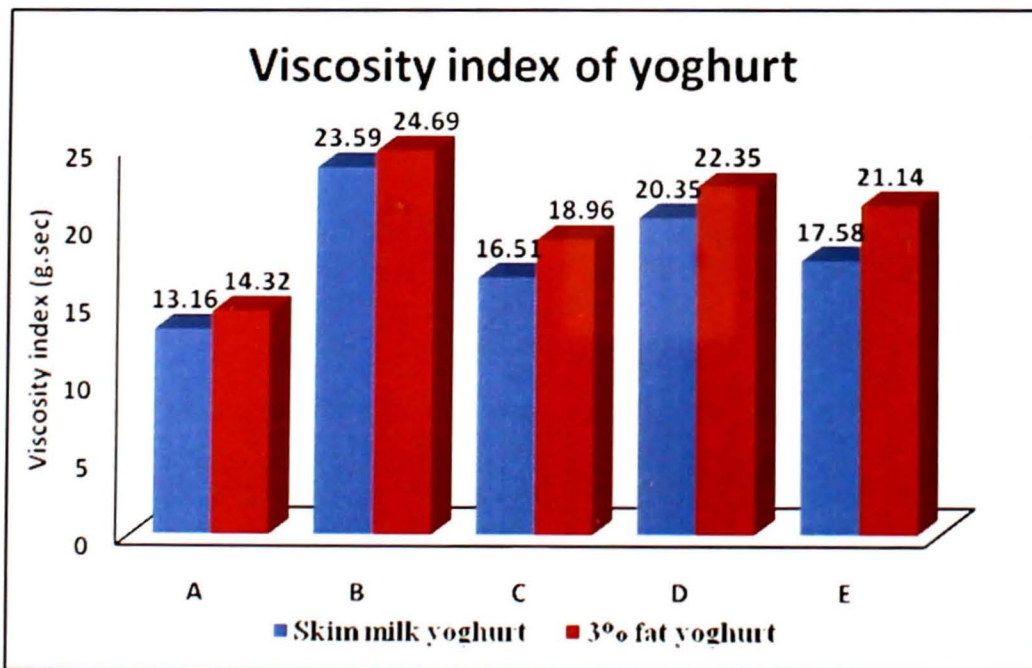
A=Control, B=Tragacanth (0.2%), C=Pectin (0.1%), D=Alginate (0.1%), E=Alginate (0.05%) + Pectin (0.05%)

Figure 4 – Effect of stabilizers on consistency of set yoghurt



A=Control, B=Tragacanth (0.2%), C=Pectin (0.1%), D=Alginate (0.1%), E=Alginate (0.05%) + Pectin (0.05%)

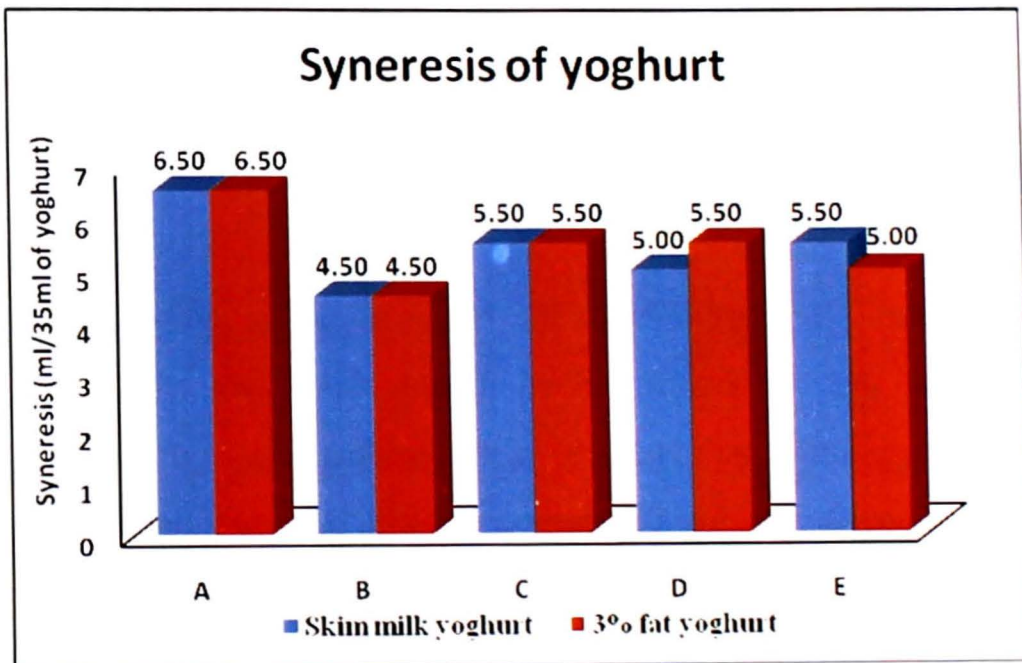
Figure 5 – Effect of stabilizers on viscosity index of set yoghurt



