EFFECT OF SELECTED EXOPOLYSACCHARIDES PRODUCING CULTURE ON QUALITY OF LOW FAT YOGHURT

THESIS SUBMITTED TO THE
NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
(DEEMED UNIVERSITY)
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

MASTER OF TECHNOLOGY
IN
DAIRYING
(DAIRY TECHNOLOGY)

BY

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KARNAL-132 001 (HARYANA), INDIA

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This is to certify that the thesis entitled, "**EFFECT OF SELECTED EXOPOLYSACCHARIDES PRODUCING CULTURE ON QUALITY OF LOW FAT YOGHURT**" submitted by **Mr. KAYUMBA FRED** towards the partial fulfilment for the award of the degree of **Master of Technology** in **Dairying (Dairy Technology)** of the **National Dairy Research Institute (Deemed University)**, Karnal (Haryana), India, is a bonafide research work carried out by him under my supervision, and guidance and no part of the thesis has been submitted for any other degree or diploma.

Dated : 16/07/11

(Dr. R. R. B. Singh)
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DEDICATED

TO

MY FAMILY

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(KAYUMBA FRED)

ABSTRACT

Some of the defects often encountered in low fat set yoghurts are high levels of syneresis, weak body and a less viscous product. Therefore an attempt has been made to produce good quality low fat yoghurt using exopolysaccharides producing cultures (EPS) which play a vital role in technological and physiological functionality of low fat yoghurt. Different levels of fat (0.5, 1.5 and 3.0%) and SNF (9 &10%) were used in conjunction with EPS producing culture for the preparation of low fat yoghurt. The ropy EPS culture was inoculated at 2% level and incubated at 37^{o}C for 7-8 hrs until pH reached 4.6. Sensory attributes, textural parameters, syneresis, viscosity, and EPS concentration were measured as quality criteria during the product development. Statistical analysis of the data revealed that the product with 1.5% fat and 10% SNF was significantly different ($p<0.05$) in terms of body and texture, overall acceptability, firmness, syneresis, EPS concentration and viscosity. The optimized product was stored along with the control (4.5% fat, and 10% SNF) under refrigeration (5 ± 1^{0}C) over a period of 12 days and analysed at 4 days interval for sensory attributes, textural parameters, syneresis, viscosity, pH, total solids, fat, Titratable acidity, proteolysis, acetaldehyde, L-and D-lactic acid, EPS concentration, SPC, coliform and yeast and moulds. The data revealed that flavour (7.93 ± 0.12), colour and appearance (8.20 ± 0.14), body and texture (7.92 ± 0.18) and overall acceptability (7.91 ± 0.11) scores were highest on the 4th day of storage. Viscosity (536.50 ± 5.19), and syneresis (6.25 ± 0.14) were found maximum on the 8th day of storage whereas consistent drop in pH and increase in Titratable acidity continued up to12th day. Proteolytic changes and acetaldehyde concentration increased throughout the storage period. EPS concentration increased significantly ($p<0.05$) during the storage and the maximum level observed was 1083.33 ± 5.46 mg/l on the 12th day. SPC decreased from initial 8.96 ± 0.03 log cfu/ml to 8.55 ± 0.02 log cfu/ml on the 12th day whereas the Coliform and Yeast & Moulds were found absent. Thus the low fat yoghurt prepared with EPS producing culture showed good consumer acceptance, increased viscosity, decreased level of syneresis and better mouth feel in comparison to control.

सारांश

प्रायः निम्न वसा युक्त योग्हर्ट में हल्का स्वरूप, ज्यादा मात्रा में पानी छोड़ना और कम गाढ़ापन जैसे प्रतिकूल लक्षण पाये जाते है। अतः इस शोध में ऐसा प्रयास किया गया जिसमें ई.पी.एस. पैदा करने वाले जामन का प्रयोग किया गया ताकि अच्छी गुणवत्ता वाले निम्न वसा युक्त योग्हर्ट का उत्पादन हो सके, जो कि मूल रूप से प्रौद्योगिकी और स्वास्थ्यवर्धक गुणों में महत्वपूर्ण होगा।

विभिन्न स्तर पर वसा {0.5,1.5 और 3.0 प्रतिशत} और वसारहित ठोस {9,10 प्रतिशत} का प्रयोग, ई पी एस विकसित करने वाले जामन के साथ मिलाकर निम्न वसा योग्हर्ट का उत्पादन किया गया। ई.पी.एस. जामन को 2 प्रतिशत के स्तर पर 37^{o}से0ग्रे0 के तापमान पर 7-8 घण्टे के लिए रखा गया ताकि पी0एच0 4.6 तक पहुंच जाए। निम्न वसा युक्त योग्हर्ट बनाने के लिए संवेदी गुण, संरचना मापक गाढ़ापन एवं ई पी एस की मात्रा को गुणवत्ता का आधार माना गया। सांख्यिकीय विशलेषण से यह ज्ञात हुआ कि 1.5 प्रतिशत वसा और 10 प्रतिशत वसा रहित ठोस से बने उत्पाद में स्वरूप एवं संरचना, स्वीकार्यता, गाढ़ापन, दृढ़ता और ई पी एस की मात्रा में महत्वपूर्ण अन्तर पाया गया। अनुकूलित उत्पाद और नियंत्रित उत्पाद {वसा 4.5 प्रतिशत, वसा रहित ठोस 10 प्रतिशत} का भण्डारण 12 दिनों के लिए प्रशीतन {5^{o}से0ग्रे0} तापमान पर किया और 4 दिनों के अन्तराल पर संवेदी विशेषताएं बनावटी गुण, गाढ़ापन, पी0एच0, कुल ठोस, वसा, अम्लता, प्रोटिओलईसिस, एसटेलंडीहाईड, एल एवं डी लेक्टिक एसिड, ई.पी.एस. मात्रा, एस0 पी0 सी0, कोलीफोर्म और यीस्ट एवं मोल्ड का विशलेषण किया गया। विवरण से यह जानकारी मिली कि भंडारण के 4 दिन बाद स्वाद {7.93±6.12}, रंग एवं रूप {8.20±0.14}, शरीर एवं बनावट {7.92±0.18} और पूर्ण स्वीकार्यता {7.91±0.12} की गणना सबसे अधिक थी। भण्डारण के 8 दिन बाद गाढ़ापन {536.50±5.91} और पानी छोड़ना {6.25±0.14} सबसे अधिक था तथा दूसरी ओर पी एच में निरन्तर पतन और अम्लता में निरंतर वृद्धि का होना अगले 12 दिनों तक जारी रहा। प्रोटियोलाईटिक/विघटित प्रोटीन की मात्रा और ऐसिटेल्डिहाइड की मात्रा भण्डारण के दौरान बढ़ती रही। ई पी एस की मात्रा महत्वपूर्ण रूप से ($P<0.05$) भण्डारण के दौरान बढ़ती रही। ई.पी.एस. की मात्रा महत्वपूर्ण से ($P<0.05$) भण्डारण के दौरान बढ़ी और 12 दिन बाद अधिकतम स्तर 1083.33±5.46 मिलीग्राम/लिटर पाया गया। एस पी सी की मात्र 12 दिन बाद प्रांभिक गणना 8.96±0.03 लोग सीएफयू/मि0लि0 से 8.55±0.002 गिरी और कोलीफोर्म और ईस्ट एवं मोल्ड की गणना पूरे भण्डारण अवधि के दौरान शून्य रही। अतः ई.पी.एस. पैदा करने वाले जामन से बना निम्न वसा युक्त योग्हर्ट नियंत्रित उत्पाद से उपभोक्ता की दृष्टि से गाढ़ेपन में ज्यादा, पानी की अच्छी पकड़ वाला और स्वाद युक्त रहा।

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LIST OF NOTATIONS AND ABBREVIATIONS USED

%	=	Percent
^{o}C	=	Degree Celsius
et al.	=	and co-workers
g	=	Gram
i.e.	=	That is
ml	=	Milliliter
cfu	=	Colony forming unit
h	=	Hour
MSNF	=	Milk solid not fat
rpm	=	Revolutions per minute
sec	=	Second
mg	=	milligram
kg	=	Kilogram
LAB	=	Lactic acid bacteria
NCDC	=	National Collection of Dairy Cultures
EPS	=	Exopolysaccharides
SPC	=	Standard Plate Count
SMP	=	Skim milk powder
cP	=	centipoise
SNF	=	Solid not fat
N	=	Normality
TCA	=	Trichloro acetic acid
pH	=	Hydrogen potential
SDL	=	Sodium Laurry sulphate
H_2So_4	=	Sulphuric acid
µg	=	Micro gram
ANOVA	=	Analysis of variance
C&A	=	Color and appearance
B&T	=	Body and texture

NS	=	Non significant
OA	=	Overall acceptability
Viz	=	Namely
TPA	=	Texture profile analysis
M Pa	=	Mega Pascal
TS	=	Total solids
WA	=	Work of adhesion
WS	=	Work of shear
B.P.	=	Boiling Point
Fig.	=	Figure
D-LDH	=	D-lactate dehydrogenase
NADH	=	Nicotinamide adenine dinucleotide
CLR	=	Corrected lactometer reading
DTM	=	Double toned milk
Vol	=	Volume
PDA	=	Potato Dextrose Agar
PCA	=	Plate Count Agar
VRBA	=	Violet Red Bile Agar
TM	=	Toned milk
SM	=	Skimmed milk
OPA	=	O-phthaldiadehyde
N	=	Normality
aW	=	Weight
ml	=	Millitre
IS	=	Indian standard
USA	=	United states of America
UV	=	Ultra violet
NAD^+	=	Nicotinamide-adenine dinucleotide
Al-DH	=	Aldehyde dehydrogenase

MW	=	Molecular weight
L-LDH	=	L-lactate dehydrogenase
D-GPT	=	D-glutamate-pyruvate transaminase
g/l	=	Gram per litre
FDA	=	Food and Drug administration
Lb	=	*Lactobacillus*
S.thermophilus	=	*Streptococcus thermophilus*
L.bulgaricus	=	*Lactobacillus bulgaricus*
X.tics	=	Characteristics
WHO	=	World health organization
Sig.	=	Significance
df	=	Degree of freedom
N		Newton
Ns		Newton per second

1.0 INTRODUCTION

Yoghurt is a fermented milk product popular all over the world. It has been known to mankind for over 6000 years. Food and Drug Administration (FDA, 1996a, b) has defined Yoghurt as the “food produced by culturing one or more of the optional dairy ingredients (Cream, milk, partially skimmed milk, and skim milk) with characteristic culture that contains the lactic acid-producing bacteria viz., *Lactobacillus delbrueckii subsp*. *bulgaricus* and *Streptococcus thermophilus*”. Generally yoghurt contains not less than 3.25% milk fat and not less than 8.25% milk solids not fat, and has a titratable acidity of not less than 0.9%, expressed as lactic acid. Low fat yoghurt and non-fat yoghurt are similar in description to yoghurt but contain 0.5 to 2% and less than 0.5% milk fat, respectively (FDA, 1996a; FDA, 1996b).

Consumer’s demand for natural, healthy, and low caloric foods is increasing. Dairy industries are looking forward for development and manufacture of low-fat /fat free fermented milk products. Fat plays an important role in the development of flavour, textural and rheological attributes in yoghurt which are the key factors affecting consumer acceptability and its marketability. The limitations of manufacturing low fat or non fat yoghurt particularly from cow milk is that, some of the important textural properties of yogurt viz., firmness and water holding capacity of curd which are responsible for smooth thick consistency essential for desirable mouth feel are often not achieved. Exopolysaccharides (EPS) produced by lactic acid bacteria have been found to over come these limitations thereby resulting in a product with minimum syneresis, improved texture and increased viscosity (Purwandari *et al*., 2007, Behare *et al*., 2010)

Exopolysaccharides (EPS) are high molecular weight carbohydrates composed of a backbone of repeated subunits of monosaccharide like, D-galactose, D-glucose and L-rhamnose, considerably at varying ratio. The EPS are made up of homopolysaccharides and heteropolysaccharides. It is used to describe both types of extracellullar polysaccharides i.e. attached as a capsule with bacterial cell wall or liberated into the medium as ropy polysaccharides (Sutherland, 1972). The quantity and the type of EPS secreted are dependent upon the species as well as the growth conditions. Dextran, mutan, and fructan are some of the examples of EPS produced by lactic acid bacteria (LAB) *Leucoconostoc masenteroides*, *Streptococcus mutans* and *Streptococcus.thermophilus,* respectively (Cerning, 1990). Gram negative bacteria like *Xanthomonas compestris*, *Acetobacter xylinum* and *Sphingomonas paucimovilis* are known to produce EPS i.e. Xanthan, Acetan and Gellan, respectively in greater quantities than LAB, and are commercially available as food additives (Harvey and McNeil, 1998). However, EPS extracted from gram negative bacteria although produced in larger quantities may not be preferred, as it is derived from non food grade organisms and cost of recovery is also high leading to un-economical production (De Vuyst *et al*., 2001). Moreover, addition of purified EPS in the food products may not have similar effects as EPS produced in situ by LAB (Duboc and Mollet, 2001). The in situ EPS production may play useful role in manufacture of a variety of cultured dairy products such as yoghurt, drinking yoghurt, cheese, cultured cream and milk based dessert (Crescenzi, 1995, Bouzar *et al*., 1997, Christiansen *et al*., 1999).

The EPS play an important role in the improvement of physical properties of fermented milks, as they act like stabilizer, viscosifier, or gelling agent and provide a product with natural thickness (Ruas-madiedo and Ryes-Gavilan, 2005). Some authors have reported direct correlation between the concentration of EPS and viscosity of fermented milk products, but no clear relationship has been demonstrated (Van Marly and Zoon, 1995, Dupont *et al*., 2000) except that if a given strain produces more EPS, the viscosity of fermented milk will increase (Ruas-Madiedo *et al*., 2002, De Vuyst *et al*., 2003). EPS producing starter culture also favours the growth of yoghurt culture i.e. combinations of ropy and non-ropy starter cultures deliver a more acceptable product (Laws and Marshall, 2001) than single strain ropy or non ropy. In addition, certain EPS produced by LAB have been shown to exhibit beneficial effects on human health such as cholesterol lowering ability, anti carcinogenic, immunomodulating, antitumoral activities and prebiotic effects. (Harris and Ferguson, 1993). In this context, EPS producing culture offer a natural and more acceptable way of making low-fat/fat free fermented milks without compromising with physico-chemical and sensory properties.

Seeing, the potential benefits of EPS producing culture and contribution of low-fat yoghurt on health and economic aspects, the present study was under taken with the following objectives:

1. To study the effect of fat and SNF levels on the textural and sensory characteristics of low fat yoghurt

2. To evaluate effect of EPS producing culture on the quality parameters of low fat yoghurt.

2.0 REVIEW OF LITERATURE

The available literatures about the study on the effect of selected exopolysacharides (EPS) producing culture on the quality of low fat yoghurt have been reviewed under the following headings:

2.1 YOGHURT

The word has its origin in a Turkish word ŋjugurtò which first appeared in the 8th century (Rasic and Kurman, 1978) deriving from the verb *yoğurtmak*, which means "to blend," a reference to how yoghurt is made. Yoghurt is one of the most popular fermented dairy products widely consumed all over the world. Itôs obtained by lactic acid fermentation of milk by the action of a starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. Bulgaricus*. The role of these two strains in yoghurt manufacture can be summarised as milk acidification and synthesis of aromatic compounds (Serra *et al.,* 2009 and Sahan *et al.,* 2008).

Yoghurt is more nutritious than many other fermented milk products and it exhibits beneficial effects on human health such as cholesterol lowering ability, anticarcinogenic, immunomodulating, antitumoral activities and prebiotic effects which are in addition to other nutrients developed during the fermentation process. Different forms of yoghurt are now available in the market like set, stirred, frozen and liquid yoghurt. To preserve its inherent quality during storage and in particular, its physico-chemical and sensory characteristics, packaging is essential (saint-eve *et al*., 2008). In recent years, there has been increasing demand for a new range of dairy products, including yoghurts which are similar to traditional products but have a low fat content.

2.1.1 Importance of low fat yoghurt

Low-fat or reduced fat yoghurt are formulated or designed so as to meet the dietary requirements of obese people at risk of cardiovascular diseases, diabetics, and persons on weight management diets (Sandrou and Arvanitoyannis 2003). With the changing lifestyle and dietary patterns, certain non communicable diseases have become major health problems worldwide. The major health consequences associated with overweight and obesity are type-2 diabetes, Coronary heart diseases, Hypertension, Gall bladder, and certain types of cancer, dyslipidemia, and insulin resistance (Ramachandran, 2004). The World Health Organization estimated that of the approximately 1.6 billion adults (age 15+) worldwide, at least 400 million adults and 180 million people were overweight and obese and had diabetes, respectively, in the year 2005 (WHO, 2006a and b).

In India, non communicable diseases caused 5.10 million deaths in the year 2002, of which cardiovascular diseases were responsible for 2.78 million deaths (Beaglehole and Yach, 2003). However, there are large disparities in cardiovascular disease mortality in different Indian states (Gupta *et al.,* 2006). The dietary factors such as high intake of fats, sugars, milk and its products and low intake of fruits and vegetables were considered responsible for cardiovascular disease mortality (Gupta *et al*., 2006)***.***

Technology exists today for producing most dairy products with lower fat percentages, but the extent to which fat can be reduced is determined principally by its effect on product quality and acceptability. Various studies have reported that fat affects both the sensory and texture properties of cultured dairy products (Lucey and Singh, 1997; Tamime and Robinson, 1999).

2.2 EXOPOLYSACCHARIDES

Exopolysaccharides (EPS) are high molecular weight carbohydrates composed of a backbone of repeated subunits of monosaccharide like, D-galactose, D-glucose and L-rhamnose, considerably at varying ratios.

Exopolysaccharides like dextran, mutan, and fructan are produced by lactic acid bacteria (LAB) normally *Leucoconostoc masenteroides*, *Streptococcus mutans* and *Streptococcus.thermophilus,* respectively (Cerning, 1990). Gram negative bacteria like *Xanthomonas compestris*, *Acetobacter xylinum* and *Sphingomonas paucimovilis* are known to produce EPS i.e. Xanthan, Acetan and Gellan, respectively in greater quantities than LAB, and are commercially available as food additives (Harvey and McNeil, 1998). However, EPS extracted from gram negative bacteria although produced in larger quantities may not be preferred, as it is derived from non food grade organisms and cost of recovery is too high leading to un-economical production (De Vuyst *et al*., 2001)

2.3 TYPES OF EPS PRODUCED BY LAB

Based on the composition of monosaccharide repeating units, EPS of LAB are described in two groups, homopolysaccharides and heterolpolysaccharides.

2.3.1 Homopolysaccharides

These are a group of polysaccharides which contain one type of monosaccharide i.e. fructose or glucose (Barker and Ajongwen, 1991). Dextran, mutan, and levan are the examples of homopolysaccharides produced by some *Lactobacillus*, *Leuconostocs*, and *Streptococcus* species of which *Leuc. dextranicum* is a well-known dextran producer (Montville *et al*., 1978)

The homopolysaccharides are synthesised by anchored or secreted transglycosylases which are able to catalyze the transfer of a corresponding glycosyl moity. Mutans are synthesized in a similar way by *S.mutans* and *S.sobrinus* (Mont Ville *et al*., 1978). However, mutans differ from dextrans in containing a high percentage of Ŭ-1, 3 linkages which are attributed to the insoluble nature of this type of polymers.

2.3.2 Heteropolysaccharides

Heteropolysaccharides are composed of different types of monosaccharide repeating units (De Vuyst *et al*., 2001). A wide range of LAB including mesophilic and thermophilic can produce heteropolysaccharides; which are composed of repeating units. The important genera for dairy applications are *Lactococcus* and *Streptococcus*. The monosaccharide compositions of the EPS are mostly glucose and glactose and small amount of rhamanose, fructose, mannose, and galactosamine (Stingele *et al*., 1996). In comparison with the homopolysaccharides, the production of heteropolysaccharide by LAB is much lower i.e. 60 to 400mg/L (Stingele *et al*., 1996). Generally, the heteropolysaccharides are synthesized intracellularly at the cytoplasmic membrane utilising sugar nucleotides as pre-cursors for the assembly of polysaccharides chains (Cerning, 1995). Heteropolysaccharides are made by polymerisation of repeating unit precursors formed in the cytoplasm (Cerning, 1990, DeVuyst Degeest, 1999). Heteropolysaccharides contains back bone of repeated sub units that are branched at positions (C_2, C3, C4, or C_6) or unbranched and that consists of three to eight monosaccharides (De Vuyst *et al*., 2001)

Table 2.1 Lactic acid bacteria which produce exopolysacharides and for which the subunit structure is known

Species and strains	Reference
L.lactis subsp.cremoris H414	Gruter *et al*, (1992)
L.lactis subsp.cremoris SBT0495	Nakajima *et al*, (1992)
L.lactis subsp.cremoris B40	Van casteren *et al*, (1998)
L.lactis subsp.cremoris B39	Van casteren *et al*, (2000b)
.lactis subsp.cremoris B891	Van casteren *et al*, (2000a)
L.lactis subsp.cremoris ARH74	Yang *et al*, (1999)
S.thermophilus CNCMI 733	Doco *et al*. (1990)
S.thermophilus EU21	Marshall *et al*. (2001b)
S.thermophilus Sfi6	Stingele *et al*. (1996)
S.thermophilus Sfi12	Lemoine *et al*. (1997)
S.thermophilus Sfi39	Lemoine *et al*. (1997)
S.thermophilus Sfi20	Navarini *et al*. (2001)
S.thermophilus OR901	Bubb *et al*. (1997)
S.thermophilus Rs	Faber *et al*. (1998)
S.thermophilus Sts	Faber *et al*. (1998)
S.thermophilus MR-1C	Low *et al*. (1998)
S.thermophilus EU20	Marshall *et al*. (2001a)
S.thermophilus S3	Faber *et al*. (2001b)
S.thermophilus SY 89 SY 102	Marshall *et al*. (2001b)
S.macedonicus Sc. 136	Vicent *et al*. (2001)
Lb. delbruekii subsp.bulgaricus rr	Robin *et al*. (1996a)
Lb. delbruekii subsp.bulgaricus 291	Gruter *et al*. (1993)
Lb. helveticus TYI-2	Faber *et al*. (2001a)
Lb. helveticus NCDO 766	Robijn *et al*. (1995b)
Lb. helveticus TN-4	Yamamoto *et al*. (1995)
Lb. helveticus Lh59	Stingele *et al*. (1997)

2.4 CHEMICAL COMPOSITION OF EPS PRODUCED BY LACTIC ACID BACTERIA

In the beginning of the EPS studies, it was found that EPS was a molecule like protein. Studies had shown that EPS had a carbohydrate structure with Ŭ- and ɓ-linkages of different types. That difference depends on the formation of main carbohydrate molecules (D-galactose, D-glucose and L-rhamnose) present in different ratios. Moreover, EPS-producing lactic acid bacteria can produce not only one type of polysaccharide but also different types of polysaccharides due to fermentation conditions. In addition, it is possible that the same strains are capable of producing high-molecular mass and low- molecular-mass EPS fractions which do not differ in monomeric composition (De Vuyst and Degeest, 1999).

