PRODUCTION OF NON-CONVENTIONAL BAKER'S YEAST USING MEMBRANE TECHNOLOGY

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

MASTER OF SCIENCE
IN
DAIRYING
(DAIRY MICROBIOLOGY)

BY

SOMNI HIMANSHU SUDHAKAR

DIVISION OF DAIRY MICROBIOLOGY,
NATIONAL DAIRY RESEARCH INSTITUTE
(I.C.A.R.)
KARNAL-132 001 (HARYANA), INDIA

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....Dedicated to
My
Beloved Parents....

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(DEEMED UNIVERSITY) KARNAL (HARYANA)
IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE

IN

DAIRYING

(DAIRY MICROBIOLOGY)

Approved by

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CERTIFICATE

This is to certify that the thesis entitled **"PRODUCTION OF NON-CONVENTIONAL BAKER'S YEAST USING MEMBRANE TECHNOLOGY"** submitted by **SOMNI HIMANSHU SUDHAKAR** in partial fulfilment of the requirement for the award of the degree of **MASTER OF SCIENCE** in **DAIRYING (DAIRY MICROBIOLOGY)** 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: 13th June, 2002

D.N. Gandhi

(D.N. GANDHI)
MAJOR ADVISOR & CHAIRMAN
(GUIDE)

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Himanshu

Dated : 11 June, 2002

(Himanshu Somni)

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Abstract

The most significant role of yeasts has been emphasized in bakery industry. The yeast ferment the sugars in dough, producing acid, alcohol, carbondioxide and many flavouring compounds. The carbondioxide produced causes dough to leaven resulting in rise in volume of dough. Conventionally, *Saccharomyces cerevisiae* is used as baker's yeast in preparation of bread/pizza bases. However, certain strains of lactose fermenting yeast also termed as Non-Conventional Baker's Yeast (NCBY) which have been found to show similar characteristics in terms of dough activity. At industrial scale, the cells are harvested from fermented medium by centrifugation. However, it has certain limitations. Membrane processes such as microfiltration (MF) and ultrafiltration (UF) have been used to remove upto 99.5% of spores and Gram-negative viable cells from milk. Concentration of bacterial cells to about 12-folds can be achieved by using UF. Moreover, report shows that retentate of different membrane modules used for separation of yeast cells were free from yeast cells. Thus, membranes can be used for harvesting NCBY from fermented broth and cells hence obtained can be used for preparation of bread and pizza base.

Amongst four strains of lactose fermenting yeast, *K. fragilis* 2C1 was selected on the basis of higher dough raising capacity. Selected strain was grown in whey based medium at 30°C/36 hrs. For harvesting the cells two types of membranes, microfiltration and ultrafiltration were used. Almost ~10 times concentration of cells could be achieved by using membranes. Bread and Pizza base prepared by NCBY were subjected to sensory evaluation. The average overall score of bread showed that the bread was liked moderately. Chemical analysis of bread revealed that the product meets the standards specified by BIS for the parameters analyzed. Pizza bases prepared by NCBY were also compared with that of the market samples and were found to be close in chemical and sensory qualities. Hence, there is a scope for improvement in the basal formulation of bread and pizza base. Keeping in view the desirable properties of NCBY in bread and pizza base making, scaling up of the production of these products at industrial scale and addition of improvers could make these products even better. Further, it also paves way to utilize whey through microbial fermentation.

सारांश

बेकरी उद्योग में यीस्ट का महत्वपूर्ण स्थान है। डो (गूंथा आटा) की शर्करा को यीस्ट, किण्वन कर अम्ल, एल्कोहोल, कार्बन डाइआक्साइड तथा अन्य फलेवरिंग यौगिकों को उत्पन्न करता है। उत्पत्त कार्बन डाइआक्साइड डो के आयतन को बढ़ाती है। परम्परागत तौर पर ब्रेड तथा पीज़ा आधार तैयार करने हेतु *सेकरोमाइसीस सरवेसि* का प्रयोग होता है। जबकि लैक्टोज किण्वन करने वाले यीस्ट के कुछ प्रभेद जोकि अपरम्परागत बेकरी यीस्ट के तौर पर भी जाने जाते हैं, डो सक्रियता सम्बन्धी लक्षण रखते हैं। औद्योगिक स्तर पर अपक्रेन्द्रन के द्वारा किण्वन माध्यम से यीस्ट कोशिकाओं को संचित किया जाता है। लेकिन इसकी कुछ सीमाएं होती हैं। माइक्रोफिल्ट्रेशन तथा अल्ट्राफिल्ट्रेशन जैसी विधियों द्वारा दूध से बीजाणुओं तथा ग्राम-निगेटिव सूक्ष्मजीवों को अलग किया जाता है। अल्ट्राफिल्ट्रेशन का प्रयोग कर जीवाणु कोशिकाओं की सान्द्रता को बारह गुना तक बढ़ाया जा सकता है। रिपोर्ट यह बताती है कि यीस्ट कोशिकाओं को अलग किये जाने वाले प्रयोगों में प्रयुक्त भित्ति माडयूल्स के परमीएट यीस्ट कोशिकाओं के अवशेष से मुक्त थे। अत: किण्वन ब्रौथ से अपरम्परागत बेकर यीस्ट को एकत्रित करने हेतु इन भित्तियों को प्रयोग किया जा सकता है तथा इनका प्रयोग ब्रेड तथा पिज़ा आधार को बनाने में किया जा सकता है।

लैक्टोज किण्वन करने वाले यीस्ट के चार प्रभेदों से के. *फ्रेजिलिस* 2सी1 को उच्च डो उठाने के आधार पर चुना गया। चयनित प्रभेदों को व्हे आधारित माध्यम में 30ीं सें.ग्रे. पर 36 घण्टों तक उगाया गया। यीस्ट कोशिकाओं को इकट्ठा करने हेतु दो तरह की भित्तियों माइक्रोंफिल्ट्रेशन तथा अल्ट्राफिल्ट्रेशन का प्रयोग किया गया। अल्ट्राफिल्ट्रेशन के प्रयोग से लगभग 10 गुनी सांद्रता प्राप्त हुई। अपरम्परागत बेकरी यीस्ट से ब्रेड तथा पिज़ा आधार किये गए तथा इनका संवेदी मूल्यांकन किया गया। समग्र स्कोर से ज्ञात होते है कि इन्हें साधारण स्तर पर पसन्द किया गया। ब्रेड का रासायनिक विश्लेषण करने पर इन्हें भारतीय मानक ब्यूरो के मानकों अनुरूप पाया गया। अपरम्परागत यीस्ट से तैयार पीज़ा आध ाारों की तुलना बाज़ार में उपलब्ध नमूनों से की गयी तथा इन्हें उनकी रासायनिक संघटन तथा संवेदी लक्षणों अनुरूप पाया गया। अत: ब्रेड तथा पीज़ा आधारों के आधारीय संरचन में सुधार की संभावना प्रतीत होती है। ब्रेड तथा पीज़ा आधारों के उत्पादन में अपरम्परागत बेकरी यीस्ट के एच्छिक गुणों को ध्यान में रखते हुए इन उत्पादों को औद्योगिक स्तर पर बनाने के प्रयास किये जाने चाहिए। इस विधि से सूक्ष्मजैविक किण्वन द्वारा व्हे की उपयोगिता बढ़ाई जा सकती हो

Chapter – I

Introduction

1. INTRODUCTION

There is a close association of yeast with man since ancient civilization. Yeasts are being used in many foods knowingly or unknowingly. They play an important role in ripening of many varieties of cheeses. A broad spectrum of fermented milk products have been developed using yeasts as one of the component of their starter microflora like kefir and koumiss. Moreover, yeasts are also used in preparation of single cell protein, supplement for protein in human and cattle feeds. The contribution of yeasts in food industry in the preparation of various alcoholic products like beer, wine, whiskey etc. is well known. However, the most widely recognized role of yeast is in preparation of bread and other similar bakery products. Baker's yeast (*Saccharomyces cerevisiae*) is considered to be indispensable tool for preparation of various bakery foods. The major role of baker's yeast in bread preparation is attributed to its leavening activity. Yeasts ferment the sugars (carbohydrates) present in dough to produce acid, alcohol and CO_2. The carbon dioxide thus produced causes the dough to rise in volume. *S. cerevisiae* has been used as conventional baker's yeast in bakery since long time. It uses molasses as a substrate for growth. However, growing demand of molasses in the industrial production of varied products has led to decrease in its availability. *Kluyveromyces fragilis* a lactose fermenting yeast had been tried alternatively for production of bread and results were comparable with that prepared from conventional baker's yeast. Hence there is a scope for scaling of the process and to develop a method for industrial scale production of these yeasts. Also there is a possibility of improving the quality of bread by supplementing lactose into formulation. This will lead to an alternative use of whey in production of NCBY. Several reports indicate beneficial effect of use of lactose in many baked goods.

At industrial scale, the yeasts cells are harvested by centrifugation from the growth medium. These cells are further concentrated by filter pressing to a desired total solids level (25-30%). However, the use of centrifugation has several limitations; the cells are subjected to mechanical stresses and the separation rate is density dependent. Alternatively, membrane processes such as microfiltration(MF) and Ultrafiltration(UF) may be used. Membrane processing in fact was developed for purpose of purification and sterilization of media. Today, it finds its application in many biochemical processes. Removal of cells or harvesting of cells is one of them. With the availability of membranes with different pore sizes, membrane suitable for specific purpose may be chosen.

Studies have shown that about 12-fold concentration of cells can be achieved by UF without any cell damage. Moreover, MF has been employed successfully to remove upto 99.5% of spores and even gram-negative pathogenic organisms from raw milk. Keeping in view large size of yeast cells as compared to bacterial cells, membrane technology can also be used for recovery of yeast cells. Since, there is a paucity of informations in this regard, the present studies have been undertaken with the following objectives :-

1. To optimize conditions for production of Non-Conventional Baker's Yeast (NCBY) by application of ultrafiltration and/or microfiltration technology using whey as basal medium.
2. To asses the viability and quality of bread and pizza base prepared by using standardized compressed yeast.

Chapter – II

Review Of Literature

2. REVIEW OF LITERATURE

2.1 SIGNIFICANCE OF YEASTS IN FOOD INDUSTRY

Yeasts, of all the various groups of microorganisms have been intimately associated with man from the dawn of his existence. The ability of certain yeasts to rapidly and efficiently convert sugars into alcohol and CO_2 has enabled this group of organisms to get association with man. Man used the fermentative ability of certain yeasts to carry out alcoholic fermentation of sugar liquid such as fruit juices, grain extracts and milk.

Industrial uses of yeasts can be classified into three groups according to the relationship of the product to the biochemistry of the organisms (Rose and Harrison, (1970).

1. **Cell constituents :-** Whole cells (fodder, food), macromolecular constituents (lipids, proteins, enzymes, nucleic acids), extraction products (coenzymes, vitamins) and breakdown products (amino acids, purines, pyrimidines).