A=Control, B=Tragacanth (0.2%), C=Pectin (0.1%), D=Alginate (0.1%), E=Alginate (0.05%) + Pectin (0.05%)

of the curd and viscosity of the product were influenced the most by tragacanth while the improvement in consistency was the best with the blend of pectin and alginate. The defect of wheying off in the product was also minimized by the addition of tragacanth (Figure 6). These observations were true even for the product with 3.0% fat. Sodium alginate and pectin helped to dispersed fat in curd and therefore cream layer formation was less in the product with these stabilizers.

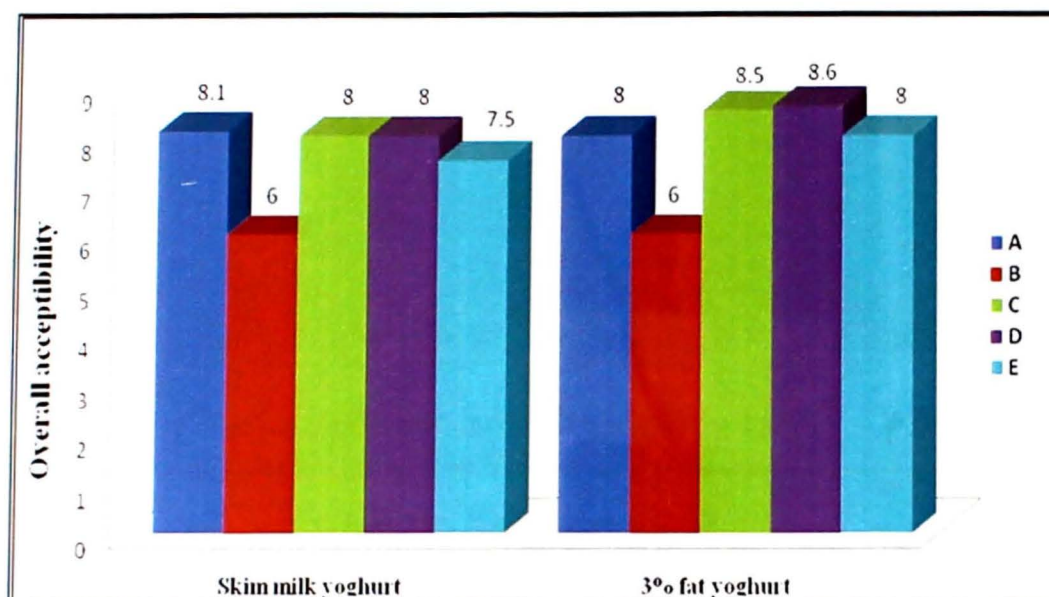
Figure 6 – Effect of stabilizers on syneresis of set yoghurt



A=Control, B=Tragacanth (0.2%), C=Pectin (0.1%), D=Alginate (0.1%), E=Alginate (0.05%) + Pectin (0.05%)

In sensory evaluation, the product with added tragacanth scored significantly less (Figure 7). The low scores were attributed to the following factors: lack of the pleasing flavour, an uncharacteristic brownish green tinge, and small undissolved gum particles in the curd matrix. Statistical analysis of the data in skim milk yoghurt presented in Table 7 shows that none of the stabilizers could improve the overall