2.5 FACTORS INFLUENCING EPS PRODUCTION BY LACTIC ACID BACTERIA

EPS production by dairy LAB varies from species to species and range in concentration between 25mg/L to 9800mg/L. The quantities are small in comparison to those of *Xanthan gum* (10-25g/L), an EPS produced by gram negative bacteria *Xanthomonas compestries*. Different media have been developed for EPS production, which include skim milk, whey based medium, semi defined medium, chemically defined medium, and basal minimum medium. It is well established that, the culture conditions and the composition (not only the carbon source) of the culture media influences the EPS yield and the molecular characteristics of the bio polymers. Several factors such as composition of the medium, temperature, and pH greatly affect the EPS production (Gancel and Novel, 1994). Most of the extra cellular polysaccharides producing organisms utilize carbohydrate as their carbon and energy source and either an ammonium salt or amino acid as their source of nitrogen (Cerning *et al*., 1992, Mozzi *et al*., 1995). Macy (1992) studied the effect of some carbohydrate and nitrogenous compounds on the EPS production by ropy lactic cultures isolated from the finished product. The cultures showed ropiness in all the aqueous solutions of lactose, maltose and casein or peptone separately or in combination but the best result was obtained with the casein and lactose combination.

Addition of glucose or sucrose to both milk and milk ultrafiltrate increased EPS production by ropy strains of *L.lactis sub sp lactis, L.lactis sub sp. cremoris* and *L.casei subsp casei* (Cerning *et al.,* 1992). *Lactobacillus delbrueckii subsp.*

bulgaricus NCFB2772 produces considerably larger amount of EPS when grown on glucose or lactose than when grown on fructose to equal cell densities and EPS have different monomer sugar composition. Regarding the temperature of EPS production by LAB, there are two views, first, EPS production is higher when the culture are incubated at sub optimal temperature i.e. 18-25^{o}C than at 30^{o}C for *mesophilic lactococci* and at 35-37^{o}C than at 42^{0}C for *thermophilic culture* (Sutherland, 1972 Macura and Townslay, 1984 Cerning *et al.,* 1992). Second, the EPS production is growth associated i.e. maximum EPS production is achieved when the cell cultures are in exponential phase (Degeest and De Vuyst, 1999, De Vuyst *et al*., 2003). The carbohydrate added to the media not only stimulates culture growth but also increase EPS production by LAB strains. However, type of sugar used in the media is largely dependent on the type of strain used and no unique sugar gives the best result because different organisms use different carbohydrates. The culturing conditions (type of sugar, temperature of incubation) must be optimised for maximal EPS production

2.6 FUNCTIONALITY OF EXOPOLYSACHARIDES PRODUCED BY LACTIC ACID BACTERIA (LAB)

Recently, the concept of a functional starter has been introduced in reference to cultures that possess properties which contribute to food safety and/ or offer one or more organoleptic, technological, nutritional, or health benefits. In this context, some EPS producing lactic acid bacteria strains could cover both technological and health aspects. Their capability to improve the rheological characteristics of fermented milks has been proven and more recently, health benefits have been attributed to some of these biopolymers.

2.6.1 Technological functionality

Exopolysaccharides act as a natural bio-thickening agent to improve the rheology of fermented milk product and act as stabilisers to limit syneresis.The functionality of EPS depends on its composition, charge, structure, spatial arrangement, rigidity and its ability to interact with milk constituents, mainly, ions and proteins. EPS influence the rheology and texture of fermented dairy products at extremely low concentration compared to externally added plant polysaccharides and no clear correlation is observed between EPS concentration and apparent viscosity of the product (Van marle and Zoon, 1995, Dupont *et al*., 2000). The difference in rheological properties of the product added with purified EPS and the product fermented with EPS producing lactic acid bacteria was observed. The milks fermented with EPS producing lactic acid bacteria showed highly cross-linked net works of proteins and EPS compared to milk added with purified EPS (Duboc and Mollet, 2001). Although, EPS concentration in fermented milk is relatively low (De Vuyst and Degeest, 1999), in situ production of EPS by ropy *Streptococcus thermophilus* improves viscosity, water retention and mouth feel of yoghurt (Marshal and Rawson, 1999, Duboc and Mollet, 2001).These EPS producing strains are viewed as an alternative to hydrocolloids as texturing agent (Wacher-Rodarte *et al*., 1993, Doleyres *et al*., 2005).

2.6.1.1 Effect on Viscosity

Exopolysaccharides produced by LAB during fermentation are known to affect cultured milk products significantly (Cerning, 1999). EPS increase viscosity, smoothness, and creaminess of fermented milk (Folkenberg *et al*., 2006). They also give characteristic slimy texture to some fermented milks such as the Scandinavian fermented milk ñVilliò. Milk products fermented with EPS producing culture have been reported to show a high viscosity compared with products produced with non-EPS producing cultures (Marshall and Rawson, 1999).

2.6.1.2 Effect on Ropiness

The presence of EPS often imparts a ropy character to the fermented product (Cerning, 1990) and EPS producing bacterial strains are often designated as ñropy strainsò. Ropiness, however, does not always correlate with the concentration of EPS which make the ropy strains an imprecise term for EPS producing strains. Products with the EPS situated in capsules closely associated with the bacteria (capsular EPS) are found to be less ropy than products with EPS distributed in the total product volume (Hassan *et al*., 1996).The effect of ropy culture on the physical characteristics of fermented milk has been extensively studied (Marshal and Rawson, 1999, Ruas-madiedo *et al.,* 2002). The products made with ropy strains have smooth body, high viscosity and less syneresis than the product made with non-ropy strains (Wacher-Rodarte *et al*., 1993).

One of the major sources of confusion in the literature is that, the term ñropyò and ôEPSò producing are used interchangeably. Ropiness is evaluated by stirring yoghurt, pulling a small portion and measuring the length of the thread formed. A higher hysterisis loop was observed in ropy yoghurt than in non-ropy yoghurt (Folken berg *et al*., 2006).The hysteresis loop indicates the level of structural breakdown during shearing. Hassan *et al*., (1996) suggested that the larger hysteresis loop in the EPS yoghurt indicates more structural break down due to disturbance of EPSï EPS interaction or EPS-protein interactions. Such interactions are absent in the EPS-negative yoghurt, which shows a smaller hysteresis area.

The product made with ropy strains have smooth body, high viscosity, and exhibit less syneresis than the product made with non-ropy strains (Wacher-Rodarte *et al*., 1993). Generally, ropy yoghurt is creamier, smooth (less granular), thicker and less firm (Folken berg *et al*., 2006). Interestingly, an EPS producing culture of *S.thermophilus* produced creamy and firm yoghurt (Folken berg *et al*., 2006). Such cultures would be commercially attractive with high ropiness.

2.6.1.3 Effect on Syneresis

Syneresis (whey separation) on the surface of set type yogurt is considered as a defect. Using ropy-EPS (ropy-exopolysacharides) producing starter cultures, syneresis could be overcome since non-EPS starter cultures had the highest level of syneresis (Amatayakul, *et al*., 2006). Many processing problems such as low viscosity and high syneresis, which occur during yogurt production, may be solved by increasing the total solids of milk or addition of stabilizers. However, fortification of milk with these ingredients may affect the taste and aroma of the final product adversely. Moreover, the use of some stabilizers are restricted or prohibited in some European countries (Amatayakul, *et al*., 2005). For this reason, EPS-producing starter cultures are preferred as they improve the rheological properties of fermented milk. The gel structure and viscosity of the products are affected by the gel formation conditions, as well as the amount and the type of the EPSs produced.

Starter culture and incubation temperature significantly ($p<0.01$) affect whey separation but the interactive effect was not significant. Yoghurts incubated at 37^{o}C and 42^{o}C as well as those incubated at 42^{o}C and 45^{o}C were not statistically different in syneresis. EPS culture and decreased incubation temperature decreased the gel syneresis. In accordance with the findings of Marshall and Rawson (1999), this might be due to high water binding capacity of EPS (Devuyst and Degeest, 1999) as well as modifications of yoghurt microstructure by EPS cultures (Hassan *et al*., 2002). In all treatments, minimum syneresis was obtained at 37^{o}C.This was in agreement with other studies which showed that a lower syneresis value (Lee and Lucey, 2004) limited rearrangement in protein net works at lower temperatures leading probably to less whey separation (Lucey, 2002).

2.6.2 Physiological functionality

The physiological functionality of these EPS carbohydrate polymers has not yet been clearly determined. It has been suggested that EPS produced by some lactic acid bacteria could exert beneficial effects on human health. Among these effects, the possibility of acting as prebiotic substrates has been demonstrated (Korakli *et al*., 2002). For fructan type of EPS, produced by one strain of *Lactobacillus Sanfranciscensis* there was evidence of *a bifidogenic* effect for the levan-type EPS produced by another strain of some species (Dal Bello *et al*., 2001). Recently scientists found some human intestinal *Bifidobacterium* isolates capable of producing EPS and some of these bacteria harbour genes relating to the synthesis of heteropolysaccharides (Ruas-Madiedov *et al*., 2007). In addition, EPS might contribute to human health as antitumar, antIulcer, immunomodulating and cholesterol lowering agents (De Vuyst and Degeest, 1999). Several studies have suggested that lactic acid bacteria and fermented dairy products have anticarcinogenic properties of *Lactococcus.lactis subsp.cremoris* (SBT0495) and when administered orally enhanced the production of specific antibodies in mice, indicating that this EPS may act as adjuvant. The yoghurt starter *L.B.delbrueckii subsp.bulgaricus* (OLL1073R-1) produces an EPS to exert a host mediated antitumor activity (Kitazawa *et al*.,1998).The water soluble EPS from kefir grains was shown to have property of retarding tumour growth when administered orally (Zubillaga *et al*., 2001). But further research is necessary in this area as a pre-requisite to employ the EPS or EPS producing lactic acid bacteria in functional foods.

2.7 APPLICATION OF EPS PRODUCING LACTIC ACID BACTERIA IN REDUCED FAT FERMENTED MILK

Milk fat contributes to the body, texture, and flavour development of dairy products. Fat reduction to satisfy consumers demand leads to textural and functional defects in low fat yoghurt, cheese, *dahi, Kefir* etc. (Mistry, 2001; Haque and Ji, 2003). In case of low fat yoghurt and *dahi*, lack of flavour, weak body and poor texture are the major problems. Mechanical breaking particularly in stirred yoghurt, strongly affects the rheology of the coagulum and favours syneresis since the net work formed by the gel is broken (Duboc and Mollet, 2001).

The consistent manufacture of good texture, mouth feel and stability is important in dairy industry. The manufacturers have used texture promoting or ropy cultures for many years particularly where addition of stabilizers is prohibited (Marshall and Rawson, 1999). These cultures may impart higher flavour intensity to the yoghurt due to carbohydrate masking the flavour, mouth feel and other attributes (Tamime and Robinson, 1999). EPS producing functional starters are particularly interesting if the amount of added solids needs to be reduced and still improve yoghurt viscosity to enhance texture and mouth feel and to avoid syneresis during fermentation or upon storage of the fermented milk products (De vuyst *et al*., 2001)

2.7.1 Low fat yoghurt

The apparent viscosity of skim milk gel made by ropy cultures increased in comparison to that made by non-ropy cultures (Tamime and Robinson, 1999). However, combining two ropy cultures for yoghurt manufacture may not always have additive effect. Marshall and Rawson (1999) found that mixing ropy and non ropy strains of *Streptococcus thermophilus* with a ropy strain of *Lactobacillus delbrueckii subsp.bulgaricus* had a greater effect on viscosity of stirred yoghurt than combining the two ropy strains. The authors suggested the interaction and co-operative growth that occur in mixed cultures would also appear to influence EPS production in yoghurt. The presence of EPS in stirred yoghurt makes the product less susceptible to mechanical damage during pumping, blending, and in filling machines (Robinson, 1981, De Vuyst and Deegest, 1999).

Although mechanical processing step do increase the syneresis of the final product, use of EPS cultures can control this defect. Hassan *et al*., (1996a, 1996b) examined the rheological and textural properties of yoghurt made with strains differentiated as encapsulated non-ropy. Yoghurt made with ropy cultures exhibited increased viscosity and shear stress values, however, differences attributed to the type of polysaccharides secretion (capsule or slime) was apparent. The presence of bacterial capsules may enhance some rheological properties such as viscosity but may weaken the gel structure. This causes lower shear stress value as compared to slime producers which produce more a stretchable gel structure (Hassan *et a.,* 1996a).

The types of EPS produced by the yoghurt bacteria also have effects on the texture and syneresis. Hassan *et al.* (1996 b) observed that Yoghurt made with encapsulated non-ropy cultures had the lowest firmness and curd tension but exhibited less syneresis than encapsulated cultures. They related lower firmness in the yoghurt made by slime producing cultures to the polysaccharides interfering with the casein structure (Hassan *et al.,* 1996b). Several microstructure studies have revealed that it is not the amount of EPS which is important to the rheological properties of fermented milk but the type and therefore the interaction of polymer with the bacterial cell and the milk proteins is important during fermentation (Marshall and Rawson, 1999). Folken berg *et al.* (2005) observed two types of microstructure in yoghurt made with different EPS producing cultures.

2.7.2 Low fat cheeses

Low fat cheeses have poor moisture retention ability. Moisture present in the cheese partially overcomes the problem of rubbery body and texture created by high casein content of low fat products (Mistry and Anderson, 1993; Mistry, 2001). Generally for cheese manufacture, the casein to fat ratio is adjusted between, 0.68-0.70% to obtain desirable textural attributes. Due to fat reduction, the casein to fat ratio is disturbed and casein contents is more in the resultant cheese consequently generating some textural defects. Low fat mozzarella cheese(s) have fewer tendencies to melt which is an undesirable attribute. These defects can be overcome by the use of additives to some extent but may not find a wider acceptance to produce a wholesome product. Incorporation of EPS strains in the starter culture retains significant amount of moisture in a variety of low fat cheese(s) that positively influence their functionality (Awad *et al.,* 2005, Zisu and Shah, 2005). Perry *et al.* (1997) investigated the influence of an EPS producing starter pair *Streptococcus thermophilus* MR-1C and *Lactobacillus delbruekii subsp. bulgaricus* MR-1R on the moisture and melting properties of low fat mozzarella cheese. Cheddar cheese made with EPS-producing *Lactococus lactis* retained more moisture (45.8-47.2%) than control made with non EPS producing strains (44.9-45.8%). The TPA revealed that cohesiveness, chewiness, and tortion shear strain values of the *Lactococcus lactis* cheeses were lower than those of control and the meltability and springiness values were higher because EPS contained cheese had more open structure which is more conducive to mastication and melting. Awad *et al* (2005) also noticed that EPS producing cultures improve texture, melting and sensory characteristics of reduced fat cheddar cheese (Awad *et al* 2005). Ropy *Lactococcus lactis subsp.cremoris* strain (JFRI) produced reduced fat cheddar cheese with moisture in non fat product and textural and melting properties similar to those of full fat type due to the ability of EPS to bind significant amount of free water. Nauth and Hayashu (2004) patented the process of manufacture of fat free cream cheese, added with ropy culture. The fat free cream cheese had firmness, consistency, and flavour comparable to conventional higher fat cream cheese.

2.7.3 Other fermented milk products

Besides yoghurt and cheese(s) the other fermented milk products in which EPS cultures have been shown to affect product rheology are *sour cream*, *kefir*, *dahi* and European cultured dairy products like *Kefir* which is traditional self carbonated slightly alcoholic fermented milk from Eastern Europe (Roginski, 1999, Tamime and Robinson, 1999). *Kefir* is prepared by inoculating kefir grains consisting of homofernentative and heterofermentative lactic acid bacteria, yeast and acetic acid bacteria. The cells are embedded in the Kefiran, a slimy polysaccharides which also has a profound effect on texture of kefir (Duboc and Mollet, 2001).

Dahi is a popular fermented milk of India, similar to yoghurt consumed almost in every house hold. The micro flora of *dahi* consists of the *Lactococcus*, *Streptococcus*, *Lactobacillus* and *Leuconostoc* singly or in combination. Low fat *dahi* made using EPS producing cultures of *Lactococcus lactis subsp.lactis (Lactococcus lactis* NCDC 191) was found to be more acceptable in terms of body, texture and flavour as compared to *dahi* made with non EPS culture NCDC 167.The microstructure studies showed that *dahi* had more open structure and pores with discontinuous which had relatively compact linear structure (Praveen, 2000)

2.8 PHYSIC-CHEMICAL, BIOCHEMICAL AND MICROBIOLOGICAL CHANGES DURING STORAGE OF YOGHURT

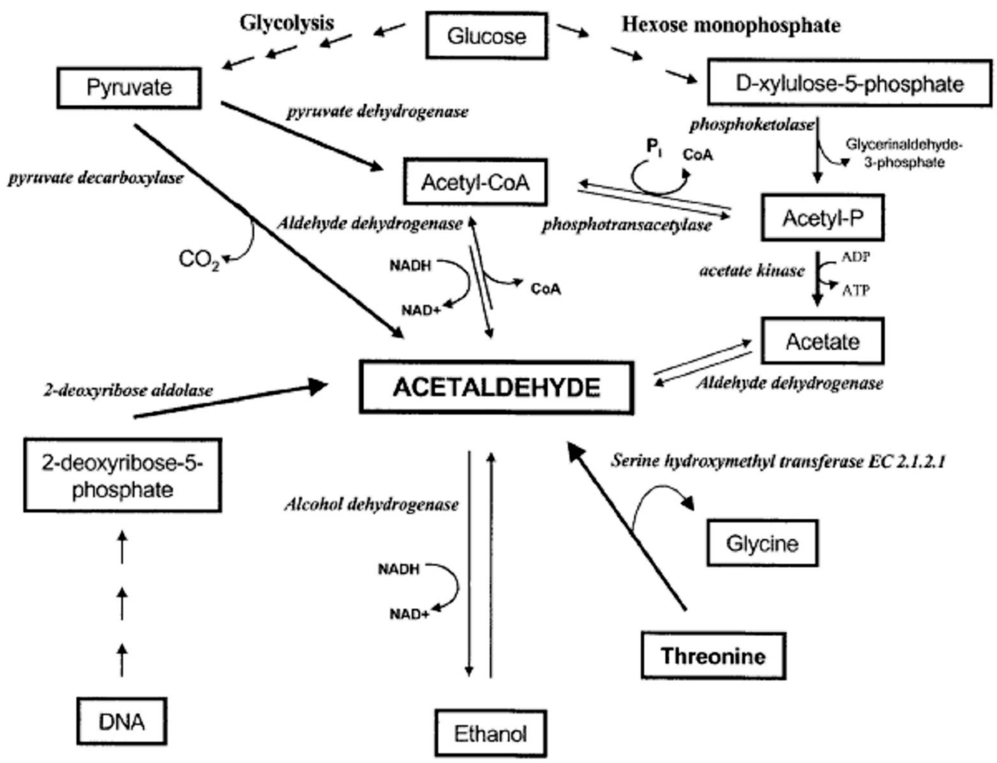
Packaging is essential for preserving the inherent quality of yoghurt during storage. Different authors studied the sorption of aroma compounds from flavoured yoghurt drink by packaging materials (polyethylene). Even at a low temperature of 4^{o}C, these authors observed a decrease in aroma compounds. Different factors can influence this sorption of aroma compounds by packaging: type of packaging material, nature of aroma compounds, composition of food matrices and external environment temperature, storage time and humidity. Regardless of packaging material, some changes in the quality characteristics of yoghurt may also appear during storage. A decrease in pH and sourness of fresh dairy products was observed during storage and was often associated with an increase in yoghurt viscosity

2.8.1 Aroma Compounds in yoghurt

Flavour is a crucial characteristic of foods as the sensory characteristics play an important role in product acceptability by consumers. In fermented dairy products, flavour perception is strongly based on the volatile components (Kalviainen *et al*., 2003). Most commercial yoghurts are produced by the action of two micro organisms, *Streptococcus thermophillus* and *Lactobacillus Delbrueckii subsp. bulgaricus*, which grow simultaneously to give the desired flavour, mouth feel and texture to the yoghurt. Several flavour compounds have been isolated from natural yogurts. The most prominent ones are mainly lactic acid which imparts an acidic and refreshing taste, and a mixture of various carbonyl compounds like acetaldehyde, ethanol, acetone, diacetyl and 2-butanone. However, among them, acetaldehyde is considered as the major flavour compound for the typical yoghurt aroma reported by several researchers (Chaves *et al*., 2002, Ott *et al*., 1997). Both the ratio and balance between these flavour compounds must be taken into account due to their effect on yoghurt flavour, for instance, the ratio between acetaldehyde and acetone of 2.8 is considered optimum (Gardini, *et al*., 1999, Chaves, *et al*., 2002). On the other hand, a greater number of volatile organic compounds identified in yogurt are not only produced by starter culture but they also originate from milk (Beshkova *et al*., 1998).

