PREPARATION OF A CEREAL BASED FOOD PRODUCT CONTAINING EXOPOLYSACCHARIDES USING LACTOCOCCUS LACTIS

A dissertation

submitted in partial fullfillment of requirement

for the award of Degree

Masters of Science

In

Microbiology

By

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CANDIDATE'S DECLARATION

I , hereby declare that the work done is being presented in dissertation entitled 'Preparation of a cereal based food product containing exopollysacharides using *Lactococcus lactis*' in partial fulfilment of requirement for the award of the degree of Masters of science in Microbiology Department of Biotechnology and Environmental Sciences , Thapar University , Patiala, Punjab is an authentic record of my own work during a period of six months from January 2013 to August 2013, under the supervision of Dr. Abhijit Ganguly , Associate Professor, Department of Biotechnology and Environmental Sciences , Thapar University . The matter embodied in this thesis has not been submitted in part or full to any other university o institute for the award of any other degree.

Place : Patiala PUNEET KAUR
Date :

CERTIFICATE

This is to certify that the dissertation entitled " Preparation of a novel food product containing exopolysaccarides by *Lactococcus lactis* " submitted by Puneet Kaur Randhawa (Reg.no. 301105011) in practical fulfilment of the requirements for the award of Degree of Masters of Sciences in Microbiology , to Thapar University , Patiala in an authentic record of student's own work carried out by her under our supervision and guidance . The report has not been submitted for the award of any other degree or certificate in this or any other University or Institute .

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Puneet Kaur Randhawa

CONTENTS

LIST OF ABBREVIATIONS

LAB Lactobacillus

MSG Monosodium glutamate

MRS De man rogosa sharpe medium

Ml Mililitres

Cfu Colony forming unit

Mm mili molar

HPLC High pressure liquid chromatography

TLC Thin layer chromatography

µl Microlitres

FOS Fructooligosaccarides

EPS Exopolysaccarides

DOUGH A 10g wheat flour : 10g V. Mungo flour

DOUGH B 10g wheat flour : 10g V. Mungo flour WITH CULTURE

DOUGH C 5g V. Mungo flour : 10g wheat flour

DOUGH D 5g V. Mungo flour : 10g wheat flour WITH CULTURE

DOUGH E 10Gg V. Mungo flour : 5g wheat flour

DOUGH F 10Gg V. Mungo flour : 5g wheat flour WITH CULTURE

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ABSTRACT

Lactic acid bacteria possess special physiological activities and regarded as safe. Therefore, various types of LAB that show EPS-producing ability is an interesting target for food industry, especially fermented foods because individual strain has specific fermentation profiles such as acid production, taste and flavour formation ability. These profiles are considered as important factors in the use of LAB as starter cultures in the production of fermented foods. In the present study, preisolated lactic acid bacteria were screened for high EPS producing strains namely, *Lactococcus lactis* . The culture media produced high amount of EPS in MRS, when inoculated and kept at 30°C for 48h with an initial pH 5 on static condition. The optimized conditions were used to prepare a fermented cereal based food product having high nutritional value. The raw material used in the food product was wheat flour and *Vigna mungo.*The EPS concentration obtained with MRS was 248.8 mg/100g sourdough and FOS with 2.2-2.6 / 100g sourdough with *L. lactis* resp. and in the food product prepared with *Vigna mungo* and wheat flour with mixed culture . In addition the sensory attributes were acceptable .The composite sourdough could be baked with FOS , EPS , fibre content and a moderate neutraceutical content .

Keywords: *Lactococcus lactis*, EPS (EXOPOLYSACCARIDE) , FOS (FRUCTOOLIGOSACCARIDE) , Sourdough .

Chapter -1 INTRODUCTION

Bread products and their production techniques differ widely around the world (*Dewettinck et al., 2008*). The objective of bread making is to convert cereal flours into attractive, palatable and digestible food. The earliest breads were unleavened or flat, but the first major technical innovation was the introduction of leavening processes, which yielded breads of superior palatability *(Chamberlain, 1975*). The foremost quality characteristics of leavened wheat breads are high volume, soft and elastic crumb structure, good shelf life and microbiological safety of the product *(Cauvain, 2003).*

The bacterial fermented products are more palatable having wide diversity of flavor, aroma, texture and acquire their characteristic properties by the action of lactic acid bacteria *(Hammes, 1990).* The microorganisms of genera Lactococcus, Lactobacillus, Leuconostoc, Streptococcus and Pediococcus are involved in these fermentations. Lactic acid bacteria (LAB) are the most important bacteria used for the production of many fermented foods. These foods include pickles, sausages, cheese, yoghurt and sourdough breads.

The use of sourdough process as a mean of leavening is one of the oldest biotechnological processes in cereal food production (*Rocken and Voysey, 1995*). The sourdough bread is prepared from a mixture of flour and water that is fermented with (LAB), mainly hetero-fermentative strains, elaborating lactic acid and acetic acid in the mixture, and hence, resulting a pleasant sour taste end product (*De Vuyst and Neysens, 2004*). The flavor of sourdough wheat bread is richer and more aromatic than yeast leavened bread (*Brummer and Lorenz, 1991).* The use of hetero-fermentative LAB results in the production of flavoring compounds like ethyl acetate, alcohols and aldehydes, whereas, homo-fermentative LAB produce diacetyl and other carbonyls. However, on the other hand, yeast fermentation results in the production of iso-alcohols only, which may contribute little towards final bread flavor *(Rehman et al., 2006*). Although, wheat and rye flours are mostly used for sourdough making but maize and rice flours could also be used (*Buttery and Ling, 1999*). The sourdough bread is characterized by a typical aroma, taste, texture and shelf life. It differs from conventional yeasted bread, because it contains a predominant bacterial flora (*De Vuyst and Neysens,2004).*

The flavor of sourdough wheat bread is richer and more aromatic than yeast leavened bread (*Brummer and Lorenz, 1991*). The use of hetero- fermentative LAB results in the production of flavoring compounds like ethyl acetate, alcohols and aldehydes, whereas, homo-fermentative LAB produce diacetyl and other carbonyls. However, on the other hand, yeast fermentation results in the production of iso-alcohols only, which may contribute little towards final bread flavor (*Rehman et al., 2006).* Although, wheat and rye flours are mostly used for sourdough making but maize and rice flours could also be used (Buttery and Ling, 1999). The sourdough bread is characterized by a typical aroma, taste, texture and shelf life. It differs from conventional yeasted bread, because it contains a predominant bacterial flora (*De Vuyst and Neysens, 2004*).

Presently, consumers intend to like a wide range of foods that are more nutritious and flavorful and have longer shelf life having no added preservatives *(Hansen and Hansen, 1996; Collar et al., 1994*). The sourdough fermentation have a number of beneficial effects that include, a prolong shelf, accelerated volume, delayed staling, improved bread flavor and nutritional value (*Thiele et al., 2002; Lavermicocca et al., 2000*). It has been reported that sourdough fermentation increases folate and thiamin contents, and decreases tocopherol and tocotrienol contents depending upon the process of production (*Kariluoto et al., 2004; Liukkonen et al., 2003).* It also improves sensory characteristics such as loaf volume, evenness of baking, color, aroma, taste and texture of breads with extended shelf life by inhibiting mold growth (*Rehman et al., 2007).*

Lactic acid bacteria (LAB) have aroused interest for their ability to secrete extracellular polysaccharides. These extracellular polysaccharides or 'exopolysaccharides' (EPS) have immense commercial value because of their industrially useful physico-chemical properties. Capacity of LAB to produce a broad range of EPS with variable composition and functionality is widening their industrial applications. Food associated lactic acid bacteria are granted the status of 'generally recognised as safe' (GRAS) and are discovered to be the suitable candidates for the production of functional EPS . These safe LAB which produce in situ novel functional EPS can escape the rigorous toxicological testing and marketing of this EPS holds huge prospects.

EPS derived from LAB play crucial role in improving the rheology, texture, mouth feel of fermented food formulations and conferring beneficial physiological effects on human health, such as antitumour activity, immunomodulating bioactivity and anticarcinogenecity. LAB can also produce a variety of functional oligosaccharides. Oligosaccharides have huge industrial applications as prebiotics, nutraceuticals, sweetners, humectants, drug against colon cancer, immune stimulators etc. . The total yield of EPS produced by the LAB depends on the composition of the medium, LAB strain and growth conditions like temperature, pH, oxygen tension and incubation period . The need of the day is to improve the productivity of EPS from LAB, reduce the cost of production for commercial viability and to produce custom made EPS with the desired functionality. The modulation of biochemical properties of EPS require a thorough understanding of its biosynthetic pathway and the relation between the structure of EPS and the functional effect provided by them after incorporation into the food matrix .

Chapter-2 REVIEW OF LITERATURE

Fermented foods are of great importance because they provide and preserve large quantity of nutritious foods with improved aroma and texture. These foods include alcoholic beverages, vinegar, pickles, sausages, cheese, yoghurts and sourdough breads. In recent years, sourdough bread has enjoyed renowned success due to increasing consumer demand in Europe for its natural taste and good health effects *(Brummer and Lorenz, 1991).* A mixture of cereals in water, result in the formation of dough, characterized by sour aroma when left alone for a while, is the first example of fermented food employed by mankind *(Hammes and Ganzle, 1998).* The sourdough is a mixture of flour and water that is fermented with lactic acid bacteria (LAB), mainly hetero-fermentative strains, elaborating lactic acid and acetic acid in the mixture, and hence, resulting in a pleasant sour taste of end product *(De Vyust and Neysens, 2004).* The sourdough fermentation is a traditional process for improving the bread quality and producing different wheat and rye breads (Thiele et al., 2002). At present the sourdough is employed in the manufacture of breads, cakes and crackers *(Ottogalli, et al., 1996).* The typical characteristic of sourdough is mainly due to its microflora, basically represented by lactic acid bacteria (LAB) and yeasts. Due to microbial community such dough is metabolically active and can be reactivated. These microorganisms ensure acid production and leavening upon addition of flour and water (*Anon., 1994).* The mechanisms of sourdough are complex (*Hammes and Ganzle, 1998*). Various flour characteristics and process parameters contribute to exercise very particular effects on the metabolic activity of the sourdough microflora. During fermentation, biochemical changes occur in the carbohydrate and protein components of the flour due to the action of microbial and indigenous enzymes. Moreover, fermentation temperature also influences the growth and metabolism of LAB and yeast *(Spicher et al., 1980).*

