BIOTECHNOLOGICAL APPROACHES FOR THE PREPARATION OF FOS BASED PREBIOTIC AND PROBIOTIC FOODS

A thesis submitted to the
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In fulfilment of the requirements for the award of
DOCTOR OF PHILOSOPHY in BIOTECHNOLOGY
by

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DECLARATION

I, Ms. Renuka B, certify that this thesis is the result of research work done by me under the supervision of **Dr. (Mrs) S. G. Prapulla**, at the Department of Fermentation Technology and Bioengineering, Central Food Technological Research Institute, Mysore. I am submitting this thesis for possible award of the degree of Doctor of Philosophy (Ph.D.) in Biotechnology from the University of Mysore, Mysore.

I further certify that this thesis or any part of it has not been submitted for award of any other degree/diploma of this or any other University.

Signature of Doctoral candidate
Date:

Signature of Guide
(S.G.Prapulla)
Date:

Counter signed by

Signature of Chairperson/Head of Department/
Institution with name and official seal

Dr. (Mrs.) S. G. Prapulla
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Fermentation Technology & Bioengineering
September, 2011

CERTIFICATE

This is to certify that the thesis entitled **"Biotechnological approaches for the preparation of FOS based prebiotic and probiotic foods"** submitted by **Ms. Renuka B** for the award of the degree of **Doctor of Philosophy (Ph.D.)** in **Biotechnology** to the **University of Mysore** is the result of research work carried out by her under my guidance in the Department of Fermentation Technology & Bioengineering, Central Food Technological Research Institute, Mysore- 570 020, India during the period 2005-2011.

S. G. Prapulla
(Guide)

Dr. (Mrs.) S. G. Prapulla
Senior Principal Scientist

Fermentation Technology & Bioengineering
September, 2011

Attendance Certificate

This is to certify that the thesis entitled **"Biotechnological approaches for the preparation of FOS based prebiotic and probiotic foods"** submitted by **Ms. Renuka. B,** for the award of the degree of **Doctor of Philosophy (Ph.D.)** in **Biotechnology** to the **University of Mysore** is the result of research work carried out by her under my guidance in the Department of Fermentation Technology & Bioengineering, Central Food Technological Research Institute, Mysore- 570 020, India. She has attended her duties during the tenure from May 2005 till date.

S. G. Prapulla

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ACRONYMS AND ABBREVIATIONS

Acronyms and abbreviations

%	percentage
~	approximately
<	less than
≤	less than or equal
>	greater than
≥	greater than or equal
°C	degree Celsius
µL	microlitre
µM	micromolar
µm	micrometer
µmol	micromoles
µs	microseconds
A°	Armstrong
ANOVA	Analysis of Variance
AOAC	The Association of Official Analytical Chemists
APEDA	Agricultural and Processed Food Products Export Development Authority
ATCC	American Type Culture Collection
a_w	water activity
bar	unit of pressure
BATH	bacterial adhesion to hydrocarbons
B_{ki}	linear regression coefficient
B_{kii}	quadratic regression coefficient
B_{kij}	cross-product regression coefficient
bp	base pair
$CaCl_2$	calcium chloride
CAGR	compound annual growth rate
CCRD	central composite rotatable design
cDNA	complementary DNA
CF	culture filtrate
CFTRI	Central Food Technological Research Institute
cfu or CFU	colony forming units
cm	centimeter
Co.	company
dATP	deoxyadenosine triphosphate
DBT	Department of Biotechnology
DFS	dynamic frequency sweep
DSS	dynamic strain sweep
DH5α	commonly used *E.coli* strain in transformation experiments
DNA	deoxyribo nucleic acid
dNTP	deoxyribonucleotide triphosphate
DVS	Direct Vat Set
e.g.	*exempli gratia*: Latin expression meaning “for the sake of example”
EC	Enzyme Commission
Eco RI	an endonuclease enzyme commonly used for restriction digestion
EDTA	ethylene diamine tetra acetic acid
et al.	Latin expression for “and others”
EU	European Union

FAO	Food and Agricultural Organization
FDA	Food and Drug Administration
FDI	Foreign Direct Investment
FOS	fructooligosaccharides
FOSHU	Foods for Specific Health Uses
FTase	Fructosyl transferase
g	gram
G*	complex modulus
g/L	gram/litre
G’	storage modulus
G”	loss modulus
GDP	Gross Domestic Product
GI	gastro-intestinal
GOS	galactooligosaccharides
GRAS	Generally Regarded As Safe
h	hour
H %	per cent cell surface hydrophobicity
H_2O_2	hydrogen peroxide
H_2SO_4	sulfuric acid
HACCP	hazard analysis and critical control point
HCl	hydrochloric acid
HDM	hydrodynamic mechanism
HELP	high electric field pulse
HFCS	high fructose corn syrup
HP	high pressure
HPLC	high performance liquid chromatography
Hunter L, a, b	Hunter values representing brightness, redness and yellowness respectively
Hz	Hertz
ICPS	International Committee on Systematics of Prokaryotes
IMARC	International Market Analysis Research and Consulting
IMFs	intermediate moisture foods
IMO	isomaltooligosaccharides
IMTECH	Institute of Microbial Technology
in vitro	Latin for “within the glass”
in vivo	Latin for "within the living"
IPTG	isopropyl-beta-thio galactopyranoside
kb	kilo base pairs
kcal	kilo calorie
KCl	potassium chloride
kDa	kilo Dalton
kg	kilogram
KH_2PO_4	potassium dihydrogen phosphate
L	litre
LAB	lactic acid bacteria
LB	Luria-Bertani Medium
log	logorithm
Ltd	limited
M	Molar
mA	milli Ampere

Mb	mega base pairs
MCA	McConkey agar
mg	milli gram
min	minutes
mL	milli litre
mm	milli meter
mM	milli molar
mm Hg	unit of pressure
MRS	de Man, Rogos and Sharpe
MTCC	Microbial Type Culture Collection and Gene Bank
N	normality or Newton
NA	nutrient agar
Na_2HPO_4	sodium biphosphate
NaCl	sodium chloride
$NaNO_3$	sodium nitrate
NaOH	sodium hydroxide
NCBI	National Center for Biotechnology Information
ND	Not detected
OD	optical density
OD	osmotic dehydration
ONPG	ortho- nitro phenol β-galactopyranoside
OQ	overall quality
OS	osmotic solution
P	probability value in statistics
Pa	Pascal
PCA	plate count agar
PCR	polymerase chain reaction
PDA	potato dextrose agar
PFGE	pulsed field gel electrophoresis
pGEM-T	a ready-to-use cloning vector
ppm	parts per million
PRESS	prediction error sum of squares
Pst I	a restriction endonuclease used for restriction digestion
PVOD	pulsed vacuum osmotic dehydration
QDA	quantitative descriptive analysis
RCT	randomized, controlled human trial
RID	refractive index detector
rpm	rotation per minutes
rRNA	ribosomal-ribo nucleic acid
RSM	response surface methodology
RT	retention time
s	seconds
SCFA	short chain fatty acids
SD	standard deviation
SEM	scanning electron microscopy
SG	solute gain
SGJ	synthetic gastric juice
spp.	species
TA	titratable acidity

TAE buffer	tris-acetate-EDTA buffer
Taq polymerase	a thermostable DNA polymerase from *Thermus aquaticus*
TE buffer	tris-EDTA buffer
tonne	1000 kg
TPA	texture profile analysis
TSS	total soluble solids
U	enzyme units
USDA	United States Department of Agriculture
UTM	universal testing machine
UV	ultra violet
v/v	volume/volume
VI	vacuum impregnation
VOD	vacuum osmotic dehydration
VOS	vacuum osmotic solution
w/v	weight/volume
w/w	weight/weight
WHO	World Health Organization
wk	week
WL	water loss
WR	weight reduction
X-Gal	5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside
XOS	xylooligosaccharides

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Abstract

The global food landscape is rapidly evolving, as consumer needs, shift with changing lifestyles. With these changes, the market for processed or ready to eat products has grown. Enhancement of these products with functional food ingredients such as prebiotics and probiotics could meet the consumers' demand for foods with benefits beyond basic nutrition. In line with this trend, the focus of the research has been on the use of beverages, traditional sweets and most ideally the fruits and vegetables as a source for delivery of prebiotics and probiotics.

Fruits and vegetables were enriched with fructooligosaccharides (FOS), a low calorie prebiotic using vacuum osmotic dehydration (VOD) process. The final product contained 8.02-17.21 g/100 g FOS without affecting the sensory attributes. Processing conditions were optimized using statically designed experiments and under the optimized conditions, the uptake of FOS in banana was found to be 9.64 g/100g of fruit pieces. Fruit juice could be considered as a novel, appropriate medium for fortification with prebiotics/probiotics. Fruit juice beverages (pineapple, mango, and orange) were fortified with FOS and the physicochemical characteristics of the fortified juices during storage have been carried out. The FOS content of pineapple, mango and orange juices was 3.79, 3.45, and 3.62 g/100ml respectively.

Catering to the needs of the present day consumers' desire to have low calorific products, *gulab jamun*, a traditional Indian sweet with FOS was prepared. In addition synbiotic *shrikhand*, milk based sweet with FOS and probiotic *Enterococcus faecium* CFR 3002 was also prepared. Studies also lead to the successful enrichment of star fruits with FOS and *Enterococcus hirae* CFR3001 and with a reference culture *Lactobaccilus salivarius* CFR 2158, to have synbiotic functional star fruits. The synbiotic star fruits contained 17.80-20.24 g/100g FOS and 138-178x10^6cfu/g probiotics. The probiotics (*E. hirae* CFR3001 and *E. faecium* CFR 3002) used in the study were isolated from fruits and vegetables and were identified at species level based on the phenotypic characteristics and 16S rRNA gene sequences.

Sensorially acceptable, physicochemically characterized fruit juice beverages, *gulab jamun, shrikhand* and fruits and vegetables enriched with FOS and/or probiotics have been the outcome of the study paving way for commercialization.

SYNOPSIS

Ph.D. Synopsis

Title: Biotechnological approaches for the preparation of FOS based prebiotic and probiotic foods

Summary and highlights of the investigation:

The development of prebiotics and probiotics during the past decade has indicated a significant shift in the food industry towards the development of 'functional foods' that promote health beyond providing basic nutrition. Prebiotics are defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that can improve the host health. The term 'prebiotic' is used to describe a variety of oligosaccharides and other food ingredients that promote the growth of beneficial gut microbes. A number of carbohydrates such as fructooligosaccharides (FOS), galactooligosaccharides (GOS) and xylooligosaccharides (XOS) have emerged as new functional food ingredients. Among which, FOS have attracted special attention as prebiotic, because of their beneficial health effects in regulating mineral absorption, lipid metabolism and serum cholesterol. These oligosaccharides improve proliferation of beneficial microorganisms in human gut by preventing colonization of pathogenic microorganisms. They have great potential for improving the physicochemical properties of foods and also functions as dietary fiber. FOS are produced by transfructosylation of sucrose using Fructosyl Transferase (FTase - EC 2.4.1.9). The FOS syrup obtained is generally a mixture of glucose (14%), sucrose (30%), 1-kestose (35%), 1-Nystose (18%) and 1-Fructofuranosyl Nystose (3%).

The use of microorganisms for the preparation of fermented milk, cereal and legume based foods has been a traditional practice since prehistoric times. Majority of the traditional Indian fermented foods are due to the involvement of mixed cultures of yeast and lactic acid producing bacteria. A number of *Lactobacillus* spp. and *Bifidobacterium* spp. have been in use as desirable probiotic cultures, so as to act as beneficial food supplements and contribute to a balanced intestinal microflora for human wellness. Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. Probiotic bacteria generally belong to the genera

Lactobacillus and *Bifidobacterium*. Some of the species belonging to the genera *Lactococcus, Enterococcus, Saccharomyces* and *Propionibacterium* are also considered as probiotics. Food products containing both prebiotics and probiotics are generally termed synbiotics, are used in non dairy products such as cereal bars, fruit and berry juices and confectionary.

The consumption of fruits and vegetables has been known to be an integral part of the human diet. The important nutrition and economic values of both fresh fruits and vegetables are well recognized. They are the ideal carriers of vitamins, essential minerals, dietary fiber, and other bioactive substances. In addition to these components, they also provide fair amounts of carbohydrates, proteins, and calories. Fruits and vegetables are thus important for our daily nutrition. India ranks second in the world in production of fruits and vegetables, accounting roughly 12% and 13%, respectively, of total global production in which, a mere 2.2% of fruits and vegetables are processed. Currently, up to 23% of the most perishable fruits and vegetables are lost during their transport through agri-food chain due to spoilage, physiological decay, water loss, mechanical damage during harvesting, packaging and transporting. India has a strong and dynamic food processing sector. Food processing adds value and enhances shelf life of perishable agro-food products. There has been a positive growth in ready to serve beverages, fruit juices and pulps, processed fruit/vegetable products (dried/preserved) and dehydrated fruits and vegetables. The food processing sector is likely to be the driving force for Indian economy. Thus, there exists a great need for processing of fresh fruits and vegetables into suitable products with higher nutritional benefits, combined with improved storage characteristics.

Commercialization has been limited by traditional processing technologies such as canning, candying and other methods. Although the use of probiotics in food is largely restricted to refrigerated dairy products, technology is developing and there are now several ways in which probiotics can be added to a number of products. Scope exists for the development of newer technologies for expanding the use of both probiotics and prebiotics into new ranges of foods and beverages and this, in turn, could provide the industry, an opportunity for growth and adding value for the manufacturer and retailer and also better health for the consumer.

Among the processing technologies, vacuum osmotic dehydration (VOD) is used in food processing sector, for enhancing the functionality through the addition of prebiotics and probiotics to fresh/cut fruits and vegetables. VOD process not only enables the storage of fruits and vegetables for a longer period, but also preserves flavour, nutritional characteristics and prevents microbial spoilage.

The work presented in the thesis describes the enrichment of fruits and vegetables with prebiotic FOS using VOD, optimization of VOD process parameters in banana, fortification of fruit juice beverages and preparation of traditional *gulab jamun* with FOS, isolation and characterization of probiotics from fruits and vegetables, incorporation of these probiotics into star fruit and *shrikhand*, a milk based dessert, along with FOS. The physicochemical and sensory attributes of prebiotic/probiotic products are also discussed.

Objectives:

I. Isolation and selection of potent probiotic cultures from fruits and vegetables
II. Characterization of selected cultures for probiotic properties
III. Incorporation of FOS and selected probiotics to fruits, vegetables and traditional sweets
IV. Standardization of the vacuum osmotic treatment parameters
V. Product characterization and storage studies

The contents of the thesis have been organized and presented in 7 chapters.

Chapter: 1

Introduction

Importance of prebiotics and probiotics, biotechnological approaches, food processing, fruits and vegetables processing industry in India, post harvest losses and preservation of fruits and vegetables, vacuum osmotic dehydration, fruits and vegetables as matrices for the incorporation of prebiotics and probiotics are presented in this chapter. Scope of the investigation is presented at the end of the chapter.

Chapter: 2

Fructooligosaccharides (FOS): A novel osmotic agent for impregnation of fruits and vegetables

A detailed study on the vacuum osmotic dehydration (VOD) of fruits and vegetables enriched with FOS is presented in this chapter. Fruits and vegetables (apple- *Malus domestica*, banana- *Musa acuminata*, jack fruit- *Artocarpus heterophyllus*, papaya- *Carica papaya*, sapota- *Diospyros digyna*, star fruit-*Averrhoa carambola*, ash gourd- *Benincasa hispida*, pumpkin- *Cucurbita pepo*, and carrot- *Daucus carota*) pieces of known dimensions were impregnated with 70 °Brix FOS syrup in the ratio of 1:5 (w/v) for 60 min at room temperature (25±2 °C) under vacuum (650 mm Hg) followed by 30 min of restoration at atmospheric pressure. The impregnated samples were further dried at 40 °C for 24 h to get 10-30% moisture content with increased shelf stability. The FOS content was found to be 8.02-17.21 g/100g product. The moisture content of FOS enriched fruits and vegetables pieces was in the range of 10-28%, and *β*-carotene content was 2116-3428 µg/100g. Scanning electron micrographs of impregnated fruits and vegetables indicated uptake of FOS in the intercellular spaces. The study clearly indicated that colour, texture and sensory attributes of the final products were not affected by the incorporation of FOS and thus, prebiotic FOS can be used as a healthier alternative for the sugar solution normally used for the preparation of osmotically dehydrated fruits and vegetables.

Chapter: 3

Vacuum osmotic dehydration of banana using FOS: Optimization of process parameters using statistically designed experiments

An experiment to determine the optimum processing conditions that would result in maximum uptake of FOS in banana during VOD was designed using statistical software (Design Expert, version 8.0.3.1). The independent process variables for the VOD were vacuum pressure (250-650 mm Hg), temperature (30-50 ^{o}C), vacuum treatment time (15-55 min), and atmospheric restoration period (10-30 min). A Central Composite Rotatable Design (CCRD) with these four factors each at five different levels, including central and axial points was employed. Experiments were carried out using 70 ^{o}Brix FOS syrup and the fruit to syrup ratio of 1:5 (w/v) in a vacuum oven. With respect to the responses *viz.,* uptake of FOS and the texture (shear strength) of the product, second order polynomial models were developed using multiple linear regression analysis. Analysis of variance (ANOVA) was performed to check the adequacy and accuracy of the fitted models. The response surfaces and contour plots showing the interaction of process variables were constructed and optimized for the desirability function for maximum FOS and minimum shear strength. A vacuum pressure of 550 mm Hg, temperature of 45 ^{o}C, vacuum treatment time of 45 min and atmospheric restoration period of 16.44 min were found to be the optimum operating conditions. With these optimized condition, the uptake of FOS was found to be 9.64 g/100g with a shear strength value of 13.42 N.

Chapter: 4; Section: 4.1

Fructooligosaccharides fortified fruit juice beverages

An attempt has been made to fortify selected fruit juice beverages (pineapple, mango and orange juice) with FOS. It was found that sucrose which is usually used as a sweetener in fruit juice beverages can be partially substituted with FOS without affecting the quality. The fruit juice beverages were evaluated for changes in physicochemical and sensory attributes during 6 months storage period at ambient (25±2 °C) and refrigeration temperature (4±2 °C). The results indicated that pH, total soluble solids, titratable acidity, colour and viscosity did not change significantly (P>0.05) during storage. The initial

FOS contents of pineapple, mango and orange juices were 3.79, 3.45, and 3.62 g/100 ml respectively. With respect to FOS content, the fruit juice beverages stored at refrigeration temperature showed 2.00-2.39 g/100 ml FOS after six months of storage, while the FOS content being 2.69-3.32, 1.65-2.08 and 0.38-0.58 g/100 ml at the end of second, fourth and sixth month of storage at ambient temperature respectively. The sensory analysis showed that the beverages were acceptable up to 4 and 6 months storage at ambient and refrigeration temperature respectively. The present study has opened up a new avenue for value addition to highly popular beverage segment.

Chapter: 4; Section 4.2

Fructooligosaccharides based traditional Indian acid coagulated milk based sweet: *gulab jamun*

Gulab jamun, a popular milk based traditional Indian sweet, is prepared by deep-frying of balls of dough in oil, followed by dipping in sugar syrup. Suitability of FOS and FOS-sucrose blend over sucrose and its effects on colour, texture, sensory and microbiological attributes of *gulab jamun* were investigated. FOS and FOS-sucrose blend did not show any adverse effect on colour values and sensory scores of *gulab jamun*. However, the increase in hardness, adhesiveness and gumminess observed were correlated to an increase in compactness of microstructure of *gulab jamun* prepared with FOS or FOS-sucrose blend as indicated by scanning electron microscopy. FOS contents of *gulab jamun* sweetened with FOS and FOS-sucrose blend were 17.58 g/100g and 9.18 g/100g respectively. The amount of FOS slightly decreased during storage for 4 days at ambient temperature (25±2 °C). The study indicated that FOS syrup could be an effective healthier alternative to sucrose (either fully or partially) in the preparation of most popular Indian sweetmeat without many changes in the physicochemical characteristics and sensory attributes of the same. Panelists have rated the product as highly acceptable, indicating the suitability and also the acceptance of FOS for the preparation of *gulab jamun*. In addition, FOS sweetened *gulab jamun* will have an added advantage over that of sucrose based *gulab jamun* from the point of low calorie and other well known health benefits such as improved mineral absorption, non cariogenicity etc.

Chapter: 5

Isolation and characterization of potent probiotics from fruits and vegetables

Lactic acid bacteria (LAB) are industrially important organisms because of their fermentative ability as well as health and nutritional benefits. Moreover, they are generally regarded as safe (GRAS) for incorporation into food products. Searching for desirable LAB isolates for the food industry, isolation of microorganisms from traditional fermented products (cereals, vegetables, milk and meat) and characterization of physiological properties are a constant effort of scientific communities around the world. In view of this, the study was carried out to isolate, characterize, and identify LAB isolates from fruits and vegetables. Selection criteria employed included the ability of isolates to withstand environmental conditions such as low pH, high bile salt concentration, and cell surface hydrophobicity, which are the fundamental characteristics of LAB isolates used as probiotics. The selected LAB isolates were able to survive (>80% and 70%) and at low pH (2.5) and relatively high bile (1.0%) concentration. These isolates showed significant antimicrobial activity against food borne pathogens and antibiogram towards a range of antibiotics. Out of 17 LAB isolates, 2 isolates (Cu8 and R32) were found to be potent probiotics based on the certain probiotic properties and were assigned to the genera *Enterococcus* on the basis of morphological, biochemical, physiological characteristics and 16S rRNA gene sequence analysis. Identification of the selected isolates was performed by partial sequencing of approximately 700 bp of 16S rDNA gene and gene fragment obtained from representative of each groups were aligned with all sequences present in the GenBank database. The two isolates were identified as *Enterococcus hirae* and *Enterococcus faecium* and the strain numbers have been assigned as CFR 3001 and CFR 3002 respectively by culture collection centre of CFTRI, Mysore, Karnataka, India.

Chapter: 6; Section: 6.1

Synbiotic functional star fruits with fructooligosaccharides and selected probiotics

An attempt was made to prepare star fruit enriched with prebiotics and probiotics to have a synbiotic product. Star fruits slices were impregnated with 70 °Brix FOS (1:5 w/v) and 1% probiotic cells (v/v) *viz., Lactobacillus salivarius* CFR 2158, a reference

culture and *Enterococcus hirae* CFR 3001, an isolate from cucumber. The moisture content, water activity, colour, texture, FOS content, viability of probiotic cells over a period of 6 months of storage at ambient temperature (25±2 °C) were carried out and scanning electron microscopic observations before and after VOD were also conducted on the initial day. Prebiotic (FOS) and synbiotic (*L. salivarius* CFR 2158, *E. hirae* CFR 3001) enriched star fruit slices showed 19.26, 20.24, and 17.80 g of FOS/100g product (initial day) respectively. The FOS content was decreased to 16.66, 18.62 and 16.09 g/100g at the end of the storage period (6 months). The viable cell count of *L. salivarius* CFR 2158 and *E. hirae* CFR 3001 was found to be $178x10^6$ cfu/g and $138x10^6$ cfu/g respectively in synbiotic star fruits up to 4 months of storage at ambient temperature (25±2 ^oC). There was slight decrease in the survivability ($34x10^6$ cfu/g and $26x10^6$ cfu/g) of probiotics (*L. salivarius* CFR 2158 and *E. hirae* CFR 3001 respectively) at the end of the storage period (6 months). The final product exhibited good synbiotic properties along with desirable textural and sensory attributes. The level of probiotic microorganisms found in prepared products falls well within the recommended range of 10^6 cfu/g probiotics in food at the time of consumption. The results indicated that the *E. hirae* CFR 3001, when compared with *L. salivarius* CFR 2158 did not show any significant difference in the overall quality of the product and it can be successfully incorporated into any fruits/vegetables without affecting quality attributes of the same. The FOS along with desired levels of probiotics in the final product did not impart any off flavour/taste and retained fresh appearance, soft texture as well as low water activity with a resultant long shelf life. These synbiotic products are expected to have higher market potential and with increasing mass market coupled with the health benefits will ultimately drive their markets.