2. **Excretion products :-** Beer, wine, cider, spirits, glycerol, CO_2.

3. **Enzyme- substrate interaction** :- Whey utilization by *K. marxianus* var. *lactis,* Maltotriose production by *Saccharomyces uvarum.*

Yeasts are commercially significant in food industry. It predominates the surface microflora of brick cheese during initial ripening stage (Olson, 1996). Yeast present may be *Debaromyces, Rhodotorula, Trichosporon* and *Candida.* Growth of yeasts serves to modify the surface of cheese. Yeasts also contribute to the final flavour of brick cheese. Yeasts are also used in manufacture of fermented dairy products (Kefir and Koumiss). A kefir grain consists of *Candida* and *Saccharomyces.* Koumiss consists *Kluyveromyces marxianus* (Vij and Gandhi, 2001).

In wine making, any yeast capable of fermenting sugar ferments glucose, fructose and mannose. Most species occurring in must (grape

must) prefer glucose i.e. they ferment glucose faster. For wine making, in contrast with media used in other branches of fermentation, grape must carries yeast flora along with it. However, in many wineries in the United States, Canada, South Africa and Australia, pure cultures are used commercially. Yeast found in grapes include *Torulopsis, Candida, Pichia, Saccharomyces* and other. However, for beer and other alcoholic beverages pure cultures of *S. cerevisiae* are used (Reed, 1987).

Another important use of yeasts is in manufacture of bakery products. It is an indispensable tool for making bakery products. Although yeast free bread (soda bread) is produced, but the bread prepared from dough leavened by yeast is the most popular. Yeast in dough produces gas that changes the dough structure by inducing physico-chemical changes in the network of gluten and other protein. In this way a well developed dough retains gas even during baking. It also influences the aroma of the final bread (Beuchat, 1987).

2.2 CLASSIFICATION OF YEASTS

Yeasts are unicellular fungi that reproduce vegetatively by budding or fission. They are uninucleated, non-motile, chemosynthetic, spherical, ovoid or elliptical in shape. The size of yeasts ranges from 2.5 to 5.0μ in width and 4.5 to 21.0μ in length (Reed and Peppler, 1973). Yeasts show great biological and biochemical diversity and undoubtedly the most important group of microorganisms exploited by man. The important yeasts of commercial interest are *Saccharomyces, Candida* and *Kluyveromyces.* Yeasts are traditionally characterized, classified and identified by morphological and physiological criteria (e.g. shape of cells, mode of sexual and asexual reproduction), anaerobic fermentation and aerobic assimilation of sugars and certain growth requirements (Walt and Yarrow, 1984).

Industrially important yeasts are classified into two classes of fungi based on their spore forming capabilities. The 'Áscomycetes', also designed as 'true yeast' and asexual 'Deuteromycetes' or 'false yeast' (Reed and Peppler, 1973). In Ascomycetes yeast, sexual reproduction is by

production of sexual spores in an ascus whereas in Deuteromycetes, sexual cycle is not present. Industrial strains such as *Saccharomyces* spp. of yeast are included among the genera of ascomycetes. These strains are widely used in baking, brewing, distilling and wine making processes. Mostly fermentation ability and exclusively single budding cells are the main features of the genera *Saccharomyces, Torulaspora, Zygosaccharomyces* and *Kluyveromyces.*

On the basis of certain activities yeasts are classified into two categories, such as (1) active yeasts and (2) inactive yeasts. Active yeasts are those used for fermentation and as a source of nutritional and flavour components. These include baker's, brewer's, distiller's and wine yeasts. Baker's yeast is available in three active forms that differ in their activity and stability. Inactive yeasts are also called as "dried yeasts' and are non-fermentative. They are predominantly used as nutritional, flavour and bulking aids (Peppler, 1979). Among these are *K. lactis*, and *K. fragilis* (Gueriviere, 1981) and *C. utilis* (Mueller, 1986).

On the basis of growth temperature, Vidal-Leiria *et al.* (1979) classified yeasts as psychotrophic growing below 24^0C, and mesophiles growing at 24^0C to 48^0C. Fernandes *et al.* (1985) isolated thermophilic yeasts, which grew well at 50^0C and had heat stability at 65^0C and 70^0C. Thermotolerant yeasts have been defined as those capable of growing at or above 45^0C and/ or fermentation at or above 40^0C. However, Hacking *et al.* (1984) observed that *Kluyveromyces* strains were more thermotolerant than *Saccharomyces* and *Candida.* Anderson *et al.* (1988) isolated thermotolerant yeasts capable of growing at a temperature more than 40^0C and found *K. marxianus* var *marxianus* to be predominant. Banat *et al.* (1992) isolated thermotolerant fermentative yeast capable of growing at 52^0C and producing ethanol at 45^0 C and 50^0C.

2.3 SUBSTRATE FOR THE GROWTH OF YEASTS

The various parameters to be considered for choice of substrate are (i) abundant availability of raw materials (ii) price, (iii) freedom from undue

toxicity (iv) carbohydrate content (v) supplementary requirement and (vi) advantage of utilization of the substrate by selected microorganisms for biomass production (Davis, 1974).

The yeasts are capable of multiplying with carbon substrates as varied as carbohydrates (monosaccharides, disaccharides, polysaccharides), hydrocarbons (gas, oil, paraffin) and lipids. Baker's yeast is generally cultivated on sugar-beet or cane molasses. However, growing demand of molasses in the industrial production of alcohol, baker's yeast, single cell protein, organic acids, and its requirement in cattle feed and tobacco industries may lead to decrease in availability of sugarcane molasses for the production of these products of industrial importance.

Since no isolated yeast can take up all these substrates, different substrates have been reported. It has been found that lactose fermenting yeast *K. fragilis* grow well in lactose whey (Shay *et al.*, 1987). Since studies have shown that lactose fermenting yeast can be used equally well for preparation, there is a lot scope of utilizing the enormous quantity of whey available through cheese and paneer in India. Whey is potential raw material for microbial biomass production, since it contains 4 to 5 percent lactose. The steady increase in consumption of dairy products and the restrictions on direct disposal of high BOD material to the environment have made feasible utilizing whey as a substrate for yeast biomass production. Yeast biomass production on whey is generally suitable for species of *Candida*, *Torulopsis*, *Kluyveromyces* (Marth, 1970; Sanderson and Reed, 1985) because of their capacity to use lactose as a carbon source.

Whey for use as a substrate for yeast production may or may not be pre-treated. The pre-treatment of whey involves the prior separation of the whey proteins from the whey. The advent of membrane technology has been a boom to economise the process for separation of many proteins (Marshall and Harper, 1988). Ultrafiltration (UF) process gives rise to production of whey permeate called 'lactose product' which can be used as a fermentation medium for the production of yeast. Ultrafiltration allows the water, soluble components like lactose, salts, and some vitamins through

the membrane, whereas milk fat, proteins, insoluble salts are retained by the membrane (Glover, 1985).

Vij (1995) reported the use of whey permeate as a medium for the growth of lactose fermenting yeast with some nutrient supplementation. The different nutrients were added such as yeast extract (0.75%), ammonium phosphate (0.5%), ammonium sulphate (0.5%), molasses (0.5%) or corn steep liquor (1%).

The maximum yeast biomass for *K. fragilis* KHF-98 was obtained with supplementation of whey permeate with (0.5%) urea and 0.02% orthophosphoric acid using 5% inoculum size with a mixing speed of 230 rpm at 30 ^{O}C (Fadel and Degheidi, 1998).

2.4 CONVENTIONAL AND NON-CONVENTIONAL BAKER'S YEAST

Commercial baker's yeast contains strains of *S. cerevisiae*, lactic acid bacteria and a low number of contaminating microorganisms. In most countries, the definition of baker's yeast is restricted to *S. cerevisiae*, a microorganism belonging to the fungal class of Ascomycetes. Cells of this yeast may be spheroidal, subglobose, ovoid, ellipsoidal to cylindrical to elongate. They occur either singly, in pairs and occasionally in short chains or in small clusters. About 3500 billion cells are contained in 0.454 kg of compressed yeast. The unique position of baker's yeast is due to two qualities: i) the dough leavening activity of baker's yeast and ii) its contribution to the taste and flavour of the finished bread. In addition to *S. cerevisiae*, there is minor group of yeast species that are part of natural sour dough include, *Candida* species, *Torulopsis* species, *Pichia* species and *Saccharomyces exigus* (Sanderson, 1984). Some of above yeasts are produced commercially on small scale as 'leavening agent'.

Although *S. cerevisiae* is traditional baker's yeast, the scientists are always interested to explore new strains of yeast species which can be used for bread making of better qualities in shortest period. Mitchell (1957) evaluated the baking properties of 75 yeast cultures and reported that

Zygomonas saccharomyces showed good baking properties as compared to *S. cerevisiae* Sankyo Co. Ltd. (1979) used *S. rosei* for high sugar bread making. Williams and Luksas (1981) prepared bread with *Candida lusitaniae* and *S. delbrueckii.* Further, Kusachi (1981) developed a lactic yeast preparation of *Kluyveromyces lactis* and *K. fragilis*. They found that addition of 2.5 per cent of this lactic yeast preparation reduced bench time of pie and snack dough by over 80 percent. Several workers also reported use of distiller's yeast for bread making (Derkanosov *et al. (*1978). In an another study, Fernandes *et al.* (1985) used a thermophilic strain of yeast *S. cerevisiae* for bread making. In these studies, dough fermentation was carried out at high temperature (41^{o}C) during bread making and various strains of sugar and lactose fermenting yeasts have been characterized by various workers. Kalinina *et al.* (1979) developed a yeast hybrid for use in liquid yeast for application in baking industry. The hybrid strain was obtained by crossing *S. cerevisiae* with *S. diastaticus*. It has advantages over the *S. cerevisiae* that it has greater fermentatioin activity and cell proliferation capacity in flour. It is acid and heat resistant and produced more aldehydes and esters which may improve bread aroma. Taya *et al.* (1984) developed a lactose utilizing hybrid strain derived from *S. cerevisiae* and *K. lactis* by protoplast fusion. Trivedi *et al.* (1984) developed a new quick rising yeast using protoplast fusion to combine two strains of yeast. Oszlayi (1983) developed an instant yeast which eliminated the normal rehydration step before bread making. Different isolates of lactose fermenting yeast of lactose fermenting yeast from milk and milk products were studied with respect to their ability to raise dough and toxicity by Vij (1995). It has found that a bread can be prepared using *K. fragilis* as comparable with that prepared by using conventional baker's yeast.