Sourdough fermenting microorganisms So far, a few less than 50 different species of LAB isolated from sourdough have been reported (*Hammes et al., 2005).* Numerous species of lactic acid bacteria occur naturally in wheat flour, including members of the genera Lactobacillus, *Pediococcus, Enterococcus, Lactococcus*, and *Leuconostoc (Hammes and Vogel, 1997)*. Most of the species of lactic acid bacteria of the genus Lactobacillus are isolated from sourdoughs *(Corsetti et al., 2001; Ottogalli et al.,* 1996). *Lactobacillus sanfranciscensis, Lb. brevis and Lb. plantarum* are the most frequent lactobacilli isolated from sourdoughs *(Bocker, Valmorri et al., 2006; Corsetti et al.,* 2003; *Corsetti et al., 2001; Gobbetti, 1998; Vogel and Hammes, 1990*). Homo-fermentative species do not produce any carbon dioxide; their function is acidification and flavor development. Although, homo-fermentative species of LAB may be used in the majority of fermented food applications but hetrofermentative species play a major role in sourdough fermentation *(Salovaara, 1998),* especially when sourdoughs are prepared in a traditional manner *(Corsetti et al., 2003; Corsetti et al., 2001*). The hetero-fermentative LAB results in better taste and flavor of the sourdough breads, because only hetero- fermentative lactic acid bacteria can produce the considerable amount of acetic acid under anaerobic conditions which are desired in sourdough (*Kosmina, 1977).*

On the other hand fermentation with homo-fermentative lactic acid bacteria results in high concentration of lactic acid, relative to acetic acid, results in mild and flat sour taste in bread (*Spicher and Rabe, 1981*). Oura et al. (1982) prepared rye bread with pure culture of hetero-fermentative bacteria *L. brevis*, this provided the rye bread with desirable aroma but not an elastic crumb. They observed an opposite effect when they used homo-fermentative bacteria (*L. plantarum*). It was concluded that in order to get satisfactory aroma and crumb characteristics, both bacterial species must incorporated. Cossignani et al. (1996) used *Lactobacillus sanfranciscensis, L. plantarum and Saccharomyces cerevisiae* for leavening wheat sourdoughs. They found that the dough's fermented with starters had more balanced microbiological and biochemical characteristics than dough's started with *Saccharomyces cerevisiae*, in which alcoholic fermentation end products largely predominated. By using starters, the greatest lactic acid bacteria cell number and acetic acid production was achieved. The starters resulted in more complete profiles of volatile compounds and greater structural stability. Starting from glucose, homo-fermentative LAB mainly produce lactic acid through glycolysis (homolactic fermentation) while hetero-fermentative LAB produce, besides lactic acid, CO2, acetic acid and/or ethanol (depending on the presence of additional substrates acting as electron acceptors (Axelsson, 1998). The LAB, both homo-fermentative and hetero-fermentative species, contribute most to the process of dough acidification, while yeasts are primarily responsible for the leavening however, the hetero-fermentative LAB also contribute partly to the leavening process (*Gobbetti et al., 1995a; Spicher, 1983*). Gobbetti (1998) reported *Lb. sanfranciscensis and Lb. plantarum* association in Italian wheat sourdough. Lb. plantarum may be superseded by another facultative hetero-fermentative species, *Lactobacillus alimentarius* in its association with *Lb. sanfranciscensis* in sourdough made from durum wheat (*Corsetti et al., 2001*). *Lactobacillus alimentarius* is capable of fermenting all four flour soluble carbohydrates (maltose, sucrose, glucose and fructose) and it is possible that this reduces direct metabolic competition with *Lb. sanfranciscensis.* Most of the *Lb. alimentarius* strains, due to a phenotypical misidentification, probably belong to *Lb. paralimentarius,* a facultatively hetero-fermentative species first isolated from Japanese sourdough (*Cai et al., 1999*).

Lactobacilus brevis and *Lactobacilus plantarum* have generally been found associated with Lactobacillus fermentum in Russian sourdoughs (*Kazanskaya, et al.,, 1983).*

Gobbetti et al. (1994a) reported that *Lactobacilus acidophilus* is common in Umbrian (Italian region) sourdoughs, even though it is rarely isolated from sourdoughs of different origin (*Infantes and Tourneur, 1991*). Corsetti et al. (2005) described a new sourdough associated species, Lb. rossiae, that seems to be wide diffused in sourdoughs of southern and central Italy (*Settanni, et al., 2005*). Lb. rossiae is often associated with the key sourdough *Lactobacilus sanfranciscensis*. *Lactobacilus rossiae* has been found in environments other than sourdough (De Angelis et al., 2006a), while no other habitat is known for *Lactobacilus sanfranciscensis* (Hammes et al., 2005). However, occurrence of lactic acid bacteria and yeasts in sourdoughs and the association between acidification and bacterial metabolism was first demonstrated in 1894 (*Hammes and Ganzle, 1998*). Association of yeasts and lactic acid bacteria are often used in the production of beverages and fermented foods (Gobbetti, 1998). The vast majority of yeasts found in sourdoughs have been allotted to the species *Candida milleri, Candida holmii, Saccharomyces exiguous and Saccharomyces cerevisiae (Hammes and Ganzle, 1998)*. Most of yeast preparations often contain LAB, especially lactobacilli rather than Pediococcus, *Lactococcus and Leuconostoc spp. (Jenson, 1998),* which contributes a little to the aroma development acidification and of dough because of the limbed processing time (*Rothe and Ruttloff, 1983).*

The microorganisms are mostly found on the glumes and glumules of caryopsis, which is usually removed during milling or during the other treatments of the grain before milling, hence, yeasts are not very abundant in refined flour (*Galli and Franzetti, 1987*). The sourdough fermentation is a complex process caused by the combined effects of the metabolism of yeasts and lactic acid bacteria. The former is mainly responsible for the leavening, while the latter acidify it. The dough in special conditions, some yeast and lactic acid bacteria act synergistically. Along with fermentation of sugars to carbon dioxide and ethanol, the yeast also produces some by-products which impart taste and flavor to the bread (*Boraam et al., 1993*). These yeasts are often associated with LAB in sourdough and yeasts/LAB ratio is generally 1:100 (*Ottogalli et al., 1996; Gobbetti et al., 1994*). The yeasts found in sourdoughs belong to more than 20 species (*Gulloet al., 2002; Stolz, 1999; Rossi, 1996*). Typical yeasts associated with LAB in sourdoughs are Saccharomyces exiguus, Candida humilis (formerly described as Candida milleri) and Issatchenkia orientalis (Candida krusei) (*Succi et al., 2003; Gobbetti et al., 1994a; Spicher and Schroder, 1978*). Other yeast species detected in sourdough ecosystem are: *Pichia anomala as Hansenula anomala, Saturnispora saitoi as Pichia saitoi, Torulaspora delbrueckii, Debaryomyces hansenii and Pichia membranifaciens (Succi et al., 2003; Foschino and Galli, 1997; Gobbetti et al., 1994a*). The presence of *Candida humilis* in sourdough was reported by Barnett et al. (2000), however the dominance of Candida humilis in sourdough is a new observation.

The variability in the number and type of yeasts species in dough are affected by many factors like dough hydration, level the type of cereal used, the leavening temperature and the sourdough maintenance temperature (*Gobbetti et al., 1994; Hardy, 1982*). Among yeasts, S. cerevisiae is most sensitive, while *S. exiguous*, or its imperfect form Torlupsis holmii is very resistant. This depends upon pH of the medium and the presence of undissociated form of acetic acid (*Lodder, 1974*). The titratable acidity and pH of the dough are important during sourdough fermentation. In the initial phase, both acidity and pH remain constant, whereas, during the intermediate phase titratable acidity increases due to the presence of yeast.

During long term fermentation phase the yeast presence becomes negative and titratable acidity and pH of the dough depend mainly on the lactic acid bacteria introduced into the system (*Mascaros et al., 1994*). The yeasts present in sourdough are only slightly influenced by lactic acid, but much more effected by acetic acid (*Schulz, 1972*). The available carbohydrates in wheat flour are maltose followed by sucrose, glucose and fructose, along with some trisacchrides like maltotriose and reffinose. The glucose increases during fermentation, whereas sucrose decreases in the presence of yeast due the reaction of invertase *(Gobbetti et al., 1994)* .The yeasts present in sourdoughs are not able to ferment maltose, a sugar common in flour. However, it can nevertheless develop because of glucose released into the medium by some lactic acid bacteria species, e.g. *L. sanfranciscensis (Foschino and Galli, 1997; Boraam et al., 1993).*

Cereal foods in its various forms are essential components of the daily diet and nutritionally, they are an important source of carbohydrates, protein, dietary fiber and many vitamins and non-nutrients (*Liu et al., 2000; Pereira et al., 2002*). The whole grains intake is reported to be associated with health benefits, including improved regulation of blood glucose level and decreased risk of diabetes cardiovascular disease, and certain cancers (Jacobs et al., 1998). Bakery products, particularly sourdough breads produced from high extraction rate flours are the best potential source of fiber to reduce cardio-vascular disease, gastrointestinal disorders and diabetes (*Pomeranz et al., 1977*) The sourdough provides aromatic and pleasing flavor, and improves overall quality and shelf life of whole grain breads (Katina et al., 2005). The sourdough fermentation has been reported to increase folate content *(Kariluoto et al., 2004; Liukkonen et al., 2003*), decrease tocopherol and tocotrienol content (*Liukkonen et al., 2003).*

Whole meal cereals are an important source of minerals such as K, P, Mg, or Zn, but mineral utilization is limited by the presence of phytic acid (*Lopez et al., 1998*). Wheat and rye contain about 2–58 mg/g phytic acid, which is localized in the aleurone layer of a kernel *(Garcia-Estepa et al., 1999*). The degradation of phytate, has repeatedly been reported in sourdough processes (*Angelis et al., 2003*). Reduction of phytic acid content during bread making depends on phytase action, meal particle size, pH, temperature, water content and fermentation time (Angelis et al., 2003). Phytate-degrading enzymes exist in cereals, yeast and lactic acid bacteria isolated from sourdoughs *(Lopez et al., 2000*). Generally, low pH favors degradation of phytic acid and optimal pH value for hydrolysis is 4.5 in wheat and rye doughs. Use of sourdoughs or acidified sponges can be adjusted to improve mineral bioavailability by increasing phytic acid hydrolysis (*Fretzdorff and Brummer, 1992*). The sourdough has also great potential to modify the macromolecules in the dough, the most well known examples being the ability of sourdoughs to reduce digestibility of starch (Liljeberg et al., 1995). The presence of lactic acid in bread, either added or formed during sourdough fermentation, has also been reported to reduce acute glyceamic and insulinaemic responses (Liljeberg et al., 1995).