2.8.1.1 Flavour Formation

Flavour formation occurs by a series of biochemical processes in which the starter cultures provide the enzymes. There are three main pathways responsible for the flavour formation that are identified as the conversions of lactose (glycolysis), fat (lipolysis), and caseins (proteolysis). In the case of glycolysis, firstly lactose is converted to lactate and then a fraction of the intermediate pyruvate is converted to various flavour compounds such as diacetyl, acetone, acetaldehyde and acetic acid. Lipolysis caused by the formation of free fatty acids can be precursors for flavour compounds such as methyl ketones, secondary alcohols, esters and lactones. Acetaldehyde (CH3CHO) a major component of typical yoghurt flavour is an organic chemical compound which is volatile at room temperature. (Miyake and Shibamoto, 1993). Acetaldehyde is produced by yoghurt bacteria, *Streptococcus*. *thermophilus* and *Lactobacillus delbrueckii*.ssp. *bulgaricus*. During milk fermentation, acetaldehyde production in mixed culture occurs immediately after milk coagulation (Beshkova, *et al*. 1998).



However, during storage, the amount of acetaldehyde decreases because of the hydrolysis by microbial enzymes in order to form other substances such as ethanol (Güler-Akın, 2005). Diacetyl and 2, 3-pentanedione increased slightly in yogurt made by mixed cultures during storage at 4^{o}C due to basal metabolic activity of lactic acid bacteria (Ott *et al.*, 1999). It has been reported by some authors that *L. delbrueckii* subsp. *bulgaricus* is a greater acetaldehyde producer than *S. thermophilus*, although other authors have reported the opposite (Ott *et al.*, 1999, Chaves *et al.*, 2002). In fact, the production of acetaldehyde by lactic acid bacteria is strain dependent (Chaves *et al.*, 2002). In general, it has been reported that non polysaccharide-producing strains of yoghurt bacteria produced high levels of acetaldehyde (37 mg/L). In contrast, ropy or viscous strains produce low levels of acetaldehyde (27.6 and 10.4 mg/L) (Ott *et al*., 2000, Bongers, *et al*., 2004). Acetaldehyde can be derived from amino acid (especially threonine, methionine and valine), nucleotide, and pyruvate metabolism in bacteria.

2.8.2 Proteolysis in yoghurt

A high amount of proteolysis in yoghurt during incubation may result in syneresis. Proteolysis in the yoghurt depends on the ratio of the two organisms used for inoculation, incubation temperature and time (Tamime and Deeth, 1980). In addition, free amino acids resulting from excess proteolysis may be precursors for production of undesirable flavours. Proteolysis occurring in yoghurt results from the symbiotic relationship between the two organisms. The proteinase of *Lactobacillus bulgaricus* hydrolyses casein to yield polypeptides which are broken down further by the peptidases of *Streptococcus thermophilus* (Tamime and Robinson, 1986).

The degree of the final amino acid content in yoghurt made from cow's milk may range from 18.7-33mg100ml^{-1}. And it is probable that the acidities of these yoghurts were 1.0-1.4g100g^{-1} lactic acid and during the storage period proteolysis of yoghurt increases and when it exceeds 0.5mg/ml, bitterness in the product was perceived. It is important that total amino acid content of yoghurt reflects a balance between proteolysis and assimilation by the bacteria (A.Y. Tamime and R.K.Robinson, 1999)

2.8.3 Lactic acid in yoghurt

Lactic acid is the end product of lactose hydrolysis. Lactose can be hydrolyzed by homo-fermentative and hetero-fermentative ways. Differences in the amount of lactic acid produced depend on the starter culture, milk type, and manufacturing and storage conditions. Furthermore, lactic acid produced is in D (-) and L (+) forms. L (+) lactic acid is produced during the early fermentation. In contrast, D (-) lactic acid production starts from about the second hour of fermentation and increases continuously. High fermentation temperature and long time storage can cause an increase in the concentration of D (-) lactic acid, thus, the ratio of L (+) / D (-) decreases. Mostly, the level of L-lactic acid is more than that of D-lactic acid and during storage period, both the L and D lactic acids increase.

World Health Organization (WHO) has reported that there is no limit for the consumption of L (+) lactic acid. On the contrary, the concentration of D(-) lactic acid that could be taken per day should be 0-100 mg/kg body weight and should not be used in infant and children diet due to low digestion (Akin, 2006). It is agreed that the typical yogurt flavour is caused by lactic acid which imparts an acidic and refreshing taste (Chaves *et al*., 2002). Besides, itṡ more important trait is that it has increasing effect on regulation of hydrolysis of casein and adsorption of some amino acids, peptides, lactose and minerals as well.

2.8.4 Microbiological evolution of lactic acid bacteria in yoghurt during storage period.

For yoghurt, the manufacture depends on a symbiotic relationship between two bacteria, *Streptococcus thermophilus* and *Lactobacillus bulgaricus,* where each species of bacterium stimulates the growth of the other. This interaction results in a shortened fermentation time and a product with different characteristics than the one fermented with a single species.

A decrease in concentration of lactic acid bacteria was observed upon storage, especially towards the end of shelf-life. Concentration of lactic acid bacteria which should be 10^{-7} cfu /gram until the end of shelf-life is registered only on the first day of product storage. This indicates a low stability of starter culture in fermented dairy products, viable bacterial cells being inhibited by the development of other micro-organisms (first enterobacteriaceae, then yeast and moulds). At the beginning, the count of lactic acid bacteria was between 10^{6} - 10^{7} cfu / gram, counts considered good for the yoghurt and towards the end of shelf-life, the count decreased to about 10^{4} cfu /gram (which is considered inadequate). It was observed that in the last phase of storage, lactic acid bacteria were inhibited by yeast and moulds. Considering that during storage, count of lactic acid bacteria decrease considerably, it is necessary to take all the measures to maintain this count at least at 10^{6}/gram. This concentration is sufficient for the product to contribute to body health and to have a therapeutic effect (Nwamaka *et al*., 2010)

3.0 Scope, Objective and Plan of Work

3.1. SCOPE

Lactic acid bacteria (LAB) play an important role in the food industry, not only because of their ability to acidify and enhance preserve foods from spoilage but also for their involvement in texture, flavour, and aroma development in the fermented food products. Yoghurt is produced by inoculation of good quality milk with *Lactobacillus delbrueckii subsp.bulgaricus* and *Streptococcus thermophilus* as starter culture in a 1:1 ratio.

Consumer demand for natural, healthy, and low caloric foods is increasing in modern times. Dairy industries are looking forward to development and manufacture of low-fat /fat free fermented milk products. Fat removal has several undesirable effects on textural properties of fermented milks such as inferior flavour, high syneresis, poor texture and rheological properties resulting in poor consumer acceptability. In this context, EPS producing lactic cultures offer a natural and more acceptable way for making low-fat/fat free fermented milks without compromising with physico-chemical and sensory properties. Also certain EPS produced by LAB have been shown to exhibit beneficial effects on human health such as cholesterol lowering ability, antcarcinogenic, immunomodulating, antitumoral activities and prebiotic effects. It was therefore that, the present research work objectives was initiated

3.2 OBJECTIVES

- ❖ To study the effect of fat and SNF levels on the textural and sensory characteristics of low fat yoghurt
- ❖ To evaluate effect of EPS producing culture on the quality parameters of low fat yoghurt.

3.3 PLAN OF WORK

3.3.1 Milk collection

Fresh cow's milk collected from the Cattle yard of NDRI-Karnal. Milk was standardised to different levels fat and SNF. The following formulations were used for yoghurt manufacture. (Table 3.1)

Table 3.1 Types of milk used for yoghurt formulation

Types of milk	Fat (%)	SNF (%)
Skimmed milk	0.5	9.0-10
Double toned milk	1.5	9.0-10
Toned milk	3.0	9.0-10
Standardised milk	4.5	9.0-10

3.3.2 Selection of culture

The selected culture in this study was collected from National Collection of Dairy Cultures (NCDC), Dairy Microbiology Division, NDRI-Karnal. The EPS producing *Lactobacillus delbruekii subsp. bulgaricus* NCDC-285, and *Streptococcus thermophilus* NCDC-401 were chosen in combination for preparation of yoghurt and mixed cultures of *Lactobacillus delbruekii subsp. Bulgaricus* and *Streptococcus thermophilus* coded NCDC-263 were used to prepare control yoghurt.

Table 3.2 List of reference cultures

EPS producing culture and control culture used	
Code number	Culture name
NCDC: 285	*L.bulgaricus*
NCDC:401	*S.thermophilus*
NCDC-263. Yoghurt culture (mixture of *L.bulgaricus* and *S.thermophilus*) as control	

3.3.3 Preparation of plain low fat yoghurt

Yoghurt was prepared by inoculating combination of cultures in the ratio of 1:1 (2% level of starter culture) from *thermophilic* group keeping one culture each in the pair from *Streptococcus* and *Lactobacillus genus*.

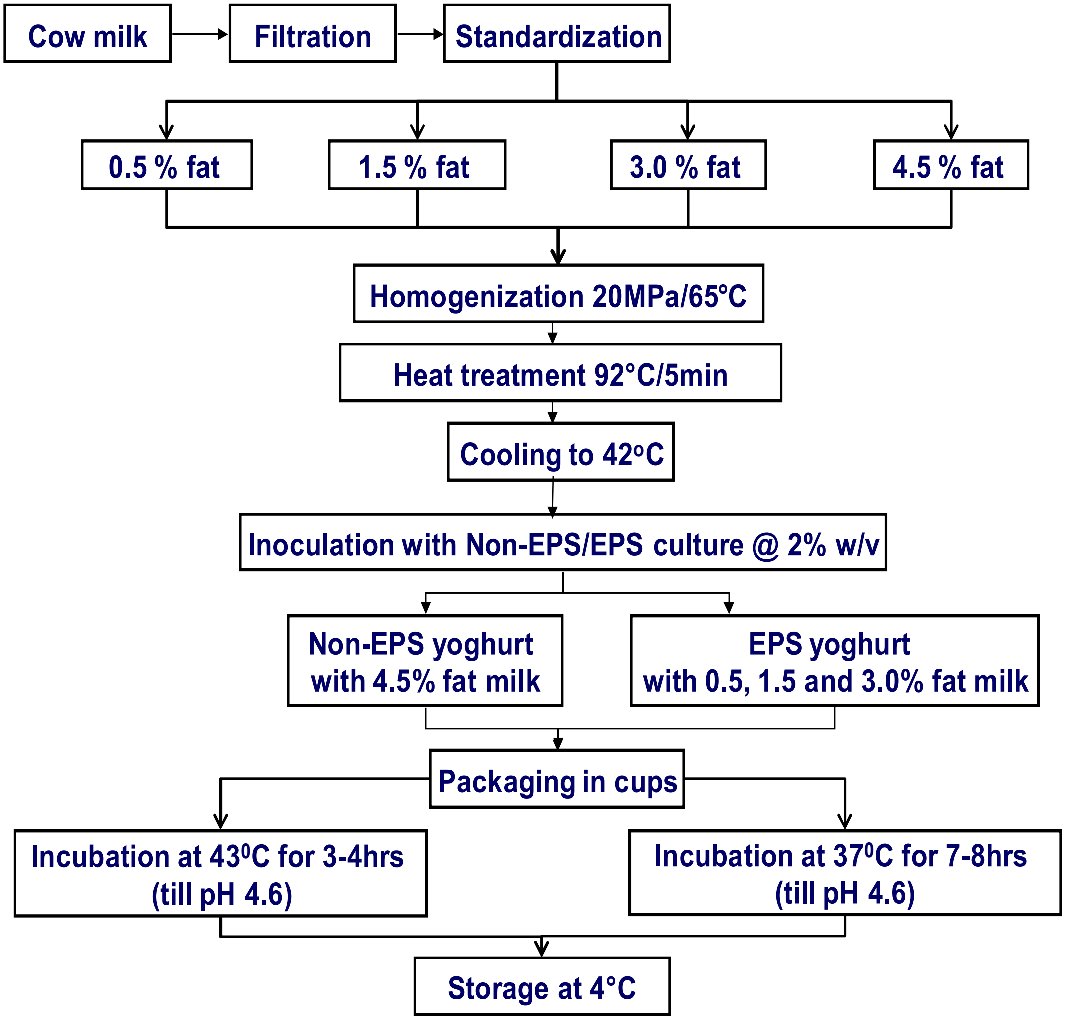


Fig 3.1: Flow diagram for preparation of Low fat yoghurt (*Mutlu et al. 2009*)

The yoghurt samples were evaluated for various sensory, physico-chemical and biochemical parameters for optimisation of the formulation. The optimised products were stored under refrigeration for shelf life studies.

4.0 Materials and Methods

4.1 MATERIALS USED

The materials used were fresh cowᦲs milk collected from Cattle Yard. Skim milk powder (SMP) and packaging cups (polystyrene cups) were obtained from the Experimental Dairy of NDRI-Karnal.

Chemicals used were Megazyme kits from Ireland (Megazyme international Ireland Ltd), extra pure phenol AR and extra pure ammonia solution from Sisco Research Laboratories PVT.LTD. (Mumbai India)

Sodium lauryl sulphate, sodium tetra borate decahydrate, Methanol from Fischer scientific Ltd., whereas, o-phthal diadehyde (OPA), ßeta-mercapto ethanol and potassium hexacyanoferrate (II) were from S D Fine-chem. limited (SDFCL). Concentrated sulphuric acid (H_2SO4) was from Merck specialities private limited (Mumbai India), whereas Trichloroacetic acid (TCA), Zinc Sulphate and Sodium Hydroxide pellets were procured from Qualigens.

4.2 METHODS USED FOR ANALYSIS OF YOGHURT

Yoghurt batches made using EPS producing culture and non EPS producing culture were analysed for physico-chemical, bio-chemical, microbiological and sensory parameters.

4.2.1 Physical properties:

4.2.1.1 Texture profile analysis (TPA)

Texture profile of yoghurt analysis (Firmness, work of adhesion, work of shear, and stickiness) was measured by $TA.XT_2^i$ Texture Analyser (stable micro system, England), fitted with 5 kg load-cell and coupled with Texture Expert-Exceed soft ware. The texture profile test of the set yoghurt (filled 80ml in 100ml glass beaker) was carried out by compression test using P25 aluminium cylindrical probe (25mm Diameter) in penetration mode with the following test conditions: Pre test speed: 1.0 mm/s, Test speed: 1.0mm/s, Post test speed: 10mm/, Rupture test speed: 4.0/mm, Distance: 25.0 mm, Force: 0.98N, Time: 5 sec, Count: 5.0, Load cell: 25 kg, Temperature: 6 ± 1^oC.

The force-time data from triplicate measuments were analysed using the Texture Exceed soft ware and the following TP parameters were worked out.

Firmness= maximum positive peak force (N)

Work of shear= Area of the positive peak i.e. the amount of force required to perform the shearing action (N.s)

Work of adhension = Area of the negative peak (N.s)

Stickiness = Maximum negative peak force (N)

4.2.1.2 Viscosity

The set yoghurt was stirred to homogeneous slurry and tempered to 20^{o}C. Apparent viscosity was measured using contraves rheomat (108 E/R.) make at different shear rate (500, 1000s^{-1}) centipoise, (cp). When constant value appeared it considered as viscosity of the sample. Each sample was measured in triplicates.

4.2.1.3 Syneresis

The syneresis of yoghurt was determined according to Kumar (2004) with minor modifications. The set yoghurt was stirred to homogenous slurry and tempered to 5^{o}C in refrigerator. The slurry was transferred to 15ml plastic centrifuge tubes up to 10ml mark. The tubes were centrifuged for 15minutes in a Remi centrifuge at a speed of 2000 rpm.The volume of clear supernatant was calculated as % syneresis in 10 ml sample.

4.2.2 Chemical analysis

4.2.2.1 Total solid (TS)

Total solid in yoghurt was measured using method described in IS: SP: 18 (Part XI), 1981. Yogurt was mixed using a spoon for 30 sec. and then approximately 3 g was transferred to a previously dried, weighed, and desiccated aluminium dish (Fisher Scientific). Sample was kept in the oven for drying at 101 ±1°C for 8 hrs. Sample was removed, and placed in desiccator for cooling and subsequent weighing. Total solid was calculated as follows:

$$\% \text{ Total solid (TS)} = \frac{\text{Dried sample with dish weight- Dish weight}}{\text{Initial sample weight}} \times 100$$

4.2.2.2 pH determination

The pH was measured using a pH meter (Volkswagen-stiftung WTW, Germany) with a glass electrode and temperature probe standardised at 20°C using pH 4.0 and 7.0 buffers.

4.2.2.3 Fat determination

The fat in yoghurt was measured using butyrometer method as described in IS: SP: 18 (Part XI), 1981. A 20g sample of well mixed yoghurt was taken in a beaker, 5ml of strong ammonia was added to the weighed sample and shaken well to make it homogeneous, Gerber sulphuric acid 10ml was taken into a butyrometer and 10.75 ml of the prepared sample of yoghurt and 1ml of amyl alcohol were added to the butyrometer, the butyrometer was stoppered and shaken slowly to mix the content. The butyrometer was centrifuged at 2000rpm for 5 minutes, removed from centrifuge and the % fat was measured. The result obtained was multiplied by the dilution factor to obtained results.

4.2.2.4 Titratable acidity

Titra**table** acidity was measured by the procedure described in IS: 1479, part1, 1960. A 10g of yoghurt sample was taken in a beaker and an equal amount of distilled water (10ml) added to it. The content was mixed well followed by addition of few drops (2-3) of phenolphtelein indicator. The sample was titrated against 0.1N NaoH till the appearance of light pink tinge, which persist for 30 seconds in the solution. The Titratable acidity was calculated by the following formula and expressed as percentage lactic acid.

$$\text{Acidity (\%lactic acid)} = \frac{9 \times V \times N}{W} \times 100$$

V=Volume of 0.1N NaOH required for titration

N=Normality of NaOH solution

W= Weight of sample (yoghurt) taken for the titration

4.2.3 Bio-chemical Analysis

4.2.3.1 Proteolysis determination

Proteolysis was determined by spectrophotometer assay using o-phthaldialdehyde (OPA) as described by Frank (1983). The OPA reagent was prepared essentially as described by Goodno et al (1981) with minor modifications. The OPA solution was prepared by mixing the following Reagents: 50ml of 100mM sodium tetra borate, 5ml of 20% sodium lauryl sulphate (SLS), 80mg of OPA (dissolved in 2ml of methanol), and 200εl of ɓ-mercaptoethanol) and diluting it to the final volume 100ml with distilled water. The reagent was prepared daily.

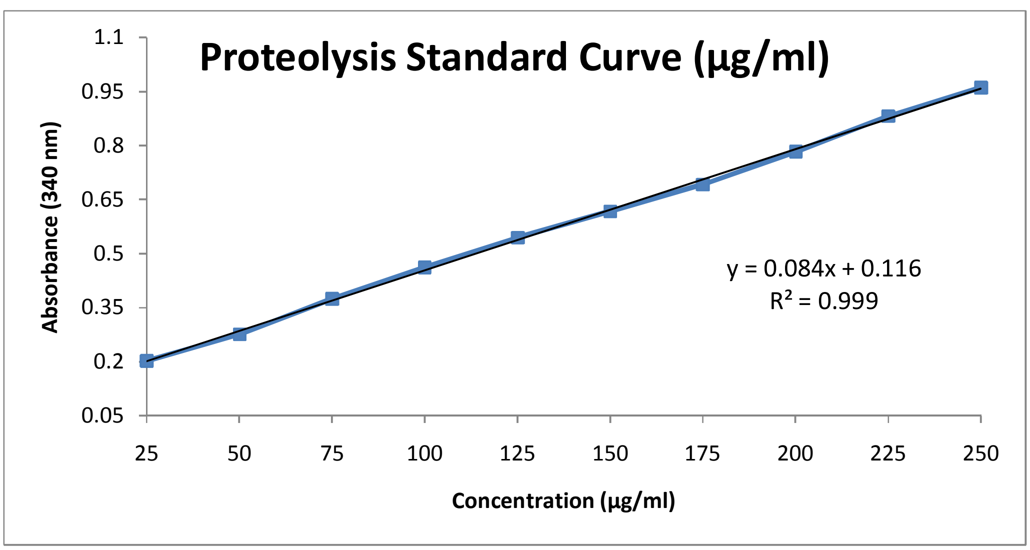
Procedure:

5gm of yoghurt was taken in plastic centrifuge tubes, closure tightened sample and centrifuged at 2000rpm for 5minutes. The supernatant (whey) was collected using syringe with needle and diluted 10 times with distilled water. The mixed whey was filtered using 0.22εm filter paper. Then, 150εl of mixed whey was taken into a quartz cuvette, 3ml of OPA solution added absorbed to it and the content mixed using vortex shaker. After holding the sample for 2minutes at room temperature. The cuvette was placed into the spectrophotometer and the absorbance value recorded at 340nm (thermo spectronic made in USA, model Genesys 10 UV).