2.5 MANUFACTURE OF BAKER'S YEAST

The commercial production of baker's yeast starts in the laboratory. A pure culture maintained in the laboratory, from which a loop of yeast cells is transferred to a small flask containing sterile medium (grain or molasses medium fortified with necessary growth factors). The flask contents are

transferred to pure culture tanks after 2 days incubation. These tanks may comprise of a series of fermentors. The growth medium is sterilized directly in the culture tanks. Contamination of the culture by other microorganisms would ruin subsequent scale-up to full size fermentation. (Beuchat, 1987)

Fermentation is conducted over a period of about 10-13 hr at $30^{o}C$. During this time nutrients are incrementally fed so that the sugar concentration is always very low, and yeast suspension is vigorously aerated. Under these conditions, the nutrients are efficiently assimilated into yeast biomass, anaerobic fermentation is discouraged, and yields are favorable (Reed, 1987). At the completion of fermentation the concentration of the yeast cells in the suspension is about 3.5-4.5%. The first step in the recovery of the yeast from the fermented wort is passing through a centrifugal separator; the second passage is preceded by a water wash of the yeast cells. Either passing through filter presses or rotatory vacuum filters may further concentrate the yeast cream. With filter presses, the yeast cream is pumped into a number of compartments separated by frames fitted with tightly woven cloth filters. A pressure of 100-150 psi is applied to reduce the water content of the yeast cream (Beuchat, 1987).

The yeast cake is prepared for packaging by first mixing small amount of water so as to adjust the solids level to a target value of about 30% and subsequently addition of emilsifiers and oils. The function of emulsifiers is to improve yeast appearance and the oils aid in yeast extrusion and cutting operations required for preparation of dry active form of the baker's yeast (Reed and Peppler, 1973).

2.5.1 HARVESTING OF CELLS BY MEMBRANE PROCESSING

Industrially two stage centrifugation is employed for separating yeast cells from fermentation broth. The centrifugation may be considered as an enhanced gravity setting device with a 'g' factor of acceleration ranging from a few hundred to many thousand times that of gravity. This has the advantages of bringing about separation much faster, in a smaller space also permitting very fine particles to be handled; control of operation made

possible. By far the most widespread industrial application of centrifuges is the separation of cream from milk. However, the majority of other applications involve separation of finely divided solids from aqueous suspension.

Continuous centrifuges, where high G- forces remove cells, are expensive to buy and operate as well as maintenance. Moreover, the production rate of a centrifuge is a function of many variables, among them square of the particle diameter, the difference in density between the particle and suspending medium, the G-forces and the viscosity of suspending medium. Thus, production rate and cell recovery are inversely proportional to each other and strongly dependent on the particle size (Cheryan, 1989). Also leads to mechanical injury to cells (Cox *et al.*, 1978).

Filtration, on other hand is not limited in throughput by particle size and separation does not decrease with decrease in particle size. Membrane filtration technology finds its application in four main areas of biotechnology viz.

1. Water treatment
2. Harvesting of cells
3. Fractionation of fermentation broths and recovery of components
4. Membrane bioreactors

Application of membranes in dairy industry involves separation of somatic cells, selective separation of bacteria, separation of whole casein, clarification of whey. Microfiltration (MF) is one of the first filtration processes, commercially developed by Sartorious-Werke in Germany in 1929. The main uses of microfilters are water purification and sterilization and microbiological and related applications, such as direct epifluorescent filter technique (DEFT) (Pettipher *et al.*, 1980) Between 93-99% of somatic cells present in raw whole milk were retained by MF membrane having pore size of 12μm (Maubois, 1997). It has been reported that 99.7% of bacteria could be removed from skimmed milk (Malmberg and Holm, 1988). Also removal of Clostridia spores was found to be 10 times better as compared to

that from bactofugation, regardless of initial counts (Piot *et al.*, 1987). Using *B. cereus* as indicator organism for efficient pasteurization, 10 times fewer counts were found in pasteurized milk after MF. Using 'Bactocatch' process for treating skim milk at 50°C, 99.95 and 99.0% population of *Salmonella* and *Listeria* respectively were reduced (Madec *et al.*, 1992).

Continuous production of concentrated cells of *Streptococcus salvarius* subsp. *thermophilus* was achieved in a continuous stirred tank reactor coupled with ultrafiltration(UF) module. Membrane processes such as MF and UF could thus be powerful techniques to concentrate starters as suggested by Amen (1982). Infact, industrial scale concentration of starters by UF has been reported by Porubcan and Sellars (1976).

Advantages of membrane separation over conventional separation and recovery techniques are simplicity of operation, separation at ambient temperature, no phase change or solvent addition, and low energy consumption especially in the case of low pressure processes of MF and UF. These advantages are particularly suited for food and biological application in which product's quality is increased when a chemical structure and activity are minimally altered (Sims and Cheryan, 1986).

For *A. niger*, the apparent maximum obtainable cell concentration was 250 g/L (Sims and Cheryan, 1987). Patel *et al.* (1987) reported that no trace of yeast cells were found in permeate of any of the different modules used to harvest yeast cells. However, cell yields or recovery is function of operating parameters and module design. (Cheryan, 1989)

2.5.2 FORMS OF BAKER'S YEAST

The baker's yeast available in the market may be in one of the four forms discussed below.

1. **Compressed Yeast :** Compressed yeast is available in 0.454 kg and 2.27 kg blocks (Beuchat, 1987). It is wrapped in wax paper and stored under refrigeration (1-5°C) until needed. Storage of the yeast under refrigeration, minimizes heat buildup, and prolongs yeast activity. Moreover, it also inhibits autolysis, another self-destructive

process whereby proteases within the yeast begin to digest cellular protein. When fresh, the yeast blocks have creamy color, and characteristically break apart cleanly. Older yeast has a more brownish color, and tends to crumble when broken apart.

2. **Active Dry Yeast :** This form of yeast posses a relatively long shelf-life. Active dry yeast is manufactured from compressed yeast. Compressed yeast is extruded and may be tumble or tunnel dried at controlled forced air-temperatures ranging from about 28°C to 40°C. The moisture content of commercially marketed ADY ranges from 7.5% to 8.3%. The addition of emulsifiers such as sorbitan esters and antioxidant such as BHA at 0.1% imparts prolonged stability to low moisture ADY.

3. **Instant Active Dry Yeast (Instant ADY) :** This type represents a definite quality improvement compared to regular ADY. Rigorous drying processes employed in producing the ADY lead some damage of yeast cell membrane and intracellular enzyme activity. The instant ADY is prepared by airlift drying yeast on metal screen or perforated plate by blowing warm air from bottom at a velocity which suspends the yeast strands in a fluid bed. An air temperature of 160 ° C can be used, but yeast temperature should be controlled to a maximum of about 40 °C.

4. **Yeast Cream :** Although compressed yeast is manufactured by concentrating the 'cream yeast' with the solids contents of about 18% to a solids content of approximately 30%, cream yeast has been made available to the baking industry in United States 1980. When stored at the recommended temperature of 2°C with agitation in the tank, it has a shelf-life of up to three weeks. It is used for the same purpose and at the same solids level as compressed yeast. The recommended exchange ratio is :

   1 lb compressed yeast = 1.7 lb cream yeast – 0.7 lb water.

   (Doerry, 1995)

2.6 EVALUATION OF BAKER'S YEAST

Baker's yeast is used for the leavening of baked goods. The usefulness of the yeast depends entirely on its ability to raise dough. Two tests are adapted namely 'fermenting power' and 'dough raising capacity'. While the responsibility of former is based on pure chemical reagents that of the latter depends on the availability of flour with identical contents of gluten with same hydration power. The results of both these tests together would enable to judge the quality of material accurately (BIS, 1988). The baker's yeast produced must be active in leavening and should possess good stability. For selection of a good baker's yeast strain, Burrows (1970) stated that five properties are necessary (a) high potential glycolytic activity, (b) ability to adapt rapidly to changing substrate (c) high invertase activity (d) high potential maltose fermentation, and (e) ability to grow and synthesize enzymes and co-enzymes under anaerobic conditions.

Salek (1983a) studied the parameters for selection of commercial strain of baker's yeast with high technological qualities. The yeast had high productivity, high growth rate, good fermentative capacity and tolerant to changes in temperature and pH. The yield and gassing power of yeast strain was also studied. In several studies, it has been reported that bread quality is also dependent on the biochemical properties of yeast (Avramanko *et al.* 1985; Oda and Ouchi, 1989). Salek (1983b) studied the criterion for evaluation of baker's yeast with respect to specific growth rate, productivity, specific rate of respiration, glucose, sucrose and maltose fermentation rate and dough raising time. According to Loest (1983) determination of fermentative capacity should be the sole compulsory test for the evaluation of baker's yeast. Tanaka *et al.* (1983) used gas retention capacity of dough in determination of baker's yeast gassing power.

In other experiment, Okagbue and Emesaiani (1987) studied the viability of yeast cells in commercial brand yeast. A count of 3.5×10^9 cfu/g was satisfactory to raise the dough while a count of 5×10^5 cfu/g in ADY sample was not sufficient to raise dough. Salii *et al.* (1990) studied the effect of

properties of compressed yeast on the bread quality. The yeast was analysed for maltase, zymase activity and fermenting power.

2.7 STORAGE STABILITY OF BAKER'S YEAST

Storage stability is an important criterion for the quality of baker's yeast. The most decisive factors influencing the keeping quality of baker's yeast are the storage temperature of yeast and type of environment.

Compressed yeast contains 68 to 72 percent moisture generally. Compressed yeast (CY) should be kept under refrigeration it being an perishable product. Peppler (1960) reported little activity loss over 19 weeks when stored at $-30^{\circ}C$. It has also been observed critically that freezing and thawing affect yeast viability (Mazur, 1970). Hautera and Lovgren (1975) reported little decrease in yeast activity after it was stored at $5^{\circ}C$ for 28 days. At $23^{\circ}C$, the fermentative activity remained constant for 16 to 18 days, while at $35^{\circ}C$, yeast activity decreased linearly with storage time and becomes zero activity after 7 to 9 days.

The decrease of fermentative activity of compressed yeast during storage is due to the breakdown of reserve carbohydrates, such as glycogen and trehalose (Eaton, 1960), an increased number of dead cells (Suomalainen, 1975) and proteolytic activity (Tokoyama and Takakuwa, 1971).

Oura and Tanner (1980) studied that the dry matter loss of compressed baker's yeast during storage at room temperature was 8 per cent and water loss was 7.4 percent. The fermentative activity dropped faster in blocks than in pieces. The storage stability of active dry yeast is significantly better than that of compressed yeast, since it contains less than 10 percent moisture. Its shelf life may be as short as 1 to 2 months to as long as one year, depending upon storage temperature and atmosphere as well as its moisture contents.

Vij (1995) reported better shelf stability of compressed yeast of the sample stored at $7^{\circ}C$ than the one stored at $30^{\circ}C$. It was found that

moisture content decreased linearly with an increased storage time. Moisture loss was higher at higher temperature of storage. Dough raising capacity of desirable level for NCBY retained upto a period of 4 weeks at low temperature than at higher temperature of storage. Viability of yeast cells also decreased rapidly at 30^{o}C (6 per cent retained after one week of storage). On the other hand 50 percent viability of the original was retained after 3 weeks of storage at 7^{o}C.