Di-Cagno et al. (2002) measured the rheology of fermented dough's by using empirical techniques and found a decrease in resistance to extension and an increase in both extensibility and degree of softening. During the sourdough fermentation different organic acids are produced. These organic acids improve the flavor of bread, help the swelling of gluten and increase gases retention, which result in products with good texture and massive volume and also function as natural dough conditioner (*Park et al., 2006*) The exopolysacchardies are microbial polysaccharides secreted extracellularly, the amount and structure of which depend on the particular microorganisms and the available carbon substrate (Korakli et al., 2001). These are produced by lactic acid bacteria during fermentation is one of the aspects of sourdough technology with the potential for the replacement of hydrocolloids. These compounds, commonly named as gums, are used as texturizing, antistaling, or prebiotic additives in bread production (*Tieking et al., 2003*).

Though the pH of a ripe sourdough varies with the nature of the process and starter culture used, but for wheat sourdoughs, it ranges from 3.5 to 4.3. The nature of the flour, in particular its ash content, has a considerable effect on acidification (*Clarke et al., 2002; Collar et al., 1994)*. The acids strongly influence the mixing behavior of dough and the dough with lower pH value requires a slightly shorter mixing time (Hoseney, 1994). This acid increases the solubility of the glutenin fraction extracted from wheat flour and also affects the swelling power of gluten (*Axford et al., 1979*). Strong flours result in superior dough handling properties, but result in unsatisfactory crumb texture and separation quality.

In comparison to bread prepared with bakers yeast, the sourdough breads are characterized by moist, dense grains and rather chewy texture (*Qarooni, 1996*). The application of sourdough to wheat breads has a positive impact on bread volume (*Clarke et al., 2004; Corsetti et al., 1998a; Collar et al. 1994a; Barber et al., 1992*) which is a primary quality characteristic of bread (*Maleki et al., 1980).* The larger loaf size produces softer bread and the sourdough breads have been shown to have lower crumb firmness values (*Clarke et al., 2004; Corsetti et al., 2000; Collar, 1994a*). The holes of relatively small size (~1 or 2 mm) are required in bakery products, whereas large voids or irregular crumb distributions are undesirable (Cauvain, 1998). An increase in the mean cell area has been demonstrated via addition of 20% sourdough (*Crowley et al., 2002).*

The optimal use of sourdough can improve the taste and flavor of the bread (Rehman et al., 2006). The flavor of sourdough wheat bread is richer and more aromatic than wheat bread, a factor that can be attributed to the long fermentation time of sourdough (*Brummer and Lorenz, 1991*). The concentration of 2-phyenylethanol, one of the most potent odorants of wheat bread crumb increases in sourdough bread crumb *(Gassenmeier and Schieberle, 1995*). The production of volatile flavor components in sourdough is strongly dependent on the starter culture, but the role played by the flour used has also been recognized (Hansen and Hansen, 1994). The main influence of microorganisms on sourdough flavor has been identified as their ability to enhance or reduce the amount of specific volatiles already present in the flour (*Czerny and Schieberle, 2002).*

In bakery products, staling indicates decreasing consumer acceptance which is caused by changes in crumb other than those resulting from the action of spoilage organisms (*Bechtel et al., 1953*). The application of lactic acid bacteria in the form of sourdough have positive effect on bread staling. One such effect is an improvement in loaf specific volume, which is associated with the reduction in the rate of staling (*Maleki et al., 1980; Axford et al., 1968*). The breads containing sourdough can decrease the staling rate as measured by differential scanning calorimetric *(Corsetti et al., 1998a; 2000; Barber et al., 1992).*

The sourdough inhibits the growth of the pathogens by synthesizing the antimicrobial compounds, like lactic acid, acetic acid, benzoic acid and hydrogen peroxide (*Park et al., 2006*). Mold growth is the most common cause of microbial spoilage and deterioration in the quality of bread during storage. Certain sourdough lactic acid bacteria and their components have an antifungal effect against various fungal species due to the production of organic acids, particularly acetic acid (Roecken, 1996). The use of lactic acid bacteria as a means of biopreservation, that is, control of one organism by another has shown very good results (Magnusson et al., 2003). Positive effects of the use of sourdough on the mold free shelf life of wheat bread have also been reported by Barber et al, (1992).

The sourdough inhibits the growth of the pathogens by synthesizing the antimicrobial compounds, like lactic acid, acetic acid, benzoic acid and hydrogen peroxide (*Park et al., 2006*). Mold growth is the most common cause of microbial spoilage and deterioration in the quality of bread during storage. Certain sourdough lactic acid bacteria and their components have an antifungal effect against various fungal species due to the production of organic acids, particularly acetic acid *(Roecken, 1996*). The use of lactic acid bacteria as a means of biopreservation, that is, control of one organism by another has shown very good results (Magnusson et al., 2003). Positive effects of the use of sourdough on the mold free shelf life of wheat bread have also been reported by Barber et al, (1992). In order to reduce the use of preservatives and treatments that may affect healthy attributes of food has led to attempts to improve bread quality and shelf life through formulation with compounds naturally occurring in foods *(Lavermicocca et al., 2000).* There is no correlation between bread shelf-life and pH level, but the type and amount of acid present may have an effect on other micro static agents (Barber et al., 1992). Employing an agar well diffusion assay it was found that the individual organic acid (acetic, caproic, proionic, butyric, n- valeric, and formic) produced by L. sanfrancisco inhibits Fusarium graminearum (*Corsetti et al., 1998b).* Other substances contributing to activity of this nature include returein, hydroxyl fatty acids, proteinaceous compounds, cyclic dipeptides, 3-phenyllactic acid, caproic acid, and diacetyl hydrogen peroxide (Magnusson, 2003). Although, antimold activities may vary greatly among the strains and are mainly detected within obligately hetero-fermentative Lactobacillus species but rate of inhibition of number of fungal species is highly strain dependent. (*Corsetti et al., 1998a).*

Flavor is simultaneous perception of taste, odors and trigeminal nerve response (*Lawless and Heymann, 1999*). The aroma of bread is one of the most important characters influencing the acceptance of a consumer (*Schiebeler, 1996*). The ingredients play a vital role in the development of characteristic flavor of the bread. Other main sources are dough fermentation and the baking process (*Hansen and Schieberle, 2005).* The flavor of bread crumb is mainly affected by enzymatic activity during the dough fermentation, whereas the flavor of bread crust is more influenced by the thermal reactions during the baking process (*Rothe, 1974).* The sourdough is mainly used to improve flavor in wheat breads (Hansen and Hansen, 1996). The flavor of sourdough wheat bread is richer and more aromatic than yeast fermented breads, due to long fermentation time of sourdough (*Brummer and Lorenz, 1991*). Some other factors also influence the production of volatile compound during production of sourdough bread including free amino acids formed during fermentation through proteolysis which influence volatile compound profile of the products (*Gobbetti et a.l, 1994b; Collar and Martinez, 1993; Spicher and Nierle, 1984)*. The production of volatiles is also influenced by proofing and baking processes (Hansen et al., 1989b). The bread flavor is composed of hundreds of volatile and non-volatile compounds, i.e. many alcohols, ketones, aldehydes, acids, esters, furan derivates, ether derivates, hydrocarbons, ketones, lactones, prazines, pyrrol derivates and sulphur compounds which serve as flavor stimuli (*Maga, 1974; Schieberle, 1996).*

Chemical analyses of flavor compounds can be combined with sensory analysis of bread. The compounds that have been positively correlated with flavor of wheat crumb are acetaldehyde, 2-methylpropanoic acid, 2/3-methyl-1-butanol, 3-methylbutanoic acid, isopentanal, 2-nonenal, benzylethanol, 2- phenylethanol, 2,3-butandione and 3-hydoxy-2-butanone, dimethyl sulphide and 2-furfural (Hansen and Hansen, 1996; Rothe, 1974). Abde-el-Malek et al (1974) concluded that micro flora involved in fermentation of bladi bread is lactic acid bacteria L. brevis and L. fermenti and yeast. The lactobacillus replaces are responsible for the typical flavor of bladi bread. In sourdough fermentation, organic acetic acid and volatile flavoring compounds produced are dependent upon the microorganisms in the dough (Hansen and Hansen, 1994a). The volatile compounds are produced both in lactic acid fermentation and in alcoholic fermentation, but the levels of these compounds are much higher in yeast fermentation (Hansen and Hansen, 1994b; Meignen et al., 2001). However, Schieberle (1996) concluded that volatiles formed do not affect the final flavor of the bread. The compounds having a high flavor dilution factor would have a significant impact on the final odor. Salovaara and Valjakka (1987) found that flour type has a considerable influence on the production of acids. The concentration of acetic acids in whole wheat flour is almost double the concentration in bread made from straight grade flour. The concentration of lactic acid is 30 to 50% higher in bread made from whole wheat flour as compared to bread made from straight grade flour.

Moreover, addition of sucrose to wheat dough stimulates both yeast and LAB growth and increases bacterial production of lactic and acetic acids (*Corsetti et al., 1994).* There is also a lack of competition between *Lactobacillus sanfranciscensis* exist for maltose consumption which is elementary for the stability of both the microorganisms for the production of good quality San Francisco French bread (Sugihara et al.,, 1970). Optimum temperature for co-culture of *C. humilis* and *Lactobacillus sanfranciscensis* are 28 and 32 ºC, respectively, but reduced production of acetate by *Lactobacillus sanfranciscensis* has been observed at 35 °C, although, both lactate and ethanol formations are not affected at this temperature (Brandt et al., 2004). In a French yeast sourdough, more than 40 flavoring components are identified: 20 alcohols, 7 esters, 6 lactones, 6 aldehydes, 3 alkanes and a single sulphur compound (*Frasse et al., 1993).*

Mold growth being the most important cause of bread spoilage, could be prevented by the use of homo and hetero-fermentative LAB (*Spicher, 1983).* This fungistatic effect is due to the production of acetic acid by LAB *(Roecken, 1996). Corsetti et al. (1998*) studied the antifungal activity of sourdough LAB. They concluded that a mixture of acetic acid, propionic acid, caproic acid and butyric acid are responsible for the activity. The caproic acid along with acetic acid plays an important role in inhibiting fungal growth. They also found that sourdough bread made from low pH and high concentration of acetic acid has large volume and lower rate of staling. Hansen et al. (1989) studied the effect of three hetero-fermentative and two homo-fermentative LAB strains on the production of organic acids in sourdough. The results show that acetic acid content is much higher in the dough acidified with hetero-fermentative strains as compared to homo-fermentative strains. The homo-fermentative strain produces only L- lactic acid, whereas other cultures produce both L- lactic acid and D- lactic acid. In another study (*Hansen and Hansen, 1994*), the effect of wheat flour types on the production of organic acid was also observed. The wheat flours have significant effect on the production of lactic and acetic acids. The highest lactic acid contents are observed in low grade and whole meal flour. The production of acetic acid depends on the starter culture used. The acetic acid is found to be 12% of the total acid contents in hetero-fermentative cultures, but it has not been detected in homo-fermentative cultures but sourdough made from straight grade flour fermented with homo- fermentative strain L. plantarum shows small amount of acetic acid. Vernochi et al. (2004) found that sourdough fermentation with C. milleri results in high amounts of acetic acid and mannitol which improves the qualitative characteristics of leavened dough and the baked products.