Figure 7 – Effect of stabilizers on overall sensory acceptability of set yoghurt



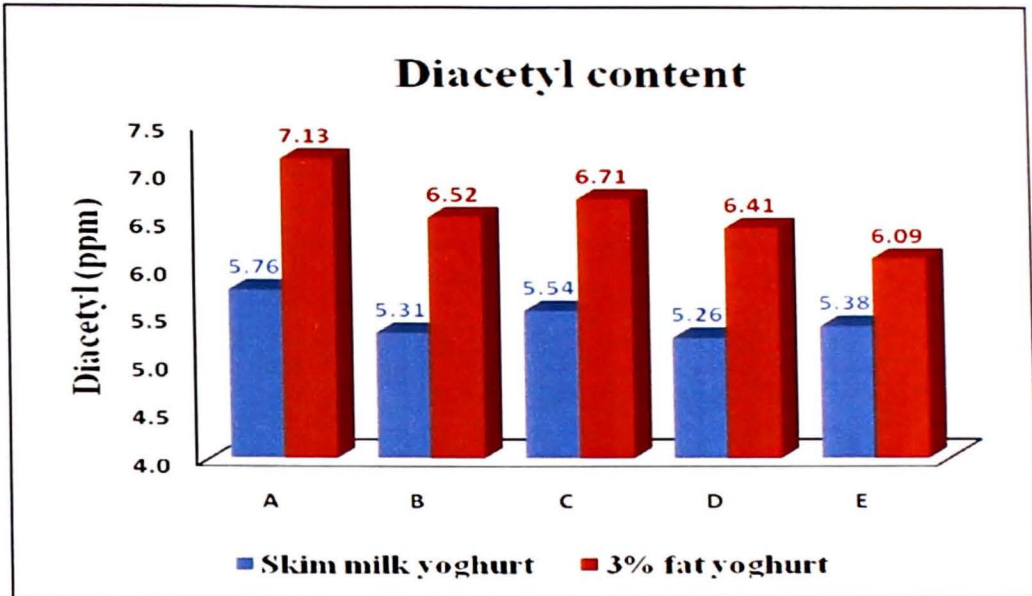
A=Control, B=Tragacanth (0.2%), C=Pectin (0.1%), D=Alginate (0.1%), E=Alginate (0.05%) + Pectin (0.05%)

sensory score. The sample with the blend of pectin and alginate too scored lower than the control. Even though rheological characteristics improved, the overall acceptability of the products was not increased by the use of stabilizers mainly because of flavour deficiency. Volker (1971) had reported that use of these additives tended to mask the flavour of the product. Main compounds contributing to the flavour of yoghurt are acetaldehyde, diacetyl and volatile fatty acids. Shukla and Jain (1986) had noticed that stabilizers decreased the production of diacetyl and volatile fatty acids.

The diacetyl contents of yoghurt added with different stabilizers are presented in Figure 8. As can be seen from the figure, all the stabilizers had a negative impact on diacetyl production. Compared to the product with 3% fat, the yoghurt prepared from skim milk had

developed lower diacetyl content. Addition of stabilizer in skim milk resulted in further reduction in diacetyl production resulting in lower

Figure 8 – Effect of stabilizers on diacetyl production in set yoghurt



A=Control, B=Tragacanth (0.2%), C=Pectin (0.1%), D=Alginate (0.1%), E=Alginate (0.05%) + Pectin (0.05%)

sensory score of the product. Lower levels of diacetyl were noticed in the product even after one week of storage (Figure 9). In yoghurt with 3% fat some improvement in overall sensory score was noticed both with pectin and sodium alginate (Table 8). This was probably because in this case negative impact of stabilizers on flavour production was felt less as fat contributed to production and perception of flavour compounds.

Change in pH is one factor which affects the perception of the flavour. The effect of stabilizers on lowering of pH during production and storage was studied and the results are presented in Figure 10. In general, all the treated samples showed slightly higher pH than the control. However, no marked difference in the rate of decrease of pH

Table 7 - Effect of different stabilizers on rheology, syneresis, diacetyl content and overall sensory score of set yoghurt from skim milk

Variables	Rheological characteristics			Syneresis (ml/35ml yoghurt)	Overall sensory score	Diacetyl content (ppm)
	Firmness (g)	Consistency (g.sec)	Viscosity index (g.sec)			
Control	127.97 ^a	348.96 ^a	13.16 ^a	6.5 ^d	8.1 ^c	5.75 ^d
Tragacanth (0.2%)	144.56 ^d ^c	483.76 ^c	23.59 ^d	4.5 ^a	6 ^a	5.31 ^{ab}
Pectin (0.1%)	130.99 ^{ab}	395.21 ^b	16.51 ^b	5.5 ^c	8 ^c	5.54 ^c
Alginate (0.1%)	135.0 ^b	471.81 ^c	20.35 ^c	5 ^b	8 ^c	5.26 ^a
Alginate + pectin (0.1%)	131.37 ^{ab}	513.13 ^d	17.58 ^b	5.5 ^c	7.5 ^b	5.38 ^b
F-value	9.68	175.56	72.21	64.05	35.23	32.36
CD^{0.05}	5.04	12.71	1.14	0.24	0.2	0.08

Note: Average of four trials; figures with different superscripts in a column are significantly different at ≤ 0.05