2.8 MANUFACTURE OF BREAD AND PIZZA BASE

Before the development of suitable ovens, the first "bread products" were most likely flat breads. These type of breads are still produced and eaten in many parts of the world (Doerry, 1995). There was gradual development in the technology of bread making. Until World War II, very limited technology for breadmaking was available. However, with the baking ingredient industry responding to the special needs of individual bakeries, there are hardly two bakeries now producing bakery foods in a same way. Nowadays, many new technologies for breadmaking are available viz. straight dough technology, sponge dough technology, water brews, sours and many others.

Despite of varied technologies available for breadmaking, the basic ingredients remain the same and form an important part of formulation resulting in the development of dough quality in terms of rheology, texture, flavour etc. the main ingredients in breadmaking and their role in dough development are :

1. Wheat flour : Flour is the most basic ingredient in all bread – like products. The type of flour used by the baker can vary not only according to its source (wheat, rye, barley etc.) but also with respect to its grind or physical shape; fine or medium grind flour, cracked or flaked grains, and in the way it is refined or separated from unwanted components of the grain : (patent flour, straight grade flour, whole meal). The type of grain product used in the manufacture of bread will not only affect the taste and texture of the baked product, but also the technology used and the final

shape and size of the food. The structure forming protein found only in wheat is called wheat gluten. Proteins present in other grains are often called gluten, too, but these proteins do not form a three-dimensional cell structure, like wheat gluten does. The gluten content of the flours used is generally 10.8-11.5 per cent.

2. Water : Although water is a major ingredient in bakery foods, there is not total agreement on how much " water quality" affects "product quality". The chemical analysis of water samples from the bakeries verified that there were significant differences in water hardness, impurities, and pH of the various samples. This finding led to the development of the first mineral yeast food. This dough additive contains the buffering salt calcium sulfate to minimize differences in water hardness and acidity (pH). The use of mineral however helped overcome differences in water quality, the effect of water on the product quality has never been fully known (Doerry, 1995).

3. Yeast : Yeast is indispensable in bread making. The yeast has following three functions:-

a) Leavening – It is actually a complex process involving interaction between dough and yeast. There are two facets of yeast leavening such as (i) carbon dioxide production (ii) carbon dioxide retention in dough system. Evolution of carbon dioxide into the dough by the action of *S. cerevisiae* on available sugars leads to the characteristic porosity of bread that enhances its palatability. This porosity is because of carbon dioxide produced by yeasts and the ability of the dough to retain the gas. In order to retain carbon dioxide produced during panary fermentation in dough systems, the gluten in the flour must be properly hydrated and attain visco-elastic property.

b) Flavor development: Fresh bread has a pleasing aroma. In bread, flavor is thought to be because of two main sources such as yeast fermentation and crust browning. Many compounds, organic esters and acids, alcohols, carbonyls are found as by-product of yeast fermentation. In addition to fermentation, bread aroma is determined by the duration,

temperature and type of baking. Browning is enhanced as a result of presence of yeast cells, which serve as a source of amino acids (El-Dash and Johnson, 1970).

c) Dough maturation; Properly matured dough is one that has optimum rheological properties such as optimum balance of extensibility and elasticity, with the result that bread will have desirable volume and crumb characteristics. Change in rheological properties of dough fermentation depends on a number of factors, including the hydration of starch, proteins of dough and activities of flour derived and added enzymes (Hautera and Lovgren, 1975).

Yeast fermentations also yield alcohol and carbon dioxide during dough fermentation. Alcohol is water miscible and influences the colloidal nature of the flour protein and alters the interfacial tension within dough. Some carbon dioxide dissolves in aqueous phase of the dough and forms weakly ionizable carbonic acids, which lower the pH of the system. *S. cerevisiae* liberates ammonia from ammonium sulphate and ammonium chloride added as yeast foods, thus liberating sulphuric and hydrochloric acid in the dough. These acids further lower the pH, which in turn, significantly influences gluten hydration and swelling, the reaction rate of enzymes in dough and various chemical reactions (Magoffin and Hoseney, 1974). It has been discovered that reductase produced by *S. cerevisiae* affect dough rheological properties by acting through intermediate substrate found in dough (Reed and Peppler, 1973).

4. Sugar : During the dough stage of sponge dough processing and in straight dough methods or any other breadmaking systems, assuming all ingredients are present, the yeast will first utilize sugar added to the dough as an ingredient. Added sucrose is almost immediately hydrolyzed into the constituent monosaccharide glucose and fructose due to the action of invertase. The yeast then ferments both simple sugars, but at differing rates; glucose is preferred and is fermented at a faster rate than is fructose. (Beuchat, 1987).

Also, with increasing production of cheese worldwide, lot of whey is produced. Whey is a rich source of lactose. Use of lactose gives several advantages in food formulations: lactose as a reducing sugar contributes favourably to the crust colour of bakery goods, it is an ideal humectant for extending the shelf life of bakery products and it contributes to desirable flavours in most baked goods (Holmes and Lopez, 1977; Vetter, 1984; Ogunriola, 1986 and Ogunriola, *et al.*, 1988). The lactose in the permeate should not be hydrolyzed completely so as to derive benefits directly related to lactose in the bread system. However, demineralization is important in preventing discoloration of the crust (dark golden brown in white pan bread) and may possibly retard the metabolic activity of the yeast cell (Ogunriola, 1993)

As per Indian standards (BIS, 1988), the ingredients that can be added to bread can be classified into two classes; i) Essential ingredients that include maida, leavening agent (baker's yeast, barm hope, fermented juice obtained from palms and lactic acid ferment), edible common salt and water. ii) Optional ingredients, which include milk and milk products, like condensed milk and milk powders, sugar and sugar products such as *gur*, jaggery, honey, malt products etc. Also it includes edible flours other than wheat flour, fat (hydrogenated vegetable oil, ghee, butter, vitamins, lecithin etc. Other group of optional ingredients includes improvers such as ammonium persulphate, potassium bromate, guar gum. Rope and mold inhibitors like propionates of calcium or sodium, acetic acid etc. may also be added at rate specified for each. Calcium or sodium salt of stearoyl 2-lactylate not exceeding 5.0g/kg of the mass of flour may be used as dough conditioner.

Pizza base is an intermediate bakery product. The ingredients used in its manufacture are same as that used in bread making, though in different proportion. The method of manufacturing is also slightly different. The dough is flattened after leavening in case pizza base making whereas in bread, the leavened dough is baked as such. Moreover, the baking

temperature in case of pizza base is also less than that used for baking of bread.

2.9 SENSORY EVALUATION OF BREAD AND PIZZA BASE

The sensory characteristics of bread include loaf volume, crumb texture, grain characters and flavour. Crust color normally is deep golden brown of the top crust to light golden brown of the side and bottom crust. Pale or grayish color indicates a lack of residual sugars that may result from over fermentation, lean formulation or inadequate amylolytic activity (Pyler, 1973). Certain factors play significant role on the sensory evaluation of bread.

Texture of the loaf crumb is a major quality factor. A soft resistant and short crumb is always preferred. Texture is greatly influenced by the grain or cell structure of the crumb. Crumb color should be soft creamy white, free from streaks and other color spots.

Aroma should be wheaty, nutty, malty and sweet diacetyl and avoid acidic, musty, ropy or rancid elements. Maga (1974) explained that bread flavour is mainly due to alcohols, aldehydes, esters, acids, ketones, lactones, phenols, ethers, amines, etc. Dough components such as flour (Hougen *et al.*, 1971), sugars, lipids and phenolic acids (Maga and Lorenz, 1973) contribute to bread aroma. Apart from these changes during fermentation (Suomalainen and Lehntonen, 1978) and baking (Hoseney, 1984) also influence the aroma of bread.

Chapter –III

Materials & Methods

3. MATERIALS AND METHODS

The current study was carried out in four phases. In the first phase, different strains of lactose fermenting yeasts were screened to select the suitable strain for production of Non-conventional baker's yeast (NCBY). In the second phase, the selected strain was harvested using membranes and effectiveness of the membranes in separating the yeast cells was evaluated. In the third phase, bread and pizza bases were prepared using NCBY. In the fourth phase, the products prepared were evaluated for sensory and chemical attributes.

3.1 MATERIALS

3.1.1 COLLECTION OF CULTURES

Three lactose fermenting yeast cultures (*Kluyveromyces marxianus)* namely, NCDC 39, NCDC 41, and NCDC 46 were procured from National Collection for Dairy Cultures, NDRI, Karnal in freeze dried form (ampoules). An isolate of cream (*K. fragilis* 2C1) was also obtained from Fermented Dairy Products Laboratory, Dairy Microbiology Division , NDRI, Karnal.

3.1.2 CHEMICALS

All chemicals and reagents used during this present investigations were of the make BDH, Glaxo, S.D. Fine chemicals, Qualigen and Hi-Media make and were of analytical grade.

3.1.3 SUBSTRATE

Whey permeate was used as a substrate . Cow milk cheddar cheese whey was collected from Experimental Dairy Plant of National Dairy Research Institute , Karnal and whey was subjected to Ultrafiltration process to obtain whey permeate. The whey permeate had following composition.

Composition of the whey permeate

Lactose (%)	5.2-5.5
Protein (%)	0.4-0.6
Ash (%)	0.49-0.52

3.2 METHODS

3.2.1 ACTIVATION OF CULTURE

The cultures (in ampoules and slant) were aseptically transferred to Yeast Lactose Broth (YLB) for activation. After growth at 30°C/24hr the cultures were streaked on Yeast Lactose Agar plate. Cultures were maintained on YLA slant and propagated in fresh media after every 14-15 days interval.

3.2.2 SELECTION OF CULTURE

Selection of culture was performed on the basis of following parameters

3.2.2.1 Fermentation of sugars

The ability to ferment sugars by yeasts was studied in the fermentation basal medium using different sugars such as glucose, maltose, lactose, sucrose and galactose at the level of three percent each.. Cells obtained from 24 h old cultures of yeasts were inoculated in the basal medium with different sugars and incubated at 30^0C for seven days. Cultures were evaluated in terms of acid and gas production in Durham's tubes.

Composition Of Basal Medium

	g or ml/L
Peptone	7.5
Yeast extract	4.5
Bromothymol blue (0.2% aqueous solution)	10.0
pH	7.0

(b) Preparation of inoculum

For the preparation of starter culture for whey fermentation, a loopful of slant culture was inoculated into sterile yeast lactose broth and incubated at 30°C for 24 h. This was then inoculated into whey medium and incubated at 30°C for 24h. The bulk culture hence obtained was inoculated @ 7.5% in 50-100 L of media.

(c) Media preparation for biomass production

Whey permeate medium (50-100 L) was supplemented with ammonium sulphate (0.5%), ammonium phosphate (0.5%) and yeast extract (0.75%). Medium was heated at 80°C for 15 min. It was allowed to cool to 30°C and then inoculated with the bulk culture.

(d) Fermentation conditions

Whey based medium (50- 100 L) was taken in a storage tank of 500 L capacity. Bulk culture of yeast was inoculated into the tank containing whey based medium and was allowed to ferment for 36 hr at 30°C. Aeration was done by using an agitator.