Chapter -3 OBJECTIVES

Few attempts in India have been made to elucidate role of EPS production in traditional sourdough . The current attempt elucidates the functional properties EPS of an indigenously isolated LAB , in sourdough . Accordingly the following objectives were framed :

1) Optimization of the developed sourdough for high production of EPS .
2) Examination of neutraceutical profile and compositionsl profile of EPS producton in sourdough .
3) To prepare a functional food containing EPS and neutraceuticals .

Chapter -4 MATERIALS AND MEATHOD

MICROORGANISM AND CULTURE CONDITION :

Isolates of LAB from a previous study (Singh et al , 2011) was screened for EPS production ability . The higher EPS production was selected as a starter culture for sourdough . The kinetics of EPS production along with the growth of *Lactococus lactis* was studied .The strain has recently been characterized as a potential producer of GABA (*Bhanwar et al ,2012*). The *Lactococcus lactis subsp lactis* overnight grown in MRS media was centrifuged at 10,000 rpm for 5 minutes at 4˚C . The supernatant was discarded , the pallet was washed 3 times with saline , it was then resuspended in phosphate buffer solution , vortexed the pallet and used for starter culture in sourdough .All the chemicals used were high in purity and were purchased from Sigma (USA) and HiMedia (Mumba , India).

FERMENTATION OF SOUR DOUGH

Plain wheat flour was used along with doughs of V. mungo flour : wheat flour was used in different concentrations with the *Lactococcus lactis* culture . The plain wheat flour dough did not show a good yield of exoplysaccaride so the sourdough with culture and a control without culture was used . The dough samples were kept under specific conditions for fermentation and 1 gram sample was taken hourly for further analysis .

DOUGH A – 10g wheat flour : 10g V. Mungo flour

DOUGH B – 10g wheat flour : 10g V. Mungo flour WITH CULTURE

DOUGH C – 5g V. Mungo flour : 10g wheat flour

DOUGH D – 5g V. Mungo flour : 10g wheat flour WITH CULTURE

DOUGH E – 10Gg V. Mungo flour : 5g wheat flour

DOUGH F – 10Gg V. Mungo flour : 5g wheat flour WITH CULTURE

MEASUREING VIABLE COUNT IN DOUGH

Sample (1g) was withdrawn after every hour from prepared dough and diluted 10 fold with sterilized PBS. After that 0.1mL of aliquot was spread on MRS agar plate and incubated for 24 h at 30°C. Then the viable colonies were counted for each dilution and expressed as log cfu/g (colony forming unit/g).

ANALYSIS OF SOURDOUGH AFTER 4 HOURS FERMENTATION

The colour , pH ash content and titrable acidity of the dough was calculated .

Wet and dry gluten

Wet and dry gluten contents of flour were estimated by following the method No. 38-10 as described in A0CC, 2000. 25 g of flour was taken in a porcelain cup. Sufficient water was added to form firm dough. Dough was hand kneaded into ball and placed it in water at room temperature for 60 min. Dough was kneaded gently in stream of tap water over bolting cloth until starch and soluble matter were removed. To determine whether gluten was starch free, one or two drops of wash water, obtained by squeezing, were dropped into clear water. The gluten thus obtained was pressed as dry as possible between the hands, and weighed as wet gluten. Then it was transferred to an air oven to a constant weight at 100 °C for 24 hours and cooled. It was weighed as dry gluten.

Moisture Content

The moisture content of flour samples was determined according to method No. 44-15 A as described in A0CC, 2000. 5 g flour was taken in a tarred crucible and dried in hot air oven (Memmert Model 200) at 100±5ºC till a constant weight. The moisture contents were calculated by the formula given below.

$$\text{Moisture (\%)} = \frac{\text{Wt. of original sample} - \text{Wt. of dried sample}}{\text{Wt. of original sample}} \times 100$$

Crude protein content

Crude protein was determined by the Kjeldahl method according to method No. 46-10 as described in AACC, 2000. The samples were first digested in digestion flask with H2SO4 in the presence of a digestion mixture for 3-4 h till the contents of digestion flask get transparent color. Samples were then diluted with distilled water up to 250 ml in a volumetric flask. The ammonia from the samples trapped in H2SO4 was liberated through distillation after adding 40% NaOH solution and collected in a flask containing 4% boric acid solution using methyl red as an indicator to determine nitrogen content in a sample by titrating against standard 0.1N H2SO4 solution. The crude protein percentage was calculated by using following formula

$$N\ (\%) = \frac{0.0014 \times \text{Vol. of 0.1N } H_2SO_4 \times 250\ \text{ml}}{\text{Vol. of diluted sample} \times \text{Wt. of original sample}} \times 100$$

Crude fat content

The method employed was that of solvent extraction using a Soxhlet extraction as described in method No. 30-10 (AOAC 2000). 2 g of flour were taken in a thimble and placed in extraction tube of Soxhlet apparatus. About 250 ml of Hexane were added in 500 ml bottom flask of the apparatus and connected to the Soxhlet apparatus. The fat was extracted by running Hexane over the sample at the rate of 3-4 drops per sec for about 5 h. The content of the flask was transferred to a pre-weighed petri dish and dried on a hot plate for 10 min at a temperature of 40-50ºC. The petri dish was cooled in desiccator and weighed. Fat percent age was calculated according to the following formula.

$$\text{Crude fat (\%)} = \frac{\text{Weight of fat in sample}}{\text{Weight of sample}} \times 100$$

Ash content

Ash content was determined by incineration of the sourdough sample at 600ºC according to method No. 08-01 as described in AOAC, 2000. 5 g oven dried sample was taken in a pre-weighed crucible and charred on a burner. Then it was ignited in a muffle furnace at 550ºC till constant weight of grayish ash was obtained. The ash of sample was calculated by using following formula.

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Acid content analysis

The total titratable acidity (TTA) of sourdough samples were determined according to method given by Lefebvre et al. (2002), using the Inolab WTW Series 720 pH meter. A 10g sample was blended with 90 ml distill water and the suspension was then titrated with a 0.1 mol/L NaOH to a final pH of 8.5. The TTA was expressed as the amount (ml) of NaOH used.

Microbial count

Samples (10 g) of the sourdough were homogenized with 90 ml PBS (0.99% w/v). Colony forming units were determined by plating serial dilutions on modified MRS agar (containing maltose) for LAB (Volgel et al., 1994). Incubation temperature was 37 °C .

DETECTION OF FUNCTIONAL COMPONENTS

Crude fiber content

Crude fiber content was determined by following the method No. 32-10 as described in AoaC, 2000. 2 g fat and moisture free sample was taken and placed in 1000 ml beaker. 200 ml solution of 1.25 % H_2SO_4 was added in the beaker. The sample was then digested by boiling for 30 min. Then it was filtered by using suction apparatus. The residue was washed with hot water until become acid free. The residue was then again transferred to 1000 ml beaker and boiled with 200 ml solution of 1.25 % NaOH for 30 min. It was again filtered and the residue was transferred to pre-weighed crucible and dried in an oven at 100 ºC for 24 h till constant weight was obtained. Then the dried residue was charred on a burner and ignited into muffle furnace at 550-600ºC for 5-6 hours, cooled in desiccators and weighed. The loss in weight during incineration represents the weight of crude fiber in sample. The crude fiber % age was calculated by using the following formula.

$$\text{Crude fiber (\%)} = \frac{\text{Weight of residue} - \text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Riboflavin Analalysis

Riboflavin content was measured in the dough by taking 0.8 ml sample and with 0.2 ml of 1m NaOH and vortex it . A 0.4 ml of resulting solution was neutralized with 1 ml of 0.1 pottasium phosphate buffer ,it was mixed by vortex and O.D at 444 nm was taken . A standard solution with different concentrations was made and a graph was plotted .

Vitamin C Analysis

1% Starch solution indicator was prepared , iodine solution with final concentration 0.5 M was also prepared and standard solution with stock 3mg /30 ml ascorbic acid solution was prepared in different concentrations , the iodine solution was titrated with the food sample and the number of drops of iodine solution were counted end point is reached when the solution turns colourless to pink . The titration was repeated with samples of unknown concentrations .

Fructoligosaccarides by HPLC

The dough (1.6 g±0.001) was weighed and put in a 50 mL round-bottomed flask. Twenty millilitres, ml 70% water solution of methanol and 0.1 g of $CaCO_3$ were added to the first extraction. Following that, an extraction was carried out at 70–75◦C under the air-condenser for 30 min. 15mL o f70%methanol was added both to the second and third extraction which lasted 30 min each. All three extracts were mixed together and centrifuged. Supernatant residue was concentrated in evaporator and was quantitatively transferred into a 5 mL measuring flask. the solution examined was diluted with acetonitrile in the proportion of 1:1, then sonicated for 15 min, filtered through the filter of 0.45 µm and injected to the chromatographic system. HPLC system (Knauer) equipped with EuroChrom 2000 data control system, RI detector, Shodex NH2P column filled with aminopropyl polymer, mobile phase acetonitrile-water mixture (67–33% v/v) with flow rate 0.8 mL/min at 20◦C was used for determination.

Exopolysaccarides by HPLC

Water soluble polysaccharides origniting form the flour (extracted from chemically acidified control doughs) and microbial EPS synthesis were purified and the accounts of EPS produced were calculated on the basis of glucose content of WPS as described previously . Caliberation of HPLC column was done with hydrolyzed stock solutions of purified EPS from sucrose-MRS-fermentations . briefly, WPS were precipitated from the watery dough extracts with ethonal for 48 h, dialyzed against double distilled water for another 48 hours (SERVA VI-SKING, 20-32 diameters 16 mm, Heidelberg, germany), and lyophilized hydrolysis of dried polysaccharide was carried out by incubation with sulphuric acid at the final concentration of 4 moles per litre during 2 hours at 80 C after neutralization with 4 moles per litre koh samples were analyzed by HPLC using a polysphere OAKC column (Kaditzky et. Al ,2008).