Chapter: 6.2

***Shrikhand:* A fermented milk based dessert enriched with fructooligosaccharides and probiotic *Enterococcus faecium* CFR 3002**

The manufacture of indigenous dairy products with low calorie prebiotic and potent probiotic will provide a successful outlet for traditional milk products, and this will provide an alternate variety to the health conscious consumers. An attempt has been

made to explore the effect of replacement of sucrose with FOS at 100% and 50% levels (50% FOS and 50% sucrose) on the physicochemical, microbiological and sensory characteristics of *shrikhand* enriched with probiotic *E. faecium* CFR 3002. The *shrikhand* enriched with FOS and blend of FOS and sucrose showed initial FOS content of 10.32 g/100g and 5.55 g/100g respectively; however it decreased to 6.16 g/100g and 1.76 g/100g during storage period of 60 days at 4±2^{o}C. The *shrikhand* showed similar values for moisture content and a_w and indicated similar trends with slight decrease in viable probiotic count. The results also indicated that the probiotics cultures remained viable (>140x10^{6} cfu/100g) during 60 days of storage which is satisfactory for the *shrikhand* to be claimed as a probiotic product.

The results indicated that the FOS can be an effective and healthier replacement to sucrose, either fully or partially for the preparation of many food items without any changes in the physicochemical characteristics and sensory attributes of the same. These prebiotic oligosaccharides have been attracting global interest due to its prebiotic properties and exhibit its properties in the final product.

Chapter: 7

Summary and conclusion

Summary, conclusion and future perspectives are given in this chapter. The thesis ends with a list of references arranged in alphabetical order.

Overall, the investigation focused on the evaluation of FOS as a novel osmotic agent for impregnation of fruits and vegetables using VOD process, followed by the optimization of process parameters using statistically designed experiments for banana. Studies are also made on the preparation and characterization of FOS based fruit juice beverages and *gulab jamun*, isolation and characterization of potent probiotic cultures from fruits and vegetables and their use in the preparation of synbiotic star fruits and *shrikhand*.

CHAPTER: 1

INTRODUCTION

1.1. Introduction

The primary role of a diet is not only to provide enough nutrients to fulfill metabolic requirements of the body but also to modulate its various functions. In recent years, a considerable growing consumer's inclination towards healthful foods has been noticed throughout the world including India. In this context, several studies to obtain foods or beverages from fruits and vegetables have been carried out. Biotechnology is driving much change, in areas as diverse as agrichemicals, plant breeding and food processing. Recent years have witnessed dramatical changes and improvements in the processing operations. Novel processing techniques are being developed. The biotechnological production of food products has been used by human beings since pre-history for the preparation of fermented beverages, breads etc. However, it was only in the last decades of 20^{th} century that the use of this technique was extended to the manufacturing of ingredients, *e.g.* flavors and oligosaccharides. An increase in the production of processed fruit and vegetable products (dried pulps, jam, and beverages), potential perspectives of control and/or treatment and extraction processes of its compounds, concomitant with the development of the processing industries, have been noticed *(Giovani et al., 2009).* In view of this, the application of biotechnology in food processing industries can make a significant difference. The 'biotechnology' concept promotes the exploitation of valuable traits for the design of more technologically robust and effective microbes with a special emphasis on probiotic lactic cultures with potentially improved biotechnological applications, paving way for the development of novel prebiotic, probiotic and synbiotic enriched fruits and vegetables and other products.

1.2. Status of food processing industry in India

The world population is estimated to reach nine billion by 2050. The Food and Agriculture Organization (FAO) estimates that, global food production needs to be increased by 70% by 2050 compared to an average 2005-07 levels to feed the rising global population. Clearly, a large part of the consumption will happen in India and China, which would require an additional 1.6 billion hectares of land to be brought into cultivation compared to the current 1.4 billion hectares being cultivated presently. This

increase in cultivable land looks improbable *(Hindu Business Line, 3 April 2011)* and thus, the demand for food would be increasing dramatically.

Currently, India is the second largest producer of food in the world and the food processing industry is one of the largest industries in India. In terms of production, consumption, export and expected growth, India is ranked fifth in the world. India's food industry is valued at US$ 180 billion of which, the food processing industry is estimated at US$ 67 billion, according to Klynveld Peat Marwick Goerdeler *(*KPMG) report on 'Food Processing and Agri Business' *(KPMG, 2007)*. The highest share of processed food is in the dairy sector, where 37% of the total produce is processed, of this only 15% is processed by the organized sector. Value addition of food products is expected to increase from the current 8% to 35% by the end of 2025. Fruit and vegetable processing, which is currently around 2% of total production in India, is very low as compared to the western countries. Taking market forces such as rising income level and changing consumer behavior due to rapid economic growth into consideration, the Agricultural and Processed Food Products Export Development Authority (*APEDA*) under the Ministry of commerce and industry, Government of India envisages a growth rate of 10% by 2015 and to 25% by 2025.

Food processing includes different methods used to transform raw ingredients into food for human consumption. The food processing industry in the country is set to ensure profitability in the coming decades. The sector is expected to attract phenomenal investments of about Rs 1,400 billion in the next decade. A Foreign Direct Investment (FDI) inflow to food processing industries has set a target of US$ 25.07 billion to be achieved by 2015. The food processing sector attracted US$ 130 million of FDI in the first eight months of the fiscal year as compared to total FDI of US$ 1.2 billion. A dominant segment of the food industry, food processing is estimated to be worth US$ 70 billion with a 32% share. It comprises agriculture, horticulture, animal husbandry, and plantation. Experts estimate the industry Grass Domestic Product (GDP) at 6-8% with value addition of food products to increase from 8% to 35% by the end of 2025. To realize India's potential in this industry, the Government has set an investment target of US$ 25.07 billion by 2015 to double India's share in global food trade from 1.6% to 3%,

increase processing of perishable food from 6% to 20% and value addition from 20% to 35%.

1.3. Status of fruits and vegetable processing industry in India

India ranks second in the global production of fruits and vegetables, next to China, and has the potential of being the biggest with the food and agricultural sector. China, India, and Brazil account for almost 30% of the world's fruit supply and most of this production is destined for domestic consumption. Fruits and vegetables are important to our health for many reasons; they are a source of fibre and are rich in vitamins, minerals, an array of phytonutrients, all of which work together either to improve our health or even prevent disease. Healthy diets, rich in fruits and vegetables may reduce the risk of many chronic diseases *(Paolo Boffetta, et al., 2010).* Most fruits and vegetables are naturally low in fat and calories and are filling.

Disaggregated data on exports of fruits and vegetables for the period 2009-2010 was Rs.5963.10 crore as against Rs.5110.61 crore for the period 2008-2009, showed 16.68% increase in export of fruits and vegetables *(India 2011- A reference Manual, 2011)*. Over the last few years, there has been a positive growth in ready-to-serve beverages, fruit juices, pulps, dehydrated and frozen fruits and vegetable products, tomato products, pickles, convenience vegspice pastes, processed mushrooms and curried vegetables etc. The domestic consumption of value added fruits and vegetable products is low compared to the primary processed food in general and fresh fruits and vegetables in particular, which is attributed to higher evidence of tax and duties including that on packaging material, lower capacity utilization, non-adoption of cost effective technology, high cost of finance, infrastructural constraints, inadequate farmers-processors linkage leading to dependence upon intermediaries. The smallness of units and their inability for market promotion are also reasons for inadequate expansion of the domestic market. In order to give fresh impetus to processing of fruits and vegetables, the Government of India in 2004-2005 had allowed 100% deduction of profit for first five years for new upcoming fruits and vegetable processing units. During 2008-2009, the Ministry of Food Processing Industries had released financial assistance of Rs. 15.84 crores to 93 fruits and

vegetables processing units in the form of 1/2 installments *(India 2011- A reference Manual, 2011).*

1.4. Post-harvest losses and preservation of fruits and vegetables

At present, the country produces 12% and 13% of world production of fruits and vegetables, respectively, whereas it shares only 1.38% in the global market, which is much less compared to the present day demand. Several efforts are being made worldwide to improve the yield/production, however post harvest loss can be a serious threat. The major losses are attributed to physiological, mechanical damage and microbial spoilage *(Flores, 2000).* The main reason for loss of fruits and vegetable is the improper storage after plucking. Most post-harvest losses in developing countries occur during transport, handling, storage, and processing. It is well established that processing treatments for fruits and vegetables that are designed to preserve them in various forms (fresh, frozen, pasteurized or dried), have an effect on organoleptic qualities such as texture, colour or flavour. Many fruits and vegetables can be processed into canned products that cater to local taste. The processed fruits and vegetables can be stored for a long time and are easy to carry and convenient to serve and they are the most seasonal crops with higher demand at present. Now, seasonality of fruits and vegetables are becoming less obvious, which is closely related with the general application of preservative technology. One of the technologies contributing to the preservation of the original properties of fruit or vegetables is 'vacuum technology', which is also called 'vacuum infusion' or 'vacuum impregnation or 'vacuum osmotic dehydration'.

1.5. Vacuum osmotic dehydration (VOD)

Increased interest in osmotic treatment stems primarily from the need to make improvements in the quality of food products. Osmotic dehydration (OD) is a widely used water removal process, wherein, fruits or vegetables are added to a hypertonic solution. Three kinds of osmotic treatments have been defined, depending on the pressure applied on the system: osmotic dehydration at atmospheric pressure (OD), osmotic dehydration at vacuum pressure or vacuum osmotic dehydration (VOD), and pulsed vacuum osmotic dehydration (PVOD) *(Fito et al., 1994).* The combination of osmotic

dehydration by vacuum impregnation (VI) could be a good choice in the development of new food products in a minimally processed way.

VOD promotes water release from a cellular material immersed in a concentrated solution, while a simultaneous external solute uptake takes place. Mass transfer occurs during this operation through different mechanisms to a different extent depending on process variables *(Andres et al., 2001)*. The action of the different mechanisms, balanced by controlling those variables, makes it possible to achieve a specific dewatering solute uptake ratio, in the final product. Mechanisms involved in mass transfer during osmotic dehydration of cellular tissues depend on the structure of the tissue (*Chiralt and Fito, 2003*). The external broken cells can be easily impregnated by the external solution, and in the intercellular spaces, bulk flow of solution, water and solute diffusion occurs. The bulk flow is promoted due to capillary pressure in processes carried out at atmospheric pressure. Nevertheless, when vacuum is applied to the system, capillary impregnation is promoted and when the atmospheric pressure is restored, pores are extensively flooded with the external solution and depending on the applied compression ratio. Mass transport in the intercellular spaces is mainly responsible for solute gain (SG). At cellular level, cell wall and membranes act as non-selective and selective barriers respectively to mass transport and the transmembrane flux is responsible for most of the cell-to-cell water transport during osmotic dehydration of tissues.

1.5.1. Mechanism of VOD

VOD leads to some advantages as compared with atmospheric dehydration. The schematic representation of VOD is depicted in Fig 1.1. The influence of vacuum treatment is very important on the kinetics of the mass transfer phenomena, especially concerning water loss (WL) and weight reduction (WR) of food during osmotic treatment. This effect of vacuum application cannot be explained only on the basis of diffusional and osmotic transport mechanisms. Therefore, a hydrodynamic mechanism (HDM) has been proposed and experimentally analyzed. Taking this new mechanism into account, a more accurate approach for the modeling of the VOD operation may be done. VOD of a porous product consists of exchanging the internal gas or liquid occluded in

open pores for an external liquid phase by the action of HDM promoted by pressure changes *(Fito, 1994; Fito and Pastor, 1994)*.

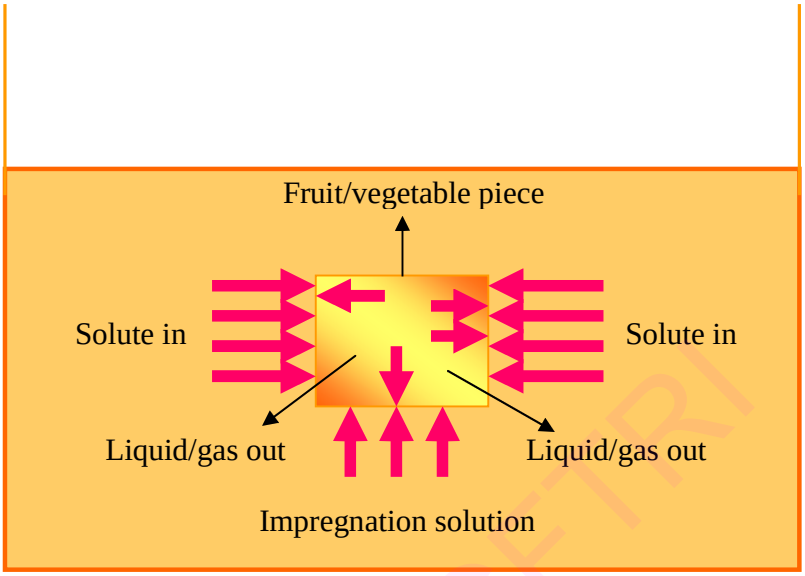

Fig 1.1 Schematic representation of VOD process

VOD is carried out in two steps after fruit/vegetable pieces immersed in a tank containing the liquid phase. In the first step, vacuum pressure is imposed on the system for a time sufficient for it to be de-aired, thus promoting the expansion and outflow of internal gas in the fruit/vegetable. Gas release takes the product pore native liquid with it. In the second step, atmospheric pressure is restored, while the product remains immersed for some time, and thus the subsequent influx of external liquid into the porous structure *(Fito et al., 2001a)*.

Many applications of VOD have been reported *(Fito et al., 2001b; Torreggiani and Bertolo, 2001; Rastogi and Raghavarao, 1996; Shi et al., 1996; Torreggiani, 1995; Fito et al., 1994; Shi and Fito, 1993).* According to Fito (1994), the main advantage of VOD over OD at atmospheric pressure lies in the increased mass transfer due to the HDM and to the corresponding increment produced in the solid-liquid interfacial surface, leading to a significant reduction in processing time. The most important HDM effect is very rapid and occurs just when atmospheric pressure is restored.

VOD can be useful in introducing dissolved or dispersed substances directly into the porous structure of the food matrix. The incorporation of functional ingredients using vacuum during osmotic process is possible to enrich the fruits and vegetables. This process is being investigated to incorporate physiological active compounds (minerals, probiotics, vitamins and firming agents such as hydrocolloids) into the structure of fruits and vegetables (*Fito et al., 2001; Fito and Chiralt, 2000*). Impregnated products can be commercialized as minimally processed fresh functional foods or can be dried osmotically or by air in order to obtain more stability (*Fito et al., 2001*).

1.5.2. VOD of fruits and vegetables

The VOD has long been used to prolong the post harvest conservation of many products: lemons *(Valero et al., 1998a; Valero et al., 1998b)*, Avocados *(Wills and Sirivatanapa, 1988)*, tomatoes (*Wills and Tirmazi, 1979)*, strawberries *(Ponnappa et al., 1993)*. The compounds used in the impregnation solution to impregnate above fruits are usually calcium salts (mostly calcium chloride) and many plant hormones (polyamines). Mujica-Paz *et al.,* (2003b) studied the impregnation properties of mango, apple, papaya, and peach at vacuum pressure. It was observed that the vacuum pressure and its application time had an important effect on the impregnation of isotonic solution in the studied fruits.

The effect of vacuum on the OD was studied by few researchers *(Giraldo et al., 2006; Barat et al., 2001; Fito et al., 2001)*. Fito *et al.,* (2001) studied the VI and OD in matrix engineering for the enrichment of fruits and vegetables (egg plant fruit and oranges) with minerals, vitamins or other physiologically active components to develop functional foods. The application of vacuum has shown beneficial effects on the structural changes of product and mass transfer kinetics leading to shorter processing times. Giraldo *et al.,* (2006) studied the osmotic dehydration process of mango fruit. The process was carried out under vacuum and also at atmospheric pressure. The greatest SG with the more heavily diluted solution was observed under vacuum. Mujica-Paz *et al.,* (2003a) reported the effect of vacuum and syrup concentration on osmotic dehydration of apple, mango and melon.

1.5.3. Osmotic solutions for fruits and vegetable processing

One of the key factors in any type of osmotic treatment is the selection of osmotic solution (OS). Three types of solutions are usually used in osmotic operations: (1) isotonic, a solution containing the same solute concentration both outside and inside the cell membrane, (2) hypotonic, a solution containing less solute molecules outside of the cell membrane than inside of it, and (3) hypertonic, a solution containing more solute molecules outside of the cell membrane than inside.

The selection of vacuum osmotic solution (VOS) should also take into consideration of the following factors: non-toxicity, good sensory characteristics, high solubility, and low cost. In general, any soluble solute or solvent that is miscible can be used as a VOS. These include starch syrup, glycerol, ethanol, polyols, lactose maltodextrin, trehalose, L-lysine, casein, monosodium glutamate, and combination of these solutes, such as glucose with sucrose, glycerol with sucrose, and sucrose with salt etc *(Ferrando and Spiess, 2001; Barbosa-Canovas and Vega-Mereado, 1996; Argaiz et al., 1994; Biswal and Maguer, 1989; Garrote and Bertone, 1989; Giangiacomo et al., 1987; Lerici et al., 1985; Hawkes and Flink, 1978; Hoover and Miller, 1975).*

Osmotic process is also affected by the physicochemical properties of the solutes employed, because differences in efficiency of dehydration arise mainly from differences in molecular weight, ionic state, and solubility of solute in water. For instance, using higher molecular weight sugars (*i.e.* lower dextrose equivalent corn syrup solids), it was possible to zero net SG *(Lazarides and Mavroudis, 1995)* and allows only migration of moisture. Other studies have confirmed that glucose resulted in higher amounts of WL and SG than sucrose *(Panagiotou et al., 2007; Garrote and Bertone, 1989; Lerici et al., 1985; Bolin et al., 1983).* Lenart and Flink, (1984) found that mixed sucrose/salt solutions gave a greater decrease in product water activity than pure sucrose solutions, although water transport rates were similar.

In most cases, low molecular weight carbohydrates are used for VOD process of fruits and vegetables because low molecular weight solutes quickly penetrate the samples: the smaller the molecular weight, the faster the diffusion (Stockes-Einstein Law). A mixture of dextrose and sucrose was found to provide the highest diffusivity of water as the dextrose concentration increased in the mixed solution. Thus, low molecular

weight solutes favour the impregnation process, whereas high molecular weight solute is helpful for the dewatering effect *(Kaymak-Ertekin and Sultanoglu, 2000)*. The diffusivity of sucrose is smaller than that of glucose because the molecular weight of glucose is about one-half of the molecular weight of sucrose *(USDA, 2003; Garrote and Bertone, 1989).* High fructose corn syrup (HFCS) solution had a diffusion coefficient 32% higher than that of a sucrose solution due to the smaller molecular dimension of the monosaccharide. Thus, fruits impregnation by HFCS had lower water activity than those treated, at the same operation conditions, with a sucrose solution because of the faster penetration rate of HFCS *(Bolin et al., 1983).* VOD in HFCS also resulted in a lower WL and a higher SG than those in the maltodextrin syrup *(Mastrocola et al., 1987)*.

Some of the OS may impart flavour of impregnated products. Corn syrups impart their characteristic flavour to delicately flavored products. Sensory study indicated that HFCS dehydrated fruit is sweeter than that treated with sucrose solution. While dextrose is a more effective osmotic agent than sucrose because of its high dehydration rate *(Kaymak-Ertekin and Sultanoglu, 2000),* sucrose solution was found to be slightly better than a glucose solution with respect to discoloration and sugar gain in a strawberry product *(Yang and Maguer, 1992)*. Sucrose, corn syrup, and concentrated fruit juices have been most commonly used in fruit VI *(Fito et al., 2000).*

Many studies have used blends of sucrose and salt in fruit and vegetable processing to obtain a maximum WL with low SG *(Sereno et al., 2001; Qi et al., 1999; Biswal and Bozorgmehr, 1992; Giangiacomo et al., 1987; Lenart and Flink, 1984; Islam and Flink, 1982)*. It was found that adding a small quantity of sodium chloride to sucrose solution tremendously increased the dewatering rate in fruits *(Sereno et al., 2001; Biswal and Bozorgmehr, 1992)*. The interaction between sucrose and salt was also found to limit the salt residue in the fruit samples. Because of its lower molecular weight, a small incremental increase in the sodium chloride concentration leads to significant change in osmotic pressure, whereas the same incremental increase in the sucrose concentration (higher molecular weight) does not. This means that diffusion coefficients are more sensitive to changes in sodium chloride concentration than in sucrose concentration *(Ade-Omowaye et al., 2002)*. A high level of sugar can reduce the taste threshold for salt. Conversely, salt can enhance the sweetness of sucrose *(Sacchetti et al., 2001)*. In general,

low salt concentrations should be used in fruit processing to avoid a significant decrease in organoleptic quality.

The usage of vacuum to develop nutritionally enriched products is relatively new in comparison with its other applications. Fito *et al.* (2001a) first evaluated the feasibility of using VI for mineral fortification of fruits and vegetables from an engineering point of view. Mathematical models were developed to determine the concentration of different minerals in VOS required to achieve a 20-25% dietary reference intake fortification in 200g of samples. Following the modeling prediction, experimental validation confirmed that VI could be an effective method for the enrichment of fruits and vegetables with minerals, vitamins or other physiologically active components. Betoret *et al.*, (2003) studied probiotic-enriched dried fruits, either with commercial apple juice containing *Saccharomyces cerevisiae*, or with whole milk or apple juice containing 10^7 or 10^8 cfu/ml of *Lactobacillus casei* (spp. *rhamnosus*) using VI technique. It was reported that dried apple samples could contain about 10^6 cfu/g *Lactobacillus casei* (spp. rhamnosus), a similar level to that in commercial dairy products.

Betalleluz-Pallardel *et al.*, (2010) reported that apple fruit can be fortified with FOS BeneoTM P95-ORAFTI (oligofructose $\geq$ 93.2% with some traces of glucose, fructose and sucrose $\leq$ 6.8%) by incorporating into the cellular structure of the apple fruit by means of VI. Aniko-Matusek *et al.*, (2008) studied the comparison of diffusion of FOS during vacuum impregnation and osmotic dehydration of apple cubes. The results indicated that the advantage of the VI rests in a higher SG of the raw material if the structure is porous, but does not speed up the diffusion, and the effective diffusion coefficient does not increase after vacuum treatment. There were no significant differences among the FOS components during OD and VI operation.

1.5.4. Factors affecting VOD

The chemical potential of the hypertonic solution is the driving force of the diffusion. The rate of the mass transport depends on many factors such as type of pre-treatments (*i.e.* conventional blanching, microwave, vacuum, high electric field pulse (HELP) and high pressure (HP) treatment), the conditions of the osmotic treatment (time, temperature, use of agitation, vacuum, ultrasound), the concentration and the quality of

the osmotic agent, use of combined solutions with salt *(Sereno et al., 2001)*, the solution-to-food ratio and the tissue structure of the food *(Fito et al., 2001; Rastogi et al., 2000; Rastogi and Raghavarao, 1994; Torreggiani, 1993).*

Three important phenomena are coupled in VOD process. They are gas out flow, deformation-relaxation of the solid matrix and liquid influx. The VOD process and the quality of finished products are determined by processing condition, including pretreatment of the samples, temperature, pressure and immersion time under vacuum, time to restore atmospheric pressure, composition and concentration of the osmotic solution, geometry of the food pieces, agitation, and solution to sample ratio. Their effects on mass transfer rate and composition of final product have been studied in several fruits, including apple *(Sereno et al., 2001; Kaymak-Ertekin and Sultanoglu, 2000; Barat et al., 1998)*, mango, kiwi *(Leunda et al., 2000)*, and banana *(Sousa et al., 1998)*. Lenart and Lewicki (1990) and Rastogi and Raghavarao (1996) found that the rate of mass transfer increased to a certain extent with an increase in concentration and temperature of the osmotic solution, above which undesirable changes in flavor, texture and color occurred.

Yang and Maguer (1992) reported that the stabilization or decrease in mass transfer occurred when the solution concentration reached 50-60%. Barat *et al.*, (2001) observed different results in apple slices subjected to VI treatment with 25-65% sucrose at 30, 40 or 50 ^{o}C, where concentration of osmotic solution did not show a significant effect on the effective diffusivity. It was explained that diffusion appeared to be hindered by unspecified active transport. Moreira and Sereno (2003), further investigated the effects of temperature, concentration, and flow rate of solution on osmotic dehydration/impregnation rate during immersion of apple cylinders in sugar solutions at $\leq$ 25 ^{o}C, and suggested that the sample SG is controlled by diffusion inside the material while WL is governed by mixed internal external flow. The influence of vacuum time and solution concentration on mass transfer and mechanical properties of osmodehydrated melon cubes has been studied (*Cristhiane et al., 2011*). Pulsed vacuum osmotic dehydration (PVOD) was carried out at 30 °C for 4 h, using sucrose solutions (40, 50 or 60 °Brix) and applying a vacuum pulse (100 mbar for 5, 10 or 15 min).