3.2.4 HARVESTING OF THE CELLS BY MEMBRANE PROCESSING

On completion of fermentation, the cells were collected in cleaned and sanitized milk cans. The cans were sanitized by steaming for 5 min. The cells were harvested using membrane filters. Two types of membranes, Microfiltration (MF) and Ultrafiltration (UF) were used. The specifications for both are as below :

Specification	Microfiltration	Ultrafiltration
Make	Alfa-Laval, Sweden	Ramicon, USA
Type	Tubular Ceramic membrane	Hollow fibre
Area	0.16 m^2	2.5 m^2
Membrane cut-off	-	50,000 dalton
Pore size	1.4 μ	-

Before starting the operation, plants were checked for all the installation and mountings viz. valves and pressure gauges as prescribed in the operation manual. Microfiltration plant was sanitized with water at 80°C temperature 15 minutes. It was then allowed to cool to temperature of operation. Prior to sanitation, the plant was cleaned using Ultrasil-25 at 75°C for 10 minutes. In case of UF, the plant was cleaned using Ultrasil-25 cleaning solution at 50°C for 15 minute. After cleaning, the fermented broth was concentrated at operating parameters specified in the Table. The biomass obtained from membrane processing of the fermentation broth was collected in sanitized milk cans (20L capacity). Finally, cleaning of membranes after operation was performed using Ultrasil-25 and Ultrasil-75 as per specification in operating manual.

Table: Operating Parameters

Parameters	**Microfiltration**	**Ultrafiltration**
Membrane type	Tubular Ceramic membrane	Polysulphone (hollow fibre)
Pore size	1.4 μ	-
Pressure (Bar)		
Feed in pr.	1.8	-
Permeate back pr.	4.1	-
Permeate pr.	3.2	1.8
Retentate pr.	1.6	0.4
Temperature	40 °C	35 °C

3.2.5 ANALYSIS OF SAMPLES

The feed i.e. fermented whey broth (FWB) and the permeate and retentate samples obtained were analyzed for viable count, total solids and biomass.

3.2.5.1 Viable Cell Count

Suitable dilutions of the sample were prepared and plated on YLA. Counts were expressed as cfu per gram after incubation at 30°C for 24 hr.

3.2.5.2 Biomass

10 ml of sample was centrifuged using refrigerated centrifuge (IEC-CU-5000) at 5000 rpm for 20 min. Centrifuged mass was suspended in minimum amount of distilled water and transferred into tared moisture dish. This was then kept in oven at 105 °C± 1°C for 24 hrs. Weight was taken again and biomass represented as gm/L.

3.2.5.3 Total Solids

5 gm of sample was taken in tared moisture dish. This was kept in an oven at 105°C ± 1 for 3hrs and weight was taken. The total solids content was represented in percentage.

3.2.6 PRODUCTION OF COMPRESSED NON-CONVENTIONAL BAKER'S YEAST (NCBY)

The biomass collected after membrane filtration was further concentrated by centrifugation. Further concentration was done by pressing the cell mass. The yeast cake obtained was collected and stored in polyethylene bags. Flow diagram for production of NCBY is given in Figure 1. It was further subjected to analysis for total viable cell count, dough raising capacity and dispersibility.

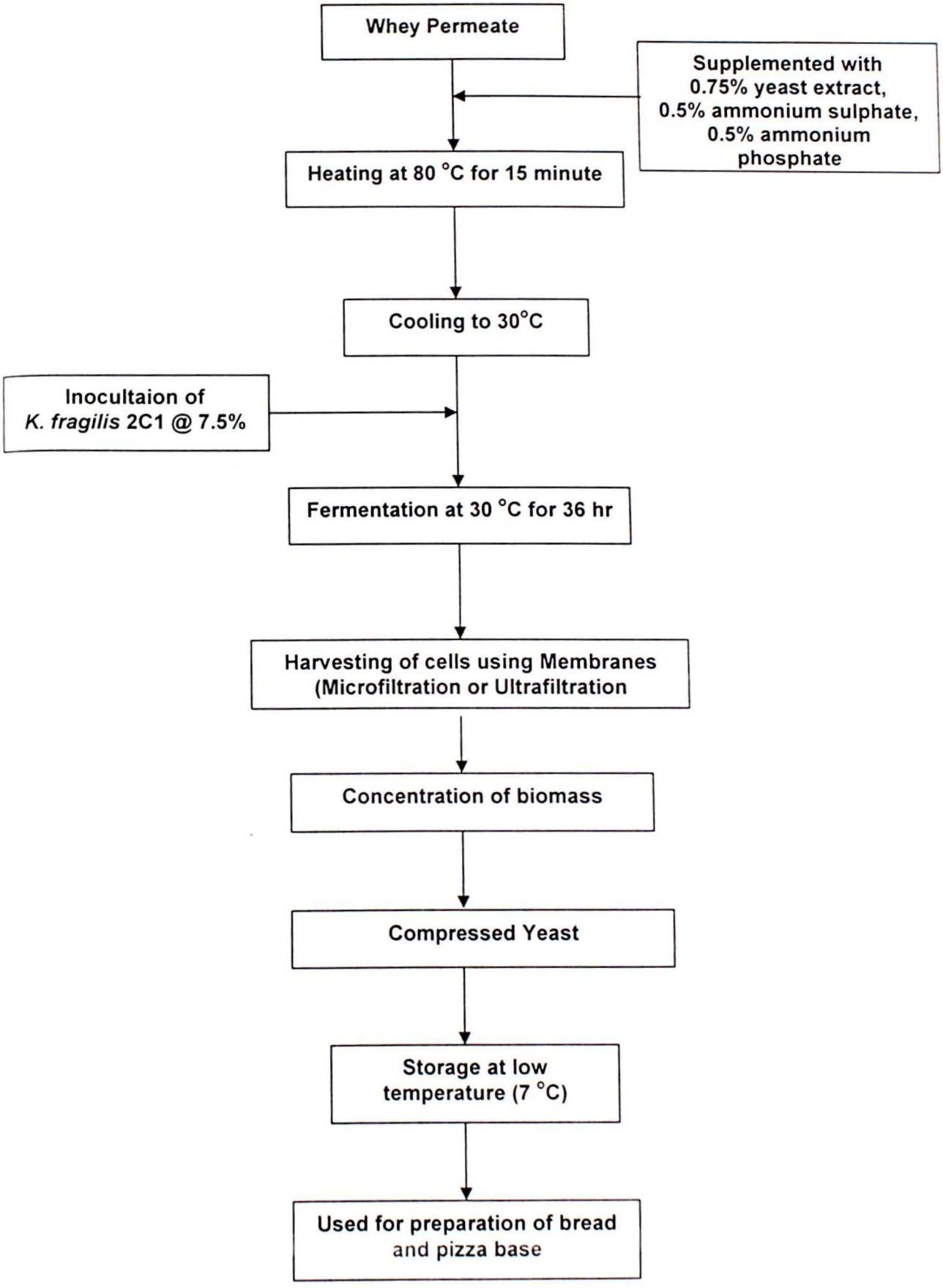


Figure 1 : Production of NCBY by using Microfiltration / Ultrafiltration

3.2.7 ANALYSIS OF COMPRESSED YEAST

The compressed and dried yeasts were analysed for various parameters such as moisture, dispersibility and dough raising capacity according to method prescribed by BIS (IS : 1320-1988).

A. **Moisture**: Moisture dish was weighed along with cover and stirring rod. 2.5 gm of compressed yeast was weighed and 5 ml of alcohol was added to it. The contents were mixed thoroughly with the help of stirring rod. The stirring rod was allowed to remain in the dish. The dish was placed in an oven at 105^{o}C ± 1 ^{o}C for 4 hr. The dish was allowed to cool in a dessicator and weighed. The moisture content was measured as given below.

$$Moisture\ (\%) = 100\ (M_1 - M_2) / (M_1 - M),$$

Where, M_1 is Weight of dish and sample before drying

M_2 is Weight of dish and sample after drying

M is Weight of dish with rod, sand and lid.

B. **Dispersibility test in water:** 20 g of compressed yeast was weighed in a beaker and mixed with 50 ml of distilled water at 40^{o}C. It was kept undisturbed for 5 minutes and then stirred for 2 minutes. 900 ml of water was taken in a 1000 ml measuring cylinder. The slurry was poured from the beaker to the water in the cylinder. 50 ml water was used to wash the beaker and transferred to the cylinder. It was then left undisturbed for 5 minutes to find out the presence of deposits.

C. **Dough raising capacity**: 4 gm of compressed yeast was added in 100 gm maida. 1gm sucrose and 55ml of water was added and kneaded well. The kneaded material was kept in a 500 ml capacity graduated cylinder and was pressed. The initial level of dough was noted. The cylinder was covered and kept at 30 ^{o}C for 1 hr and the final level of dough was noted. Dough raising capacity was determined and expressed in terms of percent rise in dough volume per hour.

3.2.8 STORAGE STABILITY OF YEAST

Samples of compressed yeast were packed in polyethythene bags to study storage stability of non-conventional baker's yeast. The samples were stored at room temperature (30^0C) and at refrigeration temperature (7^0C) for a period of 16 days. Analysis was done at 4 days interval for yeast cell counts, dough raising capacity.

3.2.9 PREPARATION OF BREAD AND PIZZA BASE BY NON-CONVENTIONAL BAKER'S YEAST (NCBY)

3.2.9.1 Preparation of Bread

Bread samples were prepared in laboratory. Slight modification was done in the bread prepared using compressed form of non-conventional baker's yeast. For non-conventional baker's yeast, half of the water in the preparation of dough was replaced with whey permeate as lactose source. The following formulation was used for preparation of bread using straight dough method. (Vij, 1995)

Bread formulation

Flour	250 g
Water	150 ml
Compressed yeast	7.5 g
Sugar	18.0 g
Salt	3.75 g
Potassium bromate	2.5 mg
Shortening (Hydrogenated Vegetable Oil)	7.5 ml
Skim milk powder	7.5 g

All the ingredients were mixed thoroughly and kneaded well to make smooth dough. This was fermented for 165 minutes at 30 ^{O}C. The dough was rounded up, flattened out and moulded. The moulded

dough was placed into a greased bread pan and proofed at 30 ^{o}C for 55 min. After proofing, the bread pan was transferred to an oven and baked at 235 ^{o}C ± 5 ^{o}C for 15 minutes. After baking, the bread loaf was removed from the pan and allowed to cool. The bread sample was packed in polyethylene bag and subjected to sensory evaluation for various attributes. Chemical analysis was done as per standards prescribed by BIS (IS: 1483-1988).