Organic acids in Sourdough

This fungistatic effect is due to the production of acetic acid by LAB (*Roecken, 1996*). *Corsetti et al.* (1998) studied the antifungal activity of sourdough LAB. They concluded that a mixture of acetic acid, propionic acid, caproic acid and butyric acid are responsible for the activity. The caproic acid along with acetic acid plays an important role in inhibiting fungal growth. They also found that sourdough bread made from low pH and high concentration of acetic acid has large volume and lower rate of staling.. The results show that acetic acid content is much higher in the dough acidified . The strain produces only L- lactic acid, whereas other cultures produce both L- lactic acid and D- lactic acid. In another study (*Hansen and Hansen*, *1994*), the effect of wheat flour types on the production of organic acid was also observed. The wheat flours have significant effect on the production of lactic and acetic acids. The highest lactic acid contents are observed in low grade and whole meal flour. The production of acetic acid depends on the starter culture used. The acetic acid is found to be 12% of the total acid contents , but it has not been detected in homo-fermentative cultures but sourdough made from straight grade flour fermented with

homo- fermentative strain L. plantarum shows small amount of acetic acid. *Vernochi et al*. (2004) found that sourdough fermentation with C. milleri results in high amounts of acetic acid and mannitol which improves the qualitative characteristics of leavened dough and the baked products.

Baking process

The sourdough was baked into cookies at optimum baking conditions .

Chapter -5 RESULTS AND DISCUSSION

MICROORGANISM AND CULTURE CONDITION :

Isolates of LAB from a previous study (Singh et al , 2011) was screened for EPS production ability . The higher EPS production was selected as a starter culture for sourdough . The kinetics of EPS production along with the growth of *Lactococus lactis* producing high amount of EPS was selected as starter culture .

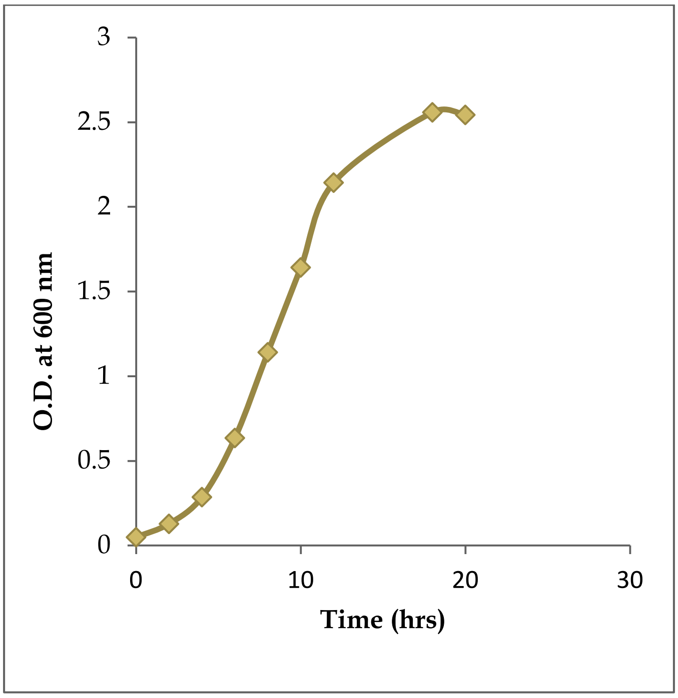
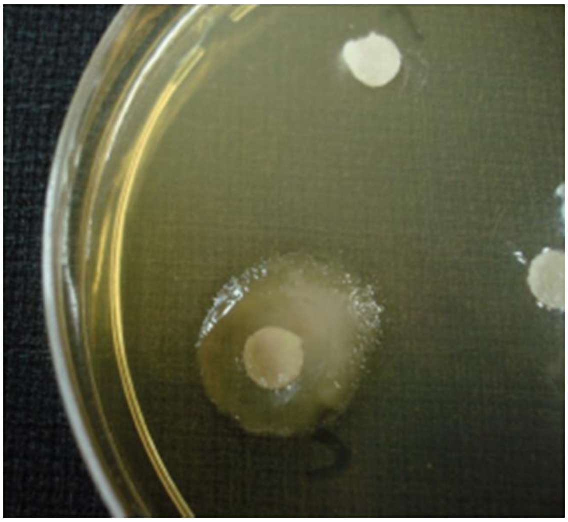


Figure 1 shows growth kinetics of l.*lactis*



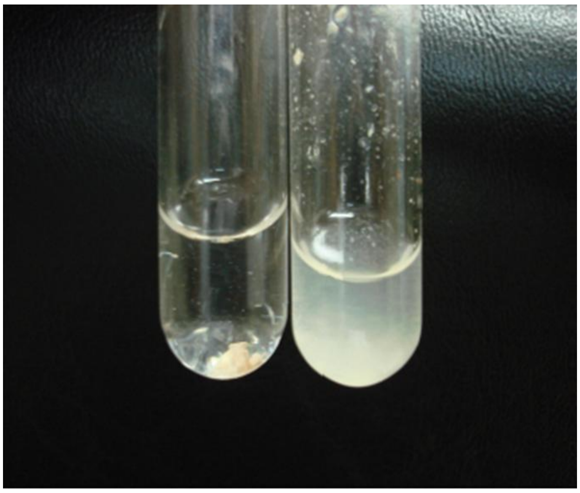


FIGURE C *Lactococcus lactis producing EPS*

PRELIMARY ANALYSIS OF THE DOUGH

The colour of the dough was creamish .the pH was observed to be 3.9-4.1 which is slightly acidic . The water absorption rate was 58.1/100ml .The titrable acidity was observed to be 13.9-22.4 ml . The texture was sticky and granular . This was compared to the plain dough , sourdough had a good aroma and a vital appearance .

RAW MATERIAL USED

AASHIRWAD AATA ,ITC LTD

Moisture content – 4.5%

Ph -7.0

Gluten content -13 %

Ashirwad aata was used with *V. mungo* flour to make the dough , which a good quality flour and the *V. mungo* flour will increase the nutritional content of the dough as the cereal products lack vitamin B .(*Bhanwar et al ,2012)*

GLUTEN CONTENT

The gluten proteins impart unique bread making properties to wheat. The content varied from 36.41%- 32.11%+ in the doughs . Dough E with 10g wheat flour and 5g vinga mungo flour showed highest gluten content . The gluten content is showed as follows :-

DOUGH	GLUTEN CONTENT
DOUGH A	33.41%
DOUGH B	32.86%
DOUGH C	32.11%
DOUGH D	36.14%
DOUGH E	35.18%
DOUGH F	34.88%

Table -1 Gluten content in sourdough

MOISTURE CONTENT

Moisture content was calculated which ranged from 10.78%- 8.88% .The highest moisture content was found in Dough A with 10.78% with minimum value of 8.88% in dough E . the table for moisture content in different doughs is as follows

DOUGH	MOISTURE CONTENT	
DOUGH A	10.78%	
DOUGH B	10.08%	
DOUGH C	10.69%	
DOUGH D	10.54%	
DOUGH E	8.97%	
DOUGH F	8.88%	

PROTEIN CONTENT

The protein content was measured by Kjeldhal meathod , which varied according to the concentration of raw material the highest protein content was found in dough A of 10.98% nd lowest protein content was found in dough F of 6.68% . The table for protein % is given as follows :-

DOUGH	PROTEIN CONTENT
DOUGH A	10.98%
DOUGH B	12.05%
DOUGH C	9.96%
DOUGH D	8.64%
DOUGH E	7.42%
DOUGH F	6.68%

Table -2 Protein content in sourdough

FAT CONTENT

The fat content was measured by Soxhlets method in which hexane was used as a solvent . The fat content varied from 1.28%- 1.40% . Dough A contained highest fat according to its concentration . The table for Fat content is given as follows :-

DOUGH	FAT CONTENT
DOUGH A	1.28 %
DOUGH B	2.76%
DOUGH C	1.54%
DOUGH D	1.88%
DOUGH E	1.95%
DOUGH F	1.65%

Table -3 Fat content in sourdough

FIBER CONTENT

The fibre content varied significantly in different flours ranging from 0.40%- 1.80%. Highest fibre content was found in Dough B .

DOUGH	FAT CONTENT
DOUGH A	1.80 %
DOUGH B	2.14%
DOUGH C	1.44%
DOUGH D	1.38%
DOUGH E	0.54%
DOUGH F	0.40%

Table -4 Fiber content in sourdough

ASH CONTENT

Ash is the mineral residue remaining after a sample has been completely oxidized in a manner such that all organic volatile material is driven off, while preventing any mineral from being lost (Posner, 1991). The values of ash content in different samples are given below , highest ash content was found in dough A .

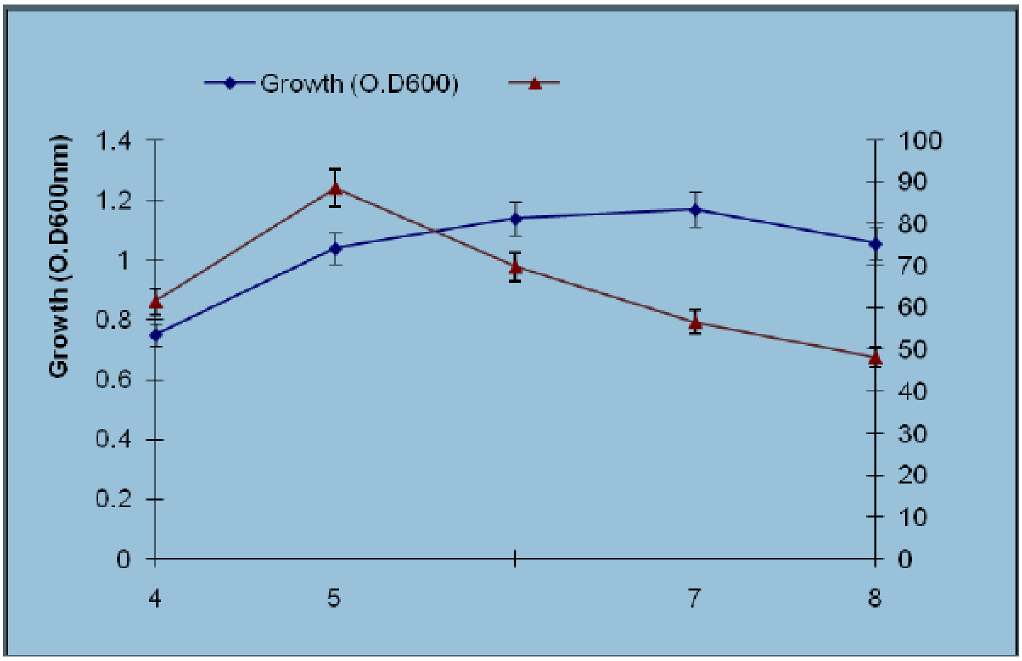
DOUGH	ASH CONTENT
DOUGH A	1.55%
DOUGH B	1.07%
DOUGH C	1.25%
DOUGH D	1.11%
DOUGH E	1.03%
DOUGH F	0.56%

Table -5 Ash content in Sourdough

VIABLE COUNT AND EPS PRODUCTION

Table 6 Microbial count in sourdough

DOUGH CONCENTRATION	MICROBIAL COUNT
DOUGH A	2.2 ×10*8 cfu/g
DOUGH B	2.0×10*9 cfu/g
DOUGH C	1.4×10*9 cfu/g
DOUGH D	2.1×10*7 cfu/g
DOUGH E	1.9×10*7 cfu/g
DOUGH F	3.1×10*7cfu/g



The growth kinetics and viable count of *L.lactis*.