4.2.3.2 Preparation of standard curve

The standard curve for proteolysis was prepared using glycine, extra pure AR (molecular weight 75.07) as standard. Standard stock solution was prepared by dissolving 50mg of glycine in 50ml of distilled water and it was subsequently diluted to different concentrations with distilled water. To 150εl of diluted standard solution, 3ml of OPA solution was added and left at room temperature for 2minutes prior to measuring absorbance at 340nm.

Fig.4.1 Standard curve for proteolysis using glycine



Y = 0.084x+0.116

Y = Reading value

x = Amount of proteolysis/peptides present in sample (mg/ml)

4.2.3.3 Acetaldehyde determination

Acetaldehyde content in yoghurt was determined using Megazyme kit as described by Beutler (1988). Sufficient quantity of yoghurt sample (10g) was taken into a 100ml volumetric flask, and 60ml of distilled water was added to it carefully 5ml of prepared carrez I solution , 5ml of carrez II solution and 10ml of NaOH solution (100mM) the content was mixed well after each addition. The volumetric flask was filled to the mark with distilled water, mixes thoroughly and filtered the content through what man number 110mm filter paper.

Table 4.1 Procedure for acetaldehyde determination

Read against air (with out a cuvette in the light path) or against water

Pipette into cuvette	Blank	Sample
Distilled water (at 25^{o}c)	2.10ml	2.00ml
Sample solution	--	0.10ml
Solution 1 (pyrophosphate buffer)	0.20ml	0.20ml
Solution 2 (NAD^+)	0.20ml	0.20ml
Mix well, read the absorbance of the solution (A_1) at 340nm after 2minutes and start the reactions by addition of		
Suspension 3(A_1-D_H)	0.02ml	0.02ml
Mix well, read absorbance of solution (A2) at the end of the reaction for (3-4minutes).If the reaction has not stopped after 5minutes, continue to read the absorbance at 2minutes intervals until the absorbance increases constantly over 2 minutes.		

Measuring blank: After calibration of spectrophotometer, read against air at 340nm. After that, remove the cuvette out of spectrophotometer and fill the cuvette with distilled water up to 2.1ml at 25^{o}C,add 0.2ml of pyrophosphate buffer and add 0.2ml NAD^+ mix well, read the absorbance of solution (A1) at 340nm after 2minutes and start reaction by addition of 0.02ml of suspension 3(A_1-D_H), mix well, read the absorbance of solution (A_2) at the end of reaction for 5minutes.If the reaction has not stopped, continue to read the absorbance at 2 minutes interval until absorbance increases constantly over 2 minutes. The procedure for blank is same as sample only that 0.10ml of prepared sample is added after filling 2ml distilled water into cuvette at 25°C.The remaining procedure is the same to blank.

4.2.3.4 Calculations

Determine the absorbance difference (A_2-A_1) for both blank and sample, and subtract the absorbance difference of the blank from the absorbance difference of the sample, there by obtaining:

The concentration of acetaldehyde which is calculated as follows

C= VxMw x æA acetaldehyde (g/L)
Ɇxdxv

V=final volume (ml)

Mw = molecular weight of acetaldehyde (g/L)

Ɇ = Extinction coefficient of NADH at 340nm

= 6300[1xmol^{-1} x cm^{-1}]

d = light path (cm)

v = sample volume (ml)

It follows for acetaldehyde:

$$C = \frac{2.52 \times 44.05}{6300 \times 1 \times 0.1} \times \text{concentration of acetaldehydes [g/L]}$$

4.2.3.5 Lactic acid determination

D- and L- lactic acids of yoghurt sample were estimated as per the following data.

4.2.3.5.1 Preparation of carrrez 1 solution

Dissolved 3.6g of potassium hexacyanoferrate (II) in 100ml of distilled water and Store at room temperature.

4.2.3.5.2 Preparation of carrez II solution

Dissolved 7.20g of zinc sulphate in 100ml of distilled water and Stored at room temperature.

4.2.3.5.3 Preparation of Sodium hydroxide (NaOH, 100mM) solution

Dissolved 4g of NaOH in 1L of distilled water and Store at room temperature.

4.2.3.6 Determination of D- and L-lactic acid in yoghurt using Megazyme kit

Accurately weighed 1g of homogenised yoghurt into a 100ml volumetric flask containing 60ml distilled water. Add to it 2ml of carrez I solution, 2ml of carrez II solution and 4ml of NaOH solution (100mM).The flask was filled up to the mark with distilled water, mixed well filtered using what man number 110mm and 0.1ml of filtrate transferred to the cuvette. (Noll,. 1988, and Gawehn, 1988)

Table 4.2. Procedure for L-lactic acid determination

Read against air (without a cuvette in the light path) or against water

Pipette into cuvette	Blank	Sample
Distilled water (at 25^{o}c)	1.60ml	1.50ml
Sample solution	——	0.10ml
Solution 1 (glycylglycine buffer)	0.50ml	0.50ml
Solution 2 (NAD^+)	0.10ml	0.10ml
Suspension 3 (D-GPT)	0.02ml	0.02ml
Mix well, read the absorbance of the solutions (A_1) after 3minutes at 340nm and start the reactions by addition of:		
Suspension 4(L-LDH)	0.02ml	0.02ml
Mix well, read the absorbance of the solutions (A2) at the end of the reaction for (10minutes).If the reaction has not stopped after 10minutes, continue to read the absorbance at 2minutes intervals until the absorbance increase constantly over 2minutes		

4.2.3.6.1 Measuring blank

After calibration of spectrophotometer, set the instrument to 340nm, fill distilled water 1.60 ml into cuvette, add glycine buffer 0.5ml, add 0.1ml of NAD^+solution, add 0.02 ml of (D-GPT) mixed well and absorbance of solutions (A1) after 3 minutes at 340nm and start reaction by addition of 0.02ml of (L-LDH) solution, mixed well and absorbance of solutions (A2) at the end of the reaction for 10minutes.If the reaction has not stopped after 10 minutes continue to read the absorbance at 2 minutes interval until the absorbance increase constantly over 2 minutes. The procedure for sample is similar to that of blank except that, for sample 1.5ml of distilled water was added into cuvette and sample solution of 0.1ml was added also.

4.2.3.6.2 Calculation for L-lactic acid:

Absorbance difference (A_2-A_1) for both blank and sample were determined and the reading of blank was subtracted from sample reading.

The concentration of L-lactic were calculated using the following formula

$$C = \frac{VxMw}{Ɇxdxv} \text{ x concentration of L-lactic acid (g/L)}$$

V = final volume (ml)

Mw = molecular weight of L-lactic acid (g/L)

Ɇ = Extinction coefficient of NADH at 340nm

= 6300[$1xmol^{-1}$ x cm^{-1}]

d = light path (cm)

v = sample volume (ml)

It follows for D-lactic acid:

$$C = \frac{2.24 \text{ x } 90.1}{6300 \text{ x } 1.0 \text{ x } 0.1} \text{ x concentration of D-lactic acid [g/L]}$$

If the sample has been diluted during preparation, the result must be multiplied by dilution factor.

Table 4.3 Procedure for D-lactic acid determination

Read against air (with out a cuvette in the light path) or against water

Pipette into cuvette	Blank	Sample
Distilled water (at 25^{o}c)	1.60ml	1.50ml
Sample solution	--	0.10ml
Solution 1 (glycylglycine buffer)	0.50ml	0.50ml
Solution 2 (NAD^{+})	0.10ml	0.10ml
Suspension 3 (D-GPT)	0.02ml	0.02ml
Mix well, read the absorbance of the solutions (A_1) after 3minutes at 340nm and start the reactions by addition of:		
Suspension 5(D-LDH)	0.02ml	0.02ml
Mix well, read the absorbance of the solutions (A2) at the end of the reaction for (5minutes).If the reaction has not stopped after 5minutes, continue to read the absorbance at 1minute intervals until the absorbance either remain the same, or increase constantly over 1minute		

4.2.3.6.3 Calculation for D-lactic acid:

Absorbance differences (A2-A1) for both blank and sample were obtained Blank reading were subtracted from sample reading. The concentration of D-lactic acid were calculated according to the following formula

$$C = \frac{V \times Mw}{Ɇ \times d \times v} \times \text{concentration of D-lactic acid (g/L)}$$

V=final volume (ml)

Mw = molecular weight of D-lactic acid (g/L)

Ɇ = Extinction coefficient of NADH at 340nm

= 6300[1xmol^{-1} x cm^{-1}]

d = light path (cm)

v = sample volume (ml)

If the sample has been diluted during preparation, the result must be multiplied by dilution factor.

4.2.3.7 Extraction and quantification of Exopolysaccharides (EPS)

4.2.3.7.1 Extraction of Exopolysaccharides (EPS)

0ne ml of yoghurt sample was taken into a 2ml size epperndof tube and boiled at 100^{o}c for 10min.The casein present in the sample was precipitated by adding 200εl of 85% trichloroacetic acid and centrifuged at 10,000rpm for 15min to remove cells and protein. The supernatant liquid was transferred into a new eperndorf tube, and 2 volume of 95% ethanol was added to the supernatant and kept over night at 4^{0}C for precipitating EPS. The EPS precipitated with ethyl alcohol was collected after centrifugation.

4.2.3.7.2 Quantification of EPS in yoghurt sample

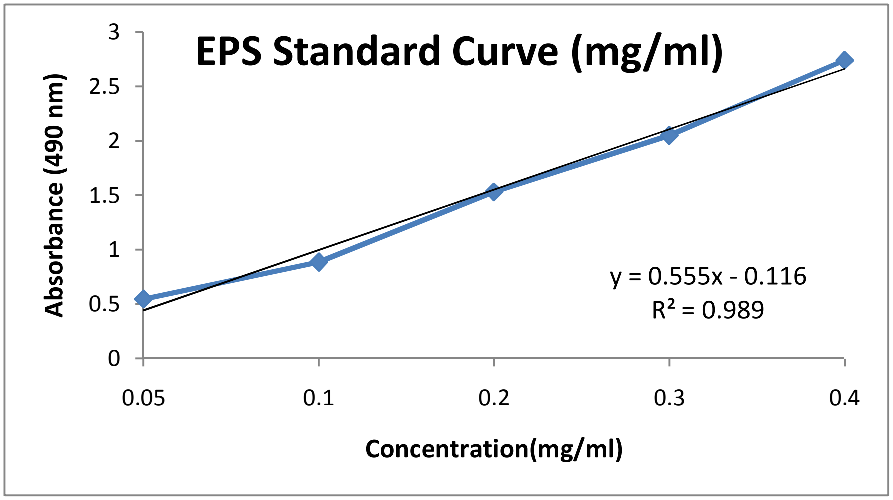
Total EPS (expressed as mg/L) in each sample was quantified calorimetrically by phenol sulphuric acid method described by Dubois *et al*., (1956).

Precipitated pellets were dissolved in 1ml of hot distilled water (55-60^{o}C). This sample solution was diluted 10 times using distilled water, and 1ml of diluted sample was taken into a glass test tube, to this were added 1ml of 5% pure phenol and 5 ml of concentrated sulphuric acid. The content was hold for 30minutes at room temperature prior measuring the absorbance. The spectrophotometer reading (Thermo spectronic made in USA, Model Genesys 10 UV) were taken at 490nm and compared with standard curve. All analysis using phenol-sulphuric acid was performed in duplicate.

4.2.3.7.3 Preparation of standard curve

The standard curve was drawn using standard stock solution of D-glucose (anhydrous) of molecular weight 180.16. A 0.5mg of D-glucose /ml of standard solution were prepared with different concentration. To 1ml of diluted standard solution, 1ml of 5% pure phenol and 5ml of concentrated H_2SO4 was added and the content was left at room temperature for 30min prior to measuring absorbance at 490nm.

Fig.4.2 Standard curve for EPS using D-Glucose (Mw180.16)



4.2.4 Microbiological Analysis

4.2.4.1 Preparation of diluents

Saline water (0.9%) was prepared using distilled water and 9ml of the diluents filled into test tubes and plugged using cotton. The plugged saline test tubes were autoclaved at 15psi for 15minutes.

4.2.4.2 Preparation of sample

Yoghurt sample was homogeneously mixed using a sterile glass rod. one gm of sample was weighed aseptically and transferred to the test tube containing sterile diluents (blank).The sample was dissolved thoroughly by shaking vigorously using the test tube shaker. This reverse as 1:10 dilution was prepared as per the need using the sterile blanks and the dilutions were utilised for plating.

4.2.4.3 Standard plate count (SPC)

The total numbers of viable bacteria in sample was enumerated by the standard plate count method using plate count agar as described by Houghtby *et al*., (1993). The plates in triplicate were incubated at 37^{o}C for 24-48hrs.The colonies were counted and expressed as log cfu/ml of low fat yoghurt.

4.2.4.4 Yeast and moulds

Potato dextrose agar was used to determine the yeast and moulds counts in sample in accordance with the method described by Houghtby *et al* (1993). Plates in triplicate were incubated at 25^{o}C for 3-5days.Colonies were counted and expressed as log cfu/ml of the sample

4.2.4.5 Coli form count

The coliform counts in the sample were determined by the standard plate count method using Violet red bile agar as described by Houghtby *et al*. (1993). Plates in triplicate were incubated at 37^{o}c for 24hrs.Colonies with dark red coloration were counted and expressed as log cfu/ml of low fat yoghurt.

4.2.5 Sensory Analysis

All the samples were drown from the refrigerator before serving to the judges. Judges were requested to assess the products in terms of general acceptability on the 9-point Hedonic scale (9 for liking extremely and 1 for dis-liking extremely). Samples were evaluated for sensory attributes such as colour and appearance, flavour, body and texture, acidity and overall acceptability by a panel of five discriminative and communicative judges from the Division of Dairy Technology and Dairy Microbiology of NDRI-Karnal.

The panellists have not been trained from the present study, but they are dairy professionals having adequate knowledge about sensory evaluation methods and the product attributes.

4.2.6 Statistical Analysis

SPSS version 13.0 (Tukey[a, b, c]) was used to study effect of fat and SNF levels on textural and sensory characteristics of low fat yoghurt

SPSS version 13.0 (Tukey[a, b, c] and Duncan[a, b, c]) was used to study effect of exopolysacharides producing culture on parameters of low fat yoghurt.

5.0 RESULTS AND DISCUSION

The present study has been broadly classified into two parts. Parts 1 of the study have focused on first objectives i.e. to study the effects of fat and SNF levels on the textural and sensory characteristics of low fat yoghurt. Whereas, second part of the study covers the second objective i.e. to evaluate effect of EPS producing culture on the quality parameters of low fat yoghurt. The results obtained in this study are presented and discussed in this chapter

The consistent manufacture of good quality yoghurts that have good texture, mouth feel and stability is important for the dairy industry. Several solutions such as increasing milk solids or addition of stabilizers were proposed to improve the texture of fermented milk products and reduce syneresis (Duboc and Mollet, 2001). However; these approaches do not address an increasing consumer demand for product with low fat. One option to meet this challenge is to take advantage of using EPS produced naturally by lactic acid bacteria as starter culture. In this investigation, an attempt has been made to develop low fat yoghurt using Exopolysaccharides producing culture (EPS) aimed at enhancing textural and sensory properties of the final product.

5.1 EFFECT OF FAT LEVELS ON SENSORY ATTRIBUTES OF LOW FAT YOGHURT

The low fat yoghurt was prepared as per the method described in Fig.3.1, with minor modifications. The sensory attributes studied during low fat yoghurt manufacture included flavour, colour and appearance (C & A), body & texture (B & T), acidity, and overall acceptability (O A). The statistical analysis for effect of fat levels on sensory attributes was carried out using SPSS version 13.0 with Tukey HSD[a, b, c]. The data (mean ± SE) for changes in sensory attributes of low fat yoghurt as a function of different fat levels is prescribed in Table 5.1**.**The ANOVA for sensory scores of low fat yoghurt is presented in Table 5.2**.** Results corresponding to Table 5.1 are also shown in Fig.5.1.

It was observed that, increasing fat had positive effect on the sensory attributes. The effect of fat levels on flavour, body and texture and overall acceptability was significant ($p<0.05$).The flavour score ranged from 7.02 to 7.58

for 0.5% and 3.0% fat yoghurt. The body and texture score ranged from 7.06 at 0.5% level to 7.74 at 3.0% fat level. The sample having 0.5% fat was different in terms of colour and appearance as compared to 1.5% ($p<0.01$) for all the sensory attributes and is increased with increase in fat content. The body and texture scores ranged from 7.06 at 0.5% fat level to 7.74 at 3.0% fat level.

Table 5.1 Changes in sensory attributes of low fat yoghurt with different fat Levels (Means ± SE)

Fat levels %	Flavour	C & A	B & T	Acidity	OA
0.5	$7.02^{a} \pm 0.12$	$7.61^{a} \pm 0.13$	$7.06^{a} \pm 0.10$	$7.07^{a} \pm 0.13$	$7.00^{a} \pm 0.12$
1.5	$7.48^{ab} \pm 0.12$	$7.77^{a} \pm 0.10$	$7.59^{b} \pm 0.11$	$7.46^{a} \pm 0.14$	$7.56^{b} \pm 0.11$
3.0	$7.58^{b} \pm 0.17$	$7.89^{a} \pm 0.08$	$7.74^{b} \pm 0.11$	$7.37^{a} \pm 0.15$	$7.53^{b} \pm 0.10$

Note: Values in the same row with similar superscripts do not significantly differ ($p<0.05$) and vice versa.

Table 5.2 ANOVA for effect of fat levels on sensory attributes of low fat Yoghurt

Parameters	Mean sum of square	df	F-value	Sig.
Flavour	3.214	2	4.627	0.012^{*}
Colour & appearance	0.725	2	1.708	0.186^{NS}
Acidity	1.464	2	2.038	0.136^{NS}
Body & texture	4.590	2	10.101	0.000^{**}
Overall acceptability	3.464	2	8.319	0.000^{**}

** indicates significant ($p<0.01$) ;* indicates significant ($p<0.05$) ;^{NS} indicates non Significant

The overall acceptability score of 0.5% fat yoghurt was significantly lower ($p<0.01$) than that of 1.5 and 3.0% fat. These findings are in agreement with the findings of Yadav *et al*., (2005) who reported that, in preparation of low-fat probiotic *dahi*, scores of body and texture, and flavour decreased significantly with the decrease in fat content. Also, Akalin *et al*., (2008) reported significant differences in the overall acceptability scores of probiotic yoghurt with decrease in fat content. Acidity development was independent of fat levels but was dependent on starter culture used, and inoculation level. There was no significant difference in acidity of the product at all fat levels.

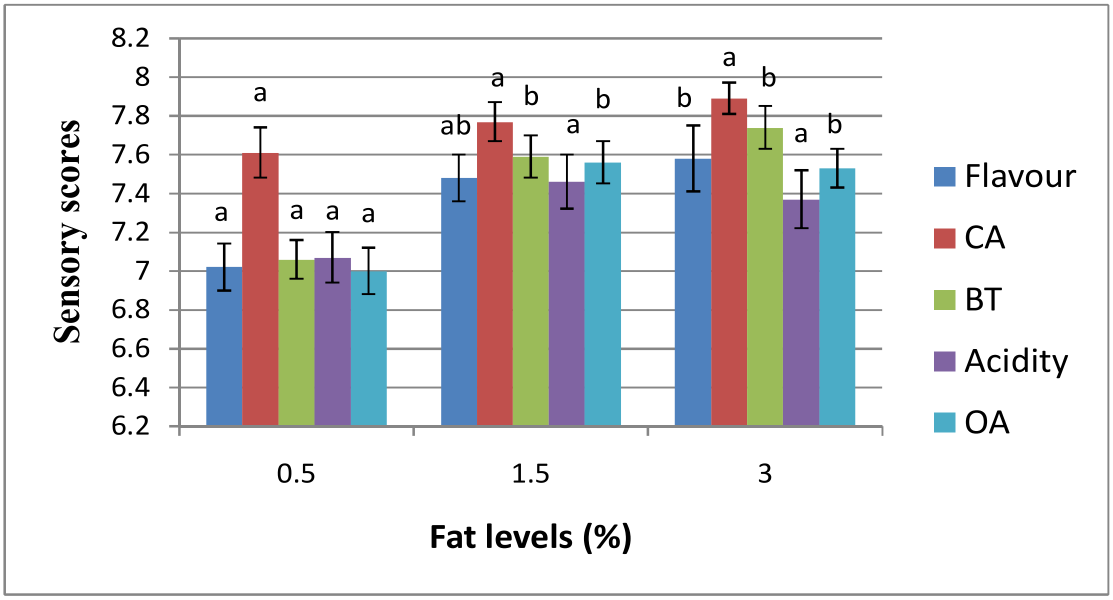


Fig.5.1: Effect of fat levels on sensory attributes of low fat yoghurt

5.2 EFFECT OF FAT LEVELS ON TEXTURAL CHARACTERISTICS OF LOW FAT YOGHURT

The texture characteristics studied during low fat yoghurt development included firmness, work of adhesion, work of shear and stick ness. Table 5.3 shows observations recorded for changes in texture characteristics of low fat yoghurt with different fat levels. ANOVA for texture characteristics of low fat yoghurt are presented in Table 5.4 and trends of results obtained have been graphically depicted in Fig**.** 5.2.