3.2.9.2 Preparation of Pizza Base

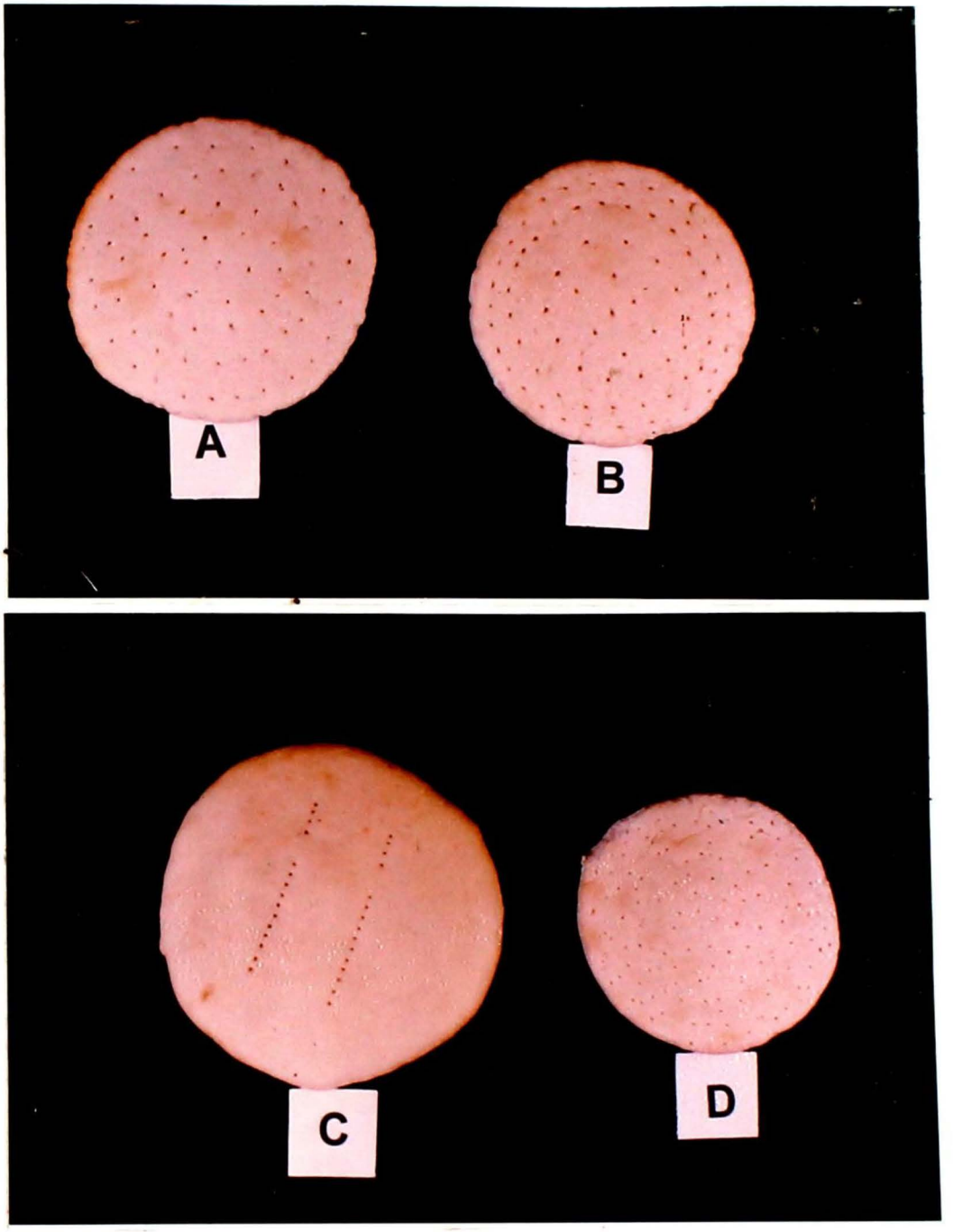
Pizza base was prepared by method prescribed in "Amul's pizza recipe" booklet. The ingredients were mixed and allowed to ferment at 30 ^{o}C for 2hr (to allow the volume to double) with intermediate punching. At the end of fermentation, the dough was flattened and rolled and then baked at 170 ^{o}C for 8min. The recipes were slightly modified for non-conventional baker's yeast. Three different level of whey permeate was added. In sample 'A', one-third of the water for dough making was replaced with whey permeate (2:1 ratio) as a source of lactose. Similarly, in sample 'B' half of the water replaced with whey permeate (1:1 ratio), and in sample 'D', all water was replaced with whey permeate. Sample 'C' was market sample (control). The samples were compared with market sample for chemical analysis and sensory analysis (Plate I).

PIZZA BASE RECIPE

Maida	500 g
Water	325 ml
Compressed yeast	8 g
Sugar	20 g
Salt	0.5 g
Shortening (Hydrogenated Vegetable Oil)	20 ml

3.2.10 CHEMICAL ANALYSIS OF THE SAMPLES

Total solids, pH, mass to volume ratio and acid insoluble ash were analysed by method described by BIS (IS : 1483-1988).



Sample A - One Third water for dough making replaced with whey permeate

Sample B - Half of water for dough making replaced with whey permeate.

Sample C - Market Sample (Control)

Sample D - All water for dough making replaced with whey permeate.

Plate - I : Samples of Pizza bases

dough was placed into a greased bread pan and proofed at 30 ^{o}C for 55 min. After proofing, the bread pan was transferred to an oven and baked at 235 ^{o}C ± 5 ^{o}C for 15 minutes. After baking, the bread loaf was removed from the pan and allowed to cool. The bread sample was packed in polyethylene bag and subjected to sensory evaluation for various attributes. Chemical analysis was done as per standards prescribed by BIS (IS: 1483-1988).

3.2.9.2 Preparation of Pizza Base

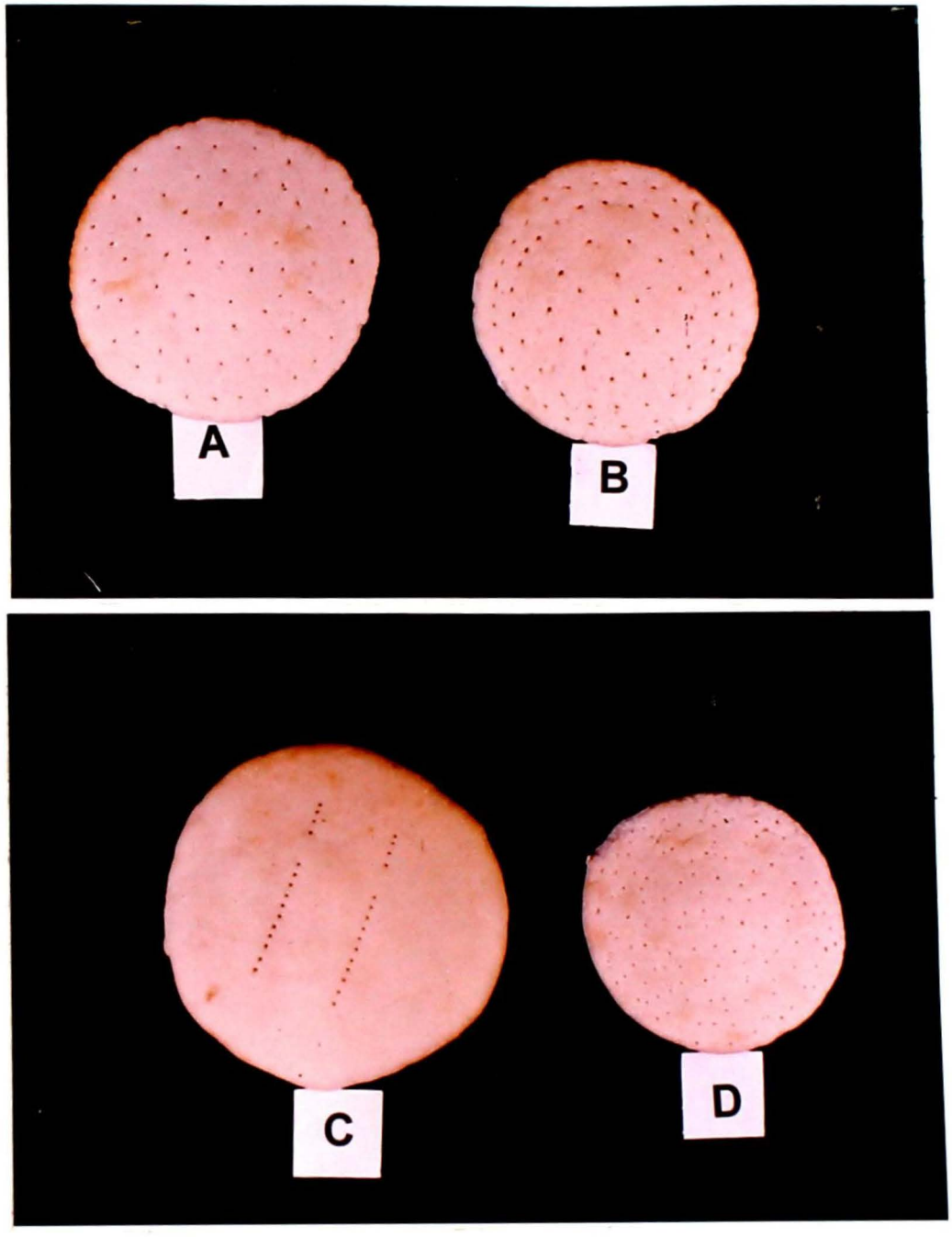
Pizza base was prepared by method prescribed in "Amul's pizza recipe" booklet. The ingredients were mixed and allowed to ferment at 30 ^{o}C for 2hr (to allow the volume to double) with intermediate punching. At the end of fermentation, the dough was flattened and rolled and then baked at 170 ^{o}C for 8min. The recipes were slightly modified for non-conventional baker's yeast. Three different level of whey permeate was added. In sample 'A', one-third of the water for dough making was replaced with whey permeate (2:1 ratio) as a source of lactose. Similarly, in sample 'B' half of the water replaced with whey permeate (1:1 ratio), and in sample 'D', all water was replaced with whey permeate. Sample 'C' was market sample (control). The samples were compared with market sample for chemical analysis and sensory analysis (Plate I).

PIZZA BASE RECIPE

Maida	500 g
Water	325 ml
Compressed yeast	8 g
Sugar	20 g
Salt	0.5 g
Shortening (Hydrogenated Vegetable Oil)	20 ml

3.2.10 CHEMICAL ANALYSIS OF THE SAMPLES

Total solids, pH, mass to volume ratio and acid insoluble ash were analysed by method described by BIS (IS : 1483-1988).



Sample A - One Third water for dough making replaced with whey permeate

Sample B - Half of water for dough making replaced with whey permeate.

Sample C - Market Sample (Control)

Sample D - All water for dough making replaced with whey permeate.

Plate - I : Samples of Pizza bases

3.2.11 SENSORY EVALUATION OF BREAD AND PIZZA BASE

Samples of bread and pizza base prepared by non-conventional baker's yeast were subjected to evaluation for various attributes, such as colour of crust, symmetry of loaf, texture and colour of crumb, flavour and taste and color, flavour, base symmetry, texture respectively. 9-point Hedonic scale was used for the sensory evaluation of the samples (Amerine *et al.*, 1965). The expression used ranged from 'like extremely' to 'dislike extremely'. The judges were asked to score the samples for attributes described above. The observations obtained were statistically analyzed using suitable statistical method. (Randomized Block Design)

Chapter – IV

Results & Discussion

4. RESULTS AND DISCUSSION

The present study was done to assess the use of membranes for harvesting the lactose fermenting yeasts from the fermentation broth and subsequently preparing compressed yeast from it. Bread and pizza base were prepared and analyzed for its various chemical attributes.