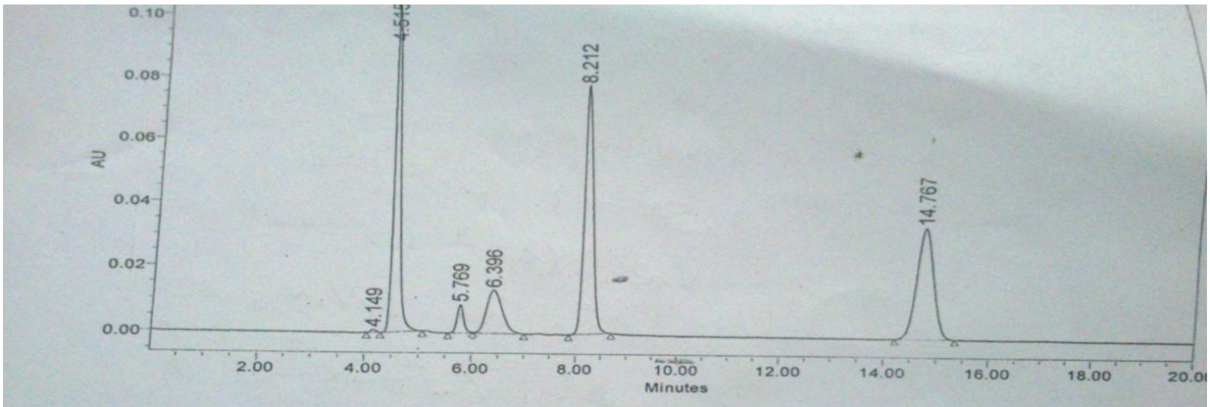
The growth curve indicated the *L.lactis* produced EPS highest EPS production was seen at the end of fermentation at 4 hours

RIBOFLAVIN ANALYSIS

The lactic acid bacteria increase synthesis of vitamins B2, B11 and B12 and the potential strategies to increase B-group vitamin content in cereals-based products, where vitamins-producing LAB have been leading to the elaboration of novel fermented functional foods.(Capozzi et al. ,2012) . The vitamin B12 content showed an increasing trend along with the fermentation time in different sourdough samples. The O.D was measured at different time intervals which showed an increasing trend in the concentration when compared to the standard . (Graphs – Annexure 1)

FRUCTOLIGOSACCARIDES AND EXOPOLYSACCARIDES

The amount of fructooligoligosaccarides were 0.3 to 0.81 g/100 g .the overall content of fructans in sourdough bread made from *V. Mungo* flour to be 2.2–2.6 g/100 g, of which the content of FOS DP 3–5 amounted to about 0.8 g/100 g. The graph is as follows in FIGURE D. The amount of exopolysaccarides were found by the amount of residual sugars in the sourdough which showed a high sugar content O.D of 2.725 . The optimum range of EPS was obtained at pH 5 . The EPS in the dough sample was 248.8 mg by the *Lactococcus lactis* culture .



	Peak Name	RT	Area	% Area	Height	Amount	Units
1	FRUCTOSE	4.149	6188	0.21	951		
2	GLUCOSE	4.515	844984	29.08	104756		
3	SUCROSE	5.769	78217	2.69	8895		
4	MALTOSE	6.396	285786	9.84	13606		
5	KETOSE	8.212	930556	32.03	78063		
6	RAFFINOSE	14.767	759544	26.14	34698		

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ORGANIC ACIDS

The organic acids like lactic acid , acetic acid and citric acid were detected as the peaks were formed when HPLC or the sourdough extract was performed . A mixture of acetic acid, propionic acid, caproic acid and butyric acid are responsible for the activity. The caproic acid along with acetic acid plays an important role in inhibiting fungal growth. The sourdough bread made from low pH and high concentration of acetic acid has large volume and lower rate of staling.. The results show that acetic acid content is much higher in the dough acidified . The strain produces only L-lactic acid, whereas other cultures produce both L- lactic acid and D- lactic acid. In another study (*Hansen and Hansen*, *1994*), the effect of on the production of organic acid was also observed.The baking properties and the aroma was not altered by the production of organic acids .

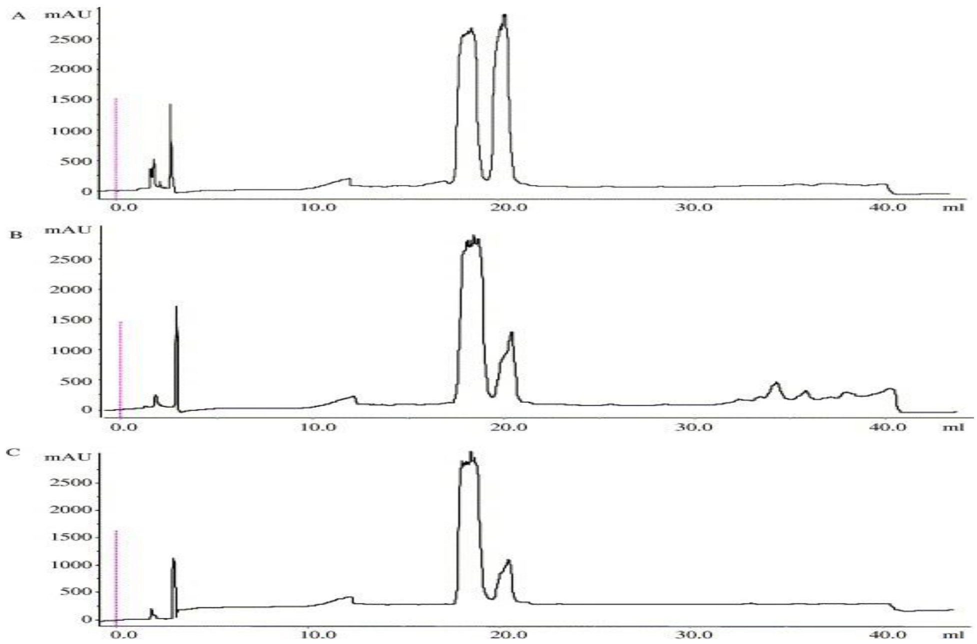
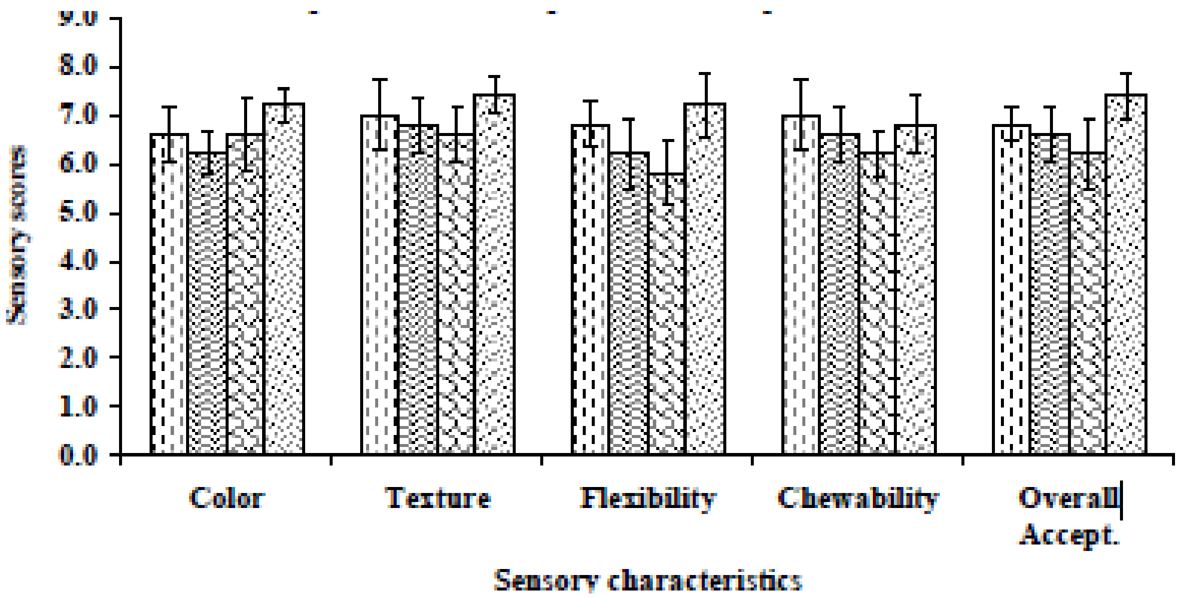


Figure E – Peaks in seen in HPLC of organinc acids

Sensory analysis

The sensory analysis for the cookies was performed , the were overall accepted.The overall score was 8 .



Chapter -6 Conclusion

In this study , a previously characterised LAB strain *L.lactis subsp lactis* was used produce EPS in dough . The strain produced high amounts of EPS in MRS as well as in dough when fermented at room temperature for 4 hours its survival was unaffected . the strain produced riboflavin and vitamin C during fermentation along with organic acids . the overall compositional profile following fermentation remained unaffected . The microbialy produced EPS improved the dough texture notably without altering its baking characteristics . The baked sourdough cookies has high sensory scores. Results of this study are encouageing for futher examining the potential of this strain as a functional starter in cereal matrixes.