Samples subjected to PVOD using 60 °Brix sucrose solution presented greater water loss, lower sugar uptake and better maintenance of fresh fruit texture throughout the process.

The effects of temperature on mass transfer kinetics can be well predicted by the Arrhenius equation. High temperature also speeds up the osmotic process, but may cause negative effects on color, texture, and flavor of samples. Optimal temperature depends on the type of the raw materials used, the type of finished product, and the speed of processing. The ratio of OS to product is an important parameter. The optimum value is usually determined by two factors: stability of the solution during processing, and the economics of transport and recycling of the solution. The high ratio increases cost and necessitates solution recycling. The effect of agitating the solution on VI processing has been investigated *(Mavroudis et al., 1998; Garrote et al., 1992; Bongirwar and Sreenivasan, 1977)*. It is clear that agitation affects WL and SG in impregnation processing *(Peanagiotou et al., 1998)*. In some cases, the advantages of agitation do not justify the cost.

Mujica-Paz *et al.,* (2002) evaluated the effect of vacuum pressure (135-674 mbar) and its application time (3-45 min) on the volume of isotonic solution impregnated in slices of mango, apple, papaya, banana, peach, and melon, and reported that vacuum pressure and time had a significant effect on the volume in all fruit slices. Mujica-Paz *et al.,* (2003) further investigated the combined effects of vacuum level (135-674 mbar) and concentration of osmotic solution (41-60 ^{o}Brix) on dehydration parameters of apple, mango, and melon. They found that the lowest final water activity level was achieved with a vacuum pressure of 674 mbar and 50 ^{o}Brix syrup in apple and 593 mbar and 57 ^{o}Brix in melon. Mujica-Paz *et al.,* (2002) upon evaluating the effect of vacuum time (3-45 min) on the volume of isotonic solution impregnated into slices of mango, apple, papaya, banana, peach, and melon. It was showed that impregnation depends significantly on the vacuum treatment time.

1.5.5. Limitations of VOD and osmotic solution

The major constraint for the industrial adoption of osmotic dehydration is the cost of osmotic solution that necessitates a proper means of its recycling. During osmotic dehydration, some of the characteristics of the solutions gets changed at the end of

process due to simultaneous leaching of color, acids, flavour and fragments from the product and the solutes in the solutions penetrating into the product, the extent of which depends upon the type of the food. To make the process feasible, the solutions are usually reconcentrated before reuse by heating or filtrating *(Valdez-Fragoso and Mujica-Paz, 2002)*. However, the reconstruction process may change the properties of the solutions. For example, heating may darken the color of the solutions, as well as generating off flavor volatiles. Care should be taken to minimize these, so that product quality is not affected by recycling the osmotic solution *(Aachary and Prapulla, 2009; Peiro-Mena et al., 2007)*.

The OS has to be concentrated in order to be recycled and this can be achieved by evaporation and/or by the addition of solute. To study the relation between water transport and membrane type, membranes of various densities, thickness and configurations were used *(Warczok et al., 2007)*. It appears that due to high solute concentration, microbial problems could be less severe in osmotic dehydration. Special attention is required for equipment design especially to handle fragile food material while having provision for automated operation control and online measurement facilities.

VOD process has been considered to improve product quality, modify product formulation, and save energy in some of the fruit and vegetable processing. By selecting appropriate process conditions, the specific application of VOD can be controlled and optimized. However, extensive studies are still required in order to fully taking advantage of its unique features and applying in large-scale industrial operations. One major issue in the large-scale industrial application of VI and any other osmotic processing is the management of the used osmotic solution at the end of the process. Potential applications of concentrated solutions include reusing as table syrups, fruit fillings, beverage bases, or syrups in canning process. From the engineering standpoint, it is possible to reuse the solution at least 20 cycles of the same recycled solutions *(Valdez-Fragoso et al., 2002; Rosa and Giroux, 2001)*. Valdez-Fragoso *et al.,* (2002) found that WL, SG, and color of dehydrated apple cubes obtained in osmotic dehydration process with reused osmotic solution were similar to those obtained with fresh osmotic solution.

Lack of knowledge relevant to the microbial safety of VI solutions and processed product is another critical aspect need to be investigated and also hinders the application

of VI technique. If raw materials are contaminated, it may contaminate VI solutions during VI processing. Further contamination would occur if contaminated solutions are to be reused.

1.6. Functional foods: Prebiotics and Probiotics

Increased consumer's interest in the health benefits of foods have led to the significant development of functional foods and nutraceuticals. Distinct changes in the understanding the role of foods in human health promotion have assumed greater importance within the last decade. The first frontier of scientific investigations has moved from the primary role of food as the source of energy and body-forming substances to the more subtle action of biologically active food components on human health. In the industrialized world, there has been an explosion of consumer interest in the active role of foods in the well-being and life prolongation as well as in the prevention of initiation, promotion and development of cancer, cardiovascular diseases and osteoporosis. As a result, the market for functional foods, or foods that promote health beyond providing basic nutrition, is flourishing *(Pisulewski and Kostogrys, 2003; Diplock et al., 1999; Berner and O'Donnell, 1998; Dimer and Gibson, 1998; Sanders, 1998).*

In Japan, regarded as the birthplace of functional food, these foods are known as "Foods for Specified Health Use" (FOSHU) and the market for these foods is significant. In total, more than 1700 functional food products have been launched in Japan between 1988 and 1998 with an estimated turnover of around 14 billion US$ in 1999 *(Menrad, 2003)*. The market was estimated to be 5 billion US$ in 2003 *(Side, 2006)* and 5.73 billion US$ in 2006, while more than 500 products were labelled as FOSHU in 2005 *(Fern, 2007)*.

The rise of functional foods has occurred at the convergence of several critical factors, such as: awareness of personal health deterioration, led by busy lifestyles with poor choices of convenience foods and insufficient exercise; increased incidence of self-medication; increased level of information from health authorities and media on nutrition and the link between diet and health; scientific developments in nutrition research; and a crowded and competitive food market, characterized by pressurized margins *(Siro et al., 2008)*. These factors have created a dynamic functional food and beverage market,

offering good prospects for growth for well-positioned food and drink manufacturers. In the year 2000, the world-wide market of functional foods generated US$ 33 billion, in 2005 this total was US$ 73.5 billion *(Justfood, 2006)*, and the market is estimated to reach US$ 167 billion after 2010, with a yearly growth potential of 10% *(Research and Markets, 2008)*. The functional foods comprise: (i) conventional foods containing naturally occurring bioactive substances (*e.g.* dietary fiber), (ii) foods enriched with bioactive substances (*e.g.* probiotics, antioxidants), and (iii) synthesized food ingredients introduced to traditional foods (*e.g.* prebiotics).

Among the functional food components, prebiotics and probiotics constitute a focus of contemporary science of human nutrition. Epidemiological studies and randomized clinical trials carried out in different countries have demonstrated or at least suggested numerous health effects related to prebiotics and probiotics consumption, such as reduction of cancer risk, improvement of heart health, stimulation of immune system, decrease of menopause symptoms, improvement of gastrointestinal health, maintenance of urinary tract health, anti-inflammatory effects, reduction of blood pressure, maintenance of vision, antibacterial and antiviral activities, reduction of osteoporosis and antiobese effects *(Grajek, 2005)*. Thus, production and marketing of prebiotics and probiotics is assuming greater challenges. In this context, biotechnology plays a key role in the functional food industry.

1.6.1. Prebiotics

A prebiotic was originally defined in 1995 as a "non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" *(Gibson and Roberfroid, 1995)*. A more recent definition states that "A prebiotic is a selectively fermented ingredient that allows specific changes; both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host wellbeing and health" *(Gibson et al., 2004)*. In other words, prebiotics are defined as non-digestible or low-digestible food ingredients that benefit the host organism by selectively stimulating the growth or activity of one or a limited number of probiotic bacteria in the colon *(Manning and Gibson, 2004; Dimer and Gibson, 1998; Crittenden and Playne, 1996).* These non-

digestible carbohydrates occur naturally in foods such as wheat, oats, bananas, asparagus, leeks, onion, garlic, chicory, and artichokes *(Van Loo et al., 1995)*. However, they are only present in small amounts. The most common prebiotic in the diet is the fibre found in fruits and vegetables. Although it is preferable to obtain prebiotics from natural sources, this may not always be practical.

Prebiotics can be incorporated into the diet through foods such as cereals and dairy products, which are supplemented with them**.** Some of the widely used prebiotics in use include inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), xylooligosaccharides (XOS), isomaltooligosaccharides (IMO) and lactulose. These substrates are fermented by particular members of the gut microbiota *(Lim, 2005)*. Prebiotics provide food for the growth of lactobacillus and bifidobacteria. The majority of studies have so far focused on inulin, FOS and GOS *(Macfarlane et al., 2008).* These saccharides have now a long history of safe use. The prebiotic potential of several other candidates has been investigated, although their classification as prebiotics is premature since the evidence at this stage is too limited. A list of these compounds is given in Table 1.1. Researchers distinguish between long chain, short chain, and full spectrum prebiotics. Inulin is a long chain prebiotic fiber. The long chain prebiotics contain 9-64 links per saccharide molecule and are digested more slowly, providing food for bacteria in the colon. Oligofructose is a short chain prebiotic, containing 2-8 links per saccharide molecule and are fermented considerably faster. A full spectrum prebiotic supplement would be something like oligofructose enriched inulin, which contains all possible saccharide links.

Table 1.1 Summary of candidate prebiotics still under investigation

Carbohydrate	**References**
Germinated barley	Kanauchi *et al.,* 1998a, 1998b, 2003
Oligodextrans	Olano-Martin *et al.*, 2000
Gluconic acid	Tsukahara *et al.*, 2002
Gentio-oligosaccharides	Rycroft *et al.,* 2001
Pectic oligosaccharides	Olano-Martin *et al.*, 2002
Mannan oligosaccharides	White *et al.,* 2002
Lactose	Szilagyi, 2002
Glutamine and hemicellulose-rich substrates	Bamba *et al.*, 2002
Resistant starch and its derivatives	Lehmann *et al.*, 2002; Silvi *et al.*, 1999; Wang *et al.*, 2002
Oligosaccharides from melibiose	Van Laere *et al.*, 1999
N-Acetylchito-oligosaccharides	Chen *et al.,* 2002
Sugar alcohols	Piva *et al.,* 1996

Source: Food Science and Technology Bulletin: Functional Foods, 2005

Recently, the World Health Organization issued a report, which advises a reduction in the overall consumption of sugars and other rapidly absorbable carbohydrates, along with an increase of daily physical activity. This recommendation was made with the expectation that this may help reduce the dramatic increase in the incidence of obesity, type 2 diabetes and related health care costs. Recently, there has been a lot of attention paid to oligosaccharides and in particular FOS present in diet. FOS has emerged as one of the important prebiotic in the functional food market. Lot of research work has been carried out on production of FOS using microbial Fructosyl Transferase (FTase) *(Sangeetha et al., 2003)*. Due to its innumerable beneficial properties to human health, FOS will continue to lead the functional food market. Since FOS is not hydrolyzed by the human digestive enzymes, it undergoes fermentation in the colon and encourages the growth of beneficial bacteria in the colon. This in turn discourages the

growth of potentially putrefactive microorganisms in the colon resulting in a healthy gut environment. FOS has been demonstrated as an effective prebiotic through both *in vivo* and *in vitro* assessments. Durieux *et al.,* (2001) have investigated the prebiotic effect of FOS by studying the metabolism of two types of chicory FOS (Fibruline Instant and Fibrulose F 97) by *Bifidobacterium longum*, *B. infantis* and *B. angulatum*.

Although FOS is present in trace amounts in natural foods, its use in different fruit and vegetable based products is an efficient way to enrich human diet. Proper choice of food vehicle and processing methods ensures its stability and bioactivity. In view of the great demand for FOS as food ingredients, scope exists for the incorporation into fruits, vegetables, beverages, confectioneries, and dairy products and this will be a continuous programme for identifying newer and novel sources. Examples of the use of FOS in food products include the following:

1. Light jam products: FOS can be used as the sole sweetening agent and gives 34% calorie reduction compared with sucrose standard. Organoleptic characteristics of the products are claimed to be very similar with the test sample, having a lower sweetness and a softer texture.
2. Ice cream: FOS can be used with inulin to replace all the sugar and reduce the fat content and give excellent mouth feel characteristics.
3. Confectionery: Hard candies, gums, and marshmallows can be made while achieving significantly reduced energy values *(Murphy, 2001)*.
4. Fruit juice beverages: FOS can be used to fortify selected fruit juice beverages to replace sucrose either fully or partially without affecting the sensory and other properties *(Renuka et al., 2009)*
5. Gulab jamun: An Indian traditional sweetmeat ‘gulab jamun’ can be enriched with prebiotic FOS *(Renuka et al., 2010)*.

1.6.1.1. Criteria for classification of a food ingredient as a prebiotic

It is necessary to establish clear criteria for classifying a food ingredient as a prebiotic. Indeed such classification requires a scientific demonstration that the ingredient (1) resists gastric acidity, (2) is not hydrolyzed by mammalian enzymes, (3) is not absorbed in the upper gastro-intestinal (GI) tract, (4) is fermented by intestinal

microflora, and (5) selective stimulation of growth and/or activity of intestinal bacteria potentially associated with health and well-being. These requirements have been classified as the three prebiotic criteria *(Gibson and Roberfroid, 1995).*

1. Resistance to digestive processes in the upper part of the GI tract.
2. Fermentation by intestinal microbiota.
3. Selective stimulation of growth and/or activity of a limited number of the health-promoting bacteria in that microbiota.

1.6.1.2. Ease of use of prebiotics

Unlike probiotics, prebiotics are non-viable and reach the colon intact and these properties make prebiotics more attractive. Their food manufacturing applications in the form of thickening agents or sweeteners make them more amenable to industrial processes. Prebiotics have a longer shelf-life and can be incorporated into a large variety of food, such as infant formulae, weaning food, cereals, confectionery, beverages, dairy products, dietary supplements etc.

1.6.1.3. Limitations of prebiotics

Possible side effects may be encountered when using prebiotics in excess and it results in an increased volume of fermentation end products. An increase in stool frequency and stool weight is often reported in human feeding trials *(Chen et al., 2001).* Consumption of a large dose (>20 g/day) of prebiotics such as inulin or lactulose may lead to a laxative effect *(Bouhnik et al., 1999).* It is critical that newly developed products are selective towards non-gas producer bacteria, as gas distension may discourage the intake of prebiotics.

1.6.1.4. Dose effects of prebiotics

Optimum doses of prebiotics have been determined for common prebiotics such as fructooligosaccharides and transgalactooligosaccharides in various populations. Doses of fructooligosaccharides administered in feeding and clinical trials range from 3-20 g/day in adults and 0.4-3.0g/day in infants *(Moore et al., 2003, Moro et al., 2002, Bouhnik et al., 1999).* These doses were fixed based on the amount of oligosaccharides

present in diet rich in vegetables *(van Loo et al., 1995).* A minimal intake of 4-10 g/day for induction of a bifidogenic effect is often suggested for prebiotics, but there is no recommended intake available.

1.6.1.5. Purity and safety of prebiotics

Due to limitations in the manufacturing process, current prebiotic preparations are generally mixtures of oligosaccharides of various chain lengths. The presence of mono- and disaccharides may hinder the specificity of the prebiotic. Chemical extraction of oligosaccharides from food may also result in undesirable color or flavor. To overcome these drawbacks, new enzymatic processes providing higher oligosaccharide selectivity and more palatable properties are developed *(Rastall and Gibson, 2002)*. The risk of bacteremia associated with prebiotics is almost negligible.

1.6.1.6. GRAS status and persistent effect of prebiotics

The recognition of GRAS status of existing prebiotics and/or their history of safe use is sufficient to guarantee their safety for public authorities, the users and the consumers *(Pascal, 2008).* The persistence of prebiotic effects when their intake is stopped has not been well established. In many feeding studies, colonic microbial changes are observed after treatment with prebiotics, but the effect generally ceased with the interruption of treatment *(Tuohy et al., 2002, Gionchetti et al., 2000).* Long-term daily intake of prebiotics seems to be necessary to achieve optimum efficiency.

1.6.1.7. Potential food applications of prebiotics

Due to the difficulties of characterizing the colonic microflora at the species level, virtually all of the data on prebiotic properties of oligosaccharides are on microflora changes at the genus level. It may, however, be desirable to develop prebiotics which are targeted at particular species of *Bifidobacterium* and *Lactobacillus*. As prebiotics exploit the use as non-viable dietary components to improve gut health, the range of foods into which they can be added is much wider than that for probiotics, where culture viability needs to be maintained. Potential applications for prebiotics as food ingredients to improve the gastrointestinal health of the consumer are beverages and fermented milks,

health drinks, bakery products, table spreads, sauces, infant formulae and weaning foods, cereals, biscuits, confectionery, cakes, desserts, snack bars, soups, salad dressings and dairy products.

It has long been thought that the gut flora of the breast-fed infant is dominated by bifidobacteria and that this is not the case for formula-fed infants *(Benno et al., 1984).* This is seen to be one reason for the improved resistance of the breast-fed infants to infections. If prebiotics could be developed with particular selectivity towards those bifidobacteria that are present in the guts of breast-fed infants, a new range of synbiotic formula foods could be envisaged. Above the age of about 55-60 years, faecal bifidobacterial counts have been shown to markedly decrease compared to those of younger people *(Kleessen et al., 1997; Mitsuoka, 1990).* This decrease in bifidobacteria is a cause for concern as the natural elderly gut flora may have become compromised through reduced bifidobacterial numbers, resulting in a diminished ability to resist colonization with invading pathogens. Prebiotics may be potentially utilized as a dietary intervention in the attempt to restore the microflora balance of the gut in the elderly population concurrently with indirectly providing antipathogenic protection.

1.6.1.8. Guidelines for the evaluation and substantiation of prebiotics

The following guidelines (Fig 1.2) have been given by FAO for the evaluation and substantiation of prebiotics *(FAO, 2007)*.

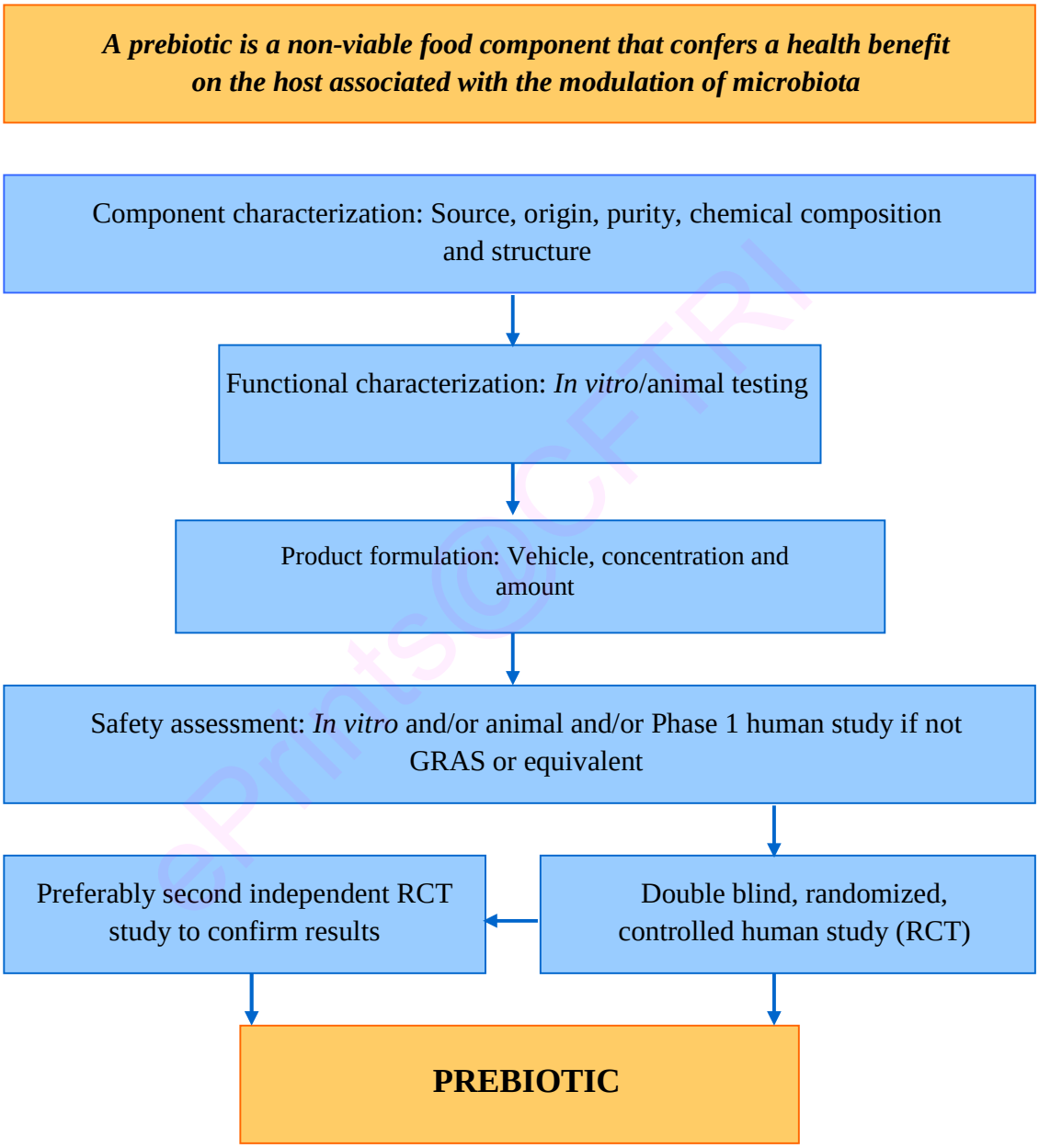

Fig 1.2 FAO guidelines for the evaluation and substantiation of prebiotics

Source: Modified based on FAO, 2007

1.6.1.9. Future prebiotic research areas

It is recognized that there are numerous potential new applications being considered for prebiotic use *e.g.* prevention and/or management of type 2 diabetes mellitus; drug bioavailability; effects on autoimmune diseases and allergy; modulation of pathogenic biofilms. There is a need for more randomized, placebo controlled clinical trials with adequate statistical power. It is recognized that prebiotics may be used in conjunction with probiotics; this is considered a synbiotic. Depending on the nature of the two components, the net effect may not be synergistic.

Research is being carried out to establish how effective prebiotic ingredients substances are in different food applications. There has been a rapid increase in the number of companies marketing such ingredients on the basis that a good balance of gut flora protects the digestive system and bowel against various cancers and other illnesses. As prebiotics are closely associated with products containing some form of fibre, product usage tends to be wide bread and baked products, breakfast cereals (such as Kelloggs Rice Krispies Multi-Grain), baby foods, dairy products and even soft drinks and pet food. However, these products are less well established than probiotics and tend to be confined to niche sectors of more established markets.

1.6.1.10. Prebiotic market

As with probiotics, the global market for prebiotics is showing a healthy growth in demand and there appears to be good scientific evidence to underpin health claims, which should further sustain growth in the future. RTS Resource Ltd calculates that the strict market for added prebiotic ingredients in functional foods, as defined, in the EU, USA and Asia, totals some 25,000 tonnes, which is forecast to rise in volume by more than 6% per year *(RTS Resource Ltd., 2008)*. Growth could occur at an even higher rate than our predictions if the industry develops more new products and markets them successfully, given legislative restrictions.

The market for prebiotics is growing rapidly from a small base. Prebiotics are mainly associated with breakfast cereals, baked goods, cereal bars and baby foods, as well as some dairy products. The probiotics market has become better established, based primarily on the launch of special yogurt and fermented milk drinks. Synbiotics are a

mixture of prebiotics and probiotics. The theory being that synbiotics load the colon with good bacteria whilst ensuring there is a plentiful supply of the right food on which to thrive. This also seems to be a rapidly growing market (Table 1.2).