4.1 SELECTION OF STRAIN

The parameters studied for selection of lactose fermenting yeast (LFY) were sugar fermentation and dough raising capacity.

4.1.1 SUGAR FERMENTATION

The four strains used in study were first checked for their ability to ferment five sugars. It is evident from Table 1 that all the strains were able to ferment all the five sugars (glucose, galactose, lactose, sucrose and maltose). Among the sugars, glucose was utilized at faster rate whereas the rate of fermentation of maltose was relatively low in terms of acid and gas production.

It has been reported that sucrose could be fermented by isolates of lactose fermenting yeasts. Also the isolates were able to ferment glucose, galactose and lactose (Vij, 1995).

4.1.2 DOUGH RAISING CAPACITY

Dough raising capacity was performed for screening purpose. The dough raising capacity of different strains used for study is shown in Fig. 2. *K. fragilis* 2C1, an isolate of cream showed the highest leavening (117%) followed by 112% rise in volume by NCDC 39. The other two standard cultures, NCDC 41 and NCDC 46 had relatively low dough leavening activity with 105% and 109% rise in volume respectively.

Table 1: **Acid and gas production from different sugars by different strains of lactose fermenting yeasts**

SUGAR	YEAST STRAINS			
	NCDC 39	NCDC 41	NCDC 46	*K. fragilis* 2C1
Glucose	++	++	++	++
Galactose	+	+	+	+
Sucrose	+	+	+	+
Lactose	+	+	+	+
Maltose	+	+	+	+

++ Fast growth

\+ Slow growth

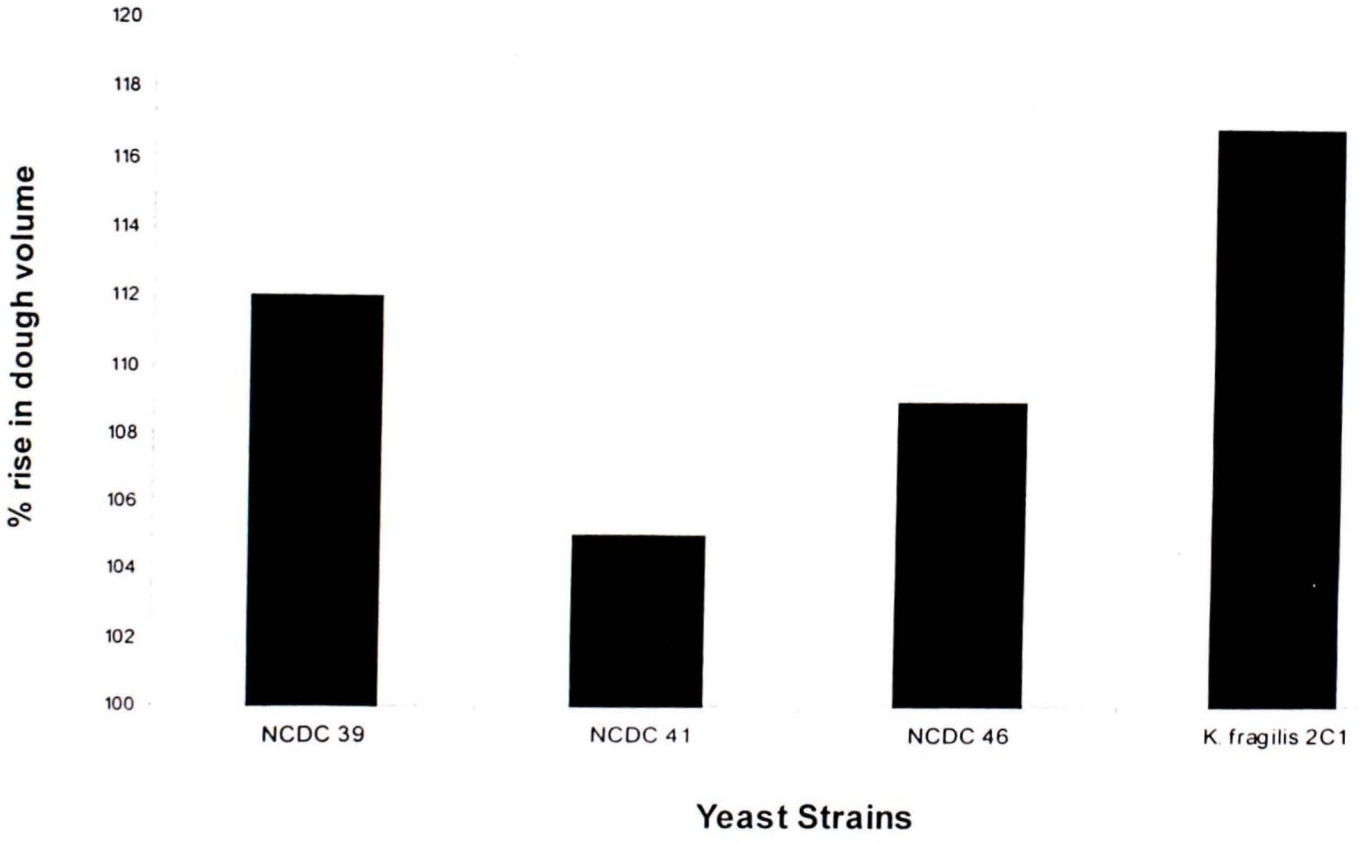


Figure 2 : Dough Raising Capacity of different strains of Lactose fermenting yeasts

As per BIS specification (IS: 1320-1988), the baker's yeast (compressed form) should have minimum 110% rise in volume. Among the cultures used for study, only two, *K. fragilis* 2C1 and NCDC 39 showed dough raising capacity sufficient to meet the BIS standards. However, there is a fall in leavening activity during storage. Hence, *K. fragilis* 2C1 was selected for further studies due to its maximum leavening activity.

Conventionally, *S. cerevisiae* is used for bread making and there are not much reports on the dough raising capacity of NCBY. A lactic yeast preparation of *K. fragilis* and *K. lactis* was developed by Kusachi (1981) and was used for snack and pie. Addition rate of 2.5% of these yeasts were found to reduce the fermentation time of these bakery products. Salek (1983a) studied the parameters for selection of commercial strain of baker's yeast with high technological qualities. The yeast had high productivity, high growth rate, good fermentative capacity and tolerant to changes in temperature and pH. Loest (1983) reported that fermentative capacity test should be the sole compulsory test for the evaluation of baker's yeast.

4.2 HARVESTING OF YEAST CELLS USING MEMBRANES

The membrane filtration processes find their application in large number of biochemical processes. This includes harvesting of cells. Membranes are in use in the dairy industry for concentration and fractionation purposes. Attempts were made to use membrane filtration processes (microfiltration and ultrafiltration) to separate lactose fermenting yeast (*K. fragilis* 2C1) from fermented whey broth (FWB). The results of the same are shown in Table 2.

In microfiltration (MF), cells were concentrated to $5.6X10^8$ cfu/ml in the retentate from the fermentated whey broth having $6X10^7$ cfu/ml. In ultrafiltration (UF), the counts were comparatively higher ($7.2X10^8$ cfu/ml) as compared to MF. From these results, it is inferred that

almost 10 times concentration of cells can be achieved using the membranes. The present observations are in line with those of Porubcan and Sellars (1976) who have observed that a culture may be concentrated varied from 2 to 12 fold by ultrafiltration. Further, a significant increase in biomass was also observed in case of UF retentate (59 g/L) as compared to MF (43 g/L) from fermented whey broth (6.8 g/L) as indicated in Table 2.

The total solids (TS) content of the permeate may be attributed to residual lactose content, salts and some other permeable nutrients. Significant increase in total solids content was observed in case of UF retentate from 6.9% to 12.5% as compared to MF retentate where the total solids content increased from 6.9% to 9.7%.

Although a very small number of cells ($2.2X10^1$ cfu/ml) was observed in case of MF permeate, however, UF permeate was free from trace of any cells. Morphological examination revealed that there were no yeast cells in the MF permeate. These results coincide with the findings of Patel *et al.* (1987) where no traces of yeast cells were found in the permeate of any of the various membrane modules used. The results showed 100% recovery of the yeast cells from the fermented whey broth. This goes in line with many studies on membranes regarding removal of bacterial cells present as contaminants in milk. 93-99% of the somatic cells present in raw milk were retained by Membralox® MF membrane having a pore size of 12μ (Maubois, 1997). Use of "Bactocatch" process for treating skim milk at $50^{\circ}C$ reduces 99.0 and 99.95% *Listeria* and *Salmonella* populations respectively (Madec *et al.*, 1992). Flora present in MF permeate were cocci and rods which might have got access in the permeate through chance contamination.

Table 2 : Composition of fermented broth, permeate and retentate of microfiltration and ultrafiltration of broth

Sample source	Microfiltration		Ultrafiltration		Fermented Broth
	Permeate	Retentate	Permeate	Retentate	
Biomass (g/L)	0.5	41	0.3	59	6.8
Total Solids (%)	6.3	9.5	5.7	12.5	7.1
Viable cells (cfu/ml)	2.2×10^{1}	5.6×10^{8}	-	7.2×10^{8}	6×10^{7}

Table 3 : Analysis of compressed yeast

Parameters	Requirement (as per BIS)	Non-Conventional Baker's Yeast
Moisture (% by mass)	73% (max)	71%
Dispersibility in water*	Satisfactory	Satisfactory
Dough raising capacity (% rise in volume)	110% (min.)	120%

* Measured as per cent rise dough volume

4.3 ANALYSIS OF COMPRESSED YEAST

The compressed yeast was prepared and analyzed for moisture, dispersibility and dough raising capacity (Table 3). The results obtained were in accordance with the BIS (IS : 1320-1988). Observations indicate that compressed NCBY is suitable for use in making bread.

4.4 STORAGE STABILITY

To study storage stability, the compressed yeast samples were stored at 7 ^{O}C and 30 ^{O}C for a period of 16 days at room temperature (30 ^{O}C) and refrigerated temperature (7 ^{O}C) and analyzed for viability of cells, dough raising capacity after an interval of 4 days.

The survivability of the culture decreased from $4.3X10^{9}$ cfu/gm to $2.2X10^{9}$ after 16 days storage at 7 ^{O}C. The increase in storage temperature proved to be more deleterious and resulted in lower viable counts of $3X10^{8}$ cfu/gm from $4.3X10^{9}$ cfu/gm after 16 days of storage at 30 ^{O}C. It is essential that a minimum dough leavening activity of 110% should be present in compressed yeast as suggested by BIS (IS: 1320 – 1988). Dough raising capacity of desirable level for Non-Conventional Baker's Yeast (NCBY) retained for up to a period of 2 weeks at low temperature of storage (7°C). Only 95% dough raising capacity was retained after one week of storage at 30 ^{O}C (Table 4).