Texture profile analysis (TPA) is well suited to the gels because it is not affected by free whey on the surface of the samples. Firmness value, i.e., the peak force obtained during penetration/compression of the probe revealed that increase of fat from 0.5% to 1.5% increased the firmness, from 1.27 N to 1.47 N respectively. (Table 5.3). Further increase in fat from 1.5% to 3.0% slightly decreased the firmness value 1.44N. The differences were however not-significant. Results obtained were in agreement with the findings of Akalin *et al*., (2008) who reported that, in the preparation of reduced-fat probiotic yoghurt, although the firmness decreased with decrease in fat content, the differences were not significant. The slight increase in firmness value of yoghurt with 1.5% fat could be due to increased milk solid not fat (MSNF) content. Similar pattern was observed with the stickiness (N), i.e., the negative peak force obtained during the withdrawal of the probe, among the three samples. The stickiness increased with increasing fat content. The stickiness of 0.5% fat yoghurt was significantly ($p<0.05$) lower than the other two samples.

Table 5.3 Changes in texture characteristics of low fat yoghurt with different fat levels (Means ± SE)

Fat levels %	Firmness (N)	Work of adhesion (N.s)	Work of shear (N.s)	stick ness (N)
0.5	$1.27^{a} \pm 0.06$	$-3.25^{b} \pm 0.03$	$0.82^{a} \pm 0.80$	$-0.36^{a} \pm 0.03$
1.5	$1.47^{a} \pm 0.12$	$-4.15^{b} \pm 0.42$	$0.01^{a} \pm 0.00$	$-0.49^{b} \pm 0.05$
3.0	$1.44^{a} \pm 0.06$	$-4.16^{b} \pm 0.41$	$0.01^{a} \pm 0.00$	$-0.48^{b} \pm 0.04$

Note: Values in same row with similar superscript letter do not significantly differ ($p<0.05$) and vice versa

Table 5.4 ANOVA for effect of fat levels on texture characteristics of low fat yoghurt

Parameters	Mean sum of square	df	F-value	Sig.
Firmness	0.072	2	2.344	0.138^{NS}
Work of adhesion	1.643	2	3.253	0.074^{NS}
Work of shear	1.312	2	1.004	0.395^{NS}
Stick ness	0.029	2	4.351	0.038^{*}
Syneresis	39.847	2	12.105	0.001^{**}
Viscosity	36474.875	2	2.988	0.089^{NS}
EPS concentration	9348.611	2	2.241	0.149^{NS}

** indicates significant at ($p<0.01$); * indicates significant at ($p<0.05$); ^{NS} indicates non significant

The work of shear(N.s), i.e., the area under the penetration cycle (down stroke), representing the amount of energy required to perform the shearing process, increased with the decrease in fat content from 1.5 and 3.0% to 0.5% but between 1.5% and 3.0% work of shear was same. The value of work of shear of 1.5 and 3.0% was significantly lower ($p>0.05$) than that of 0.5% fat yoghurt.

The work of adhesion (N.s) of 3.0% fat yoghurt was higher than that of 0.5 and 1.5% but, the differences were not significant.

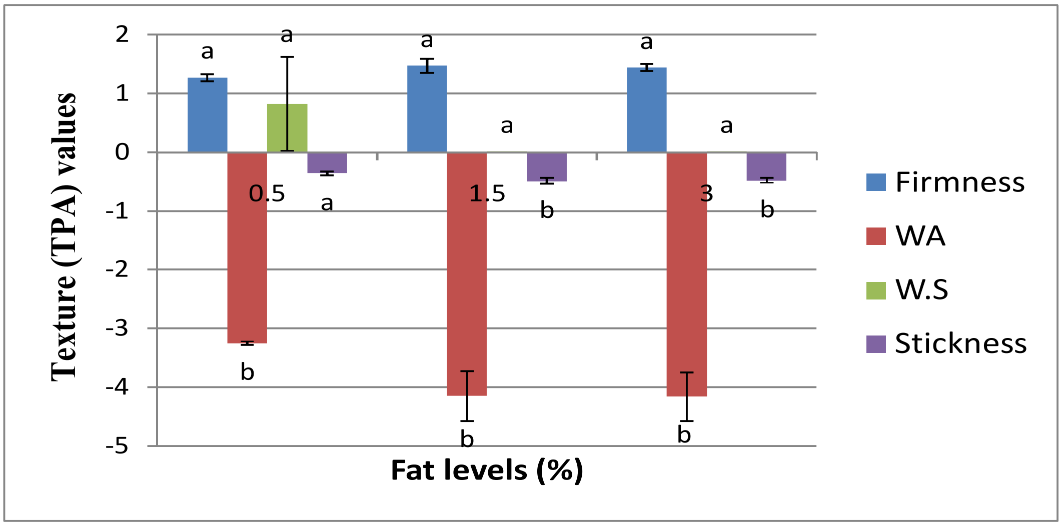


Fig. 5.2: Effect of fat levels on textural characteristics of low fat yoghurt

5.3 EFFECT OF FAT LEVELS ON PHYSICAL AND BIOCHEMICAL PARAMETERS OF LOW FAT YOGHURT

The physical and Biochemical parameters studied during low fat yoghurt development included: syneresis, viscosity and EPS concentration.

5.3.1 Effect of fat levels on syneresis

Syneresis or whey separation is regarded as a defect on the surface of set yoghurt, and other set-style fermented dairy products. The syneresis values in set yoghurt increased with decrease in fat content. It was observed that, the sample with 0.5% fat showed significantly ($p<0.05$) higher syneresis value 9.17% as compared to 1.5% and 3.0% fat (Table 5.5) Results corresponding to Table 5.5 are also shown in Fig.(5.3 & 5.4). Akalin *et al*. (2008) reported similar findings and observed increased syneresis in reduced-fat probiotic yoghurt than the full-fat one. Antunes *et al*., (2004) also reported that fat-free set yoghurt showed considerable wheying off when not stabilized by other ingredients. This may be explained by the fact that fat globules get entrapped physically within the gel network resulting in increasing number of pores within the gel matrix occupied by fat globules, thus leading to prolonged gelation and more difficult whey drainage. Also fat has a role in gel structure formation due to development of multiple interactions between fat globules, whey proteins, and casein micelles (Sodini *et al*., 2004).

5.3.2 Effect of fat levels on viscosity

Viscosity of yoghurt made with 0.5% fat decreased with the decrease in milk fat (533.00cp**)** compared to1.5% fat (646.75cp) and 3.0% fat (682.25cp) (Table 5.5). However viscosity values were not significant**.** Akalin *et al*., (2008) also reported that the viscosity of probiotic yoghurt decreased with decrease in fat content. Similar observations were made by De-Lorenzi *et al*., (1995) and Shaker *et al*., (2000).

5.3.3 Effect of fat levels on EPS concentration

EPS producing culture are used to improve physical properties of yoghurt. In the present study the highest EPS concentration was noted in the sample with 1.5% fat (826.76mg/L) the sample with 3.0% fat had the lowest EPS concentration (752.11mg/L) (Table 5.5) however there was no statistically significant difference between the samples with regard to EPS concentration. This shows that higher fat content does not favour the growth of EPS in yoghurt. These observations are in agreement with the findings of Gursoy, *et al.,* (2010).

Table 5.5 Changes in physical and Biochemical parameters of low fat yoghurt With different fat levels (Means ±SE)

Fat levels %	Syneresis%	Viscosity(cp)	EPS concentration (mg/L)
0.5	$9.17^{b} \pm 0.95$	$533.00^{a} \pm 64.64$	$767.18^{a} \pm 43.92$
1.5	$4.58^{a} \pm 0.52$	$646.75^{a} \pm 64.61$	$826.76^{a} \pm 23.31$
3.0	$4.83^{a} \pm 0.47$	$682.25^{b} \pm 60.53$	$752.11^{a} \pm 32.82$

Note: Values in same row with similar superscript letter do not significantly differ ($p<0.05$) and vice versa

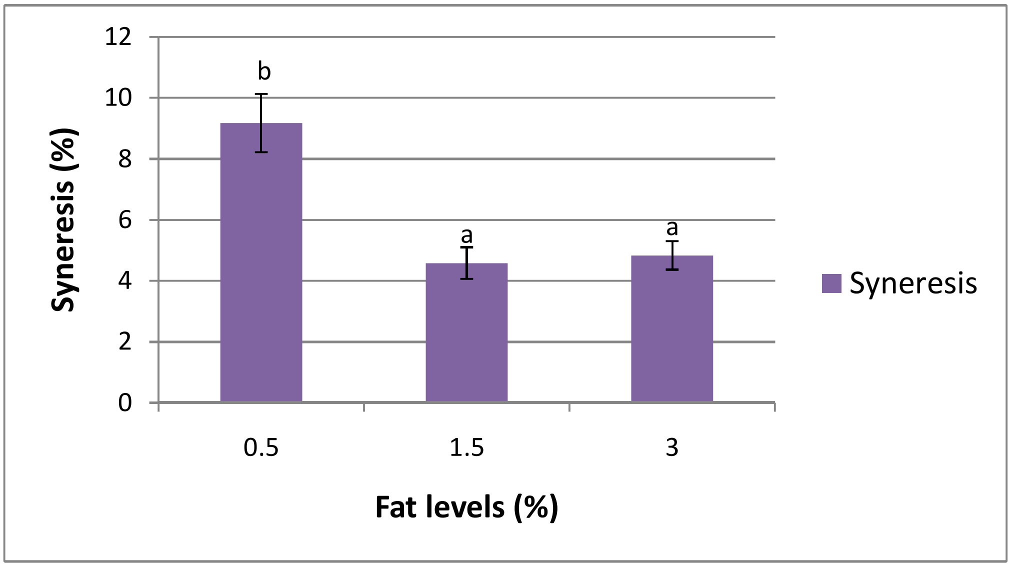


Fig.5.3: Effect of fat levels on syneresis of low fat yoghurt

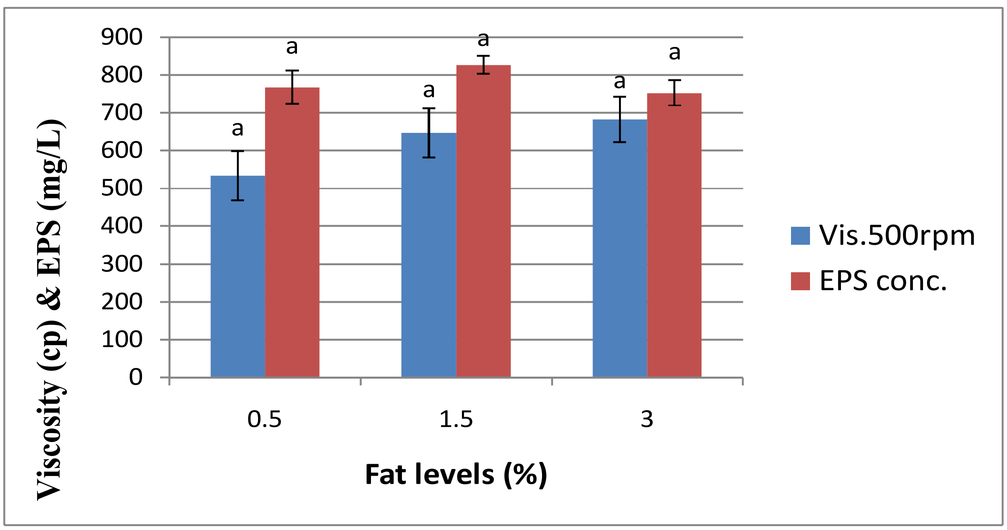


Fig.5.4: Effect of fat levels on viscosity and EPS concentration of low fat yoghurt

5.4 EFFECT OF SNF LEVELS ON TEXTURAL PARAMETERS AND SENSORY ATTRIBUTES OF LOW FAT YOGHURT

5.4.1 Effect of SNF levels on sensory attributes of low fat yoghurt

It were observed that, with increase in SNF level from 9% to 10%, flavour of the product increased significantly ($p<0.01$) from 7.14 to 7.58 according to point hedonic scale. (Table 5.6) and corresponding results are shown on (Fig.5.5) this could be attributed to higher lactose content in higher SNF samples responsible for more lactic acid production and enhanced flavour perception. Also, lower acceptability of the sample with 9% SNF could be due to longer lag phase and uncompleted growth of *Lactobacillus bulgaricus* which didn't provide enough time for aroma production in these samples. The observed results are in agreement with findings of Tamime and Robinson, (1999).

The overall acceptability of the yoghurt increased from 7.21 to 7.52 and the values were significantly different (p<0.05) due to increase SNF level from 9% to 10%. This may be due to ability of high level of SNF to reduce wheying off and increase in flavour of the product. Colour and appearance, body and texture and acidity of the sample with different SNF levels were not significant different.

Table 5.6 Changes in sensory attributes of low fat yoghurt with different SNF levels (Means ± SE)

SNF Levels %	Flavour	C & A	B & T	Acidity	O A
9.0%	$7.14^{a} \pm 0.12$	$7.77^{a} \pm 0.09$	$7.37^{a} \pm 0.10$	$7.17^{a} \pm 0.12$	$7.21^{a} \pm 0.10$
10%	$7.58^{b} \pm 0.11$	$7.74^{a} \pm 0.08$	$7.56^{a} \pm 0.09$	$7.43^{a} \pm 0.10$	$7.52^{b} \pm 0.09$

Note: Values in same row with similar superscript letter do not significantly differ (p<0.05) and vice verse

Table 5.7 ANOVA for effect of SNF levels on sensory attributes of low fat yoghurt.

Parameters	Mean sum of square	df	F-value	Sig.
Flavour	5.311	1	7.646	0.007^{**}
Colour & appearance	0.025	1	0.059	0.808^{NS}
Acidity	1.776	1	2.472	0.119^{NS}
Body & texture	1.001	1	2.204	0.141^{NS}
Over all acceptability	2.629	1	6.313	0.014^{*}

** indicates significant at (p<0.01); * indicates significant at (p<0.05)] ^{NS} indicates non significant

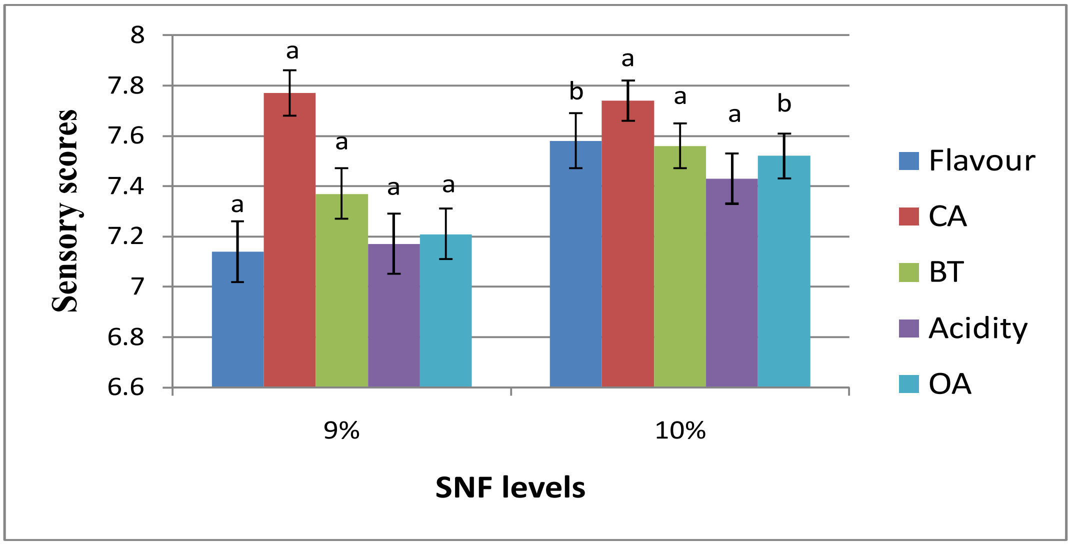


Fig. 5.5: Effect of SNF levels on sensory attributes of low fat yoghurt

5.4.2 Effect of SNF Levels on Textural Parameters of Low Fat Yoghurt

Effect of SNF levels from 9 to 10%, shows significant increase in firmness, adhesiveness, and stick ness of the product regardless of the amount of fat added to the samples. Therefore firmness, work of adhesion and stickiness were 1.34, -3.65, -0.42 respectively for 9% SNF sample and the respective values for other attributes increased significantly ($p<0.05$) to 1.45, -4.05 and -0.47 in 10% SNF sample (Table 5.8) and corresponding results are shown on (Fig.5.6). These findings were in line with the observation of Mohammed *et al.*, (2004) who reported that high total solids in yoghurt increases firmness of the gel and decreases syneresis.Work of shear were not significantly different between two levels of SNF 0.01 N.s and 0.54 N.s

Table 5.8 Changes in texture parameters of low fat yoghurt with different SNF levels

SNF levels %	Firmness (N)	WA (N.s)	WS (N.s)	STICKNESS (N)
9.0	$1.34^{a} \pm 0.04$	$-3.65^{a} \pm 0.24$	$0.01^{a} \pm 0.001$	$-0.42^{a} \pm 0.03$
10.0	$1.45^{b} \pm 0.09$	$-4.05^{b} \pm 0.38$	$0.54^{a} \pm 0.54$	$-0.47^{b} \pm 0.05$

Note: Values in same row with similar superscript letter do not significantly differ ($p<0.05$) and vice versa

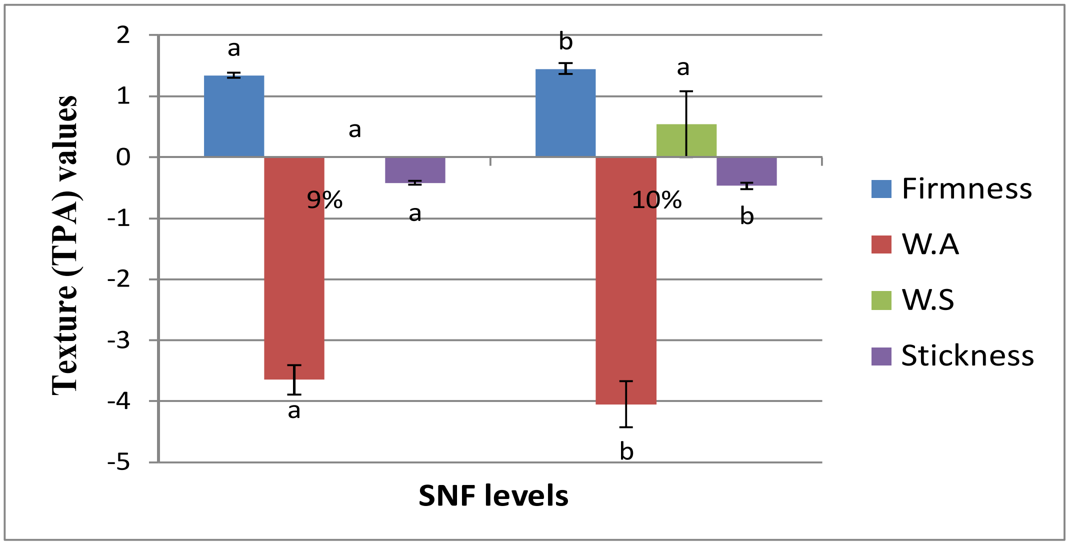


Fig. 5.6: Effect of SNF levels on textural parameters of low fat yoghurt

5.5 EFFECT OF SNF LEVELS ON PHYSICAL AND BIOCHEMICAL PARAMETERS OF LOW FAT YOGHURT

The physical and biochemical parameters studied during the manufacture of low fat yoghurt included: Syneresis, Viscosity and EPS concentration.

5.5.1 Syneresis

It was observed that an increase in SNF level from 9 to 10% led to decrease in syneresis from 6.5% to 6.22% respectively (Table 5.9) and corresponding results are shown on (Fig. 5.7). However, there were not significantly different. It is a well known that, high SNF level improves texture of yoghurt and decrease wheying off significantly. However in our studies due to small difference between SNF levels used, (1%) there were no significant difference among samples.

5.5.2 EPS concentration

EPS producing culture were used to improve physical properties of the yoghurt samples. In this study, it was observed that, there was significant different ($p<0.05$) between samples made with 9% SNF 746.57mg/L and sample made with 10% SNF 817.46mg/L (Table 5.9) and corresponding results are shown on (Fig. 5.8).This indicated that, increase in SNF level from 9 to 10,favoured the production of EPS in yoghurt.

5.5.3 Viscosity

The content of SNF directly affected viscosity of yoghurt. It was observed that both increase in SNF levels from 9-10 %, there was increase in viscosity of yoghurt from 167.25cp to 215.83cp which was significantly different (p<0.01). (Table 5.9) and corresponding results are shown on (Fig. 5.8). There is dependence of total solids on yoghurt gel formation that could be attributed to differences in casein micelle composition.

It were thus noticed that the viscosity was higher in yoghurt made with 10% SNF compared 9% SNF. This indicated that increase in SNF levels increases the viscosity of yoghurt which is in agreement with the findings of several researchers. According to Rawson and Marshal, *Streptococcus thermophilus* are the most germs incriminated in the production of exocellular texturising agents called Exopolysaccharides that might interact with the protein content of milk and increase the viscosity and rheological quality of the products, so due to this when there is increase in SNF levels from 9% to 10% there is increase in protein content of the yoghurt and when Exopolysaccharides interact with this high level of protein increases viscosity of the product.

Table 5.9 Changes in physical and biochemical parameters of low fat yoghurt with different SNF levels (Means ± SE)

SNF levels %	Syneresis (%)	EPS conc.(mg/L)	Viscosity(cp)
9.0	$6.50^{a} \pm 0.65$	$746.57^{a} \pm 29.76$	$167.25^{a} \pm 11.52$
10	$6.22^{a} \pm 1.06$	$817.46^{b} \pm 23.20$	$215.83^{b} \pm 9.65$

Note: Values in same row with similar superscript letter do not significantly differ (p<0.05) and vice versa.