Both prebiotics and probiotics represent different but potentially exciting parts of the market for healthy food and drink. All the signs are that consumers will continue to favour products with specific health benefits and this factor should ensure that these sectors continue to enjoy above average growth. However, as can be seen in the history of the development of probiotic dairy drinks, the consumer must feel both comfortable and somewhat knowledgeable about the products and their benefits before markets can develop. Perhaps the most significant barrier to future growth, therefore, is legislation. New EU legislation is likely to curb the use of specific health claims that lack the scientific evidence to back them up.

Table 1.2 Information on suppliers of probiotics and prebiotics

Company	Description	URL
BioGaia	*L. reuteri* culture comes in three different, producer-friendly forms: freeze-dried powder, freeze- dried DVS (Direct Vat Set) granules, and frozen pellets	www.biogaia.com
Bio K +	Producer and seller of probiotic mix including *L. acidophilus* and *L. casei*	www.biokplus.com
Chr. Hansen	The "nu-trish" brand probiotic culture range consists of Probio-Tec, Yo-Fast, and other nu-trish culture blends with a well-defined viscosity profile that ferment quickly	www.chr-hansen.com
Cerbios-Pharma	Producer of *Enterococcus* LAB SF 68	www.cerbios.ch
Danisco	The company supplies probiotic cultures for foods and supplements, as well as natural food protectants	www.danisco.com
Danone	Producer of several brands of fermented dairy products containing probiotics	www.danone.com
DSM	The Lafti line of probiotics is formulated for stability, survivability, and concentration, and includes *L. acidophilus* (Lafti L10), *L. casei* (Lafti L26), and *Bifidobacterium* (Lafti B94)	www.dsm.com
GTC Nutrition	NutraFlora short-chain fructo-oligosaccharides (scFOS) are a cane sugar or beet sugar–derived natural prebiotic fiber	www.gtcnutrition.com
Lallemand	The company delivers probiotics and bio-supplements to the nutraceuticals, functional-foods, and pharmaceuticals industries	www.lallemand.com

Continuation of **Table 1.2** Information on suppliers of probiotics and prebiotics

Company	Description	URL
National Starch	The Hi-Maize brand corn-based resistant starch has multiple benefits, including acting as a prebiotic for digestive health	www.hi-maize.com
Orafti	BeneoSynergy1 is the unique, patented oligofructose-enriched inulin prebiotic used in the landmark SynCan project on synbiotics and colon cancer	www.orafti.com
Proctor & Gamble	"Align" is a probiotic supplement contain *B. infantis* 35624	www.alignGi.com
Sanofi-Aventis	Producer of *Bacillus clausii* strains O/C, NR, SIN, and T, marketed in Europe, Asia, and South America as Enterogermina	www.sanofi-aventis.com
Sensus	Frutafit inulin and Frutalose fructooligosaccharides are soluble dietary fibers with bifidogenic/prebiotic properties, suitable for a variety of food systems to enrich fiber, reduce calories, and replace sugars and fats	www.sensus.us
Solvay	Producer of lactulose (Duphalac) for treatment of constipation and hepatic encephalopathy	www.solvay.com
Valio	The *Lactobacillus rhamnosus* GG probiotic is the most researched in the world and was recently licensed to Dannon for the U.S. yogurt market. The Gefilus family containing LGG is marketed worldwide	www.valio.fi
VSL Pharmaceuticals	VSL#3 is a mixture of eight strains with 450 billion live bacteria per packet	w ww.vsl3.com
Winclove	The company sells mixtures of probiotic strains for different indications	www.winclove.com

1.6.2. Probiotics

The definition of the term probiotic has evolved through the years. Probiotics are live microorganisms administered in amounts that positively affect the health of the host *(Sanders, 2003; FAO/WHO, 2002).* The first recorded probiotic was fermented milk for human consumption. After that, probiotics became popular with animal nutrition. The role of fermented milk in human diet was known even in vedic times. The natural microbiota of human intestine consists of more than 400 different strains of bacteria, numbering about 100 billion. Majority ($\geq$95%) of this microbial population inhabits the healthy colon.

Probiotic microorganisms majorly belong to the genera, *Lactococcus, Lactobacillus, Bifidobacterium, Saccharomyces, Bacillus, Enterococcus, Pediococcus, Leuconostoc (Prado et al., 2008; Shah, 2007; Isolauri et al., 2004)*. Probiotics of the *Lactobacillus* genus accounted for the largest share, representing 61.9% of total sales in 2007. Lactic acid bacteria (LAB) constitute a diverse group of organisms providing considerable benefits to humankind. LAB occur either as natural inhabitants of the intestinal tract or as fermentative LAB used in food industry, imparting flavor, texture and possessing preservative properties. Beyond these, some species are administered to humans as live microbial supplements, which positively influence our health mainly by improving the composition of intestinal microbiota. *Lactobacillus acidophilus, L. casei, L. lactis, L. helviticus, L. salivarius, L. plantarum, L. bulgaricus, L. rhamnosus, L. johnsonii, L. reuteri, L. fermentum, L. delbrueckii, Streptococcus thermophilus, Enterococcus faecium, E. faecalis, Bifidobacterium bifidum, B. breve, B. longum* and *Saccharomyces boulardii* are the commonly used probiotics *(Gibson et al., 1999).*

Microorganisms must fulfill a number of criteria related to safety, functional effects and technological properties to achieve a probiotic status *(FAO/WHO, 2001)*. From the safety point of view, the probiotic microorganisms should not be pathogenic, have no connection with diarrhoeagenic bacteria and no ability to transfer antibiotic resistance genes, as well as be able to maintain genetic stability. To be recognized as functional food components, they should demonstrate the following properties: acid and bile stability, resistance to digestive enzymes, adhesion to intestinal surface, antagonistic activity against human pathogens, anti-carcinogenic and anti-mutagenic activity,

cholesterol-lowering effects, stimulation of the immune system without inflammatory effects, enhancement of bowel motility, maintenance of mucosal integrity, improvement of bioavailability of food compounds and production of vitamins and enzymes *(Ouwehand et al., 1999)*. Probiotics must have good technological properties so that it can be manufactured and incorporated into food products without loosing viability and functionality or creating unpleasant flavors or textures.

1.6.2.1. Probiotic products

The global market for probiotic ingredients, supplements, and foods was worth $14.9 billion in 2007 and reached US$16 billion in 2008. Estimates target a total of US$19.6 billion on sales in 2013 at a compound annual growth rate (CAGR) of 4.3%. Probiotics are available in a variety of food products and supplements. A probiotic may be made out of a single bacterial strain *(Gilliland and Speck, 1977)* or it may be a consortium as well. Probiotics can be in powder form, liquid form, gel, paste, granules or available in the form of capsules, sachets, etc. Food products containing probiotics are in general, almost exclusively dairy products; milk and yogurt - due to the historical association of LAB with fermented milk. A total of 78% of current probiotic sales in the world today are delivered through yogurt. A spectrum of probiotics is reported in probiotic products available in market (Table 1.3).

Table 1.3 Examples of probiotic strains in products

Strain (alternative designations)	**Product Name**	**Manufacturer**
Bifidobacterium animalis DN 173 010	Activia	Danone/Dannon
Bifidobacterium breve Yakult	Bifiene	Yakult
Bifidobacterium infantis 35624	Align	Procter & Gamble
Bifidobacterium lactis HN019 (DR10)	Howaru Bifido	Danisco
Enterococcus LAB SF 68	Bioflorin	Cerbios-Pharma
Escherichia coli Nissle 1917	Mutaflor	Ardeypharm
Lactobacillus casei DN-114 001	Actimel, DanActive	Danone/Dannon
Lactobacillus casei F19	Cultura	Arla Foods
Lactobacillus casei Shirota	Yakult	Yakult
Lactobacillus johnsonii La1 (Lj1)	LC1	Nestlé
Lactobacillus plantarum 299V	GoodBelly, ProViva	NextFoods Probi
Lactobacillus reuteri ATTC 55730	Reuteri	BioGaia Biologics
Lactobacillus rhamnosus ATCC 53013 (LGG)	Vifit and others	Valio
Lactobacillus rhamnosus LB21	Verum	Norrmejerier
Lactobacillus acidophilus CL1285 & *Lactobacillus casei* Lbc80r	Bio K+	Bio K+ International
Tested as mixture: *Lactobacillus rhamnosus* GR-1 & *Lactobacillus reuteri* RC-14	FemDophilus	Chr. Hansen

Fruit juices, desserts, and cereal-based products may be other suitable media for delivering probiotics *(Cargill, 2009)*. Indeed, technological advances have made possible to alter some structural characteristics of fruit and vegetables matrices by modifying food components in a controlled way such as pH modification, fortification of culture media, among others *(Betoret et al., 2003)*. This could make them ideal substrates for probiotics, since they already contain beneficial nutrients, such as minerals, vitamins, dietary fibres, and antioxidants, while lacking the dairy allergens that might prevent consumption by certain segments of the population *(Sheehan et al., 2007)*.

1.6.2.2. Status of probiotics in India

In India, probiotics are often used as animal feed supplements for cattle, poultry and piggery. This requirement is also met by importing probiotics from other countries. It is rarely used for human beings - Sporolac, *Saccharomyces boulardii* and yogurt (*L. bulgaricus* + *L. thermophilus*) are the most common ones. Sporolac is manufactured using *Sporolactobacilli. Lactobacilli* solution is an example of a probiotic, usually given to pediatric patients in India. The latest and recent addition to the list of probiotics in India is ViBact (which is made up of genetically modified *Bacillus mesentricus*), which acts as an alternate to B-complex capsules. In India, only sporulating lactobacilli are produced and they are promoted with some of the antibiotic preparations. Probiotics should not be considered a panacea for health, but can be incorporated into a balanced and varied diet to maximize good health. In general, the country is bound to become a fertile seedbed for all types of probiotic products in the foreseeable future.

1.6.2.3. Indian Council of Medical Research (ICMR) guidelines for probiotics

International guidelines *(Reid, 2001; Sanders and Huisint Veld, 1999)* on probiotics in food broadly specify the kind of tests that may be required to determine the safety and to assess the health claim of a probiotic product in food. The regulatory mechanism for probiotics differs from country to country and also even within a country *(Patel et al., 2008).* Therefore, a holistic approach is needed for formulating guidelines and regulations for evaluating the safety and efficacy of probiotics in India which should be in consonance with current international standards. Indian Council of Medical

Research (ICMR) along with the Department of Biotechnology (DBT) has formulated guidelines for evaluation of probiotics in food in India.

Keeping in view the above, a task force was constituted by ICMR, comprising of experts from varied fields to develop guidelines for evaluation of probiotics in food in India. The task force took into consideration the guidelines available in different parts of the world *(EFFCA, 2008; Food and Drug Administration, 2006; FAO/WHO, 2002)* and deliberated on the various aspects to be covered *(FAO/WHO, 2001).* The guidelines will help the regulatory authority for evaluating probiotic products in India. The parameters include identification of stain, *in vitro* screening for probiotic characteristics, animal studies to establish safety and *in vivo* animal and human studies to establish efficacy. It also includes requirements for labeling of the probiotic products. The following guidelines set forth by ICMR are meant to be followed for a strain or food to be termed as 'probiotic' for marketing in India.

1.6.2.3.1. Genus, species and strain identification

Effects of probiotics are strain specific. Strain identity is important to link a strain to a specific health effect as well as to enable accurate surveillance and epidemiological studies. Both phenotypic and genotypic tests should be done using validated standard methodology. Nomenclature of the bacteria must conform to the current, scientifically recognized names as per the International Committee on Systematics of Prokaryotes (ICPS). The current molecular techniques used for identification include PCR based techniques; 16S rRNA sequencing and DNA finger printing techniques like ribotyping and Pulsed Field Gel Electrophoresis (PFGE). It is recommended that probiotic strains in use in India should be deposited in internationally recognized culture collection/repositories.

1.6.2.3.2. *In vitro* tests to screen potential probiotic strains

The following *in vitro* tests with standard methodology are recommended for screening putative probiotic strains: Resistance to gastric acidity, bile acid resistance, antimicrobial activity against potentially pathogenic bacteria, ability to reduce pathogen adhesion to surfaces and bile salt hydrolase activity. These tests are based on the hostile

gut environment which they mimic under *in vitro* conditions. The cultures evaluated as probiotics based on these tests should be subjected to preclinical validation in appropriate animal models before clinical trials are conducted in human subjects.

1.6.2.3.3. *In vivo* safety and efficacy studies in animal models

Assessment of the acute, subacute and chronic toxicity of ingestion of extremely large amounts of probiotics should be carried out for all potential strains. Such assessment may not be necessary for strains with established documented use. To substantiate *in vitro* effects, appropriate, validated animal models must be used first, prior to human trials.

1.6.2.3.4. Evaluation of safety and efficacy of probiotics for human use

In recognition of the importance of assuring safety, even among group of bacteria that are Generally Recognized as Safe (GRAS), probiotics strains needs to be characterized at a minimum with the following tests: (1) Determination of antibiotic resistance patterns, (2) assessment of undesirable side-effects, (3) if the strain under evaluation belongs to a species that is a known mammalian toxin producer or of hemolytic potential, it must be tested for toxin production and hemolytic activity respectively, and (4) assessment of lack of infectivity by a probiotics strain in immune compromised individuals would be an added measure.

The principal outcome of efficacy studies on probiotics should be proven with similar benefits in human trials, such as statistically and clinically significant improvement in condition, symptoms, signs, well-being or quality of life, reduced risk of disease or longer time to next occurrence or faster recovery from illness. Each of the parameter should have proven correlation with the probiotics tested. Probiotics delivered in food may not be tested in Phase 3 studies (effectiveness), unless the product makes a specific health claim wherein it becomes imperative to generate the required evidence necessitating carrying out Phase 3 studies. If a probiotic food has a record of documented long and safe use outside the country, the data regarding this could be reviewed and deemed as sufficient to allow its marketing within the country.

1.6.2.3.5. Effective dosage of probiotic strains, labeling requirements and manufacturing and handling procedures

The minimal effective dose or the level of viable cells of the probiotic strain in terms of cfu/ml/day in the carrier food that demonstrates general health promoting functions or well being or specific health claims in target population should be clearly indicated. In addition to the general labeling requirements under the food laws, the following information should also be mentioned on the label *(Saldanha, 2008)*: (1) Genus, species and strain designation following the standard international nomenclature, (2) the minimum viable numbers of each probiotic strain should be specified at the level at which efficacy is claimed and at the end of shelf-life, (3) evidence-based health claim(s) should be clearly stated, (4) the suggested serving size to deliver the minimum effective quantity of the probiotic related to the health claim, and (5) proper storage conditions to be mentioned. Adequate quality assurance programmes should be in place. Good manufacturing practices should be followed in the manufacture of probiotic foods. The codex general principles of food hygiene and guidelines for application of Hazard Analysis and Critical Control Point (HACCP) should be followed.

A very promising area in the development of enhanced functional food ingredients is the development of synbiotics. These are defined as a combination of a probiotic and a prebiotic *(Gibson and Roberfroid, 1995)* and there has been a lot of recent interest in the concept. There seems little doubt that synbiotics will result in elevated levels of bifidobacteria due to the prebiotic component. A comparative *in vitro* study *(Bielecka et al., 2002)* of several strains of *Bifidobacterium* and FOS and inulin concluded that strains of *B. longum, B. catenulatum* and *B. animalis* grew best on FOS with much lower growth rates seen on inulin. The selected synbiotics were then fed to rats and faces analyzed for bifidobacteria, coliforms and total cell counts. Rats that were fed FOS and the synbiotics displayed higher levels of bifidobacteria and lower coliform levels. The synbiotic preparation was, however, no more effective than the prebiotic. The ability of FOS to promote survival of the particular species of *Bifidobacterium* was not evaluated; however, FOS has been reported to increase bile resistance in bifidobacteria *(Perrin et al., 2000). L. reuteri* has been investigated as a component in a synbiotic with soygerm powder *(de Boever et al., 2001).* Soygerm powder contains a few prebiotic

oligosaccharides studied by others *(Rycroft et al., 2001)*, but it is also a source of isoflavones, which are believed to be protective against some forms of estrogen-related cancer, osteoporosis and cardiovascular conditions. Soygerm powder (4 g/l) increased resistance of *L. reuteri* to bile salts. In addition, the lactobacilli cleaved the isoflavone glycosides to liberate the aglycone isoflavone, increasing its bioavailability *(Izumi et al., 2000).*

One of the principal benefits of synbiotics is believed to be increased persistence of the probiotics in the GI tract. A synbiotic preparation of *L. acidophilus* and FOS has been studied in an *in vitro* model of the human gut *(Gmeiner et al., 2000).* The model used was the simulated human intestinal microbial ecosystem popularly known as SHIME reactor and the synbiotics resulted in higher levels of lactobacilli (an increase of 0.89 log) in the vessel corresponding to the ascending colon. An increase in bifidobacteria was seen in the vessels corresponding to the ascending (1.27 log), transverse (0.9 log) and descending (0.47 log) colon, presumably due to the prebiotic component of the synbiotics. Increases were also seen in levels of propionate and butyrate and in β-galactosidase. Persistence of a particular ingested strain of probiotic, *B. lactis* Bb-12, as a synbiotic preparation with GOS has been investigated using randomly amplified polymorphic DNA genotyping *(Alander et al., 2001).* A total of 30 healthy volunteers consumed prebiotics, probiotics or synbiotics in a yoghurt medium for two weeks. Volunteers consuming each supplement showed increase in LAB and all of the volunteers taking the probiotic (or synbiotic) had showed the presence of *B. lactis* in the faecal flora. No increase in survival of the *B. lactis* due to GOS was seen; however, as pointed out by Alander *et al., B. lactis* had very good survival properties to begin with. The development of synbiotics might be more important for strains of probiotic with poorer survival properties. The technological properties of bacteria play a very significant role in the production of probiotics *(Saarela et al., 2000)*.

The ability of a synbiotic preparation with *Bifidobacterium* with GOS to protect against *Salmonella* infection in mice has been investigated *(Asahara et al., 2001).* Mice were treated with streptomycin to compromise the gut flora by selective removal to undetectable levels of bifidobacteria, lactobacilli and enterobacteria. Feeding with *B. breve* at 10^8 cfu/mouse/day or the synbiotic preparation, which additionally contained

galactooligosaccharides at a concentration of 2-50 mg/mouse/day, resulted in re-colonisation of the gastrointestinal tract with *B. breve*. Mice fed the probiotic and synbiotic displayed reduced faecal excretion of *Salmonella enterica* serovar Typhimurium after pathogen challenge. In addition the synbiotic blocked extra-intestinal translocation of the pathogen, whereas galactooligosaccharides alone did not.

Research on probiotics, prebiotics and synbiotics is promising. However, further research is needed to substantiate preventive and therapeutic health benefits, mechanism of action, optimal intake, duration of treatment, selection of specific probiotic strains for a targeted outcome and mode of delivery. In the meantime, health professionals can tailor nutrition guidance by matching products with a specific strain for a client specific condition or by offering guidance about these products as components for healthy diets in general.

The consumption of foods containing prebiotics and probiotics shows a progressive increase in the last decade due to changes in habits and trends of consumers attracted by the benefits from the consumption of this new functional food. Nowadays the development of fruits and vegetables with prebiotics and probiotics is a topic of high interest for the health conscious consumers. However, the available information is very limited.

1.6.3. Fruits and vegetables as matrices for prebiotic and probiotic fortification

Plant tissues are multiphase systems with an intricate internal microstructure formed by cells, intercellular spaces, capillaries and pores. Edible portions of most fruit and vegetables are composed of fleshy parenchyma cells, which form the bulk of the softer parts of plants. Nutrients of importance to man are frequently stored in these thin-walled living cells. Despite the differences in composition and structure of the cell walls of various plant tissues, the limiting pore diameters appear to be similar *(Carpita et al., 1979).* Also, an intact plant cell wall constitute an extremely effective physical barrier against attack by microorganisms since its pores are far too small to permit bacteria (size range ~0.1 to 5 μm), yeasts (size range ~5 to 30 μm), mould spores (size range ~3 to 9 μm) and even viruses (size range ~0.02 to 0.3 μm) to penetrate through the protoplast *(Alzamora et al., 2000).* Penetration of microorganisms through the cell walls, therefore,

would require physicochemical or enzymatic degradation and/or alteration of wall structures. In porous fruits and vegetables not the wall pores but the intercellular spaces may play a major role regarding the penetration of microorganisms. These intercellular spaces are commonly referred to as 'pores'. Intercellular air spaces are common in parenchymatous tissue and have been estimated to be 20-25% of the total volume in apple, 15% in peach, 37-45% in mushroom, and 1% in potato. For instance, mature cells of apple parenchyma tissues may be 50-500 μm in diameter with interconnecting air spaces ranging from 210-350 μm across *(Lapsley, 1992).*

Rodriguez (1998) conducted basic impregnation studies with different microorganisms (*Saccharomyces cerevisiae*, *L. acidophilus* and *Phoma glomerata*) to evaluate their penetration by VI into a porous fruit tissue (Granny Smith apple). When compared microbial counts of fresh apple and apple treated under atmospheric conditions, it could be observed that the simple soaking renders a significant increase in microbial counts. This highlights the fact that capillary force and superficial adherence are very important factors that cannot be neglected in any modeling approach of immersion and impregnation operations. The model proposed by Roa *et al.*, (2001) was used for predicting microorganism incorporation into vegetable tissues. These authors simplified the model previously developed by Fito (1994) by accomplishing direct experimental determination of the volumetric fraction of sample occupied by the impregnating solution as a result of HDM.

The fortification of apple cylinders with *Bifidobacterium* spp. ''Bb12'' (Christian Hansen Corp.) by applying VI was investigated by Maguina *et al.*, (2002). Viability evaluation of *Bifidobacterium* spp. in apple pieces stored in anaerobiosis at 4°C for 12 days revealed that viable populations decreased only by a log cycle after the sixth day, and remained in that level until the end of storage. Probiotic-enriched dried apple by VI was developed by Betoret *et al.*, (2003). Apple cylinders were impregnated either with commercial apple juice containing *S. cerevisiae* or with whole milk or apple juice containing 10^7-10^8 cfu/ml of *L. casei* (spp. *rhamnosus*). At the end of the storage, *L. casei* concentration in dried product was greater than 10^6 cfu/g, being very similar to the levels usually found in commercial probiotic dairy products.

Incorporation of carbohydrates into fruits and vegetables are rather simpler and has been widely employed in the production of dehydrated fruits and vegetables by VI or OD techniques. The use of prebiotic oligosaccharides as an osmotic solution for the dehydration process is scarce. Froseth and Creedon (2003) patented a process for preparing dried infused food pieces comprising: a food body including, at least in part a fruit component; fruit infused inulin content ranging from about 1% to about 35% wherein the inulin has a DP ranging from about 2-9; a moisture content ranging from about 5% to about 25%; and, a_w ranging from about 0.25 to 0.75. The infusing step includes the sub-step of holding the mixture of inulin containing syrup and food pieces for sufficient time to allow osmotic equilibrium to form the inulin infused food product and a spent inulin syrup.

Ramesh *et al.,* (2004) prepared prebiotic enriched fruits and vegetables by OD using FOS syrup produced by the action of FTase enzyme on table sugar solution. The novelty of the process is that it uses FOS syrup for the enrichment of fruit and vegetables. The final product contains about 12-14 g of FOS/100 g product. The authors claimed that FOS enriched fruits and vegetables can be used as topping for ice-cream, biscuits, chocolates, breads, sweetened puffs and buns. It can also be used in Indian traditional sweets dishes such as *payasam* in addition to raisins and nuts. The final product exhibits functional properties like non-cariogenicity, low calorific value and other prebiotic properties. The product might improves mineral absorption, reduces the total cholesterol and triglyceride levels in the body. Another novelty of the process is the value addition to fruits and vegetables. Also, use of FOS syrup improves the texture, colour, taste and shelf life of the product. Presence of FOS also reduces the chance of microbial contamination to the product to a considerable level. The FOS syrup used for OD also gets enriched by the leached out nutrients *(Ramesh et al., 2004).* Recently, Anikó-Matusek *et al.,* (2008) studied how the different oligosaccharide components of FOS diffuse during VI and osmotic dehydration of apple cubes. The study demonstrated that there is great potential for developing high quality, FOS fortified fresh-cut apples using VI technology, thus further enhancing the health benefit of the apples.

In the similar direction, the present investigation is paying awareness to the health conscious consumers, a new approach of prebiotic and probiotic enriched foods.