Figure F -Cookies made by Sourdough after fermentation with *Lactococcus lactis*

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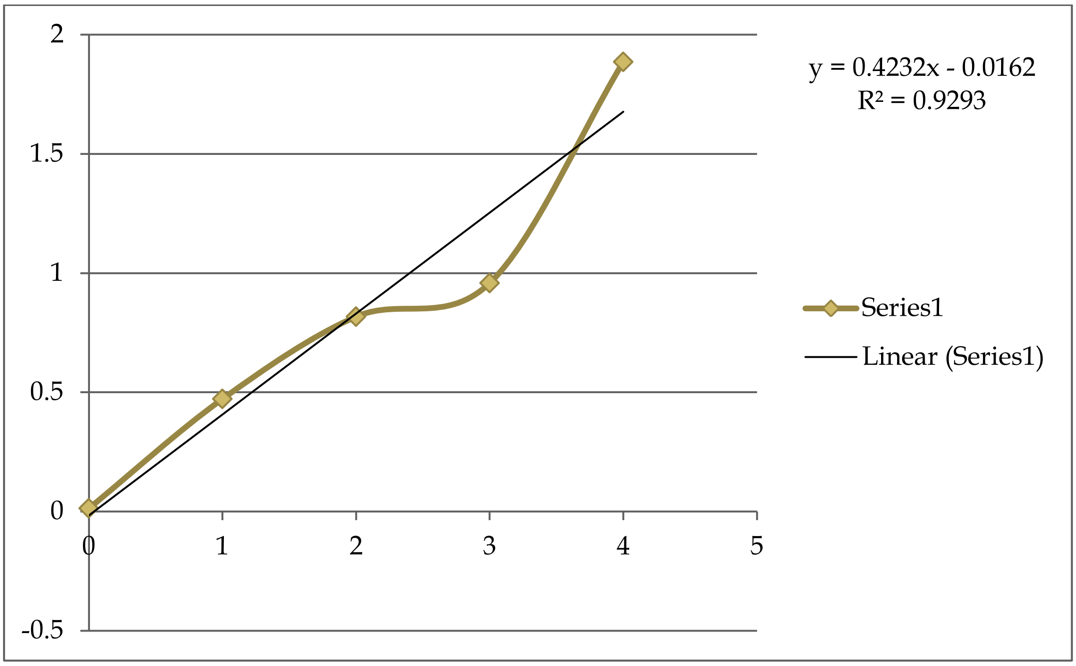
ANNEXURE 1



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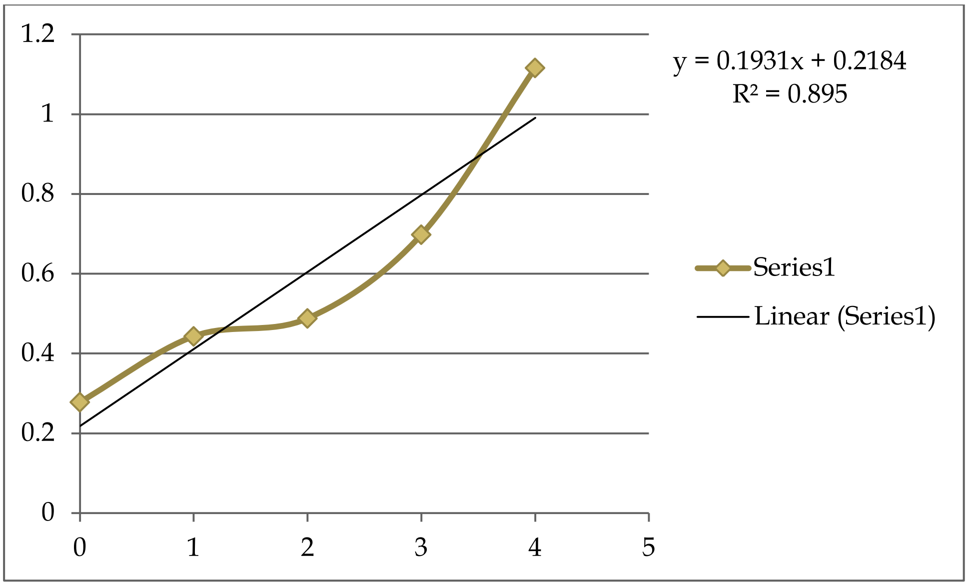

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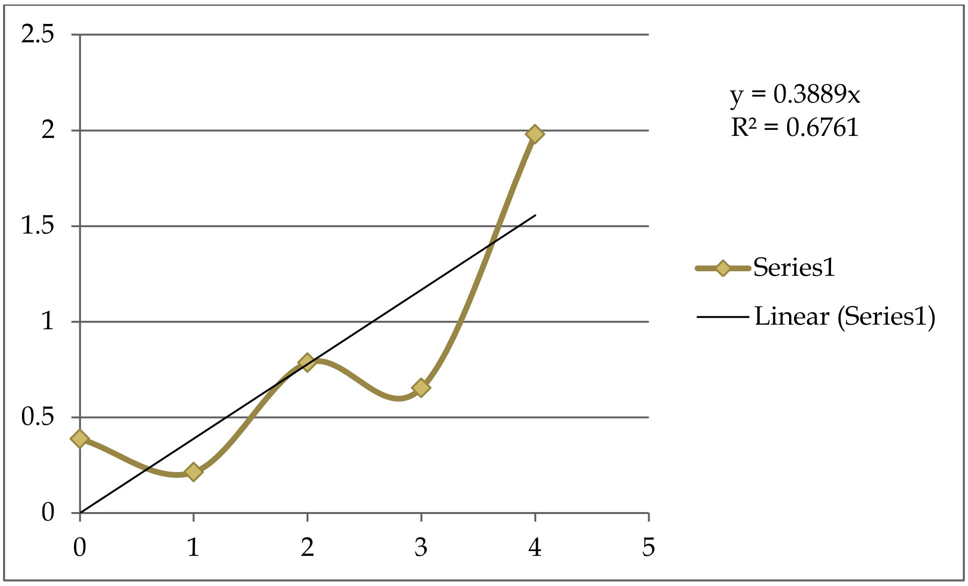

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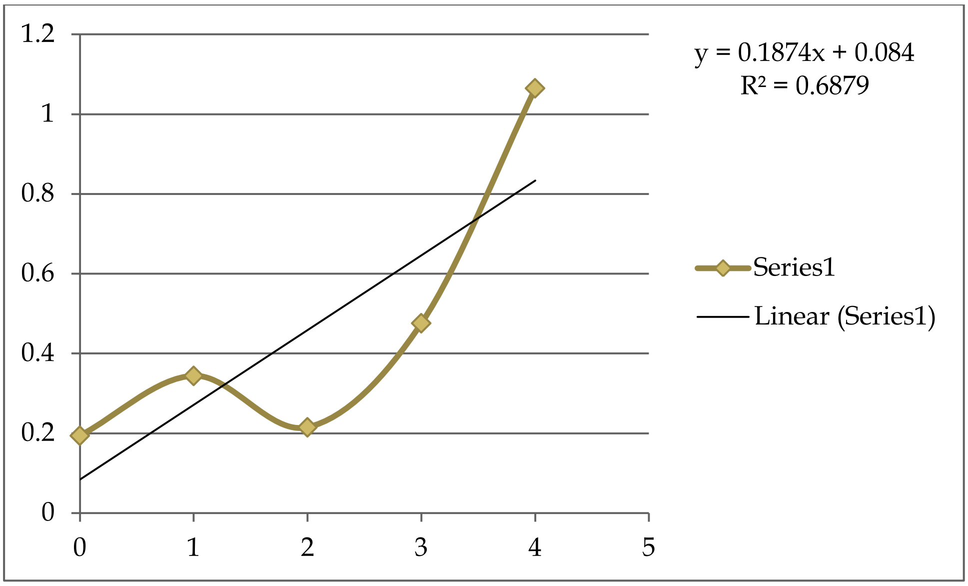
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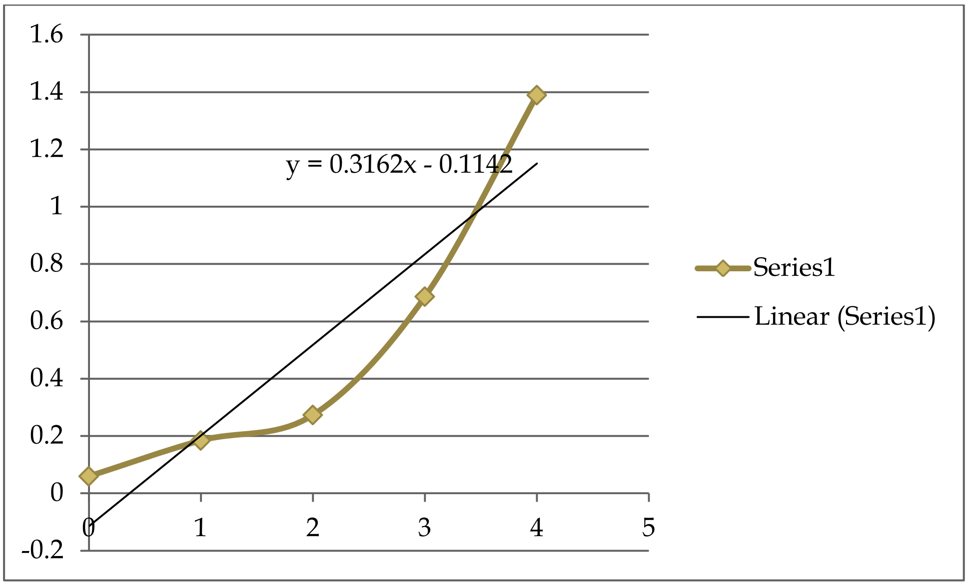
0
0
1
2
3
4
5
Series1
Linear (Series1)

DOUGH E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1.4
1.2
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0.6
0.4
0.2
0
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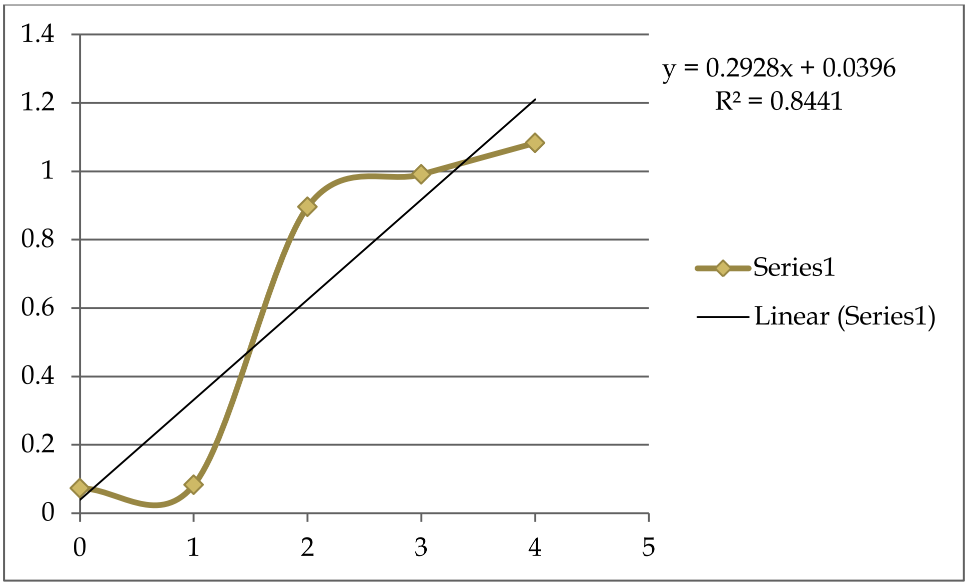
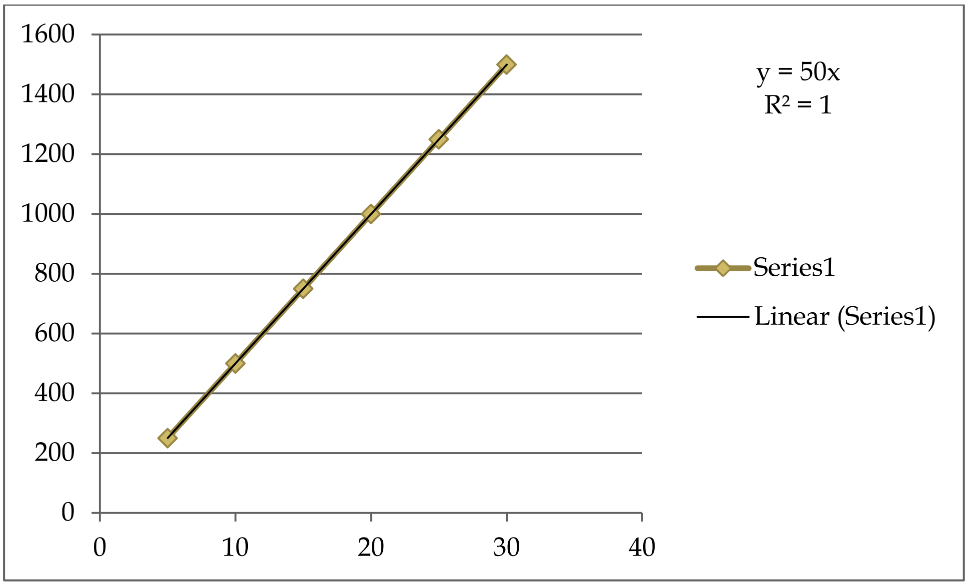
1
2
3
4
5
Series1
Linear (Series1)