Table 5.10 ANOVA for effect of SNF levels on physical and biochemical parameters of low fat yoghurt

Parameters	Mean sum of square	df	F-value	Sig.
Firmness	0.200	1	06.539	0.025^{*}
Work of adhesion	2.824	1	5.591	0.036^{*}
Work of shear	1.298	1	0.993	0.339^{NS}
Stick ness	0.047	1	7.110	0.021^{*}
Syneresis	1.681	1	0.511	0.489^{NS}
Viscosity	208981.125	1	17.119	0.001^{**}
EPS concentration	22614.264	1	5.421	0.038^{*}

** indicates significant at (p<0.01); * indicates significant at (p<0.05); ^{NS} indicates non significant

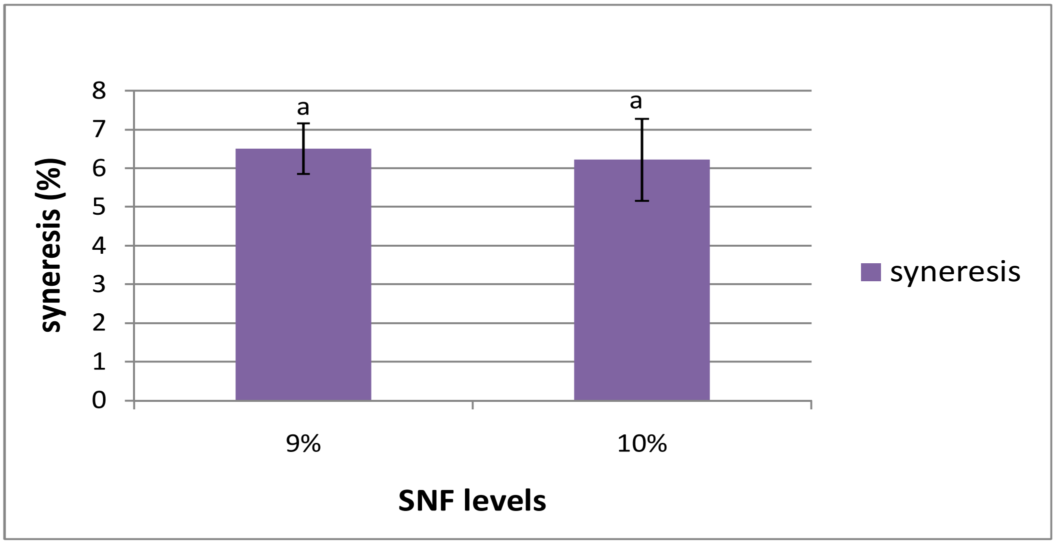


Fig.5.7 Effect on syneresis of low fat yoghurts

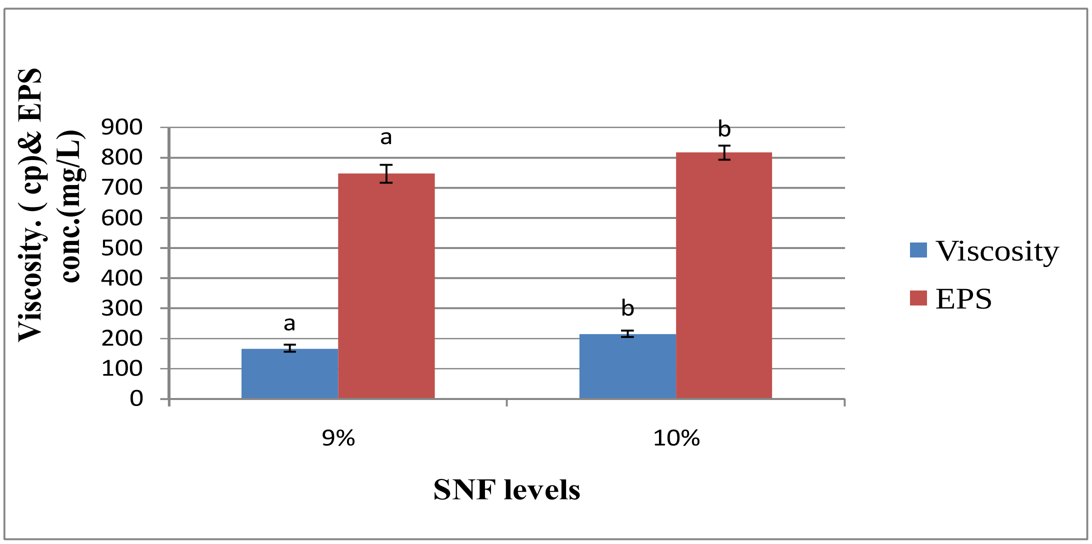


Fig.5.8: Effect of SNF levels on viscosity and EPS concentration of low fat yoghurt

Table 5.11. ANOVA for interaction effect of (fat *SNF) levels on physical and biochemical parameters of low fat yoghurt

Parameters	Mean sum of square	df	F-value	Sig.
Firmness	0.052	2	1.689	0.226^{NS}
Work of adhesion	1.711	2	3.386	0.068^{NS}
Work of shear	1.310	2	1.002	0.396^{NS}
Stick ness	0.016	2	2.352	0.137^{NS}
Syneresis	3.097	2	0.941	0.417^{NS}
Viscosity	2539.125	2	0.208	0.815^{NS}
EPS concentration	16911.419	2	4.054	0.045^{*}

* indicates significant at (p<0.05); ^{NS} indicates non significant

The interaction effect of fat and SNF levels was found to be significant (p<0.05) for EPS production which means a very slight increase in SNF levels 1%, increase EPS production in yoghurt. Increasing total solids in yoghurt EPS interact with lactose content which enhances lactic acid production and because of this production of EPS increases because of having favourable conditions.

5.6 EFFECTS OF STORAGE PERIOD ON THE QUALITY PARAMETERS OF LOW FAT YOGHURT

The EPS yoghurt (1.5% fat and 10%SNF) and control yoghurt (4.5% fat and 10% SNF) were stored at refrigeration temperature and studied for sensory, physico-chemical, biochemical, and microbiological parameters for 12 days of storage and analysis was done at 4 days interval.

5.6.1 Effect of storage on sensory attributes of low fat yoghurt

The sensory attributes studied during storage of low fat yoghurt included flavour, colour and appearance (C & A), body & texture (B & T), acidity and overall acceptability (O A).

5.6.1.1 Flavour

The results of sensory data on flavour indicated that, yoghurt made with EPS strains received higher aroma scores than control (p<0.05). Yoghurt made with ropy EPS-producing strains received high aroma scores on 0 day 7.82 and

7.93 on 4^{th} day as compared to 8^{th} day and 12^{th} day of storage with score of 7.27 respectively

The flavour scores during first four days of storage were significantly higher ($p<0.05$) than those at day 8 and 12 days. The aroma scores of all samples were increasing from 0 day to 4^{th} day of storage and then there ware decrease. This could be related to the development of acidity and decrease in acetaldehyde content of the samples. The sample made with EPS^+ strains had flavour characteristics in comparison to control and were preferred by the panel of judges. The Panellist preference did not change significantly between 8^{th} day and 12^{th} day of storage (Table 5.12) and corresponding results are shown on (Fig 5.9)

5.6.1.2 Colour and appearance

There was no significant difference in colour and appearance score of samples between 0 day and 4 day of storage but significant different ($p<0.01$) were observed on 8 and 12days of storage. Significant lower colour and appearance scores for sample during advanced storage periods (8 and 12 days) could be ascribed to acidity development in samples which leading to wheying off which were perceived as a defect and negatively influence acceptability (Table 5.12) and corresponding results are shown on (Fig. 5.10)

5.6.1.3 Body and texture

Results showed that body and texture scores were 7.75, 7.92 & 7.35 on 0day, 4th day, and 8^{th} day of storage respectively. There were significantly different ($p<0.05$) compared to scores obtained on 12^{th} day of storage. Good body and texture is in comparison to hydration and stabilisation abilities of casein micelles in coagulum attributed better texture of yoghurt (Tamime & Robinson, 1983), while decrease in body and texture score on 12^{th} day of storage 7.13 could be due to high syneresis and increase in acidity thus affecting body and texture of the product (Table 5.12) and corresponding results are shown on (Fig. 5.11)

5.6.1.4 Acidity

The product were having high sensory score for acidity up to 4 day of storage 7.8 on 0 day & 7.6 on 4^{th} day which significantly higher ($p<0.05$) from acidity score recorded on 8 &12 days of storage (Table 5.12) and corresponding results are shown on (Fig. 5.12). The acidity scores on day 8 and day 12 were not significantly different compared to EPS yoghurt which were rated better by the panellists through out the storage duration.

5.6.1.5 Overall acceptability

The higher scores for overall acceptability were observed on 0 day 7.83 & 4 day 7.91 which were however not significantly different from each other. Decrease in overall acceptability scores were recorded 8^{th} day 7.41 and 12^{th} day 7.38.Lower overall acceptability of the products could be ascribed to factors like development of syneresis, high acidity and decrease in aroma compounds of the product during the last days of storage period .EPS yoghurt were how ever rated better than control through out the storage period (Table 5.12) and corresponding results are shown on (Fig. 5.13)

Table 5.12 Effect of storage period on sensory attributes of low fat yoghurt

(Means ± SE)

Days of storage				
Parameter	0	4	8	12
Flavour	$7.82^{a} \pm 0.14$	$7.93^{a} \pm 0.12$	$7.27^{b} \pm 0.23$	$7.27^{b} \pm 0.24$
Colour & appearance	$8.16^{a} \pm 0.12$	$8.20^{a} \pm 0.14$	$7.79^{b} \pm 0.23$	$7.51^{b} \pm 0.14$
Body & texture	$7.75^{a} \pm 0.21$	$7.92^{a} \pm 0.18$	$7.35^{ab} \pm 0.24$	$7.13^{b} \pm 0.24$
Acidity	$7.8^{a} \pm 0.13$	$7.6^{ab} \pm 0.18$	$7.04^{b} \pm 0.30$	$7.08^{b} \pm 0.25$
Overall acceptability	$7.83^{ab} \pm 0.14$	$7.91^{a} \pm 0.11$	$7.41^{b} \pm 0.21$	$7.38^{b} \pm 0.21$

Note: Values in same row with similar superscript letter do not significantly differ ($p<0.05$) and vice versa

Table 5.13 ANOVA for effect of storage period (days) on sensory attributes of low fat yoghurt

Parameters	Mean sum of square	df	F-value	Sig.
Flavour	1.645	3	3.730	0.017*
Colour & appearance	1.428	3	4.333	0.009**
Body & texture	1.747	3	3.461	0.023*
Acidity	1.910	3	3.066	0.036*
Overall acceptability	1.005	3	2.758	0.052NS

** indicates significant at ($p<0.01$); * indicates significant at ($p<0.05$); NS indicates non significant

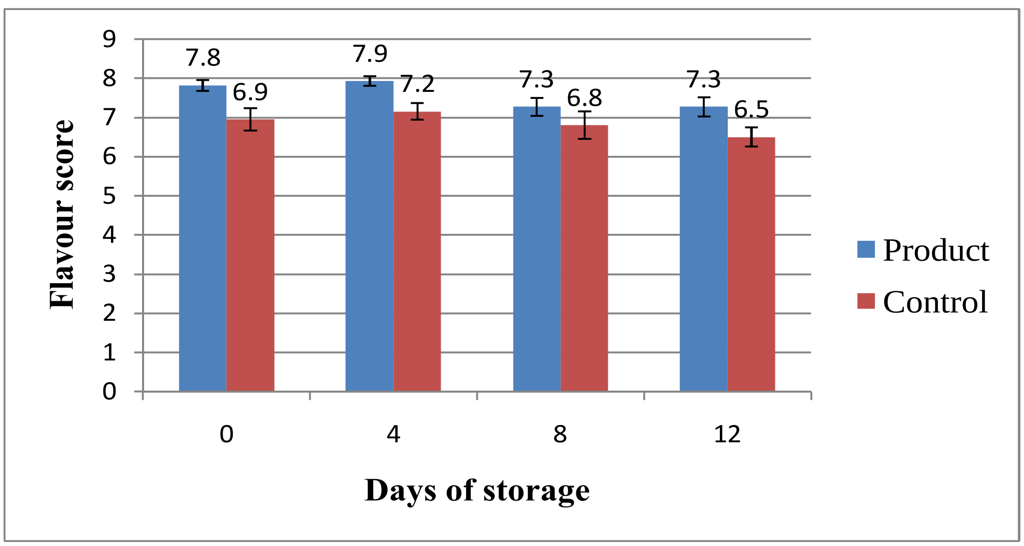


Fig.5.9: Effect of storage period on flavour of yoghurt

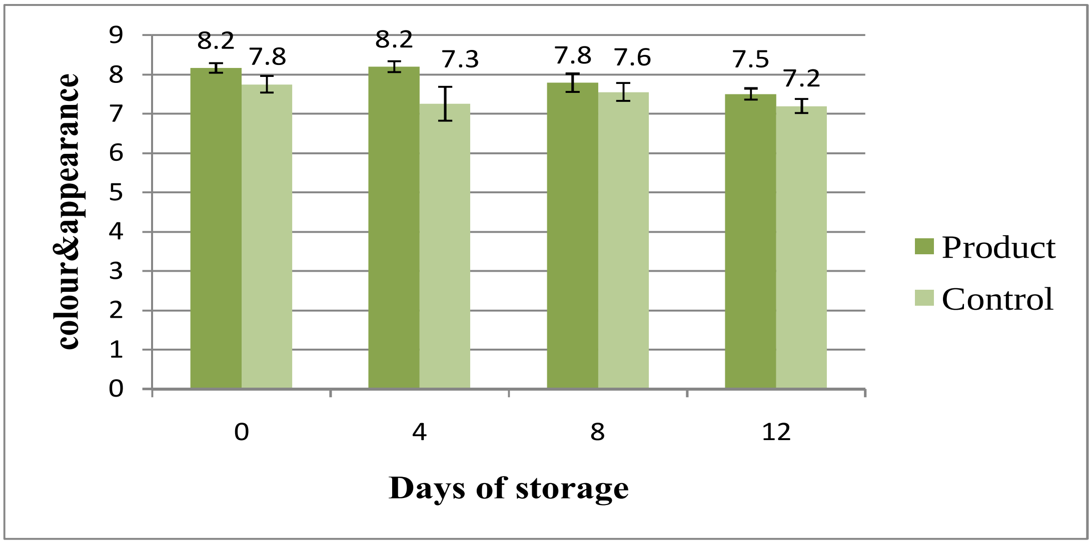


Fig.5.10: Effect of storage period on colour and appearance of yoghurt

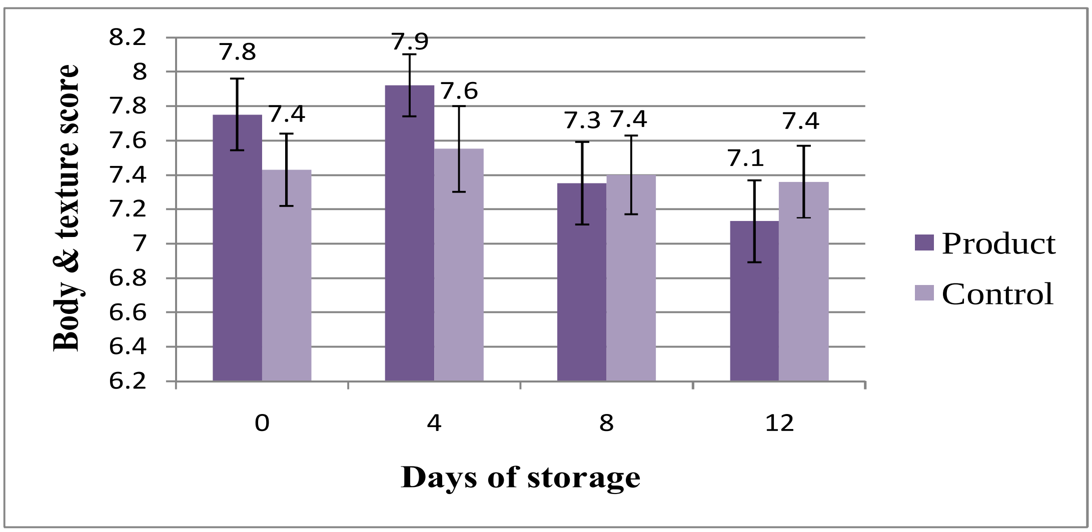


Fig.5.11: Effect of storage period on body and texture of yoghurt

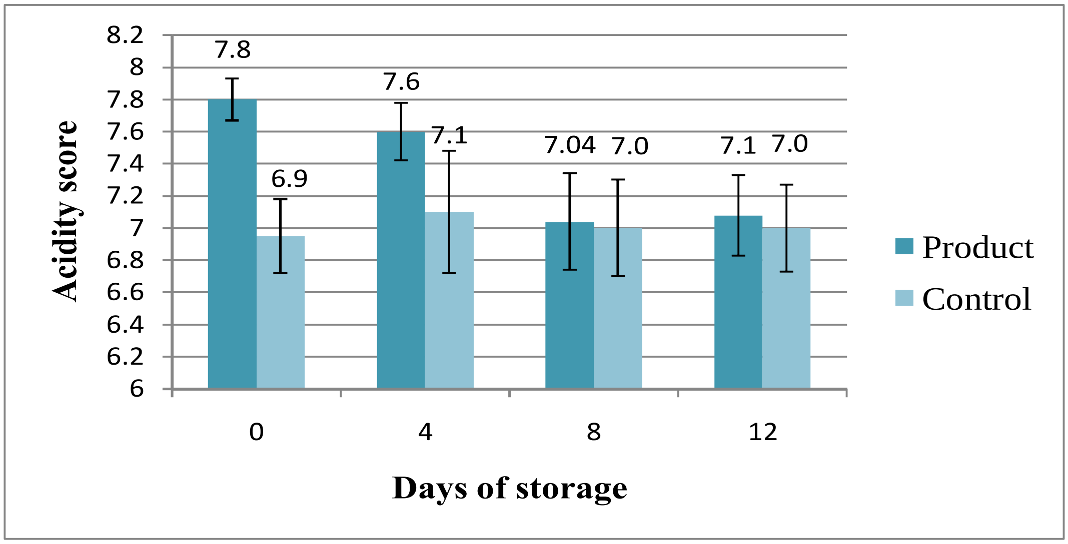


Fig.5.12: Effect of storage period on acidity of yoghurt

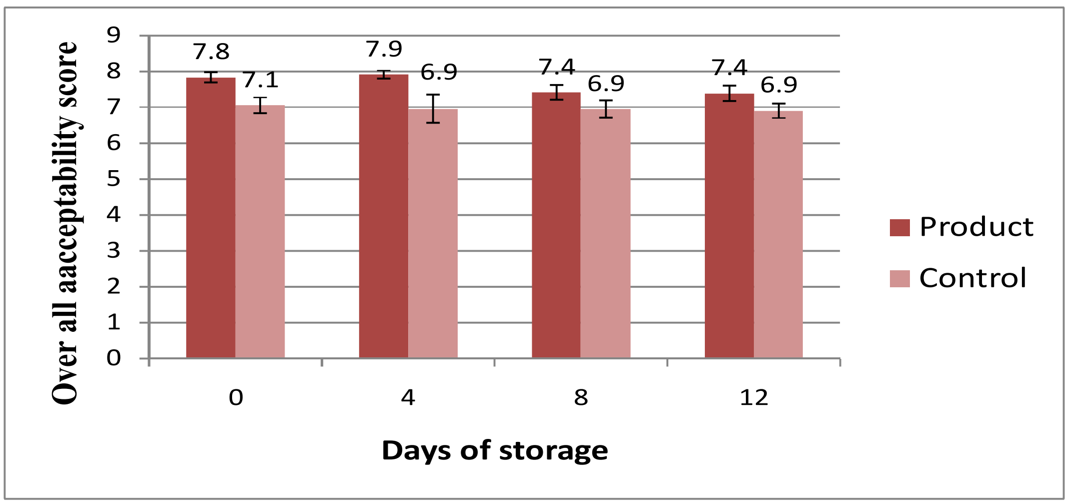


Fig.5.13: Effect of storage period on over all acceptability of yoghurt

5.7 EFFECT OF STORAGE ON VISCOSITY AND SYNERESIS OF LOW FAT YOGHURT

The physical parameters studied during storage of low fat yoghurts include viscosity and syneresis.

5.7.1 Viscosity

The viscosity observed on day 8 (536.50 cp) and day 12 (415.25 cp) were significantly different ($p<0.01$) with each other and also with values observed on day 0 and day 4.It was observed during storage that, viscosity increased up to 8 days of storage .The viscosity of EPS yoghurt were 482.83cp on day 0 and 499.17cp of 4 days there were not significantly different.

This increase in viscosity during storage is in agreement with the findings of Mutlu (2009).Several authors have reported that EPS in milk products fermented with EPS-producing culture drastically influence the viscosity of the end product. Our results however indicated that there was no correlation between viscosity and EPS production (Table 5.14) and corresponding results are shown on (Fig 5.14). The highest apparent viscosity value did not correspond to the highest EPS yield. These results corroborate with findings of De Vuyst *et al*., (2003) who reported that although fermentation with *S.thermophilus* LY03 produced large amounts of EPS with high molecular mass, it resulted in relatively thin yoghurt. It is known that, depending on the decrease in pH, the protein-protein interactions and the consequent low protein rearrangements in the acid casein gels continue during cold storage (Ozer *et al*., 1997). In this case, during and after fermentation, the development of gel stiffness was stimulated.