The increase in storage temperature proved detrimental and resulted in gradual loss of viability and dough raising capacity which can be attributed to sensitivity of cells to higher temperature of storage. Present findings were also comparable with the studies conducted by (Hautera and Lovergren, 1975) who reported a little decrease in yeast activity when stored at 5^{0}C for 28 days, whereas at 35 ^{O}C, it decreased linearly with the storage. Vij (1995) reported that dough raising capacity of desirable level for NCBY was retained upto a period of 4 weeks at low temperature than at high temperature of storage.

Table 4 : Storage study of compressed yeast

Parameter	Storage temperature							
	30°C				7°C			
	Days interval				Days interval			
	4	8	12	16	4	8	12	16
Viability (cfu/gm)	$10X10^8$	$6X10^8$	$4X10^8$	$3X10^8$	$3.3X10^9$	$2.7X10^9$	$2.4X10^9$	$2.2X10^9$
Dough leavening *	112	95	92	90	118	116	114	109

* Measured as per cent rise dough volume

4.5 CHEMICAL ANALYSIS OF BREAD AND PIZZA BASE

The bread prepared by using NCBY (*K. fragilis* 2C1) was subjected to chemical analysis for various attributes. A sample of bread procured from market was also analyzed for the same attributes. Finally, both were compared with the standard parameters laid down by BIS (IS: 1420- 1988).

On similar lines, chemical analysis of the pizza bases prepared was done. The observations were compared with that of market sample. Since standards for pizza base were not available to compare, the attributes were compared with market sample.

4.5.1 CHEMICAL ANALYSIS OF BREAD

In the bread prepared using NCBY, half of the water for dough making was replaced with whey permeate as a source of lactose. Use of lactose gives several advantages in food formulations. Lactose being a reducing sugar contributes favourably to the crust colour of bakery goods. It is an ideal humectant for extending the shelf life of bakery products and it contributes to desirable flavours in most baked goods and enhances their specific volume (Holmes and Lopez, 1977; Vetter, 1984; Ogunriola, 1986 and Ogunriola *et al.*, 1988)

The results of chemical analysis of bread are presented in Table 5 . The various attributes analyzed were volume/mass ratio, pH, total solids and acid insoluble ash. It may be seen that the volume to mass ratio for market bread was 5.1 which was slightly higher than that of bread prepared using NCBY (4.9). However, both these samples had mass to volume ratio higher than that specified by BIS.

An insignificant difference was observed in total solids content of both the samples as indicated (63% in bread from NCBY and 65% in market sample). However, both the samples were satisfactory in terms of the BIS standard (Table 5).

Table 5 : Chemical analysis of bread

Parameter	Market sample	Bread from NCBY	BIS specification
Total solids (% by mass)	65	63	60 (min.)
Acid insoluble ash (on dry basis)	Absent	Absent	0.1
pH	5.6	5.2	5.0-6.0
Volume/Mass ratio	5.1	4.9	2.5 (min.)

Table 6 : Chemical analysis of pizza base

Parameter	Sample A [1]	Sample B [2]	Sample C [3]	Sample D [4]
Total solids (%)	51	51	53	56
Acid insoluble ash (on dry basis)	Absent	Absent	Absent	Absent
pH	5.3	5.5	5.6	5.3
Volume/mass ratio	2.7	2.7	2.9	2.8

[1] sample A, One-third of the water replaced with whey permeate and prepared using NCBY;

[2] sample B, Half of the water replaced with whey permeate and prepared using NCBY

[3] sample C, Market sample (control)

[4] sample D, All water was replaced with whey permeate and prepared using whey permeate

Further the pH for the bread permissible according to BIS ranges from 5.0 – 6.0. pH is an important criterion for the quality of the bread with optimum pH in the range 5.2-5.6. A lower pH such as 4.8 would normally affect the activity of the baker's yeast (Ogunriola, 1993). Both the samples had a pH falling within this range (5.6 for market sample and 5.2 for bread prepared using NCBY). Moreover, acid insoluble ash was absent in both cases. To meet the standards, the acid insoluble ash should not exceed 0.1% (on dry basis). Thus, bread prepared using NCBY satisfied the standards of BIS in terms of chemical quality for the parameters analyzed.

4.5.2 CHEMICAL ANALYSIS OF PIZZA BASE

The effect of supplementation of whey permeate as a source of lactose was studied. Whey permeate was added at three different levels for the preparation of dough for pizza base. Prepared pizza bases were analyzed for various chemical parameters such as total solids, pH, acid insoluble ash and volume/mass ratio and the findings are presented in Table 6. It was noted that there was no significant difference ~~was observed~~ between market sample and bases prepared by NCBY in terms of chemical attributes.

The pH of the pizza base prepared from NCBY supplemented with whey permeate (sample A, B, D) ranged between 5.3-5.5 whereas the market pizza base sample (sample C) had a pH of 5.6. Similarly, the total solids content of pizza bases prepared using NCBY ranged between 51 –56% while it was 53% in market sample. Volume / mass ratio was of value 2.7-2.8 for pizza base samples (A, B, D) against 2.9 for market sample (sample C). Acid insoluble ash was absent in all the samples.

These results indicate that pizza base prepared by NCBY had chemical quality at par to that of market sample.

4.6 SENSORY EVALUATION OF BREAD AND PIZZA BASE

The bread and pizza base prepared were subjected to sensory evaluation by a panel of 11 experts on 9 point Hedonic scale. Statistical analysis was performed on the data obtained from sensory evaluation.

The bread was evaluated for various attributes viz. color of crust, symmetry of loaf, texture, color of crumb, flavour and overall score and compared with market sample. Statistical analysis was done on the data obtained. Average score with standard deviation for the bread from NCBY and market sample is given in the Table 7 for different characteristics evaluated. Critical difference was observed in the scores of experimental bread and market sample. However, on comparison with market sample bread, average overall score of bread for various characteristics showed that bread was liked moderately.

The three different samples of pizza base prepared were also subjected to sensory evaluation on 9 point Hedonic scale(Table 8). The data obtained on various attributes (flavour, base symmetry, color, texture) was analyzed statistically using same technique as used for bread.

Among pizza bases prepared by supplementing different levels of whey permeate using NCBY, no significant difference was observed in various parameters. This indicated that all the bases were of equally good quality. Although, a significant difference was noticed between the market sample and pizza bases prepared using NCBY, these pizza bases were liked moderately by judges.

Lactose has been implicated in the desirable crust colour, texture and flavor of bread (Holmes and Lopez, 1977; Vetter, 1984). In a study by Ogunriola (1992), hydrolyzed lactose syrup (HLS) at different levels was added to bread formulation.. 50% HLS added bread scored highest score. This higher score could be attributed to added lactose. It was concluded from the study that lactose in the permeate should not be completely hydrolyzed so as to derive benefits directly related to lactose in the bread system.

Table 7 : Sensory evaluation of bread

Parameters	Average Score ± std dev.					
	Color of crust	Symmetry of loaf	Texture	Color of crumb	Flavour	Overall score
Market sample	8.2±0.31	8.3±0.43	8.3±0.33	8.3±0.37	8.3±0.41	8.4±0.40
Bread from NCBY	7.4±0.36	7.1±0.42	6.9±0.41	7.1±0.33	7.0±0.35	7.2±0.32
Critical difference	0.378	0.367	0.78	0.457	0.434	0.432

Table 8 : Sensory evaluation of pizza base

Parameters	Flavour	Base symmetry	Colour	Texture	Overall score
Sample A [1]	7.2±0.38	7.3±0.31	7.2±0.35	7.0±0.31	7.3±0.35
Sample B [2]	6.9±0.33	7.1±0.44	7.1±0.37	7.1±0.41	7.1±0.43
Sample C [3]	7.5±0.34	7.7±0.38	7.8±0.40	7.7±0.40	7.9±0.37
Sample D [4]	7.0±0.41	6.9±0.40	7.0±0.31	6.9±0.39	7.1±0.35
Critical difference	0.364	0.4504	0.387	0.386	0.386

[1] sample A, One-third of the water replaced with whey permeate and prepared using NCBY;

[2] sample B, Half of the water replaced with whey permeate and prepared using NCBY

[3] sample C, Market sample (control)

[4] sample D, All water was replaced with whey permeate and prepared using whey permeate

Chapter – V

Summary & Conclusions

5. SUMMARY AND CONCLUSIONS

The present investigation was taken to explore the feasibility of using membranes to harvest the cells from the fermented whey broth. Keeping in view the ability of lactose fermenting yeast to leaven the dough in preparing bread, a strain of lactose fermenting yeast (NCBY) was selected for bread and pizza base making. Sensory evaluation and chemical analysis of finished products was carried out under the present study.

5.1 Amongst the chosen lactose fermenting yeasts, *K. marxianus* (NCDC 39, NCDC 41, NCDC 46) and *K. fragilis* 2C1, *K. fragilis* 2C1 was selected on the basis of higher dough leavening activity of 117% for further studies.

5.2 For harvesting of cells from the fermented broth, two types of membranes viz. microfiltration (MF) and ultrafiltration (UF) were used. Nearly 10 times concentration of the cells could be achieved. The concentration factor in case of UF was slightly higher than the MF. However, certain contaminants present in fermented whey broth could also be removed to some extent in the permeate using MF.

5.3 In view of total solids content, viable cell count and dough raising capacity obtained in the yeast biomass by membrane processing, the yeast biomass can also be used as yeast cream in large scale production of bread.

5.4 Storage of compressed form of NCBY at high temperature ($30^{o}C$) proved more deleterious on the leavening activity as compared to NCBY stored at $7^{o}C$. The stored yeast showed desirable activity even after 2 weeks of storage at $7^{o}C$.

5.5 Chemical analysis of bread revealed that the product meets the standards specified by BIS. Pizza base were compared with market sample and were found to be close to market sample.

5.6 Sensory evaluation by experts adjudged the products (bread and pizza bases) to be of moderate liking. No significant difference was observed between the pizza bases products prepared using different levels of whey permeate.

It can be concluded from the present study that application of membranes for separating cells from fermented whey broth could be a promising technology. Using centrifuges (2 stage) a total solids content of around 18% is achieved in industrial preparation of compressed form of baker's yeast. By applying membrane technology, the processes could be made continuous and concentration close to that obtained by centrifugation can be achieved.

Although, the bread prepared using whey permeate had significant difference in sensory attributes compared to market sample, there is scope for improvement in the basal formulation of bread. Scaling up of the production of this bread at industrial scale and addition of improvers could make this product even better. Similarly, there is a scope to improve the formulations of pizza bases prepared by using NCBY.

Thus, this study envisages the scope for further improving the quality of bread and pizza bases prepared from NCBY. Further, it also opens a new avenue for whey utilization at large scale in the production of NCBY.

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