DOUGH F

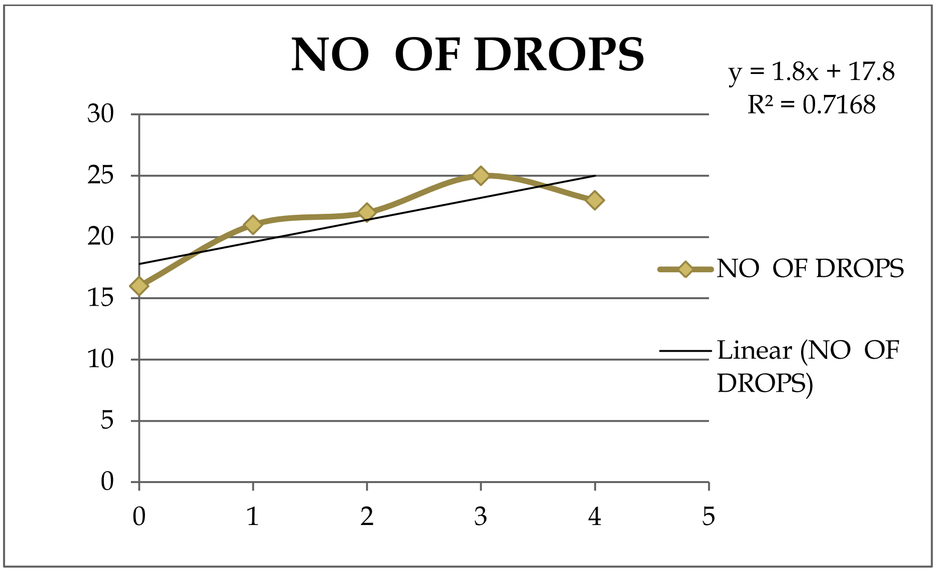


STANDARD CURVE FOR RIBOFLAVIN



ANNEXURE 2

DOUGH A



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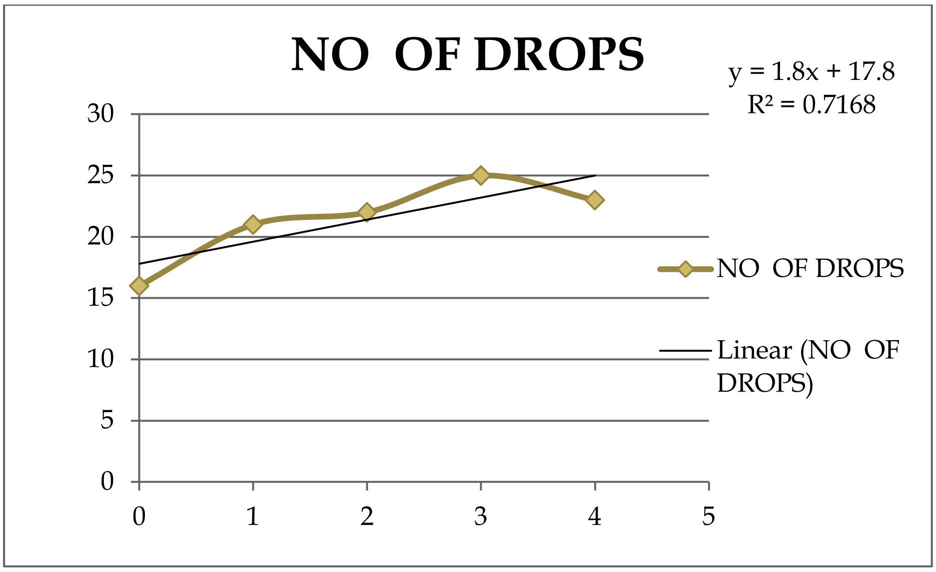
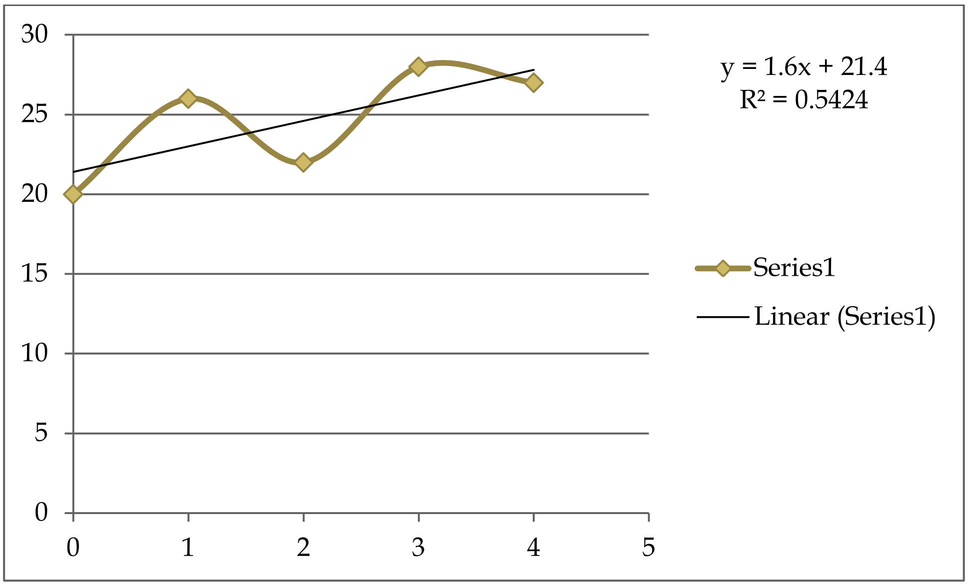


DOUGH C



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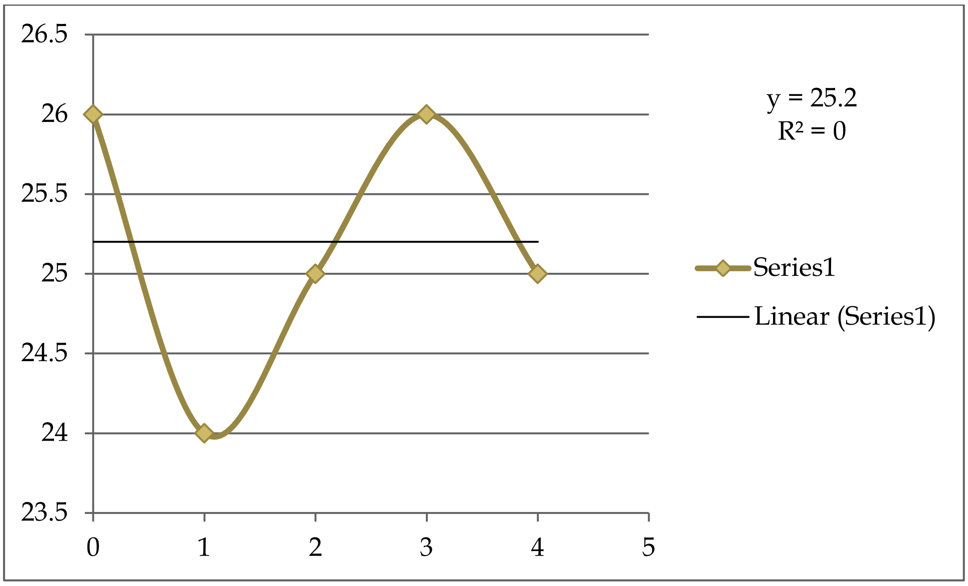

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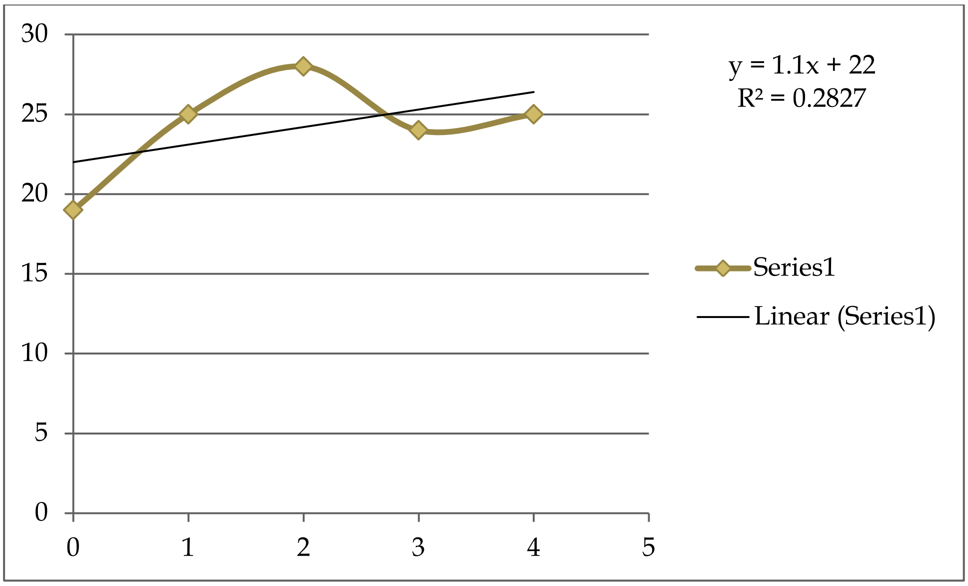

DOUGH F