Figure 9 - The effect of different stabilizers on diacetyl content of set yoghurt during storage

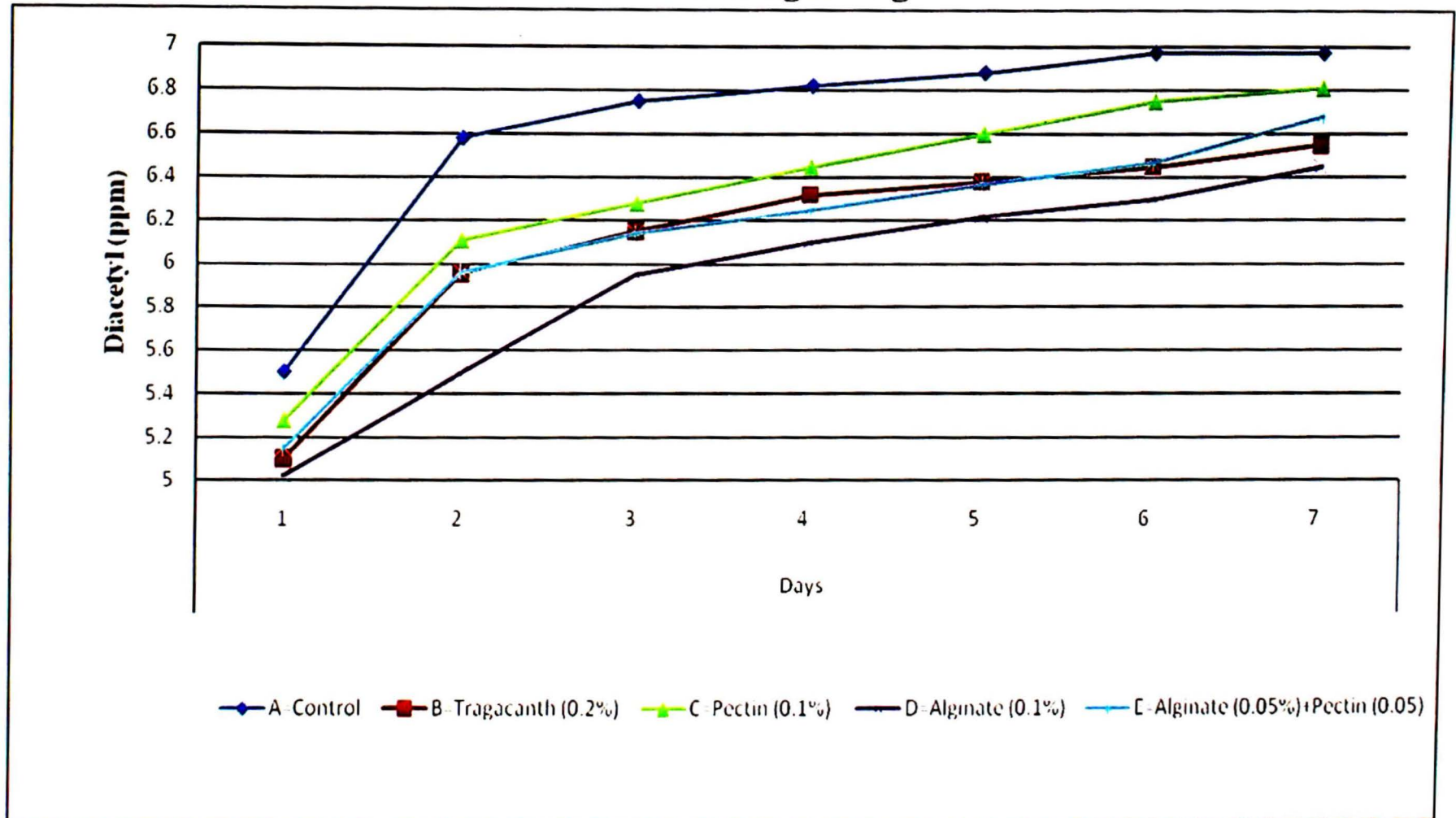
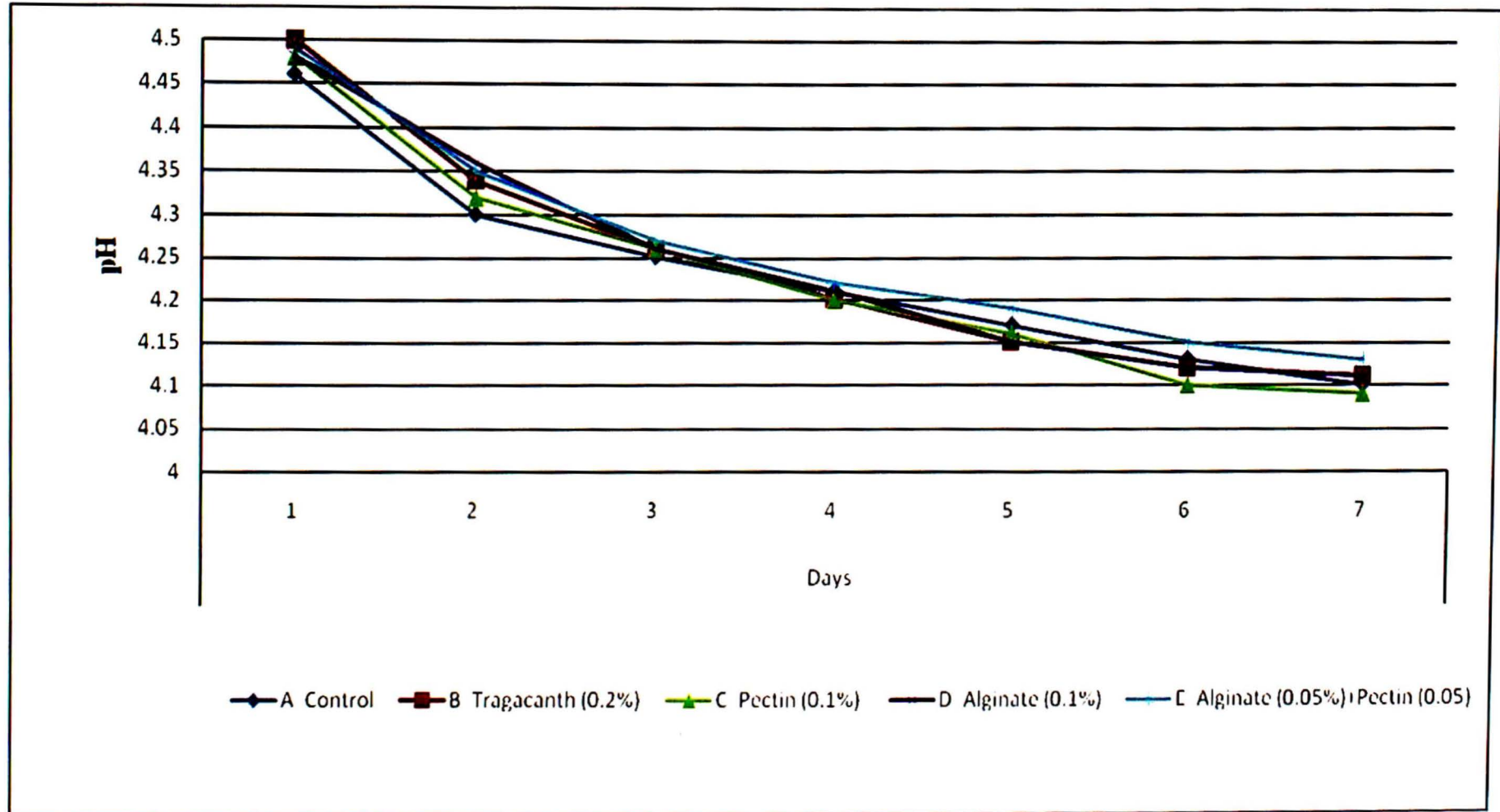