1.7. Scope of the work

The research program on ***'Biotechnological approaches for the preparation of FOS based prebiotic and probiotic foods'*** is based on the following facts.

1. Fructooligosaccharides (FOS), produced from transfructosylation of sucrose using FTase, have a great potential as an osmotic agent to incorporate into fruits and vegetables. However, the replacement of sucrose syrup, a commonly used osmotic solution with FOS needs to be studied **(Chapter 2)**.
2. Detailed studies on the vacuum osmotic dehydration (VOD) parameters for the production of FOS enriched fruits/vegetables are needed. Optimum experimental conditions have to be established to ascertain the maximum FOS intake in the product **(Chapter 3)**.
3. With the changing pace of human activity and changing life styles, the demand for the readymade fruit juice is increasing. Fruit juice beverages fortified with minerals and vitamins are abundantly available. FOS fortified fruit juice beverages is a new approach and a detailed study to establish their functional properties is required **(Chapter 4: Section 4.1).** The consumption of sweets is an integral part of the Indian dietary system. The enrichment of '*gulab jamun*' with FOS, a low calorie prebiotic as an effective replacement of sucrose either fully or partially is to be investigated **(Chapter 4: Section 4.2)**.
4. Though, there are number of sources available for the isolation of probiotics, fruits and vegetable are also a rich source for probiotic strains, which are most suitable for food industry. The potency of the probiotic organisms isolated from fruits and vegetables needs to be evaluated for further use **(Chapter 5)**.
5. There is a great potential for synbiotic products and traditional Indian dairy products for innovation and value addition. Synbiotic (FOS along with probiotics) fruits **(Chapter 6: Section 6.1)** and synbiotic *shrikhand* would provide health benefits to the consumers **(Chapter 6: Section 6.2)**, and improve the scale of economy in the food and/or dairy industry.

The specific aspects of the thesis are presented under the following headings

Chapter 1: Introduction

Chapter 2: Fructooligosaccharides: A novel osmotic agent for impregnation of fruits and vegetables

Chapter 3: Vacuum osmotic dehydration of banana using fructooligosaccharides: Optimization of process parameters using statistically designed experiments

Chapter 4:

Section 4.1: Fructooligosaccharides fortified fruit juice beverages

Section 4.2: Fructooligosaccharides based traditional Indian acid coagulated milk sweet; *gulab jamun*

Chapter 5: Isolation and characterization of potent probiotics from fruits and vegetables

Chapter 6:

Section 6.1 Synbiotic functional star fruits with fructooligosaccharides and selected probiotics

Section 6.2 *Shrikhand:* A fermented milk based dessert enriched with fructooligosaccharides and probiotic *Enterococcus faecium* CFR 3002

Chapter 7: Summary and conclusion

Bibliography

CHAPTER: 2

FRUCTOOLIGOSACCHARIDES: A novel osmotic agent for impregnation of fruits and vegetables

2.1. Introduction

Changing concepts in nutrition and diet over the years have led to the development of "functional foods" that promote health beyond providing basic nutrition *(Klaenhammer and Kullen, 1999)* and/or reduce the risk of diseases. Functional foods are better understood as a concept rather than a well-defined group of food products *(Alzamora et al., 2005)*. These foods are enriched with physiologically active components such as prebiotics, probiotics, vitamins, and minerals. A number of carbohydrates such as fructooligosaccharides (FOS), galactooligosaccharides (GOS) and xylooligosaccharides (XOS) have emerged as new functional food ingredients. Among which, FOS have attracted special attention as prebiotic, because of their well studied health effects in regulating mineral absorption, lipid metabolism and serum cholesterol *(Sangeetha et al., 2005b; Katapodis et al., 2004; Cuervo et al., 2004; Moore et al., 2003)*. FOS improve proliferation of beneficial microorganisms in human gut by preventing colonization of pathogenic microorganisms. They have great potential for improving the physicochemical properties of foods and functions as dietary fiber *(Gibson and Roberfroid, 1995)*.

The consumer demand for functional foods is on par with the demand for higher quality fruits and vegetables. Fruit and vegetable consumption has been shown to be an important part of any diet leading towards good health. Consumption is generally based on locally and seasonally available foods, since expensive imports cannot be afforded or for the lack of the necessary storage facilities for perishable products. Fruits and vegetables in general contain more than 75% water and are prone to fast spoilage, if not stored properly. Even proper storage fails to preserve the fruits for a long period unless they are treated with hypertonic/hypotonic solutions to get dehydrated or intermediate moisture foods (IMFs). It is at this juncture that, vacuum osmotic dehydration (VOD), a well known method of osmotic treatment of foods can be used as an effective tool for enhancing the functionality and storage parameters of fruits and vegetables *(Fito and Chiralt, 2000; Martinez-Monzo et al., 1998)*.

VOD leads to some advantages as compared with atmospheric osmotic dehydration, since a faster dehydration as well as controlled impregnation of active compounds in to the food matrix can be achieved with this treatment *(Chiralt et al.,*

2001; Fito et al., 2001a; Fito et al., 2001b). The influence of vacuum on the kinetics of the mass transfer phenomena, especially concerning weight loss and solid gain is very important during osmotic treatment. The effect of vacuum application can be explained on the basis of hydrodynamic mechanism (HDM).

The use of vacuum in osmotic dehydration allows an increase in the rate of water, weight loss and solid gain *(Sereno et al., 2001; Rastogi and Raghavarao, 1996)* and introduces controlled quantities of a solution in to the porous matrix of fruits and vegetables *(Fito et al., 2001)*. VOD not only enables the storage of fruits and vegetables for a longer period, but also preserves flavour, nutritional characteristics and lessens the extent of microbial spoilage by promoting fast compositional changes *(Zhao and Xie, 2004)*.

The VOD process results in dehydrated or IMFs products. IMFs are those with a water activity (a_w) of 0.65 to 0.90 and moisture content ranging from 10% to 40%. The osmotic solutions such as sugars and salts solutions used during VOD increases the stability of these foods. IMFs offer advantages both to consumers and processors as this process requires less energy when compared with other methods such as canning, drying, freezing or refrigeration *(Raoult-Wack, 1994)*. These foods can be consumed without further processing as they bear soft texture with considerable shelf stability because, the water is not available to support microbial growth as it is bound physically and chemically with the osmotic solute.

Sucrose is the most widely used osmotic solution in a majority of the studies on impregnation and osmotic dehydration. Fruit/vegetable products with sucrose, glucose, fructose, corn syrup, minerals, nutraceuticals, sorbitol, glycerol etc as added food ingredients are well known *(Chafer et al., 2001)*, but there are hardly any report on the use of FOS as an osmotic agent. Hence, there exists scope for the impregnation of fruits and vegetables with FOS to give value addition to the product. Generally FOS is a mixture of short chain fructans having a low calorific value (1.5 kcal/100 g) and is about one-third as sweet as sucrose *(Yun, 1996)*.

The present chapter details the effective use of FOS as a healthier and low calorie replacement to sucrose for the development of shelf stable prebiotic enriched fruits and vegetables.

2.2. Materials and methods

2.2.1. Preparation of fructooligosaccharide (FOS) syrup

The production of FOS was carried out in two stage continuous process. In the first stage, Fructosyl Transferase (FTase - EC 2.4.1.9) was produced by submerged fermentation *(Sangeetha et al., 2003)* using *Aspergillus oryzae* MTCC 5154, a soil isolate from our laboratory. The culture was maintained on potato dextrose agar (Himedia Laboratories Ltd, India) slants at 4 °C. The inoculum for the production of FTase was developed by transferring a loop of mycelium from a five day old slant of *A. oryzae* MTCC 5154 into inoculum medium (1% sucrose, 0.2% yeast extract, 100 ml medium per 500 ml flask, pH 5.50). The flasks were incubated at 30±1 °C on a rotary shaker (Emenvee Rotary Shaker 48N3, Pune, India) at 250 rpm for 24 h. The culture fluid was separated from mycelia by filtration and was used as the source of FTase, without further purification *(Sangeetha et al., 2005a)*.

In the second stage, FOS syrup (30 L) was produced by transfructosylation of sucrose (60 °Brix) using FTase at enzyme to substrate ratio of 1:9 (v/v) in an indigenously designed and fabricated bioreactor at 55 °C for 18 h *(Sangeetha et al., 2005b)*. The resultant FOS syrup of 58°Brix (Refractometer; Range: 58-92 °Brix, No: 94478, Erma, Japan) was a mixture of glucose (14%), sucrose (30%), 1-kestose (35%), 1-nystose (18%) and 1-fructofuranosyl nystose (3%). The syrup was concentrated to 70 °Brix (10 L water evaporation per hour using 0.5 kg/cm^2 steam pressure at 55 °C) using agitated thin film evaporator (M/S. Chemetron corporation, model: 04-012, USA) and stored at 4 °C until use.

2.2.2. Vacuum osmotic dehydration (VOD) of selected fruits and vegetables with FOS

The fruits and vegetables were purchased from the local super market (Table 2.1), Mysore, India. Care was taken to use fairly uniform sized fruits and vegetables. The VOD experiments were carried out in a 1 L glass bowl. The selected fruits and vegetables were washed, peeled, cut either into 1 cm cubes (apple, papaya, sapota, ash gourd, pumpkin) or 0.6 cm thick slices (banana, star fruit, carrot), and were water blanched at

80-90 °C for 2-3 min. The process flow chart for the preparation of FOS enriched fruits/vegetables is depicted in Fig 2.1.

Table 2.1: The fruits and vegetables used in the VOD process

Sl. No.	Fruits / Vegetables	
	Common Name	**Scientific Name**
1	Apple	*Malus domestica*
2	Banana	*Musa acuminate* Colla
3	Jack fruit	*Artocarpus heterophyllus*
4	Papaya	*Carica papaya*
5	Sapota	*Manilkara zapota*
6	Star fruit	*Averrhoa carambola*
7	Ash gourd	*Benincasa hispida*
8	Pumpkin	*Cucurbita pepo*
9	Carrot	*Daucus carota*

The fruits and vegetable pieces with an initial moisture content of 67 to 92% were immersed in FOS syrup (70 °Brix), at a fruit/vegetable to FOS ratio of 1:5 (w/v) (to make sure that the fruits/vegetables were immersed completely in the FOS syrup) and subjected to vacuum at 650 mm Hg pressure (85.5% vacuum drawing out 650 mm Hg from the standard atmospheric pressure of 760 mm Hg) (Vacuum oven with glass window PEW, Model No. 132 VG, M/S. Industrial and Lab Tools Corporation, Chennai, India) for 60 min at ambient temperature (25±2 °C). The atmospheric pressure was restored for 30 min and at the end of treatment time, the fruits and vegetable pieces were separated from FOS syrup by straining. The FOS adhered to the surface of the fruit/vegetable pieces was removed using adsorbent paper followed by drying in a hot air oven (The Andra Scientific Company Ltd., Andra Pradesh, India) at 40 °C for 24 h to obtain a product with 10-30% moisture. Fig 2.2 shows the photograph of the FOS enriched fruits and vegetable pieces.

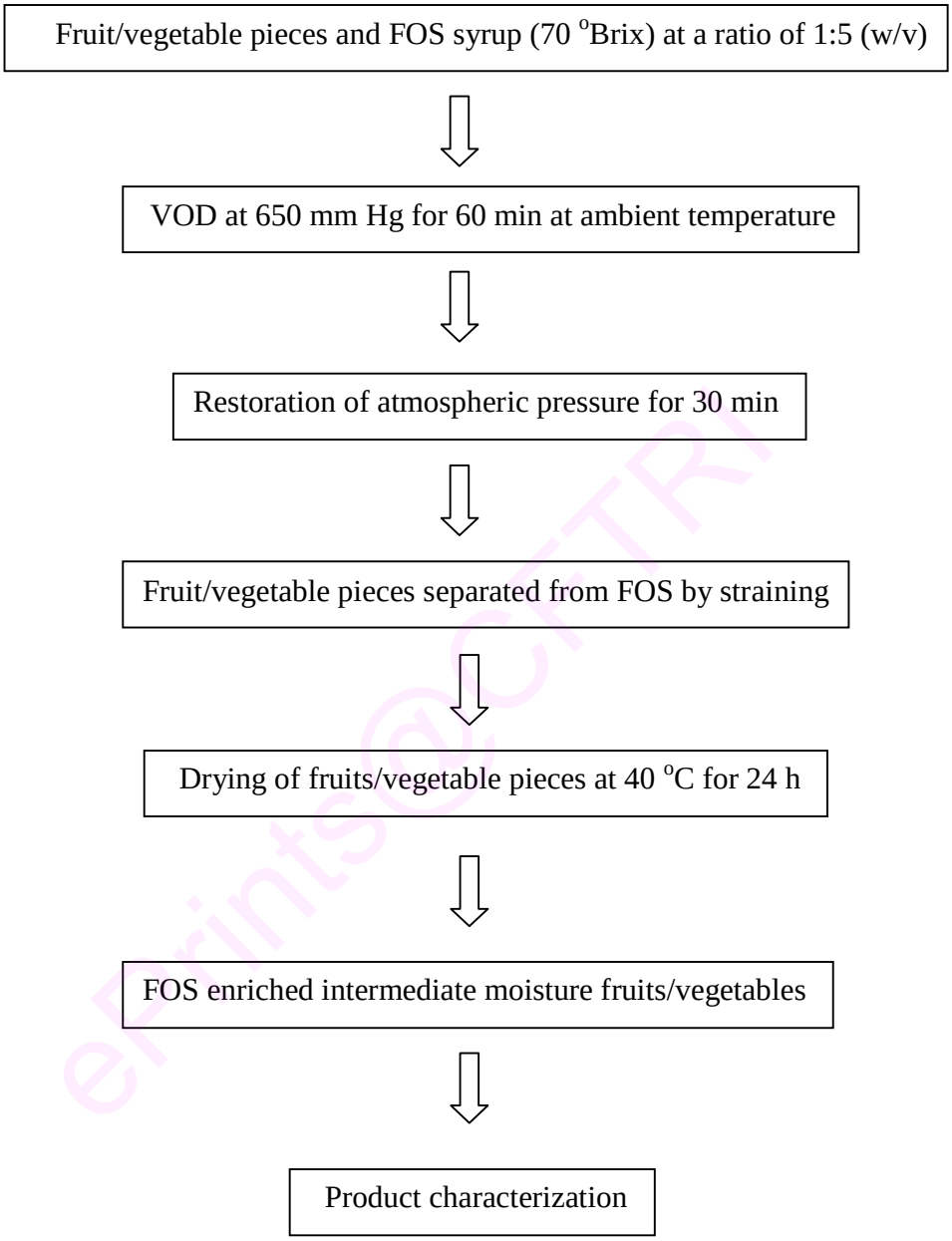

Fig 2.1 Process flow chart for the preparation of fruits and vegetables enriched with FOS

Fig 2.2 FOS enriched fruits and vegetables

2.2.3. Characterization of FOS enriched fruits and vegetable pieces

2.2.3.1. Estimation of moisture content and water activity (a_w)

Moisture content was determined by drying the samples at 90 ^oC in an oven until constant weight was reached *(AOAC, 1980)*. Moisture determinations were made in triplicates and average values are presented. Water activity values of FOS enriched fruits/vegetable pieces were estimated using Novasina Thermoconstanter (Type-TH/RTD, No-750, Switzerland).

2.2.3.2. Colour measurement of fresh and FOS enriched fruits and vegetable pieces

The colour values of fresh and FOS enriched fruits and vegetable pieces were measured using a Hunter Lab colour measuring system (Lab scan XE, Hunter Ass. Lab, Virginia, USA), using the *L* a* b** colour scheme. Wavelengths of the visible light range

between 400-700 nm, view angle of 2° and the most commonly used illuminant 'C' were used to analyze the *L**, *a** and *b** values. The *L**, *a** and *b** values represent brightness/darkness, green/red and yellow/blue respectively *(Krishnamurthy and Kantha, 2005)*. Colour values presented are an average of three readings.

2.2.3.3. Texture analysis of fresh and FOS enriched fruits and vegetable pieces

A universal testing machine (UTM; LLOYD, LR 5K) at 50 mm/min cross head speed with a load cell of 50 kg weight was used to measure the shear strength of fresh and FOS enriched fruits and vegetable pieces. The test cell consists of thin stainless steel blade with an equilateral triangular hole (mounted to the cross head) and is led through a narrow slit between two shear bars *(Krishnamurthy and Kantha 2003)*.

2.2.3.4. *β*-carotene

The amount of *β*-carotene in fresh and FOS enriched fruits and vegetables pieces was estimated using acetone extraction followed by separation in an adsorption column and subsequent measuring of the colour intensity at 450 nm using a UV/Visible spectrophotometer (Shimadzu, UV-1601) and compared with that of a *β*-carotene reference standard. The amount of *β*-carotene was expressed as µg/100ml of sample *(Ranganna, 2003)*.

2.2.3.5. Estimation of FOS content

The FOS enriched fruits and vegetables pieces (5 g) were taken randomly and macerated with 5-10 ml of triple distilled water using mortar and pestle. The extract was centrifuged at 8000 rpm for 20 min and filtered through 0.45 micron membrane filters. The filtrate was appropriately diluted with triple distilled water and analyzed for FOS content by HPLC (SCL-6B, Shimadzu, Japan) equipped with a refractive index detector RID 6B (Shimadzu, Japan) using an Aminopropyl column 250×4.6 mm SS Excil amino 5 µm (SGE, Australia). Appropriately diluted reaction mixture was injected using HPLC injector syringe (Hamilton, Nevada, USA). The analysis was carried out at room temperature (25±2 °C) using acetonitrile: water (70:30 v/v) as the mobile phase at a flow rate of 1.0 ml/min *(Sangeetha et al., 2002)*.

The FOS obtained was compared with FOS standards: 1-kestose, 1-nystose and 1-fructofuranosyl nystose from Wako Pure Chemical Industries, Ltd. (Gift from Dr. Sosaku Ichikawa, Osaka, Japan) and analytical grade glucose and sucrose were also used as reference standards. Data acquisition was done by AIMIL chromatography data station (AIMIL, New Delhi, India) and processed on computer using WINACDS software (AIMIL, New Delhi, India). The final FOS content was expressed as the percentage conversion based on initial sucrose concentration. The final FOS content of VOD fruits and vegetables was expressed as g/100g product.

2.2.3.6. Scanning electron microscope (SEM) analysis of fresh and FOS enriched fruits and vegetables pieces

Structural analysis of the fresh and FOS enriched fruits and vegetable pieces were carried out using SEM (Leo 435 VP, Leo Electron Microscopy Ltd. (Zeiss), Cambridge, U.K) to confirm the uptake of FOS. The sample fixation was carried out by immersing fruit and vegetable sections in glutaraldehyde (2%) for 24 h in 0.1 M phosphate buffer (pH 7.2 to 7.4) at 4 °C. Samples were dehydrated by sequential immersion in ethanol solutions [(30%, 50%, 70%, 80% (v/v)] for 15 min each followed by immersion in 95% ethanol for 20 min and were dried in a desiccator. Polaron E5100 SEM coating system was used for gold coating. The samples were coated with 3 A° thickness gold for 2 min at 20 mA current. Gold-coated samples were examined at 150x and 300x magnifications.

2.2.3.7. Microbiological analysis of FOS enriched fruits and vegetable pieces

Microbial load of FOS enriched fruits and vegetable pieces was determined by plating a known volume of serially diluted samples on plate count agar (PCA), potato dextrose agar (PDA) and McConkey agar (MCA). The PDA plates were incubated at 25±2 °C for 48 h. The PCA and MCA plates were incubated at 37 °C for 24 h. The colonies were counted and expressed as cfu/g of samples. The experiments were performed in triplicate and the average results are reported.

2.2.3.8. Sensory analysis of fresh and FOS enriched fruits and vegetable pieces

Sensory analysis was carried out using hedonic scale consisting of 10 points (1-10), where 9-10 = excellent, 7-8 = very good, 5-6 = good, 3-4 = fair, 1-2 = poor *(Sidel and Stone, 1993)*. An internal panel of nine expert members evaluated the products for colour, aroma, flavour, texture and overall acceptability.

2.2.3.9. Statistical analysis

Statistical analysis was carried out using a one-way analysis of variance (ANOVA), Origin 6.1 Software. Means were separated using the least significant difference test. Significance was defined at $P<0.05$.

2.3. Results and Discussion

2.3.1. Effect of FOS enrichment on moisture content and water activity (a_w) of fruits and vegetables

Moisture content is an important parameter, which plays a major role in maintaining the freshness of fresh fruits and vegetables. However, the high moisture content and high a_w make them more susceptible to spoilage. In order to circumvent this, drying is widely used for getting shelf stable products. Moisture content of fresh and FOS enriched fruit and vegetable is shown in Fig 2.3. Moisture content of FOS enriched apple, sapota and pumpkin was found to be in the range of 20-30%, where as in banana, jack fruit and papaya, it was 14%. The star fruit, ash gourd and carrot contained 11.14, 17.46 and 18.94% moisture respectively. The replacement of native liquid or air in the pores of the fruits and vegetable is achieved by an external solution *i.e.* FOS syrup (70 °Brix) during VOD and thus, moisture content and water activity of fruits and vegetable pieces changed markedly after applying vacuum during the process. The FOS syrup used is hypertonic and thus, the native liquid would have been expelled, partially during process. FOS enriched fruits and vegetable pieces were dried at 40 °C in order to retain some of the desirable attributes of fruits and vegetables. The final moisture content of FOS enriched fruits and vegetables at the end of 24 h drying was found to be less than 30%, which is a characteristic of IMF.

It is generally known that water removal by osmotic dehydration is a relatively slow process. Hence the vacuum was used during the osmotic dehydration process to enhance the rate of removal of water content from fruits and vegetables. Fito (1994) reported that vacuum operation significantly increased the water loss rate compared to that obtained at atmospheric pressure.

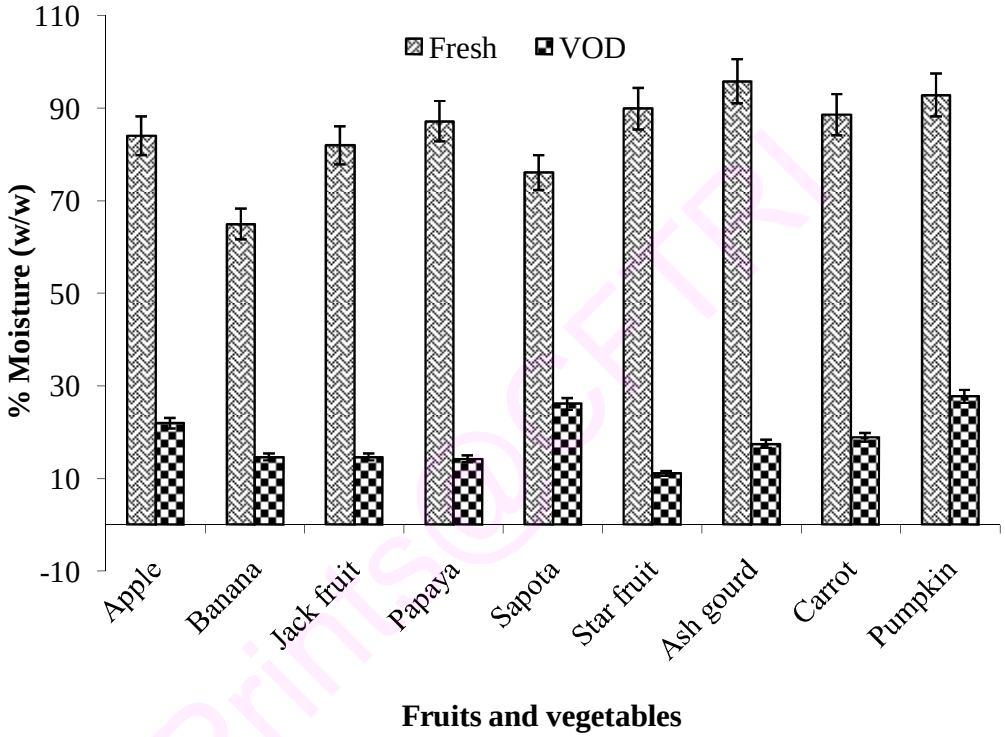

Fig 2.3 Moisture content of fresh and FOS enriched fruits and vegetables

It has been reported that the reduction of moisture content (% dry basis) was much higher when the concentration of the syrup used was higher. It is also said that the increase in osmotic syrup concentration increases diffusional changes and the osmotic pressure exerted on the fruit cell structure, consequently results in greater moisture reduction *(SAS, 1993)*. A combination of hypertonic FOS syrup (70 ^{o}Brix) and the application of vacuum (650 mm Hg), promotes hydrodynamic gain of FOS syrup into tissue pores of fruits and vegetables.