5.7.2 Syneresis

Whey separation is an important factor affecting consumer acceptability of yoghurt. Yoghurt made with EPS producing culture showed low levels of syneresis compared to control (Table 5.14) and corresponding results are shown on (Fig 5.15). This could be due to capability of EPS for water ï binding ability in the protein matrix of yoghurt Amatayakul *et al*., (2006). Duboc and mollet, 2001 reported that, in yoghurt, casein precipitates because of hydrophobic interactions and forms a continuous net work with cavities filled with serum and bacterial cells. It is due to anenvelop of EPS surrounding the bacterial starter strains, by which ropy cells attach to the protein matrix via a web of filaments. La Torre *et al*., 2003 and Guzel-Seydim *et al*., 2005 obtained similar results in set type yoghurt.

Higher levels of syneresis not significantly different from each other were observed on day 8 (6.25%) and 12 day (5.62%) of storage. These values and values recorded on day 4 (3.37%) were however significantly different ($p<0.01$) from the syneresis value recorded at the beginning of storage. Yoghurt made with EPS strains showed significant lower level of whey separation than those made with non EPS strains. These were in agreement with findings of Mutlu *et al.* (2009).

Table 5.14 Effect of storage period on viscosity and syneresis of low fat yoghurt (Means ±SE)

Days of storage				
Parameter	0	4	8	12
Viscosity (500cp)	482.83^{a} ± 1.69	490.17^{a} ±16.69	536.50^{b} ± 5.19	415.25^{c} ± 7.07
Syneresis (%)	2.175^{a} ± 0.04	3.375^{b} ± 0.27	6.25^{c} ± 0.14	5.63^{c} ± 0.07

Note: Values in same row with similar superscript letter do not significantly differ (p<0.05) and vice versa

Table 5.15 ANOVA for effect of storage (days) on physical properties of low fat yoghurt

Parameters	Mean sum of square	df	F-value	Sig.
Firmness	0.010	3	1.243	0.311^{NS}
Work of adhesion	0.379	3	1.980	0.137^{NS}
Work of shear	3.78E-006	3	0.657	0.585NS
Stick ness	0.003	3	1.077	0.373^{NS}
Viscosity (500rpm)	7490.561	3	27.840	0.000**
Syneresis	10.917	3	141.145	0.000**

** indicates significant at (p<0.01); ^{NS} indicates non significant (in all levels)

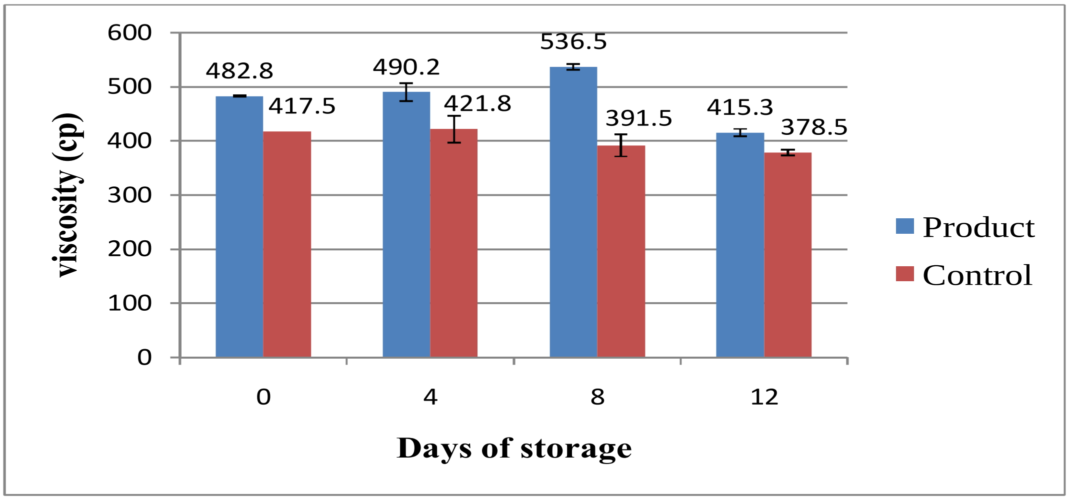


Fig.5.14: Effect of storage period on viscosity of low fat yoghurt

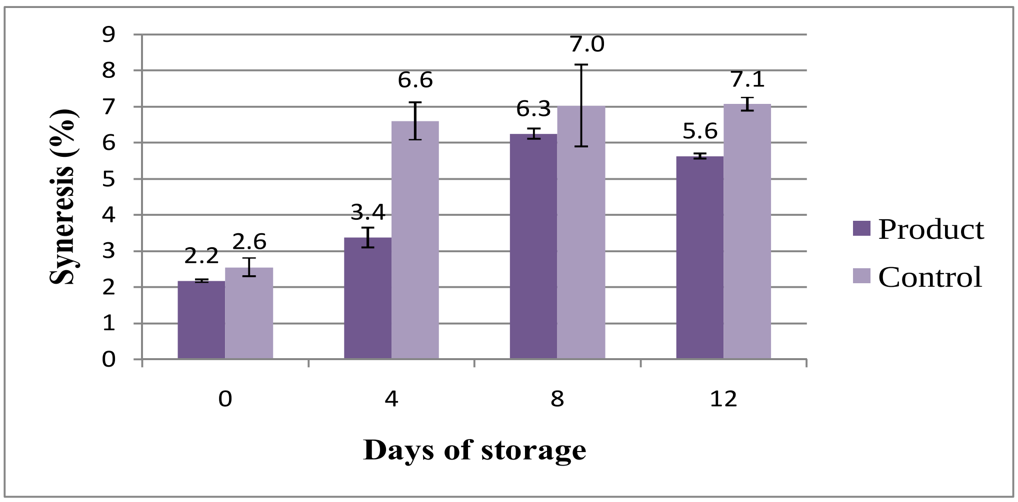


Fig.5.15: Effect of storage period on syneresis of yoghurt

5.8 EFFECT OF STORAGE ON TEXTURAL PARAMETERS OF LOW FAT YOGHURT

The textural parameters studied during storage of low fat yoghurt include firmness, work of adhesion, work of shear and stickiness.

Texture profile analysis (TPA) revealed that none of the four textural parameters studied differed significantly during the storage (Table 5.16) and corresponding results are shown on (Fig. 5.16 & 5.17). This indicates that effect of storage period on TPA parameters of yoghurt was very small which can not be detected by the instrument. As can be seen from (Fig. 5.15) the firmness (N) of yoghurt made with ropy EPS were less compared to control yoghurt made with non EPS producing culture. This is due to high amount of ropy EPS produced by combined strains and these observations are in agreement with the findings of Hassan *et al*., (1999) who reported the same. The EPS could interfere with the association between casein micelles resulting in a less firm coagulum. The incompatibility between the EPS and proteins may thus be the explanation. The EPS and proteins have like charges at pH values above the iso-electric point of the protein (de Kruif & Tuinier, 2001), as is noticed in yoghurt. The incompatibility between EPS and proteins may result in depletion-induced attraction of casein micelles by EPS, leading to the formation of an acid milk gel filled with EPS (De Kruif &Tuinier, 2001, Tolstoguzov, 1997).This in turn may cause a difference in a protein aggregation mechanisms between yoghurt made using Non-EPS and EPS producing starters leading to differences in the structure of the protein net works.

Table 5.16 Effect of storage period on textural parameters of low fat yoghurt (Mean ± SE)

Days of storage				
Parameter	0	4	8	12
Firmness	$1.15^{a} \pm 0.03$	$1.23^{a} \pm 0.02$	$1.18^{a} \pm 0.023$	$1.19^{a} \pm 0.03$
Work of adhesion	$-2.35^{b} \pm 0.16$	$-2.66^{b} \pm 0.14$	$-2.16^{b} \pm 0.09$	$-2.42^{b} \pm 0.17$
Work of shear	$0.01^{a} \pm 0.00$	$0.009^{a} \pm 0.00$	$0.009^{a} \pm 0.00$	$0.009^{a} \pm 0.00$
stickiness	$-0.28^{b} \pm 0.02$	$-0.32^{b} \pm 0.01$	$-0.27^{b} \pm 0.01$	$-0.30^{b} \pm 0.22$

Note: Values in same row with similar superscript letter do not significantly differ ($p<0.05$) and vice-versa

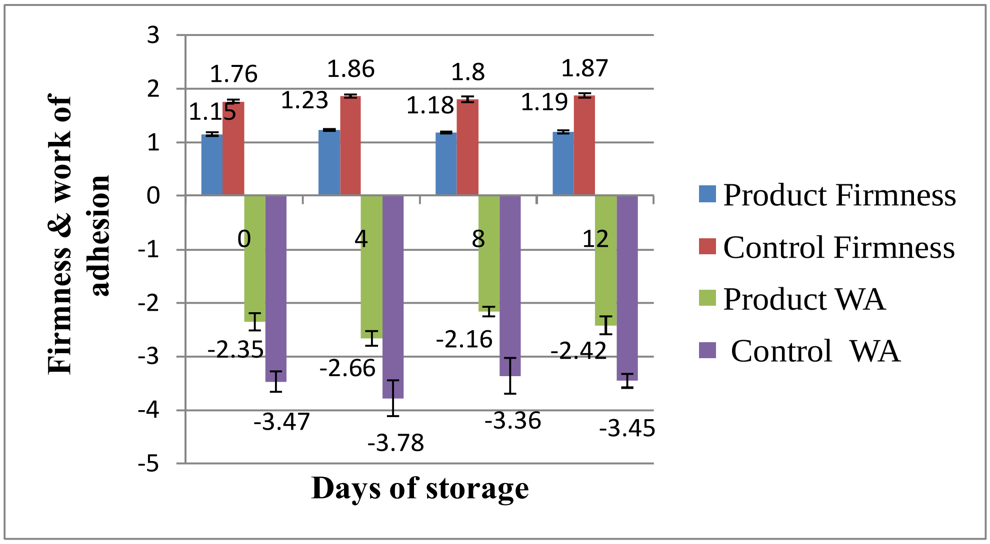


Fig. 5.16: Effect of storage period on firmness and work of adhesion of yoghurt

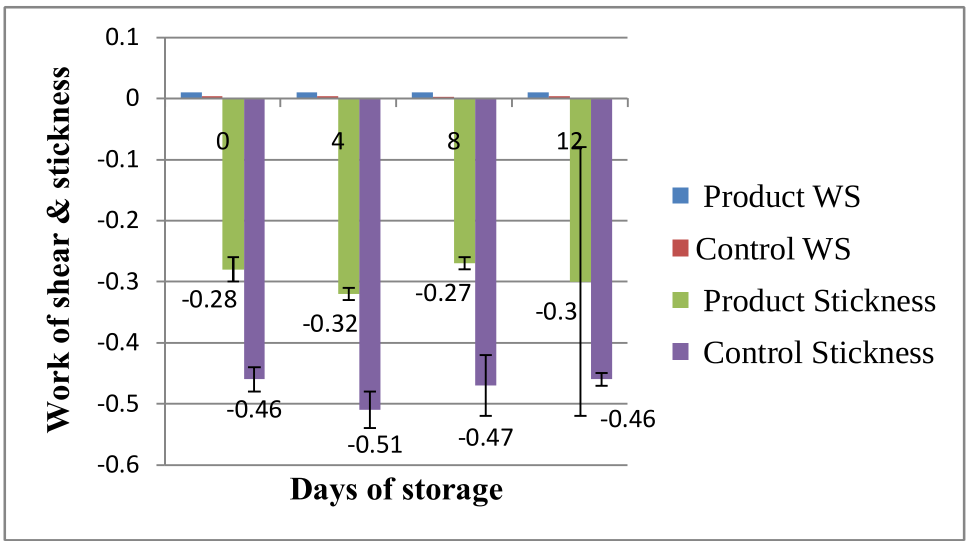


Fig.5.17: Effect of storage period on work of shear and stickiness of yoghurt

5.9 EFFECT OF STORAGE PERIOD ON CHEMICAL PARAMETERS OF LOW FAT YOGHURT

The chemical parameters studied during storage of low fat yoghurts included pH, titratable acidity, fat and total solid (TS).

5.9.1 pH

The pH value of 0 day sample was 4.50 which dropped to 4.39 after 4 days. The two values were however not significantly different from each other.pH values recorded on day 8 (4.26) and day 12 (4.18) were also not significantly different. The pH values of the first half of the storage period were however significantly higher ($p<0.01$) than the values observed in the second half. The pH of yoghurt decreased continuously through out storage period (Fig. 5.18). These observations are in agreement with observation reported by Sokolinska *et al*., (2004) who indicated that, the pH values decrease during the manufacturing process from the time it was inoculated with bacterial cultures to time when it was manufactured. This is because during inoculation with starter bacteria, fermentation takes place by converting lactose in milk to lactic acid so this lactic acid decreased pH as well as increasing titratable acidity. Moreover, according to Luquet (1990) lactic strains have the ability to ferment lactose into lactic acid, with an increase of acidity and decrease in pH of fermented milk, which reveals the influence on the composition of the inoculum on the rate of bacteria growth such as *Streptococcus thermophilus*. The findings are in agreement with findings of Ozer and Atasoy (2002) who reported that, acid production is directly related to lactose metabolism by yoghurt starters and amino acids.

5.9.2 Titratable acidity

During storage, we noticed a remarkable increase in titratable acidity at the period of storage progress from 0 day to day 12 (Table 5.17) and corresponding results are shown on (Fig. 5.19).The titratable acidity in fresh sample were 0.95% which increased but not significantly different value of 1.0% after 4days.The titratable acidity on subsequent days i.e. day 8 and day 12 were significantly different from the value recorded during first 4days of storage but were not significantly among them selves. The mean level of acidity for control sample was higher than EPS yoghurt. This suggests that a considerable amount of glucose and galactose produced by EPS culture might be used for the production of polysaccharide materials rather than lactic acid (Ozer & Atasoy, 2002). Chograni *et al*., (2008) reported that, there is a proportional relationship between the rate of inoculation strains and acidity, the degree of acidity increases proportionally to inoculation rate. Increasing fat content lead to a slight decrease in titratable acidity of yoghurt, but it was found to be insignificant in control which was having high fat content.

Table 5.17 Effect of storage period on chemical parameters of low fat yoghurt

(Mean ± SE)

Days of storage				
Parameter	0	4	8	12
Total solids (%)	11.32^{a} ± 0.02	11.46^{a} ±0.05	11.66^{a} ±0.17	11.50^{a} ± 0.25
pH	4.50^{b} ± 0.00	4.39^{b} ± 0.00	4.26^{a} ± 0.05	4.18^{a} ± 0.01
Fat (%)	1.53^{a} ± 0.02	1.48^{a} ± 0.02	1.48^{a} ± 0.03	1.51^{a} ± 0.02
Titratable acidity (%)	0.95^{a} ± 0.00	1.00^{ab} ± 0.00	1.16^{bc} ± 0.04	1.21^{c} ± 0.06

Note: Values in same row with similar superscript letter do not significantly differ ($p<0.05$) and vice versa

Table 5.18 ANOVA for effect of storage period (days) on chemical Parameters of low fat yoghurt

Parameters	Mean sum of square	df	F-value	Sig.
Total solids (T.S)	0.058	3	0.807	0.524^{NS}
pH	0.057	3	30.247	0.000**
Fat (%)	0.002	3	1.286	0.344^{NS}
Titratable acidity (%)	0.048	3	10.838	0.003**

** indicates significant at ($p<0.01$); ^{NS} indicates non significant (in all levels)

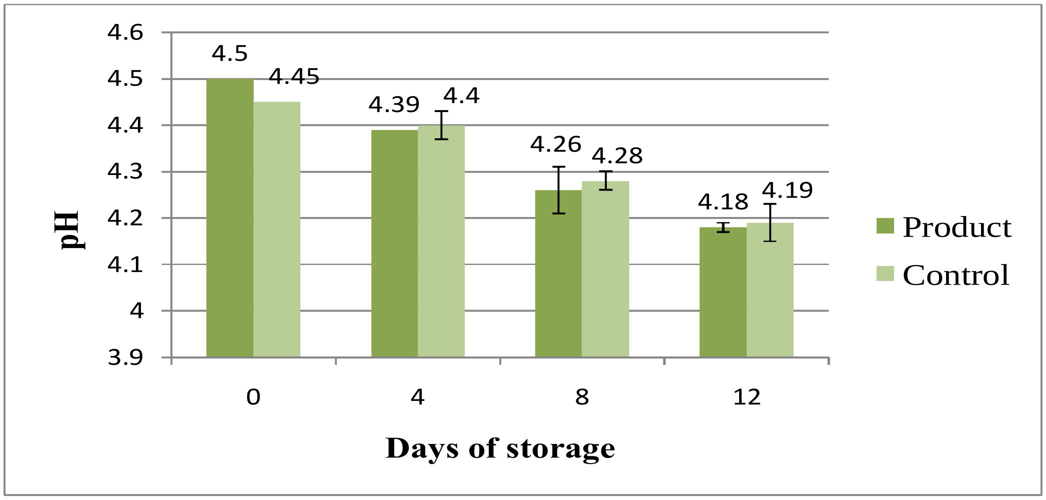


Fig.5.18: Effect of storage period on pH of low fat yoghurt

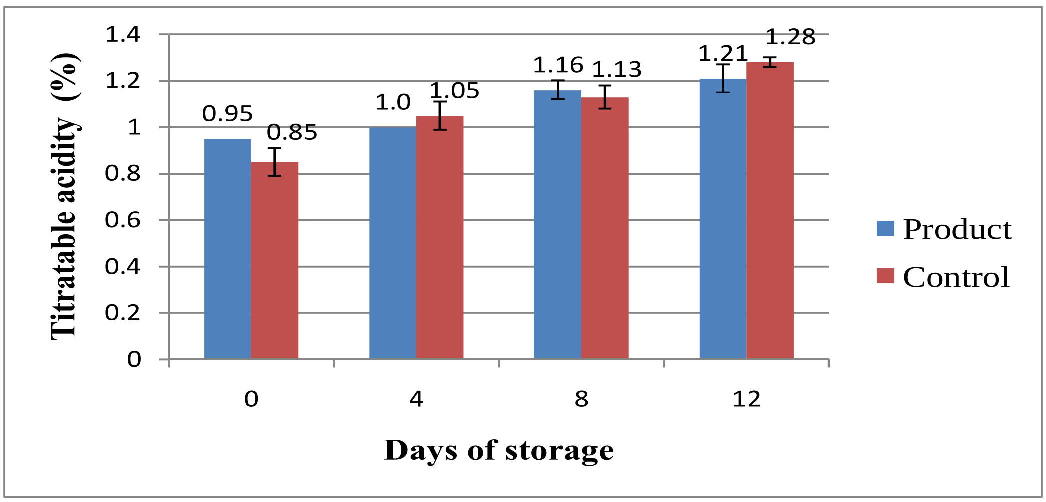


Fig.5.19: Effect of storage period on titratable acidity (% LA) of yoghurt

5.10 EFFECT OF STORAGE ON BIOCHEMICAL PARAMETERS OF LOW FAT YOGHURT

The biochemical parameters studied during storage of low fat yoghurt included proteolysis, acetaldehyde, L- and D-lactic acid and EPS concentration.

5.10.1 Proteolysis

Proteolysis increased through out the storage duration in EPS yoghurt. Between 0day and 8th day, there was no significant difference in Proteolytic changes. The significant differences ($p<0.05$) were however observed between 0day 2.08 µg/ml and 12day (2.58 µg/ml) (Table 5.19) and corresponding results are shown on (Fig 5.20) the observations were in agreement with observations of Guzel *et al*., (2005) who reported that, proteolysis of yoghurt made with both ropy

(EPS) and non ropy cultures, showed increase in proteolysis up day 14 of storage. As can be seen in Fig. 5.20, control yoghurt showed higher level of proteolysis than EPS yoghurt. Dutta *et al.,* (1971) also reported that yoghurt bacteria have slight Proteolytic activity; however, *L.bulgaricus* has slightly higher Proteolytic activity than *S.thermophilus*. In this study, proteolysis in yoghurt sample was lower than threshold level of bitterness (0.5mg/ml).

5.10.2 Acetaldehyde

Acetaldehyde is considered as the major flavour component of yoghurt. During the storage, it were observed that, acetaldehyde content of EPS yoghurt increases from 0 day up to 12 day of storage (1.25, 1.38,1.23 & 1.89mg/L at 0,4,8 and 12 day respectively) (Table 5.19) and corresponding results are shown on (Fig. 5.21) but on the 8^{th} day, there was slight decrease in acetaldehyde level which was non statistically significant. Highly significance difference ($p<0.01$) was observed on 12^{th} day of storage.

Acetaldehyde content in yoghurt samples was high in EPS yoghurt than control which might be due to difference in storage time and temperature. With longer incubation time, yoghurt micro organisms were able to produce more acetaldehyde which is the most important flavour substance of yoghurt.Threonine aldolase converts threonine to glycine and acetaldehyde. Increasing incubation temperature results in decreasing production of acetaldehyde (Wilkins *et al*., 1986).There was a significant difference ($p<0.01$) in acetaldehyde content between 0day and 12^{th} for yoghurt samples, which shows that acetaldehyde content in yoghurt increased up to the end of storage. This is due to alcohol dehydrogenase produced by yoghurt cultures which do not convert acetaldehyde to ethanol during storage. This might be due to short period of storage or capability of bacteria strains used to enhance flavour compounds in EPS yoghurt.