Table 8 - Effect of different stabilizers on rheology, syneresis, diacetyl content and overall sensory score of set yoghurt with 3% fat

Variables	Rheological characteristics			Syneresis (ml/35ml curd)	Overall sensory score	Diacetyl content (ppm)
	Firmness (g)	Consistency (g.sec)	Viscosity index (g.sec)			
Control	145.44 ^b	362.52 ^a	14.32 ^a	6.5 ^d	8 ^b	7.13 ^e
Tragacanth (0.2%)	150.64 ^c	510.00 ^d	24.69 ^e	4.5 ^a	6 ^a	6.52 ^c
Pectin (0.1%)	139.08 ^a	409.19 ^b	18.96 ^b	5.5 ^c	8.5 ^c	6.71 ^d
Alginate (0.1%)	143.70 ^b	495.17 ^c	22.35 ^d	5.5 ^c	8.6 ^c	6.41 ^b
Alginate + pectin (0.1%)	138.99 ^a	538.87 ^e	21.14 ^c	5 ^b	8 ^b	6.09 ^a
F-value	5.08	479.47	135.17	86.75	78.71	246.91
CD^{0.05}	4.31	8.38	0.83	0.19	0.30	0.06

Note: Average of four trials; figures with different superscripts in a column are significantly different at ≤ 0.05

Figure 10 - The effect of different stabilizers on change in pH of set yoghurt during storage



during storage was noticed.