The water holding capacity of FOS is similar to that of sucrose *(Sabater-Molina et al., 2009)* and hence, the resultant a_w of FOS enriched fruits and vegetables was in the range of 0.67-0.86, which is a characteristic of IMF (Table 2.2) The application of vacuum during osmotic process decreases a_w in the minimal process of fruits *(Alzamora and Gerschenson, 1997)*. With the decrease in moisture content, the a_w of the final product also decreased, resulting in shelf stable FOS enriched fruits and vegetables.

2.3.2. Effect of FOS enrichment on colour of fruits and vegetables pieces

Appearance and colour are the most important quality characteristics of foods, which play an important role in consumer acceptability. The colour of foods is due to the presence of naturally occurring or added pigments, or even developed as a result of complex chemical and biochemical (enzyme or non-enzymatic browning) changes occurring during processing. The FOS enriched fruits and vegetable pieces showed a slight decrease in L^* values after VOD (Fig 2.4a). The FOS enriched sapota and ash gourd did not show much difference in L^* value when compared with fresh sapota and ash gourd, indicated fresh appearance. The decrease in L^* value (<50%) of fruits and vegetables observed may be due to the transparency gained because of air loss and total or partial substitution of air spaces present in the pores, by FOS.

The a^* values of jackfruit and papaya after VOD with FOS indicates that, the colour did not change significantly (P>0.05) and had retained fresh appearance which could probably enhance the consumer acceptability. Apple and ash gourd showed negative a^* value, indicated slight brownish tinge. Where as, the jack fruit and papaya showed similar trend in a^* value. The slight decrease (sapota, carrot and pumpkin) and increase (banana and star fruit) in a^* value was also observed (Fig 2.4b). The FOS enriched fruits and vegetables had yellowish tinge as indicated by the positive b^* value (Fig 2.4c).

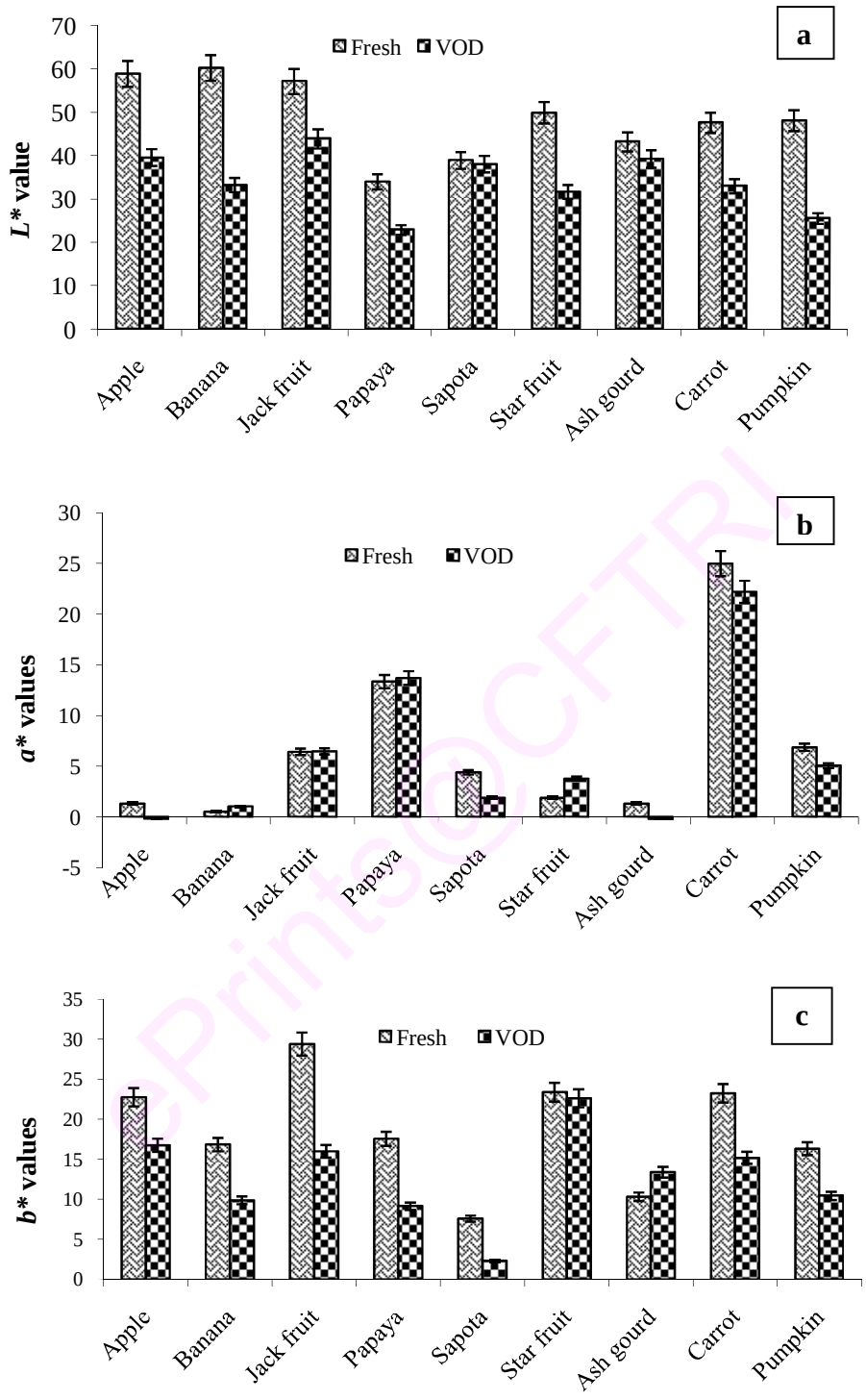

Fig 2.4 Colour values [(a) *L** value; (b) *a** value and (c) *b** value] of fresh and FOS enriched fruits and vegetables

The results indicated that there were no observable changes in the colour of the finished product after enrichment with FOS, though a slight increase or decrease in *L*a*b** values was observed in comparison with that of fresh fruits and vegetables. It is reported that, for light colored fruits sensitive to enzymatic browning, air leaves the pores of the fruits during vacuum treatment, reducing the oxygen concentration in the sample tissue. This results in slowed down oxidative reaction rates, which in turn would lead to the final product with a good natural colour *(Alzamora et al., 2000)*. In addition, it was found that the colour of the osmotic solution has an impact on the colour of final products due to the filling of the pores with the solution, especially in case of light colored products *(Xie and Zhao, 2003a).*

2.3.3. Effect of FOS enrichment on texture (shear strength) of fruits and vegetable pieces

Fruits and vegetables are important components of a healthy diet, and their textural characteristics are important quality attributes in determining consumer choice. Tissue structure and how the tissue reacts to applied force during shear or cutting test determines the textural characteristics of fruits and vegetables. The texture quality of FOS enriched fruits and vegetables can be significantly influenced by the type and concentration of osmotic solution used. Shear test determines the resistance offered by a food sample against cutting or shearing and is widely used on account of its good reproducibility of the results *(Szczesniak, 1990)*.

The shear values of FOS enriched fruits and vegetable were significantly different ($P<0.05$) in comparison with that of fresh fruits and vegetable pieces as shown in Fig 2.5. The shear strength of FOS enriched fruits and vegetable pieces were similar to that of reported values for the well studied IMF *(Engelen et al., 2003)*. In the present study, the FOS enriched banana, jackfruit, papaya, sapota, star fruit and carrot showed an increase in shear strength (6.74 N, 38.5 N, 15.55 N, 7.28 N, 17.6 N and 33.05 N respectively), when compared to that of fresh fruits and vegetables.. The observed increase in shear strength could be due to the air-liquid exchange during the vacuum operation *(Alzamora et al., 2000; Alzamora et al., 1997)*. In contrast to general increasing trend, the shear values of ash gourd, apple and pumpkin were decreased (30.6 N, 2.9 N and 20.75 N

respectively) after VOD. It is well established that the texture properties, in general are dependent on the behavior of cellular matrix and soluble solid phase inside the tissue *(Contreras et al., 2005; Gabas et al., 2002)*. Vision, audition, touch and kinesthesis sensations are involved in texture perception during food selection and later during consumption. In line with the above argument, the final products were found to be highly acceptable by the sensory panelists.

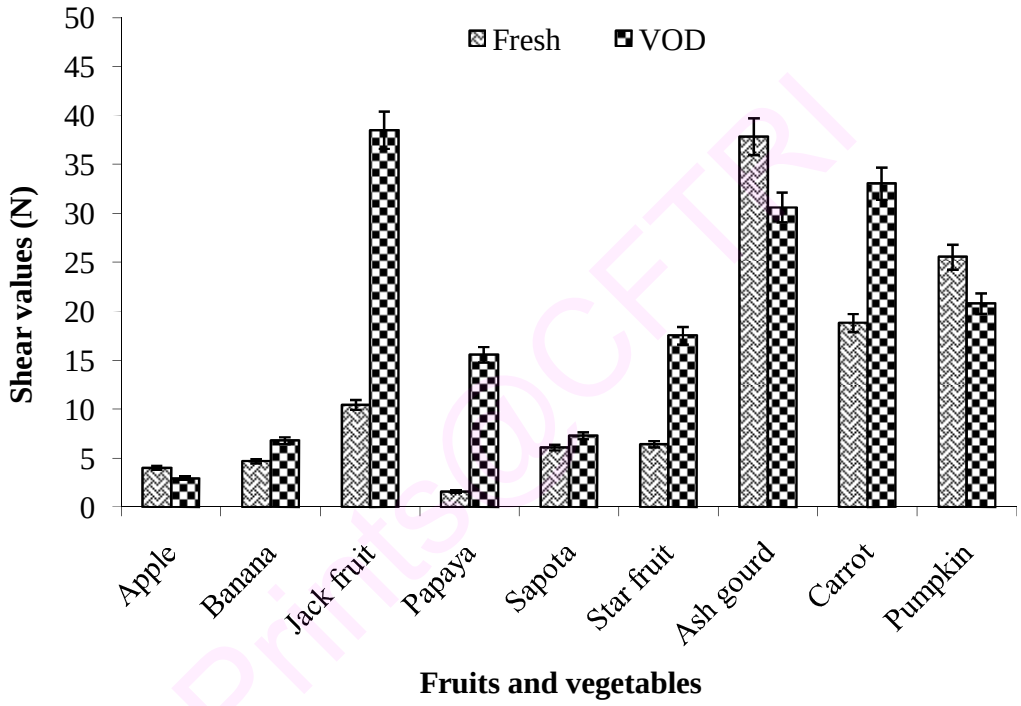

Fig 2.5 Texture of fresh and FOS enriched fruits and vegetables

2.3.4. *β*-carotene content

Carrot, pumpkin, jackfruit, and papaya are rich source of carotenoids, especially *β*-carotene and other carotenoids that are well known precursors of vitamin A. The retention of *β*-carotene is of utmost importance not only from the point of preserving the appearance of the finished product, but also from the point of nutritional and dietary value of the product *(Ong and Chytil, 1983)*.

β-carotene content of fresh and FOS enriched fruits and vegetable pieces are shown in the Fig 2.6. There was 60-70% retention of *β*-carotene in FOS enriched fruits

and vegetables. The observed loss of *β*-carotene could also be due to the probable leaching of carotenoids to water during blanching. The carotene bound proteins are water soluble and blanching the fruits and vegetables for 3-5 min could result in release of carotene from the bound protein matrix into water. The finished product with retention of 60-70% of *β*-carotene would find greater acceptability by the health conscious consumer and thus, an enhanced market potential.

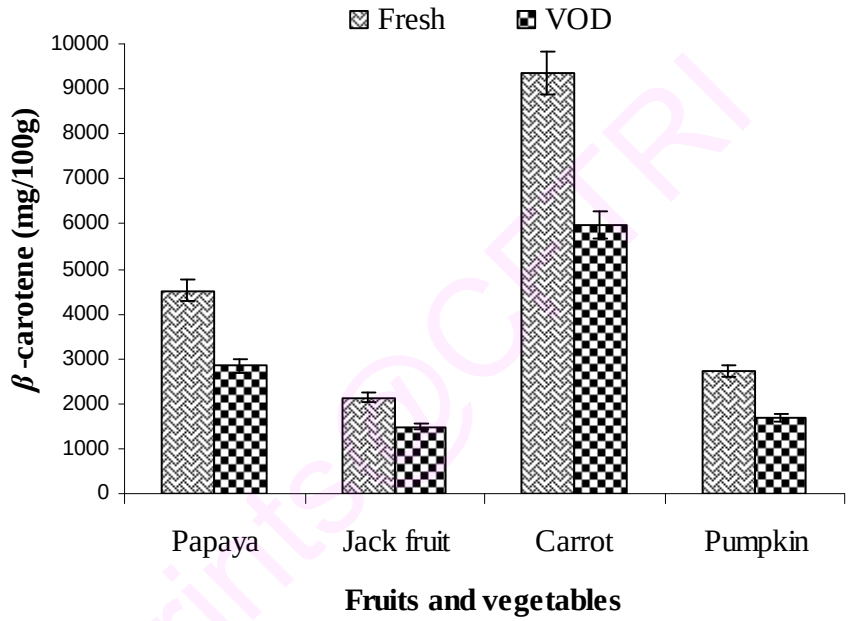

Fig 2.6 *β*-Carotene content of fresh and FOS enriched fruits and vegetables

2.3.5. FOS uptake by fruits and vegetables during VOD

The incorporation of solute/any food ingredient into the food system to a certain extent to changes their nutritional and functional property without modifying its integrity is possible through VOD. The quantity of external liquid impregnated in the food structure during VOD depends mainly on vacuum pressure and its application time. The FOS content of VOD fruits and vegetable pieces is shown in Table 2.2. The uptake of FOS ranged between 8.02-17.21 g/100g of the final product, depending on the structure of fruits and vegetables used**.** The uptake of FOS is more in apple, star fruit and ash gourd when compared to that of banana, papaya and carrot. The increased surface area

and the biological microstructural characteristics of fruit and vegetable tissue *(Shi and Fito 1993)* are some of the factors, which facilitates better uptake of FOS *(Gras et al., 2003)* during VOD. The FOS uptake could also accelerated by the coupling of HDM with the diffusional phenomena promoted by concentration gradients, due to capillary effect *(Fito, 1994; Fito and Pastor, 1994; Fito et al., 1994)*. Application of vacuum pressure facilitates the outflow of internal gas or liquid and their efficient substitution by the external liquid, thus improving the gain of external solute, FOS in the present study. Based on the results, it can be inferred that there were no observable or significant changes in the various quality parameters such as colour, texture and sensory attributes of the final product due to FOS enrichment. The enriched fruits and vegetable pieces were shelf stable, with prebiotic properties and could be consumed without further preparation.

Table 2.2 Water activity, FOS content and microbial load of VOD fruits and vegetables

Fruits/vegetables	**Water activity (a_w)**	**FOS (g/100g)**	**Microbial load (cfu/g)**
Apple	0.86±0.01	16.55±3.00	$2x10^3$
Banana	0.66±0.02	08.49±2.00	Nil
Jackfruit	0.69±0.03	09.47±2.00	Nil
Papaya	0.71±0.01	8.02±1.00	$1x10^3$
Sapota	0.75±0.01	10.32±3.00	$2x10^3$
Star fruit	0.67±0.01	17.21±2.00	Nil
Ash gourd	0.78±0.02	11.78±3.00	Nil
Carrot	0.74±0.01	08.43±2.00	$3x10^3$
Pumpkin	0.81±0.03	09.14±2.00	$8x10^3$

Values are an average of three readings

2.3.6. Scanning electron microscopic (SEM) observation of fruits and vegetable pieces

SEM studies were carried out to understand the effect of FOS impregnation on fruits and vegetables structure. The variations in microstructure of fresh and FOS enriched fruits and vegetable pieces are shown in Fig 2.7. Intercellular spaces of fresh fruits and vegetable are clearly seen [Fig 2.7 (a), (b) and (c)] and these spaces were filled with FOS [Fig 2.7 (d), (e) and (f)] in case of impregnated fruits and vegetables pieces, indicated that the external liquid infused the cellular cavity through the permeable cell wall by replacing intracellular water, thus avoiding the cell wall deformation *(Albors et al., 1996)*.

Accumulation of FOS in the narrowest zone of the intercellular spaces is probably due to the pressure gradients created during the vacuum application during the process *(Betoret et al., 2003)*. The reduction in the cell shape and size could probably the resultant of loss of native liquid during VOD. A thick middle lamella between cells was observed. This could be the reason for the observed differences in shear values (Fig.2.5). This thick material could be due to the concentrated sugars or polymeric compound, which may be formed by interactions of middle lamella pectin with solute present in the impregnation solution. Moreno *et al.*, (2000) reported similar phenomenon in strawberries.

The type of osmotic solution used in VOD process affects the microstructure of fruits and vegetables *(Martinez-Monzo et al., 1998)*. Cell shrinkage is reported to increase when sucrose is used; however, the cells have been found to retain its original form with the use of glucose *(Muntada et al., 1998; Monsalve Gonzalez et al., 1993)*. The FOS syrup used for impregnation of fruits and vegetables is a mixture of glucose (14%), sucrose (30%) and oligosaccharides (56%). The glucose and sucrose components could have been favorable in retaining the microstructure of the end product in spite of the use of considerable vacuum (650 mm Hg).

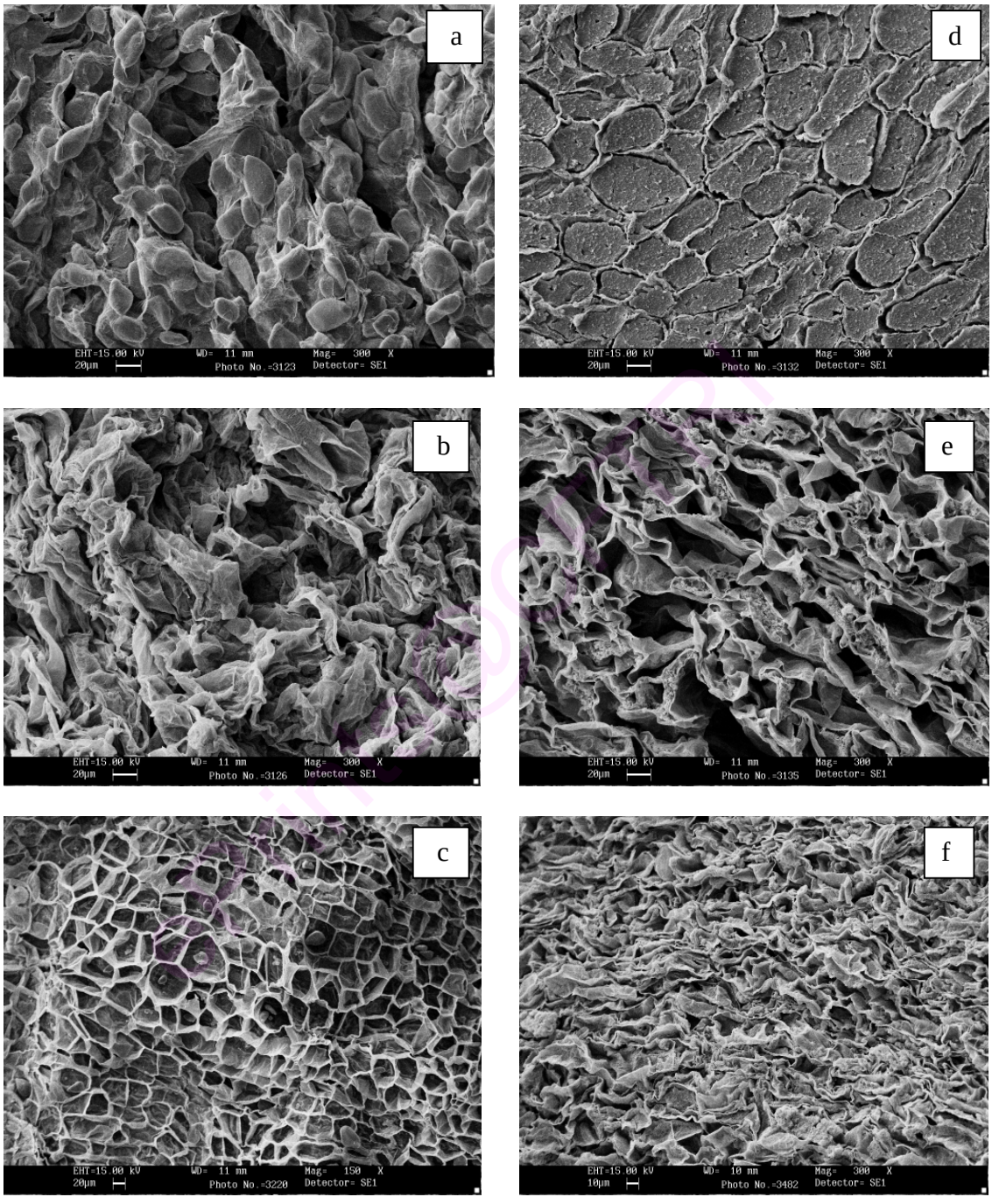

Fig 2.7 Scanning electron micrographs of fresh banana (a), jackfruit (b) and carrot (c) and FOS enriched banana (d), jackfruit (e) and carrot (f).

2.3.7. Microbiological analysis of FOS enriched fruits and vegetable pieces

The presence of microorganisms like yeast and moulds beyond a certain number in food is harmful. The microbial loads of FOS enriched fruits and vegetable pieces are given in Table 2.2. Vacuum application results in expelling the internal gas or liquid, which are replaced by the osmotic solution (FOS syrup) on vacuum release *(Andres et al., 2001)*. This results in lower moisture content, concomitant with lower $a_{w,}$ which in turn reduces the chances of microbial spoilage.

No colonies were detected in case of banana, jackfruit, star fruit and ash gourd. However, a few colonies were observed on PCA plates in case of apple, papaya, sapota, carrot and pumpkin. No coliforms were observed in any of these FOS enriched fruits and vegetable pieces. Incorporation of anti-browning agent, pH reducers, firming agents and antimicrobials to the fruits and vegetables for extending shelf life and enhancing microbial safety have been reported *(Betoret et al., 2003; Gras et al., 2003; Xie and Zhao, 2003a; Fito et al., 2001a)*. The present study has successfully shown the use of FOS for developing nutritionally enriched, shelf stable fruits and vegetable pieces with considerable shelf stability.

2.3.8. Effect of FOS enrichment on sensory attributes of fruits and vegetable pieces

FOS enriched fruits and vegetable pieces showed good sensory acceptance for all the sensory attributes evaluated. Slightly lower scores were observed in banana and carrot, but with average scores, above the limit of acceptability. However, all the FOS enriched fruits and vegetables pieces were found to be acceptable based on the overall quality (Table 2.3).

Table 2.3 Sensory attributes of fresh and FOS enriched fruits and vegetables

Fruits/ vegetables	Colour		Flavour		Texture		Overall Acceptability*
	C*	T**	C*	T**	C*	T**	
Apple	9.00±0.25	7.50±0.16	7.00±0.22	7.00±0.16	9.00±0.25	8.00±0.16	8.00±0.22
Banana	9.00±0.16	7.00±0.22	7.00±0.26	7.00±0.16	9.00±0.22	5.50±0.26	7.50±0.26
Jackfruit	9.00±0.22	9.00±0.25	9.00±0.25	9.00±0.22	8.00±0.16	5.00±0.25	8.00±0.16
Papaya	9.00±0.25	9.00±0.26	8.00±0.16	8.50±0.16	8.00±0.22	7.50±0.16	8.00±0.25
Sapota	8.50±0.25	8.00±0.25	8.00±0.16	8.00±0.25	9.00±0.26	8.50±0.22	8.50±0.25
Star fruit	8.50±0.16	8.00±0.16	7.50±0.25	7.50±0.16	8.00±0.25	7.50±0.25	8.50±0.22
Ash gourd	9.00±0.16	7.50±0.16	9.00±0.25	8.00±0.16	9.00±0.16	8.50±0.22	8.50±0.16
Carrot	9.00±0.22	8.50±0.22	8.00±0.16	7.00±0.25	8.00±0.25	6.00±0.16	7.50±0.22
Pumpkin	9.00±0.22	9.00±0.26	9.00±0.25	7.50±0.25	8.00±0.25	8.00±0.25	8.50±0.16

C* Fresh fruits and vegetables: T** FOS enriched fruits and vegetables

*Minimum acceptable score is 7.00

CHAPTER: 3

VACUUM OSMOTIC DEHYDRATION OF BANANA USING FRUCTOOLIGOSACCHARIDES: Optimization of process parameters using statistically designed experiments

3.1. Introduction

India, with a production of 44.04 million tonnes of fruits from an area of 3.72 million hectares is the second largest producer of fruits after China. Banana (*Musa acuminate* Colla) is one of the world's most important food crops in the tropical and sub tropical regions where it constitutes a major staple food crop for millions of people. It is grown in Maharashtra, Tamil Nadu, Gujarat, Karnataka, Andhra Pradesh, Kerala, Assam, and Bihar in an area of 565 thousand hectare with production of 19.19 million tonnes *(Singh, 2007)*. Banana is one of the important fruits grown in Karnataka, cultivated in 52,613 hectares, with a production of 1.3 million tonnes and a productivity of 24.6 tonnes per hectares, which is below the national average of 30.6 tonnes per hectares.