5.10.3 L-and D-lactic acid

EPS yoghurt showed significant increase ($p<0.01$) in both L-and D-lactic acid up to 4^{th} day of storage after which the increase was not significant for the rest of the storage period (Table 5.19) and corresponding results are shown on (Fig 5.22 & 23). Higher concentration of L-lactic acid was observed on 4^{th} day (85.46 mg/L) and lowest concentration was on 12^{th} day (80.10 mg/L) while high concentration of D-lactic acid was observed on 12^{th} day (40.45 mg/L) and lowest concentration on 0 day of storage (19.78 mg/L). The control sample were having high level of L-and D-lactic acid as compared to EPS yoghurt. This increase in both L-and D-lactic acid is associated with high fermentation temperature and long time storage can cause an increase in the concentration of D (-) lactic acid, thus, decreasing the ratio of L (+) / D (-) lactic acids

Mostly, the concentration of L-lactic acid is more than that of D-lactic acid and during storage period, both L and D lactic acid increases. This is in agreement with the findings of Akin (2006) who reported that concentration of D (-) lactic acid that could be taken per day should be 0-100 mg/kg body weight and should not be used in infant and children diet due to low digestion. World Health Organization (WHO) has reported that there is no limit for the consumption of L (+) lactic acid. It is agreed that the typical yogurt flavour is caused by lactic acid which imparts an acidic and refreshing taste Chaves, *et al*., (2002)

5.10.4 EPS concentration

The EPS concentration in the sample decreased from initial 857.35mg/L (0 day) to 808.10 mg/L (4^{th} day), the values being not significantly different. A significant drop were observed on 8^{th} day (723.87mg/L) which increased significantly to 1083.33mg/L on 12^{th} day ($p<0.01$) (Table 5.19) and corresponding results are shown on (Fig. 5.24).This slight decrease in EPS concentration on 4^{th} and 8^{th} day during first week of storage may indicate the activity of enzymes capable of degrading the EPS (Deegest *et al*., 2002).But highly significant increase ($p<0.05$) were observed on 12^{th} day of storage. These are in agreement with the findings of Amatayakul *et al*., (2006) who reported increase in EPS content during 28 days of storage while Doleyres *et al*., (2005) found the content to be stable during 4 weeks of storage period. The EPS concentration in yoghurt made with EPS producing strains in our study ranged from 723.87 mg/L to 1083.33 mg/L (Table 5.19) wile in control range from 568.5 to 609.01 mg/L. Other researchers have reported EPS concentration in fermented milk product to be ranging from (40 to 400mg/L Marshall & Rawson 1999, De Vuyst *et al*., 2003). There could be several possible reasons for differences between these studies and our results including the use of different strains. The level of inoculation of starter cultures, the differences in fermenting conditions and the methods of isolation, purification and quantification of EPS (Amatayakul *et al.,* 2006).

Table 5.19 Effect of storage period on biochemical parameters of low fat Yoghurt (Means ± SE)

Days of storage				
Parameter	0	4	8	12
Proteolysis (µg/ml)	$2.08^{a} \pm 0.07$	$2.26^{ab} \pm 0.08$	$2.27^{ab} \pm 0.09$	$2.58^{b} \pm 0.09$
Acetaldehyde (mg/L)	$1.25^{a} \pm 0.01$	$1.38^{a} \pm 0.03$	$1.23^{a} \pm 0.10$	$1.89^{b} \pm 0.03$
L-lactic acid (mg/L)	$76.09^{a} \pm 0.18$	$85.46^{b} \pm 1.8$	$81.30^{ab} \pm 0.42$	$80.10^{ab} \pm 1.38$
D-lactic acid (mg/L)	$19.78^{a} \pm 1.8$	$37.48^{b} \pm 2.3$	$39.24^{b} \pm 1.57$	$40.45^{b} \pm 1.98$
EPS conc.(mg/L)	$857.35^{a} \pm 38.92$	$808.10^{ab} \pm 24.44$	$723.87^{b} \pm 5.46$	$1083.33^{c} \pm 5.46$

Note: Values in same row with similar superscript letter do not significantly differ ($p<0.05$) and vice versa

Table 5.20 ANOVA for effect of storage periods (days) on biochemical and microbiological parameters of low fat yoghurt

Parameters	Mean sum of square	df	F-value	Sig.
Proteolysis (µg/ml)	0.134	3	5.559	0.023*
Acetaldehyde (mg/L)	0.289	3	31.428	0.000**
L-lactic acid (mg/L)	44.643	3	10.393	0.004**
D-lactic acid (mg/L)	283.158	3	22.569	0.000**
EPS concentration (mg/L)	70842.006	3	43.473	0.000**
SPC (cfu/ml)	0.115	3	63.471	0.000**

** indicates significant at ($p<0.01$); * indicates significant at ($p<0.05$)

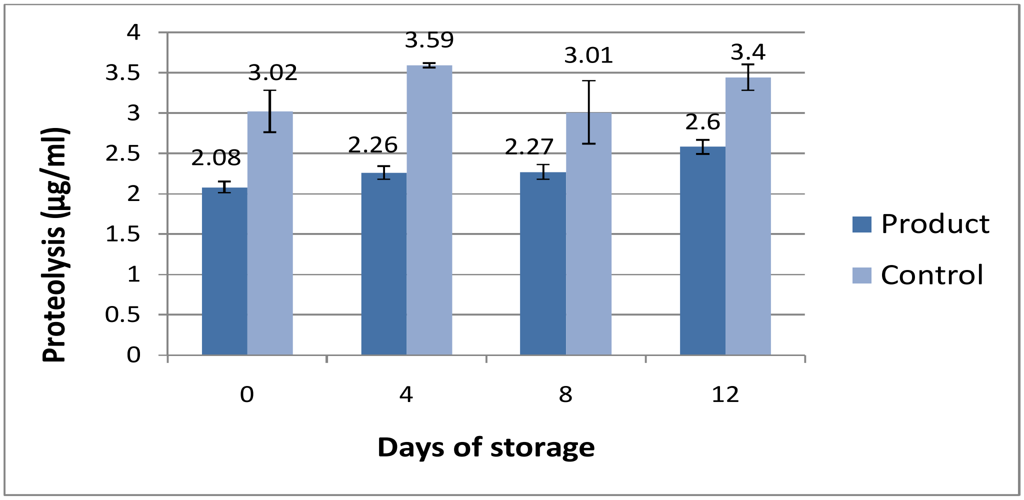


Fig.5.20: Effect of storage period on proteolysis (µg/ml) of yoghurt

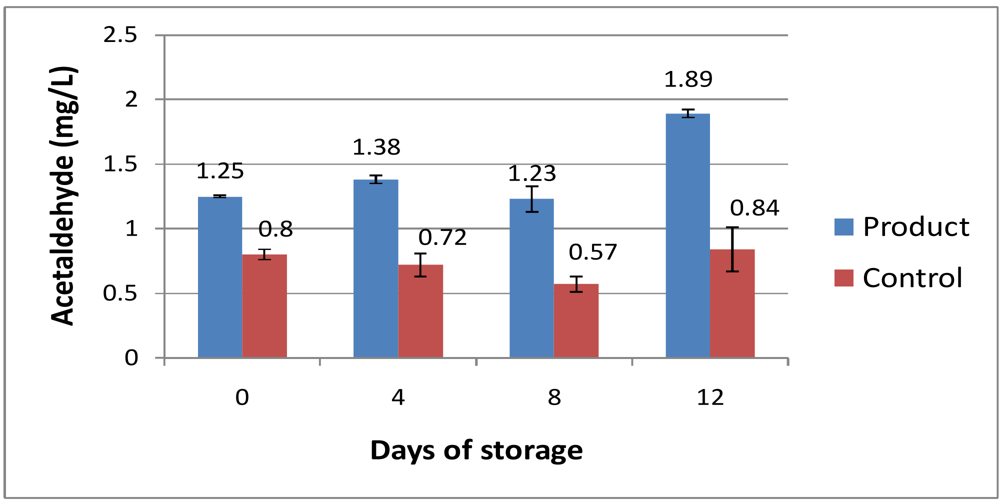


Fig.5.21: Effect of storage period on acetaldehyde (mg/L) content of yoghurt

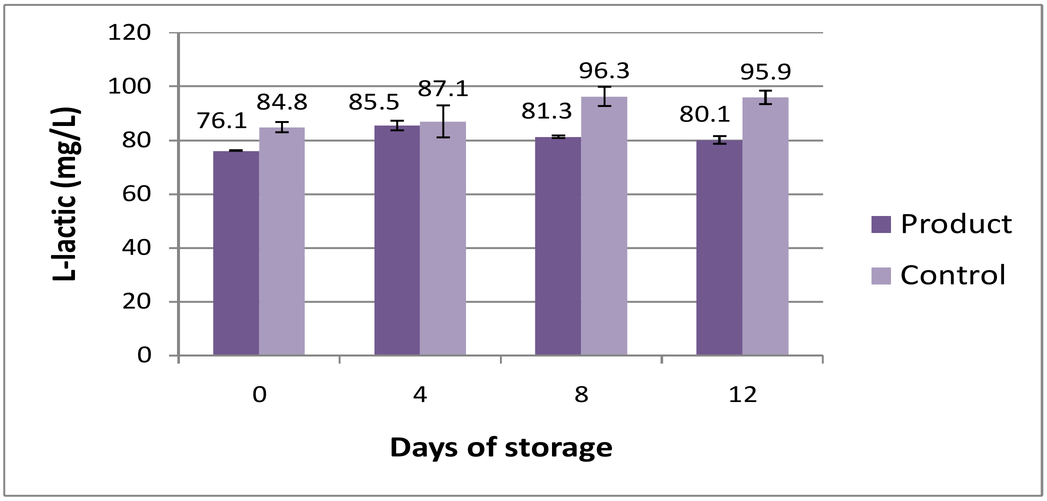


Fig. 5.22: Effect of storage period on L- lactic acid (mg/L) of yoghurt

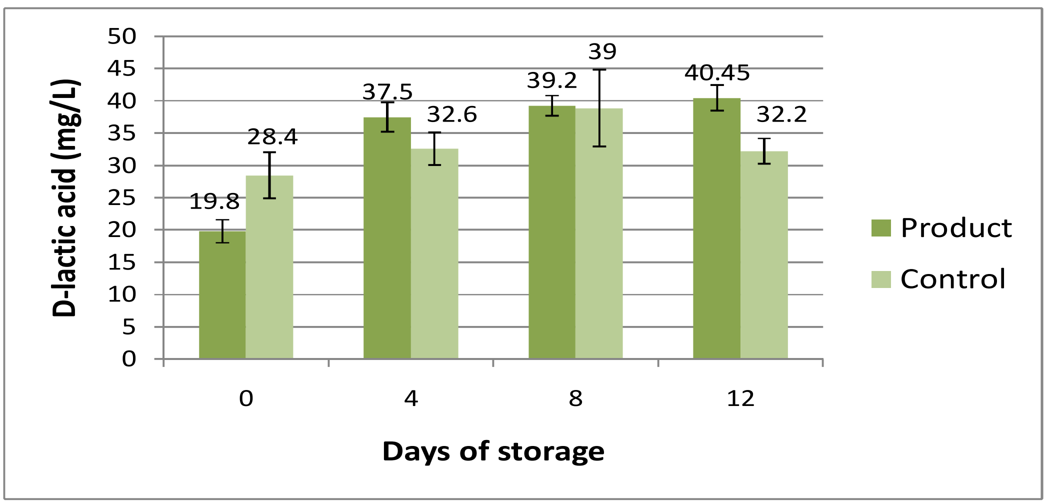


Fig. 5.23: Effect of storage period on D- lactic acid (mg/L) of yoghurt

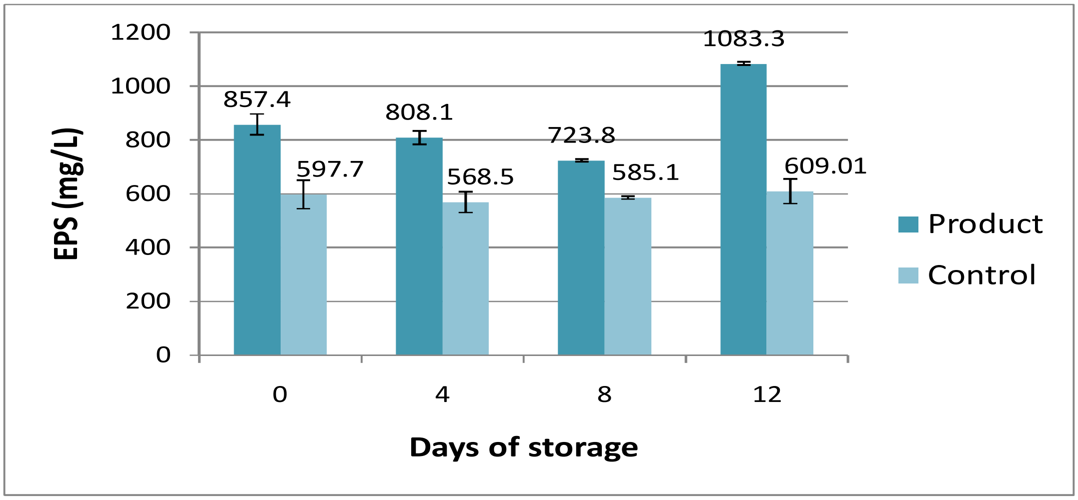


Fig. 5.24: Effect of storage period on EPS concentration (mg/L) of yoghurt

5.11 EFFECT OF STORAGE ON MICROBIOLOGICAL QUALITY OF LOW FAT YOGHURT

The microbiological parameters studied during the storage of low fat yoghurt included standard plate count, coliform, yeast and mould

5.11.1 Effect of storage on standard plate count (SPC)

Standard plate count (SPC) decreased over the entire storage duration (Table 5.21) and corresponding results are shown on (Fig 5.25). On 8th day and 12th day the decrease in cfu/ml indicated low stability of starter culture in fermented dairy products, viable bacteria being inhibited by development of high acidity. At the beginning, the SPC count were 8.96 log cfu/ml which is considered good for yoghurt, at the end of shelf life SPC was very low, i.e. 8.55 log cfu/ml (Table 5.21) which is considered inadequate. Similar results have been reported by Guler-Akin and Akin (2007) who noted that, both *L.bulgaricus* and *S.thermophilus* count decrease by about 1 log cycle during storage but in our case, decrease was less than 1 log cycle. The SPC count in fresh sample were 8.96 log cfu/ml which decreased to not significantly different level of 8.93 log cfu/ml.The SPC count on day 8 (8.71log cfu/ml) and day 12 (8.55 log cfu/ml) were significantly lower than those observed during in first four days.

Table 5.21 Effect of storage at (4-5°C) on Standard plate count (SPC) (log cfu/ml) in low fat yoghurt (mean ± SE)

Days of storage				
Parameter	0	4	8	12
SPC (log cfu/ml)	$8.96^{a} \pm 0.03$	$8.93^{a} \pm 0.03$	$8.71^{b} \pm 0.01$	$8.55^{c} \pm 0.02$

Note: Values in same row with similar superscript letter do not significantly differ (p<0.01) and vice versa

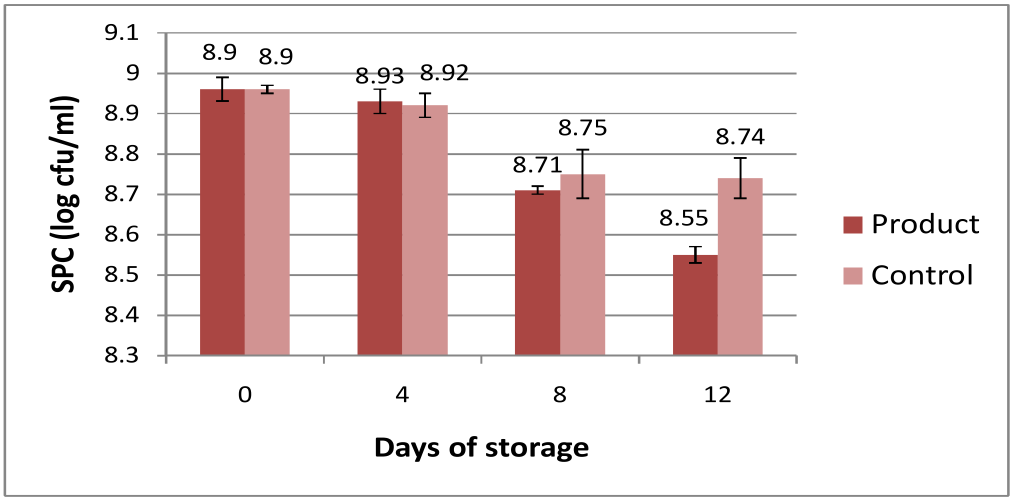


Fig. 5.25 Effect of storage period on standard plate count (SPC) (log cfu/ml) in yoghurt

5.11.2 Effect of storage period on yeast and mould count

Yeast and moulds have the ability to grow in acidic environment and are the main causative organisms for spoilage of fermented milk products. Owing to its acidic environment, low fat yoghurt prepared and stored for 12 day period was not favourable to the growth of yeast and moulds due to hygienic practices applied

5.11.3 Effect of storage period on coliform count

The microbiological analysis for coliforms count for all the samples had shown that there were no coliforms present in any of the samples through out the storage period. The presence of coli forms indicates unhygienic practices during product manufacture. Absence of coliforms in our samples indicated hygienic practices followed during the product manufacture and storage period.

6.0 Summary and Conclusion

The present study was undertaken with two objectives, the first objective was to study the effect of fat and solid not fat (SNF) levels on quality of low fat yoghurt and second objective was to study the effect of selected Exopolysaccharides (EPS) producing cultures on quality parameters of low fat yoghurt. The salient findings of the study are given below.

- Three fat levels and two SNF levels were used to prepare yoghurt with medium and low fat using a modified method. A total of three different types of milk i.e. milk having 0.5% fat and 9-10% SNF; 1.5% fat having 9-10% SNF, 3.0% fat having 9-10% SNF, were used to make yoghurt before optimisation of the final product.
- Culture combinations used were EPS producing cultures (NCDC: **285** & NCDC: **401**).
- Three types of milk i.e. milk with 0.5, 1.5 and 3.0% fat and 9-10% SNF were inoculated with EPS producing culture @ 2% in the ratio of 1:1 and incubated at 37^{o}C for 7-8hrs. The standardised milk was inoculated with NCDC: **263** @ 2% level and incubated at 43^{o}C to prepare control yoghurt.
- Based on sensory attributes, yoghurt with1.5% fat scored highest in terms of body and texture and overall acceptability (7.59 & 7.56) while, yoghurt with 0.5% fat scored least (7.06 & 7.00) according to 9 point hedonic scale. Also Syneresis was less in 1.5% compared to 0.5% fat (4.58 %, & 9.17 %).
- Studies on different levels of SNF used before optimisation revealed that 10% SNF showed higher scores for flavour and overall acceptability (7.58 ± 0.11 & 7.52) compared to 9% SNF (7.14 & 7.21) according to 9 point hedonic scale. Also, yoghurt with 10% SNF, showed higher firmness (1.45N), EPS concentration (817.46mg/L), Viscosity (215.83cp) as compared to 9% SNF with 1.34N firmness, 746.57mg/ml EPS and 167.25cp viscosity.
- Out of the three types of milk used with different levels of fat and SNF inoculated with EPS producing cultures, low fat yoghurt prepared from milk with 1.5% fat and 10% SNF showed better technological and sensory properties compared to yoghurt prepared from milk with 0.5% fat with 9-10% SNF levels and yoghurt with 3% fat with 9-10% SNF levels.

- Non EPS producing culture (NCDC: **263**) were used to prepare control yoghurt with 4.5% fat and 10% SNF and were compared with the final optimised product during storage studies
- Low fat yoghurt with 1.5% fat and 10% SNF were selected for storage studies based on sensory, rheological and biochemical parameters of yoghurt.
- Low fat yoghurt showed increase in viscosity from 0day (482.83 cp) to 8th day of storage (536.50cp), while syneresis level was high on 8th day (6.25%).
- The maximum titratable acidity and decrease in pH were observed on 12th day (1.21% and 4.18% LA) respectively.
- Biochemical parameters like proteolysis, acetaldehyde, EPS concentration and D-lactic acid were maximum on 12th day (2.58µg/ml, 1.89mg/L, 1083.33mg/L & 40.45mg/L) respectively while L- lactic acid was maximum on 4th day (85.46mg/L)
- The minimum (SPC) was observed on 12th day of storage (8.55 log cfu/ml) Yeast and mould counts were nil in all the samples throughout the storage of the product.

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Annexure 1

Division of Dairy Technology
NATIONAL DAIRY RESEACH INSTITUTE
Karnal (Haryana)

Score Card for Sensory Evaluation of low fat yoghurt

Product particulars: ____________ **Date: _________**

Kindly evaluate the given samples of yoghurt for flavour,colour and appearance, Body and texture, Acidity, and overall acceptability using the following 9-point hedonic scale and enter the score for each sample in the space provided in the below table.

Hedonic ratings	**Score**
Like Extremely	9
Like Very Much	8
Like Moderately	7
Like Slightly	6
Neither Like nor Dislike	5
Dislike Slightly	4
Dislike Moderately	3
Dislike Very Much	2
Dislike Extremely	1

Sensory attributes	**Sample code**				
Flavor					
Color and appearance					
Body and texture					
Acidity					
Overall acceptability					

Remarks (if any):

____
____
____

Signature: ________________

Division of Dairy Technology
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CERTIFICATE

This is to certify that the final three copies of the thesis entitled, "**EFFECT OF SELECTED EXOPOLYSACCHARIDES PRODUCING CULTURE ON QUALITY OF LOW FAT YOGHURT**" submitted by **Mr. KAYUMBA FRED in partial fulfilment of the award of the degree of Master of Technology in Dairying (Dairy Technology) of the National Dairy Research Institute (Deemed University), Karnal (Haryana), India**, have included all corrections and suggestions made by the External Examiner and the Advisory Committee members during the viva-voce examination under my supervision and guidance.

Dated: 16/07/11

(Dr.R.R.B.Singh)
Major Advisor & Chairman
(Guide)

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