It is concluded from these observations that the beneficial effect of gum tragacanth, pectin and alginate on quality of set skim milk yoghurt through improvement in rheological properties is off set by their negative impact on flavour of the products. However, in set yoghurt with 3% fat, improvement in overall quality was achieved by use of pectin or sodium alginate, at a level much lower than the permitted one.

Chapter- 5

Summary & Conclusions

CHAPTER 5

SUMMARY AND CONCLUSION

Yoghurt is a fermented milk product obtained by lactic acid fermentation through the action of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Yoghurt is claimed to be highly nutritious and easily digestible as compared to milk due to the predigested nutrients by bacterial starter. In addition to good flavour, proper body and texture and minimum wheying off are the essentials to good quality set yoghurt. These characteristics are influenced by milk composition, heat treatment during processing, the starter culture selected for fermentation and the type of stabilizers used. Stabilizers are very small particles of hydrophilic materials and function as thickeners or gelling agents.

The present investigation was to find out the feasibility of using permitted stabilizers in yoghurt and to fix their optimum levels to obtain a product with better consumer acceptability without addition of milk solids. Efforts were made to investigate the effect of different stabilizers and their combinations on physico-chemical properties of set yoghurt, like firmness, consistency, viscosity index, syneresis, pH and flavour development especially diacetyl production.

Gum tragacanth, CMC sodium salt, carrageenan, sodium alginate, high methoxy pectin, guar gum, xanthan gum and locust bean gum have been tried in present study. Though, as per PFA all these are permitted upto a maximum level of 0.5% in yoghurt, none of them, could be used at such high concentration for improving the appearance and texture of

set yoghurt. Among these, guar gum, locust bean gum and xanthan gum were found unsuitable for set yoghurt preparation even at low concentration (0.1%) as they caused casein destabilization upon their addition to milk and that eventually resulted in distinct curd settling. CMC showed marked negative impact on viscosity index and syneresis of yoghurt. Among all the combinations of stabilizers tried, only the sample prepared using a blend of sodium alginate and pectin appeared to have improved rheological properties.

Gum tragacanth, pectin, alginate and a blend of alginate and pectin imparted maximum positive effect on rheology and syneresis of yoghurt at concentrations of 0.2%, 0.1%, 0.1% and 0.1% respectively. The maximum improvement in firmness (144.56 Vs 127.97 g), viscosity index (23.6 Vs 13.16 g.sec) and syneresis (4.5 Vs 6.5 ml/35ml yoghurt) was found in the sample treated with tragacanth, while maximum consistency (513.13 Vs 348.96 g.sec) was noticed in the sample prepared with the blend of alginate and pectin. Alginate and pectin and their blend improved the dispersion of fat in curd and therefore reduced cream layer formation in yoghurt with 3% fat. The overall effect of these gums on sensory score was evaluated. None of the stabilizers could improve the overall sensory score in skim milk yoghurt. The product with added tragacanth scored significantly less, mainly because of flavour defect.

Compounds contributing to the flavour of yoghurt are mainly acetaldehyde, diacetyl and volatile fatty acids. All the stabilizers had negative impact on diacetyl production. Compared to the yoghurt with 3% fat, the yoghurt prepared from skim milk had lower diacetyl content. Addition of stabilizers in skim milk resulted in further reduction in diacetyl production resulting in lower sensory score of the product.

In yoghurt with 3% fat, some improvement in overall sensory score was noticed both with pectin and sodium alginate. This was probably because in this case the negative impact of stabilizers on flavour production was felt less as fat contributed to production and perception of flavour compounds.

Change in pH is one factor which affects the perception of the flavour. The effect of stabilizers on lowering of pH during production and storage was studied. In general, all the treated samples showed slightly higher pH than the control. However, no marked difference in the rate of decrease of pH during storage was noticed.

It is concluded from these observations that the beneficial effect of gum tragacanth, pectin and alginate on quality of set skim milk yoghurt through improvement in rheological properties is off set by their negative impact on flavour of the products. However, in set yoghurt with 3% fat, improvement in overall quality was achieved by use of pectin or sodium alginate, at a level much lower than the permitted one.

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