Banana has high nutritional value, being rich in starch, sugars, besides vitamin A, C and minerals such as potassium, phosphorus, calcium, sodium, and magnesium *(Borges, 1997)*. Banana is one among the most widely consumed fruits in the world in its fresh form and is also consumed in various other forms like dried banana, also called banana "chips". Banana is used in the preparation of banana puree as the basis for baby food, desserts, sweets, and for dairy products *(Deka and Choudhury, 2006)*. Banana has a commercial value for its highly appreciated sensorial characteristics, and hence occupies an important place for its multifaceted application, but is constrained by a short post-harvesting life at room temperature. The short life span, apart from resulting in increased fruit losses, also imposes restrictions on commercialization and transportation. There exists a scope for the development of various processing operations for making different products with an extended shelf life. Therefore, a need was felt to evaluate a simple and an industrially feasible vacuum osmotic dehydration (VOD) for the development of shelf stable nutritionally enriched banana slices. Osmotic dehydration (OD) is relatively a slow process and hence, in order to accelerate the mass transfer, the vacuum pressure *(Rastogi et al., 1997; Fito, 1994)* and ultrahigh hydrostatic pressure *(Rastogi and Niranjan, 1998)* have been used.

The application of vacuum with the osmotic solution as a preliminary step allows obtaining a faster processing with minimal heating and increased solute impregnation in osmotic treatments *(Chiralt et al., 1999; Fito and Chiralt, 1997)*. This is especially desirable for improving sensory properties of the finished product. Viscosity and

concentration of the osmotic solution greatly affect the product response to solute impregnation and osmo-dehydration kinetics *(Giraldo et al., 2003; Barat et al., 2001; Chafer et al., 2001)*, as well as the final ratio of water loss and solute gain in the product, which in turn has a great influence on product characteristics.

The effect of several factors including the type and concentration of osmotic agents *(El-Aouar et al., 2006; Collignan and Raoult-Wack, 1994; Lerici et al., 1985)*, processing temperature and time *(Biswal and Bozorgmehr, 1992; Lenart and Flink, 1984)*, agitation *(Mavroudis et al., 1998)*, food matrix to solution ratio *(Vijayanand et al., 1995)* and raw material characteristics *(Mavroudis et al.,1998)* on OD of fruits, vegetables, meat and fish have been investigated. Investigations on factors affecting OD of a specific food product provides valuable information on the most important processing variables and their levels prior to optimization studies *(Ozen et al., 2002)*.

Statistical designs are powerful tools that can be used to account for the main as well as the interactive influences of different process parameters on the OD process. The OD requires that, it should be optimized with the purpose of diminishing costs and undesired effects in the resulting product. There are many optimization methods such as conventional graph method *(Wanasundara et al., 2002; Mettler and Seibel, 1993; Gurmail et al., 1990; Ylimaki et al., 1988)*, the improved graph method *(Garrote et al., 1993; Fichtati et al., 1990; Floros and Chinnan, 1988)*, the desired function method and the procedure of extended surface *(Guillou and Floros, 1993)* are successfully applied in optimization process.

Response surface methodology (RSM) is a useful statistical tool for industrial applications to optimize reaction conditions, an effective statistical tool and widely used in process optimization, which includes experimental design, model fitting, validation and condition optimization *(Cornell, 2002)*. A modeling approach based on an empirical model obtained by RSM and investigation on influence of the ingredients concentrations on a wide range of mathematical parameters has been reported *(Ferreira et al., 2007; Haminiuk et al., 2007; Biazus et al., 2005; Higuti et al., 2004)*. RSM presupposes the use of an experimental design to investigate the functional form of the process or system which involves one or more response variables that are influenced by various factors, or independent variables. The main advantage of RSM is the reduced number of

experimental runs that provide sufficient information for statistically valid results. RSM is faster and more informative than the classical one-variable-at-a-time approach or the use of full factorial designs. RSM guides, in process optimization by providing the maximum or the minimum of the responses, if they exist, or determining a region in the total space of the factors wherein, certain desirable operating conditions are satisfied *(Myers, 1971)*.

The effect of sugar concentration, temperature and time on the OD of apples using RSM was studied by Quintero-Ramos *et al.,* (1993). Vijayanand *et al.,* (1995) followed the same statistical approach to optimize the processing conditions of OD of cauliflower. Corzo and Gomez (2004) optimized the OD of cantaloupe using the desired function methodology of response surface optimization. The present chapter details the effect of operating conditions like vacuum pressure, temperature, vacuum treatment time and atmospheric restoration time on the uptake of fructooligosaccharides (FOS) and the texture (shear strength) of banana slices during VOD.

3.2. Materials and methods

3.2.1. Preparation of FOS syrup

The detailed protocol for the preparation of FOS syrup is given in the section 2.2.1.

3.2.2. VOD of banana slices with FOS

Bananas (*Musa acuminate* Colla) were purchased from a local super market (Mysore, Karnataka, India). Fruits of uniform maturity from the same bunch were peeled and cut into slices of 6 mm thickness. The selection of banana in the present study is based on the previous observation (Chapter 2) on FOS uptake. The study mainly focused on the maximum uptake of FOS in banana. The average moisture content of the banana was found to be 65 to 67±2% (wet basis). A known quantity (100 g) of banana slices, immersed in FOS syrup (70 ^{o}Brix; 500 ml) in a glass bowl was placed in the vacuum oven (Vacuum oven, Model No. 132 VG, M/S. Industrial and Lab Tools Corporation, Chennai, India) as per the experiment design. Detailed sample preparation is given in

section 2.2.2. Experiments were randomized in order to maximize the effects of unexplained variability in the observed responses due to extraneous factors.

3.2.3. Texture (shear strength) and FOS content of banana slices

The measurement of shear strength and estimation of FOS content of the banana slices are given in section 2.2.3.3 and 2.2.3.5 respectively.

3.2.4. Experimental design and statistical analysis

The RSM was used to estimate the main effects of process variables on uptake of FOS (g/100g) and shear strength (N) of the banana slices during VOD. Vacuum pressure (x_1), temperature (x_2), vacuum treatment time (x_3) and atmospheric restoration time (x_4) were selected as independent variables based on the preliminary trials to fix the experimental domain.

A Central Composite Rotatable Design (CCRD) was used to examine the response pattern and to determine the optimum synergy of the variables *(Cochron and Cox, 1957)*. The design included 31 experiments and was adopted by adding 7 (n_o) central points and 8 (λ=2) axial points to 2^4 full factorial design. The centre runs provided a means for estimating the experimental error and a measure of lack of fit. The axial points were added to the factorial design to provide for estimation of curvature of the mode. The four process variables such as vacuum pressure (250-650 mm Hg), temperature (30-50 ^oC), vacuum treatment time (5-55 min) and atmospheric restoration time (5-30 min), at five levels with 7 replicates at the center for the VOD of banana slices are given in Table 3.1. Treatment schedule for the chosen CCRD is shown in Table 3.2. Experiments were randomized to maximize the effects of unexplained variability in the responses because of extraneous factors and influences.

Table 3.1 Different process variables in coded and un-coded terms chosen for the Central Composite Rotatable Design (CCRD)

Independent variable	Range and levels					Mean	Standard deviation
	-2	-1	0	+1	+2		
Vacuum pressure (mm Hg)	250	350	450	550	650	450	87.99
Temperature (^{o}C)	30	35	40	45	50	40	4.40
Vacuum treatment time (min)	15	25	35	45	55	35	8.80
Restoration time (min)	10	15	20	25	30	20	7.91

A second-order polynomial equation was adapted to fit the experimental data given in Table 3.2. Two models of the following form were developed to relate to the two responses (Y) such as FOS uptake and shear strength of product to the four process variables (x):

$$Y_k = B_{k0} + \sum_{i=1}^{4} B_{ki}\, x_i - \sum_{i=1}^{4} B_{kii}\, x_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} B_{kij}\, x_i\, x_j$$

Where Y_k= response variable (Y_1= uptake of FOS; Y_2= shear strength) and x_i represent the coded independent variables (x_1= vacuum pressure, x_2= temperature, x_3= vacuum treatment time and x_4 = restoration time) and B_{ko} is the value of the fitted response at the center point of the design i.e., at point (0,0,0), $B_{ki,}$ B_{kii} and B_{kij} are the linear, quadratic and cross-product regression coefficients respectively.

Table 3.2 Central composite rotatable design (CCRD) with experimental values of response variables

Run order	x_1: Vacuum pressure (mm Hg)	x_2: Temperature (^oC)	x_3: Vacuum time (min)	x_4: Restoration time (min)	Y_1: FOS uptake (g/100g)	Y_2: Shear strength (N)
1	350 (-1)	35 (-1)	25 (-1)	15 (-1)	7.50	22.70
2	550 (+1)	45 (+1)	45 (+1)	25 (+1)	10.03	17.70
3	450 (0)	40 (0)	35 (0)	30 (+2)	6.91	20.31
4	350 (-1)	35 (-1)	45 (+1)	25 (+1)	7.56	12.50
5	450 (0)	30 (-2)	35 (0)	20 (0)	5.71	22.45
6	350 (-1)	45 (+1)	25 (-1)	15 (-1)	5.76	18.70
7	450 (0)	40 (0)	35 (0)	20 (0)	6.42	14.70
8	550 (+1)	45 (+1)	45 (+1)	15 (-1)	9.61	12.80
9	550 (+1)	35 (-1)	25 (-1)	25 (+1)	7.08	20.65
10	450 (0)	40 (0)	55 (+2)	20 (0)	10.00	11.20
11	450 (0)	40 (0)	35 (0)	20 (0)	6.23	16.90
12	450 (0)	40 (0)	35 (0)	20 (0)	6.15	17.00
13	450 (0)	50 (+2)	35 (0)	20 (0)	7.27	22.89
14	350 (-1)	45 (+1)	45 (+1)	25 (+1)	8.54	17.85
15	450 (0)	40 (0)	35 (0)	10 (-2)	6.50	20.98
16	550 (+1)	45 (+1)	25 (-1)	15 (-1)	6.91	16.15
17	550 (+1)	45 (+1)	25 (-1)	25 (+1)	7.37	23.50
18	450 (0)	40 (0)	35 (0)	20 (0)	6.03	16.30
19	350 (-1)	35 (-1)	25 (-1)	25 (+1)	7.24	17.90
20	550 (+1)	35 (-1)	45 (+1)	25 (+1)	6.86	16.00
21	650 (+2)	40 (0)	35 (0)	20 (0)	7.49	23.40
22	450 (0)	40 (0)	35 (0)	20 (0)	6.46	15.00
23	350 (-1)	45 (+1)	25 (-1)	25 (+1)	5.93	29.76
24	550 (+1)	35 (-1)	45 (+1)	15 (-1)	6.66	22.55
25	250 (-2)	40 (0)	35 (0)	20 (0)	7.04	22.50
26	350 (-1)	45 (+1)	45 (+1)	15 (-1)	8.57	14.15
27	550 (+1)	35 (-1)	25 (-1)	15 (-1)	6.76	20.35
28	450 (0)	40 (0)	35 (0)	20 (0)	6.41	10.80
29	450 (0)	40 (0)	35 (0)	20 (0)	6.56	10.80
30	350 (-1)	35 (-1)	45 (+1)	15 (-1)	7.76	20.00
31	450 (0)	40 (0)	15 (-2)	20 (0)	7.91	17.90

Mathematical models were evaluated for each response by means of multiple regression analysis. Modeling was started with a quadratic model including linear, squared and interaction terms. The significant terms in the model were found by analysis of variance (ANOVA) for each response. Significance was judged by determining the probability level that the F-static (F-value) calculated from the data for which the index was less than 5%. Model adequacies were checked by R^2, adjusted-R^2, predicted-R^2 and prediction error sum of squares (PRESS) *(Myers and Montgomery, 1995)*. The model with comparable adjusted R^2 and predicted R^2, a low PRESS and high adequate precision values was selected. Residual analysis was conducted to validate the assumptions used in the ANOVA. This included calculating case statistics to identify outliers and examining diagnostic plots such as normal probability plots and residual plots.

To deduce optimum process conditions, response surface and contour plots were generated for different interaction of any two independent variables, while holding the value of the third variable as constant (at the central value). Optimization of the process was aimed at finding the levels of independent variables, *viz*., vacuum pressure, temperature, vacuum treatment time and restoration time which would give maximum possible FOS uptake and minimum shear strength of the product. RSM was applied to the experimental data using commercial statistical package, Design-Expert Version 8.0.3.1 (Trial Version, Stat-Ease Inc., Minneapolis, MN). The same software was used for the generation of response surface plots, superimposition of contour plots and optimization of process variables. Maximization and minimization of the polynomials thus fitted was performed by desirability function method.

3.2.5. Optimization

During optimization of the VOD process, response variables describing the measures of the systems and the quality characteristics are to be optimized in conjunction. One of the responses (FOS uptake) is to be maximized while the other (shear strength) is to be minimized. This could complicate the process of optimization of the several possible approaches like constrained optimization, superimposition of contour diagrams of response variables etc. A desirability function that combines all the

responses into one measurement was developed for the criteria of maximizing FOS uptake and minimizing shear strength of the banana slices.

3.2.6. Statistical analysis

Statistical analysis was carried out using a one-way analysis of variance (ANOVA).

3.3. Results and discussion

3.3.1. Fitting Models

Experimental data (Table 3.2) were used to assess the effect of process variables on the two responses by fitting suitable models. ANOVA was conducted to determine the adequacy of the model and significance of process variables on each response. The model for FOS uptake was found to have a large R^2 value of 0.985 and adjusted and predicted R^2 value of 0.972 and 0.938 respectively. The model for texture (shear strength) was found to have a large R^2 value of 0.891 and a reasonably good adjusted and predicted R^2 value of 0.795 and 0.671 respectively. Adequate precision value is a measure of signal to noise ratio. A ratio greater than 4 is desirable. The precision value for FOS and texture was found to be 34.221 and 13.476 respectively, which indicated adequate signal. The models were highly significant (P<0.0001) and the lack of fit was insignificant (0.514 and 0.926 for FOS uptake and texture respectively) which implied that the models adequately represented the relationship between responses and factors.

Multiple linear regression analysis of the experimental data yielded second order polynomial models for predicting FOS uptake and texture values as assumed at the beginning of the study. Statistical significance of all the main effects, linear, quadratic and interaction effects for each response are shown in Table 3.3.

Table 3.3 ANOVA indicating the variables as linear, quadratic and interaction terms and coefficients for the prediction models

Source		FOS			Texture/Shear strength		
	Df	**Co-efficient**	**Sum of Squares**	***P*-value Prob > F**	**Co-efficient**	**Sum of Squares**	***P*-value Prob > F**
Model	14	6.322	38.709	< 0.0001	14.414	525.541	< 0.0001
x_1: Vacuum pressure	1	0.138	0.459	0.0027	-0.086	0.177	0.8367
x_2: Temperature	1	0.351	2.954	< 0.0001	-0.048	0.056	0.9076
x_3: Vacuum treatment time	1	0.634	9.652	< 0.0001	-2.065	102.341	<0.0001
x_4: Atmospheric restoration time	1	0.079	0.150	0.0593	0.297	2.112	0.4795
x_1x_2	1	0.489	3.822	< 0.0001	-1.048	17.556	0.0532
x_1x_3	1	-0.060	0.058	0.2271	0.810	10.498	0.1261
x_1x_4	1	0.107	0.185	0.0388	0.221	0.783	0.6652
x_2x_3	1	0.658	6.917	< 0.0001	-0.941	14.175	0.0791
x_2x_4	1	0.060	0.058	0.2271	2.848	129.732	< 0.0001
x_3x_4	1	-0.019	0.006	0.6998	-1.210	23.426	0.0283
$x_1\wedge^2$	1	0.261	1.953	< 0.0001	1.926	106.031	< 0.0001
$x_2\wedge^2$	1	0.068	0.131	0.0766	1.856	98.461	< 0.0001
$x_3\wedge^2$	1	0.683	13.37	< 0.0001	-0.174	0.870	0.6485
$x_4\wedge^2$	1	0.121	0.421	0.0037	1.349	52.065	0.0024
Residual	16		0.584			64.475	
Lack of Fit	10		0.367	0.5143		24.127	0.9266
Pure Error	6		0.216			40.349	
Cor Total	30		39.294			590.016	
R^2		0.985			0.891		
Adj R^2		0.972			0.795		
Predi R^2		0.938			0.671		
PRESS		2.412			193.888		
CV%		2.653			10.999		

Values of "Prob > F" less than 0.05 indicate model terms that are significant. In this case x_1, x_2, x_3, x_4, x_1x_2, x_1x_4, x_2x_3, x_1^2, x_3^2, x_4^2 and x_3, x_1x_2, x_2x_4, x_3x_4, x_1^2, x_2^2, x_4^2 were found to be the significant model terms for FOS uptake and shear strength respectively. The insignificant effects were also carried forward for modeling, as it was found that some of the insignificant terms for one response were significant for the other. ANOVA showed that the lack of fit was not significant for the response surface models at 95% confidence level. The experimental results were analyzed through RSM to obtain an empirical model for the best response. The mathematical expression of relationship to the response with variables (coded factors) is shown below:

$$Y_1 = 6.323 + 0.138 * x_1 + 0.351 * x_2 + 0.634 * x_3 + 0.079 * x_4 + 0.489 * x_1 * x_2 - 0.06 * x_1 * x_3 + 0.108 * x_1 * x_4 + 0.658 * x_2 * x_3 + 0.06 * x_2 * x_4 - 0.019 * x_3 * x_4 + 0.261 * x_1^2 + 0.0676 * x_2^2 + 0.684 * x_3^2 + 0.121 * x_4^2$$

$$Y_2 = 14.414 - 0.086 * x_1 - 0.048 * x_2 - 2.065 * x_3 + 0.297 * x_4 - 1.048 * x_1 * x_2 + 0.81 * x_1 * x_3 + 0.221 * x_1 * x_4 - 0.941 * x_2 * x_3 + 2.848 * x_2 * x_4 - 1.21 * x_3 * x_4 + 1.926 * x_1^2 + 1.856 * x_2^2 - 0.174 * x_3^2 + 1.349 * x_4^2$$

Where Y_1 and Y_2 represent FOS uptake (g/100g) and texture (shear strength, N) respectively, and x_1, x_2, x_3, and x_4 are the coded values of the test variables, vacuum pressure (mm Hg), temperature (^oC), vacuum treatment time (min), and atmospheric restoration time (min) respectively.

3.3.2. Response surface and contour plots

The FOS uptake of the banana slices varied from 5.71 to 10.03 g/100g and the shear strength, an indicator of the texture of the banana slices varied from 10.80 to 29.76 N in the experimental domain depending upon the process conditions. To visualize the combined effects of the two factors on the response, response surface and contour plots were generated for each of the fitted models as a function of two variables, keeping the other two variables (central values) constant. The response surface curves were plotted to determine the optimum level of each variable for maximum response. The response surface curves for FOS uptake are shown in Fig 3.1, 3.2, 3.3, 3.4, 3.5 and 3.6. The response surface curves for shear strength are shown in Fig 3.7, 3.8, 3.9, 3.10, 3.11 and 3.12.

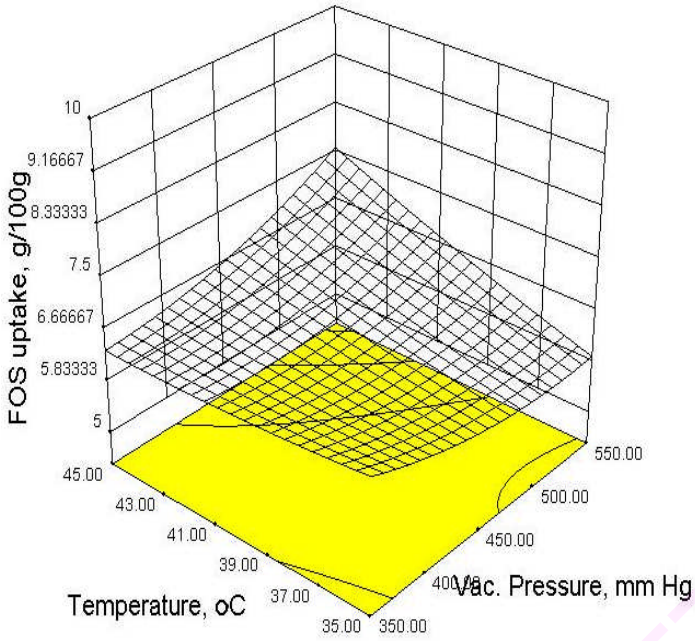

Fig 3.1 Response surface plots for FOS uptake in banana slices as a function of temperature and vacuum pressure

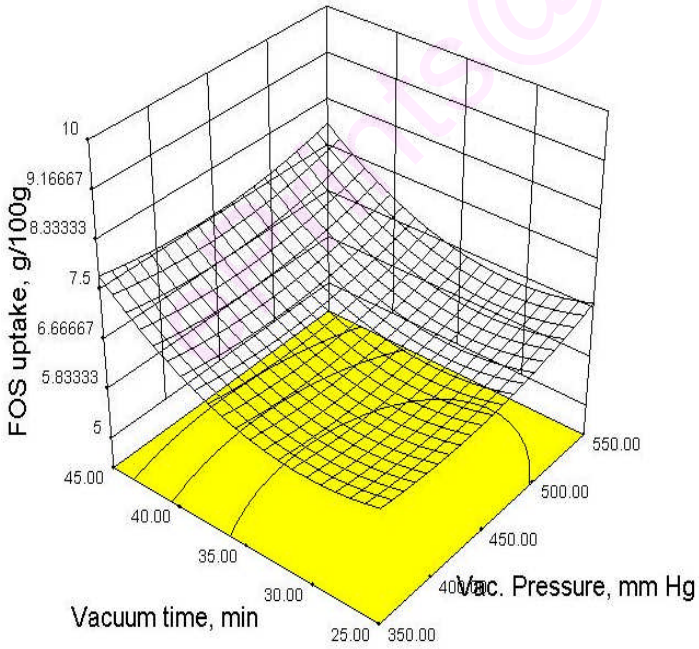

Fig 3.2 Response surface plots for FOS uptake in banana slices as a function of vacuum treatment time and vacuum pressure

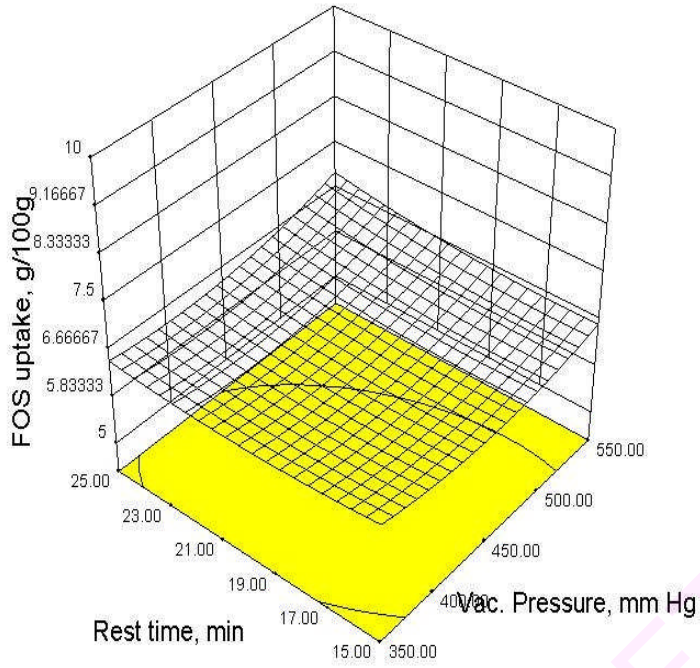

Fig 3.3 Response surface plots for FOS uptake in banana slices as a function of atmospheric restoration time and vacuum pressure

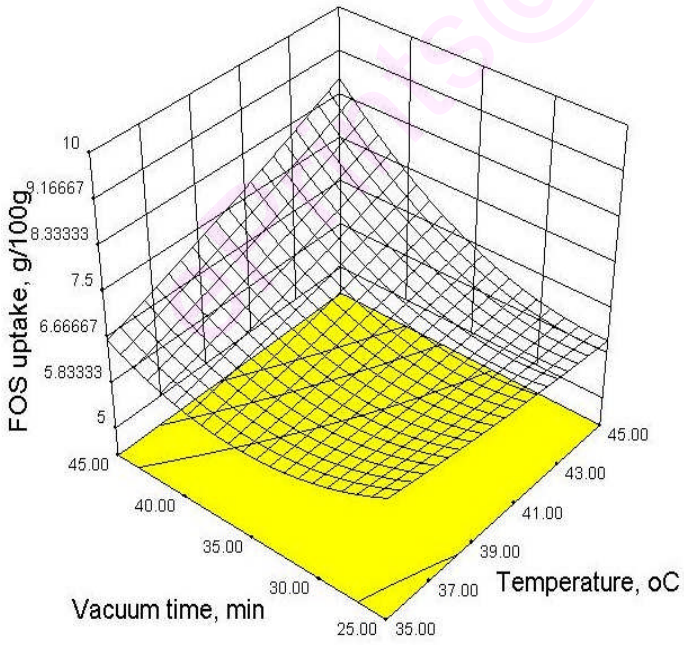

Fig 3.4 Response surface plots for FOS uptake in banana slices as a function of vacuum treatment time and temperature

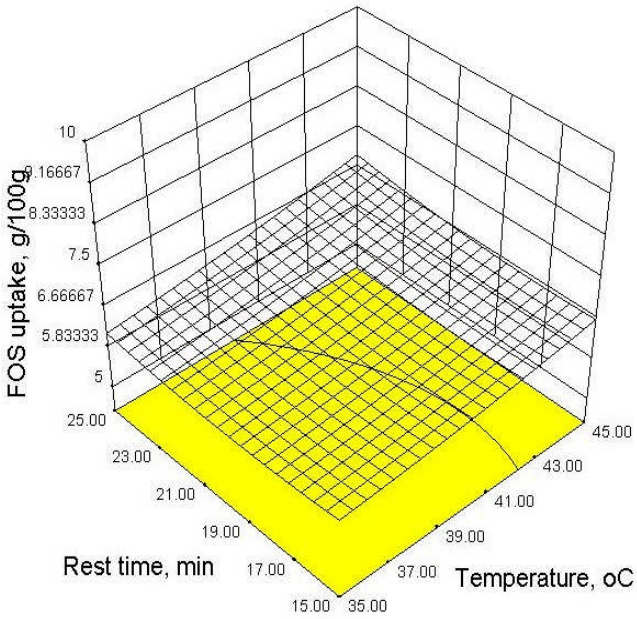

Fig 3.5 Response surface plots for FOS uptake in banana slices as a function of atmospheric restoration time and temperature

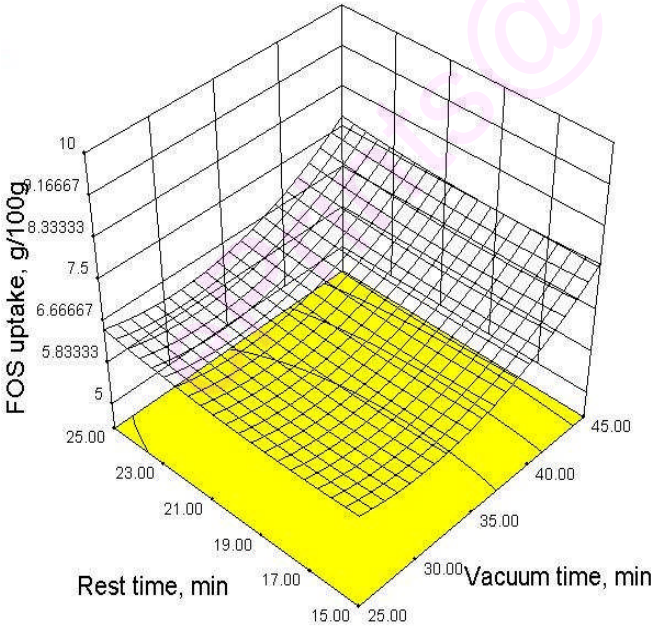

Fig 3.6 Response surface plots for FOS uptake in banana slices as a function of atmospheric restoration time and vacuum treatment time

The response surface curves, Fig 3.1 to Fig 3.6 indicate the interaction between the variables and FOS uptake. Fig 3.1 indicates that at lower temperature, vacuum pressure has a negative effect on uptake of FOS, where as the trend reverses at higher temperature. Also, at lower pressures increase in temperature is less significant where as at higher pressure FOS uptake increases with temperature. Fig 3.2 indicates that high pressure and longer vacuum treatment time result in better FOS uptake. However, the uptake beyond 35 min of treatment time is responsible for higher uptake as indicated by a steeper gradient. Fig 3.3 shows a similar effect of high temperature and higher vacuum treatment time resulting in better uptake of FOS. The effect of atmospheric restoration time with respect to vacuum pressure, vacuum treatment time and temperature are brought about in Fig 3.4, 3.5 and 3.6. For any restoration period, application of higher vacuum pressure aids in better FOS uptake even though at any given pressure, the atmospheric restoration time did not appreciably increase the uptake. Vacuum treatment time beyond 35 min result in higher uptake. Higher temperature results in better uptake for any given duration of the restoration period and for any given temperature, atmospheric restoration time did not significantly increase the uptake.

The response surface curves, Fig 3.7 to Fig 3.12 indicate the interaction between the variables and shear strength. Fig 3.7 shows that lower shear strength of the banana slice favors medium temperature and vacuum pressure. An increase or decrease in the values of temperature and pressure adversely affect the texture of the banana slices by increasing its shear strength. Fig 3.8 shows that in the longer vacuum treatment time and medium pressure range of 450 to 500 mm of Hg is preferable to get a product of lower shear strength. Fig 3.9 indicates that, vacuum treatment time has a significant effect on the shear strength of the banana slices. Longer vacuum treatment time has resulted in banana slice of lower shear strength across the temperature domain. The effect of atmospheric restoration time with respect to vacuum pressure, vacuum treatment time and temperature are brought about in Fig 3.10, 3.11 and 3.12. Vacuum pressure in the range of 450 to 500 mm Hg results in banana slices of lower shear strength compared to higher or lower pressures. Longer vacuum treatment time results in a banana slice of better texture (low shear strength) for any given period of atmospheric restoration time. High

temperature and longer atmospheric restoration time is not preferred as it results in a banana slice of very high shear strength.

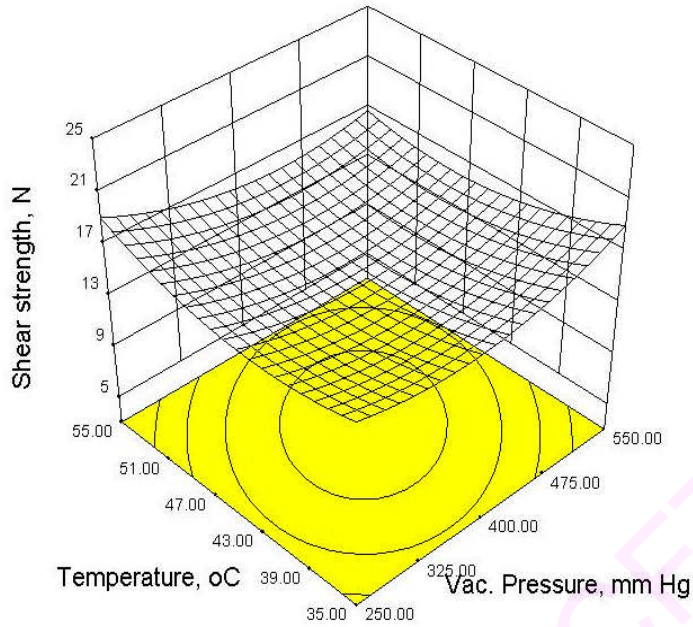

Fig 3.7 Response surface plots for shear strength of banana slices as a function of temperature and vacuum pressure

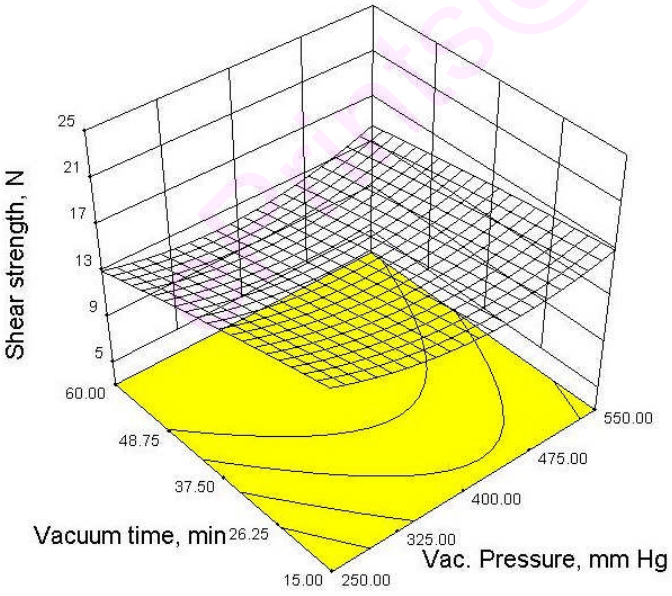

Fig 3.8 Response surface plots for shear strength of banana slices as a function of vacuum treatment time and vacuum pressure

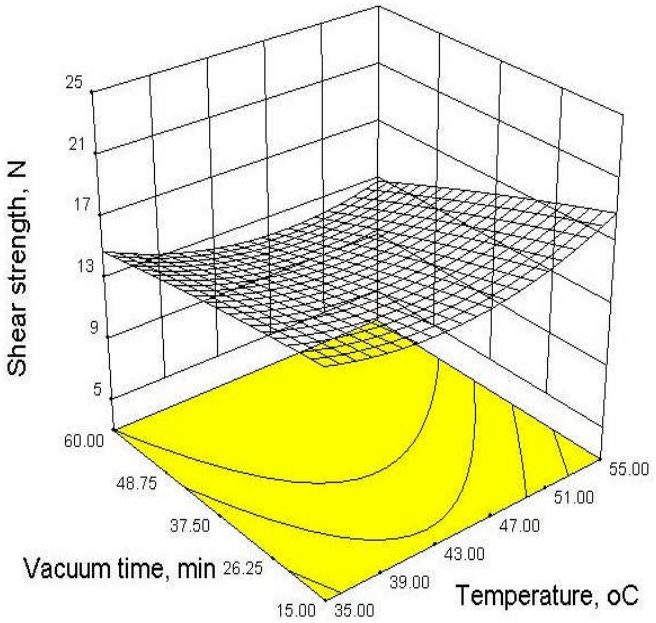

Fig 3.9 Response surface plots for shear strength of banana slices as a function of vacuum treatment time and temperature

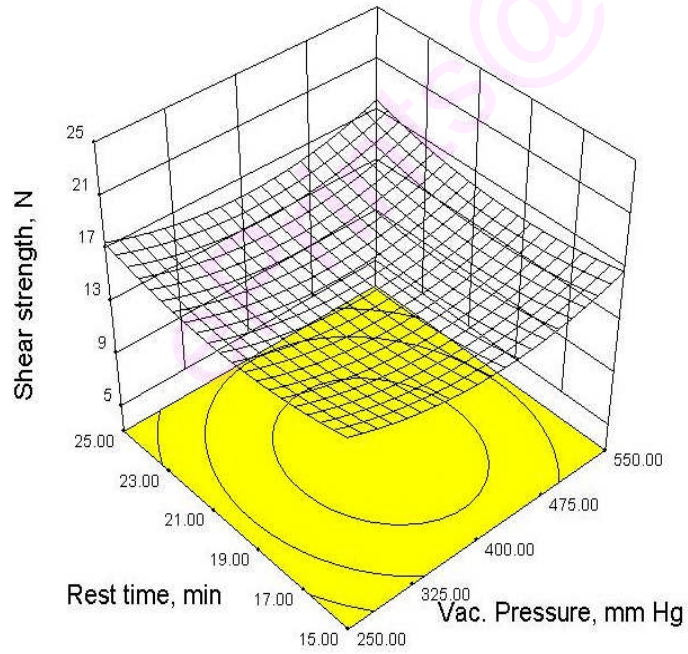

Fig 3.10 Response surface plots for shear strength of banana slices as a function of atmospheric restoration time and vacuum pressure

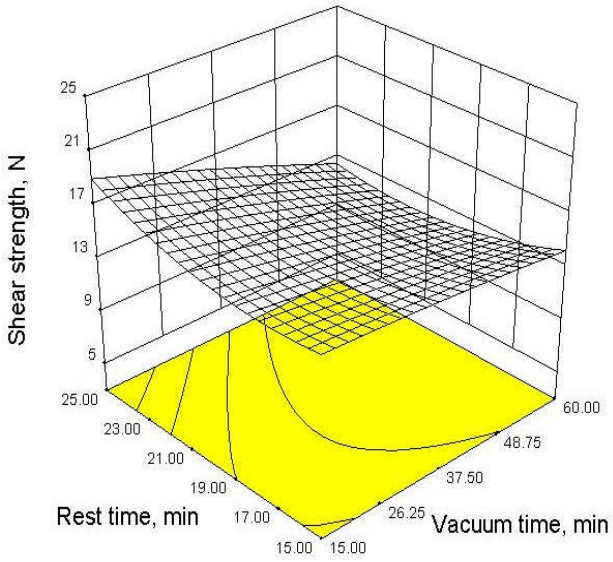

Fig 3.11 Response surface plots for shear strength of banana slices as a function of atmospheric restoration time and vacuum treatment time

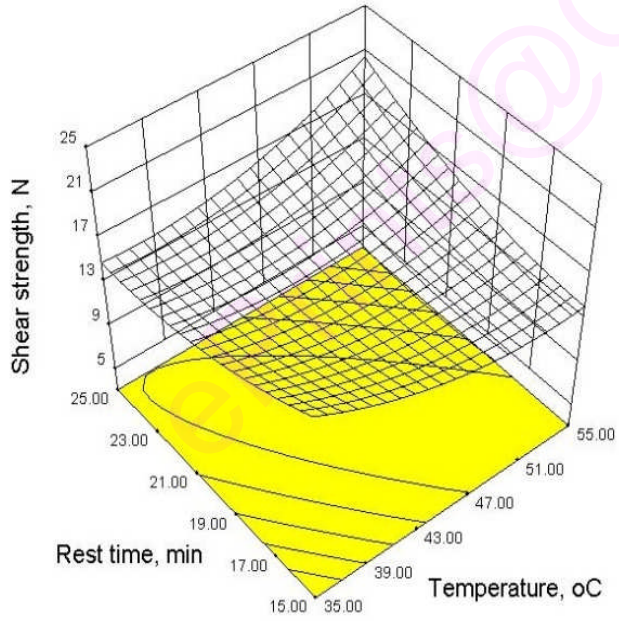

Fig 3.12 Response surface plots for shear strength of banana slices as a function of atmospheric restoration time and temperature

In view of these conflicting and competing kind of influences of process variables on the responses, optimization was based on a desirability function. The desirability was to maximize the FOS content in the final product while minimizing the shear strength, from the point of sensorial acceptability.

3.3.3. Optimization

Optimum condition for VOD of banana slices was determined to obtain maximum FOS uptake and minimum shear strength. Second order polynomial models obtained in this study were used for each response in order to determine the specified optimum conditions. In this study, vacuum pressure, temperature, vacuum treatment time and atmospheric restoration time were selected in the range of 250-650 mm Hg, 30-50 ^{o}C, 15-55 min and 10-30 min respectively.

Table 3.4 shows results from validation experiments to be in good agreement with predicted values. By applying desirability function method, three solutions were obtained for the optimum covering the criteria. The first one is 550 mm Hg for vacuum pressure, 45 ^{o}C for temperature, 45 min for vacuum treatment time and 16.44 min for atmospheric restoration time. The second is 542.07 mm Hg for vacuum pressure, 45 ^{o}C for temperature, 45 min for vacuum treatment time and 20.56 min for atmospheric restoration time. The third one is 500 mm Hg for vacuum pressure, 45 ^{o}C for temperature, 44.99 min for vacuum treatment time and 15 min for atmospheric restoration time. The results indicated that the desirability value for the first solution was greater than the second and third solution. So the factor level obtained at the first solution was selected as the optimum.

The suitability of the model equations for predicting optimum responses at the optimum conditions of 550 mm Hg for vacuum pressure, 45 ^{o}C for temperature, 45 min for vacuum treatment time and 16.44 min for atmospheric restoration time of processing time would result in FOS uptake of 9.64 g/100g and shear strength of 13.42 N. These results were compared to the calculated FOS uptake (9.44 g/100g) and shear strength (13.79 N). Experimentally determined values for FOS uptake and shear strength of the banana slices under the optimum conditions were very close to the predicted values (Table 3.4). Calculated values were within the experimental limits; therefore, the fitted

models allow the prediction of the FOS uptake and decreased shear strength in banana slices by VOD within the studied experimental conditions. It should be considered that the final decision of the optimal conditions depends on the cost, the product quality and the consumer expectations.

Table 3.4 Experimental conditions and observed response values of validation trials

Experiment No.	Vacuum pressure (mm Hg)	Temperature (^{o}C)	Vacuum time (min)	Restoration time (min)	Predicted		Experimental	
					FOS (g/100g)	Shear strength (N)	FOS (g/100g)	Shear strength (N)
1	550.00	45.00	45.00	16.44	9.44	13.79	9.64	13.42
2	542.00	45.00	45.00	20.56	9.48	14.63	9.45	14.40
3	500.00	45.00	44.99	15.00	9.01	12.66	9.16	14.25

RSM and graphical optimization methods were effective in locating optimum processing conditions for VOD of banana slices. RSM has been successfully applied to optimize food processing operations. It can be used in solving problems that concern ingredients and/or processing conditions as variables. The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple factors and their interactions. Also, the study of the individual and interactive effects of these factors will be helpful in effort to find the target value. Therefore, RSM provides an effective tool for investigating the aspects affecting the desired response if there are many factors and interactions in the experiment. To optimize the process, RSM can be employed to determine a suitable polynomial equation for describing the response surface *(Yin et al., 2009; Deshpande et al., 2008)*. RSM used in the present study determining the optimum operating conditions that yield maximum FOS uptake (9.64 g/100g) and minimum shear strength (13.79 N) in VOD of banana slices. The optimum conditions obtained are only for banana fruit and the conditions obtained may or may not be suitable for other fruits. The FOS content of other fruits studied is well within the expected result. Therefore, the optimization of the process parameters for the maximum uptake of FOS was carried only for banana. Analysis of variances showed that the effects of all the process variables were statistically significant for FOS uptake. Second order polynomial models were obtained for predicting FOS uptake and shear strength of banana slices. This methodology could therefore be successfully employed to study the importance of individual, cumulative and interactive effects of the test variables in vacuum osmotic processes.

CHAPTER: 4; SECTION: 4.1

FRUCTOOLIGOSACCHARIDES FORTIFIED FRUIT JUICE BEVERAGES

4.1.1. Introduction

Fruits are one of the five major food groups in food pyramid. India is one of the largest and most varied fruit producing nations in the world, accounting for about 10.4% of all fruits and nearly 40% of tropical fruits produced globally *(FAO)*. The major fruit growing states include, Uttar Pradesh, Karnataka, Tamil Nadu, Maharashtra, and Gujarat. The major tropical fruits grown in India include banana, mango, guava, pineapple, papaya, lychee, with lesser product of sapota, jackfruit, phalsa, annona and ber. The 3 major tropical fruits (mango, pineapple, and guava) accounted for 33% of total fruit production in 2004.

Indian consumers are becoming increasingly aware of the positive health aspects of fruit consumption. A diversified diet and interest in healthier eating has led to increased consumption of fruits. The consumption of fruits in India is anticipated to increase by 4% per year according to projected growth rates for income, population, and trends in food preferences. This would result in demand for fruits reaching 66 million tonnes by 2015, with the bulk of this demand growth for tropical fruit. India can meet this increased demand by identifying and adopting measures to reduce wastage and improve domestic productivity of fruit. Consumption of processed fruits, juices, is expected to increase rapidly, particularly due to projected income growth. Of the total processed fruit production in the country about 45% is contributed by mango-based products and 15% by pineapple-based products.

Fruit juices are an important source of energy in the form of sugars; glucose, fructose and sucrose being the most abundant in fruit and fruit products. The world trade in fruit juices is said to be highly competitive. The observed growth in the fruit juice market is expected to prevail in the coming years. The statistics has shown that a beverage fruit blend, among others is a growing section of the beverage market. This has attracted the attention of fruit growers, fruit juice distributors and processors to meet the demand and is making a profit over the pulpy juices and fruit juice blends. Fruit juice beverages with neutraceuticals have carved niche place in the fruit juice market *(Stack, 1995)*.

Consumer trends with respect to food choice are changing due to the increasing awareness of the link between diet and health *(Mark-Herbert, 2004)*. The consumption of

foods and beverages containing functional prebiotics and probiotics is a growing global consumer trend *(Verbeke, 2005)*. Consequently, the global functional food market is thriving with recent estimates indicating up to a $50 billion annual share *(Stanton et al., 2005)*.

Fruit based beverages are becoming popular in comparison to synthetic or aerated drinks due to their pleasing flavour and nutritional characteristics. Therefore, there is great scope for preparation of fruit juice and other fruit based beverages as they quench thirst and also help as a source of energy. Ready to serve beverages from tropical fruits such as orange, mango and pineapple are very popular due to their unique color, flavor and consumer preference. The juices, either made from juice concentrates and the corresponding recovery aromas, or made as single strength juices, are annually produced in high amounts. They are sweetened with the natural sweetener sucrose unless specified as a 'lite' beverage in which case they may contain a permitted synthetic sweetener. The nutritional quality of these beverages is improved by supplementing with neutraceutical components.

Fruit juices are generally viewed as an appropriate medium for fortification, because they have an established market sector as functional drinks through sales of calcium and vitamin fortified juices and are consumed regularly. Traditional nutritional ingredients (*e.g.* vitamins, minerals) used to fortify functional foods are widely recognized and accepted by consumers as being healthy *(Breithaupt, 2001)*. Fortification with novel functional ingredients such as probiotics *(Luckow et al., 2006)*, prebiotics like fructooligosaccharides (FOS) is a recent development in this direction. The present chapter details the value addition of selected conventional tropical fruit juices by fortification with FOS followed by analysis of the physicochemical and sensory attributes of these beverages, over a storage period of 6 months.

Preparation of FOS based beverage concentrate *(Ramesh et al., 2004)*, spread *(Prapulla et al., 2002)* and honey-like product *(Sangeetha et al., 2003)* has been successful and the processes have been patented. On similar lines a study has been taken to fortify selected fruit juice beverages.

4.1.2. Materials and Methods

4.1.2.1. Preparation of FOS syrup

The detailed protocol for the preparation of FOS syrup is given in the section 2.2.1.

4.1.2.2. Preparation of fruit juices

Ripe fruits of pineapple (*Ananas comosus* L. Merr), mango (*Mangifera indica*) and orange (*Citrus sinensis* L.) were procured from the local fruit market (Mysore, Karnataka, India). The fruits were washed, peeled, crushed and passed through a pulper to obtain pulp. In the case of oranges, the fruits were peeled and passed through a screw type juice extractor to obtain orange juice.

4.1.2.3. Preparation of fruit juice beverages with varying ratio of FOS and sucrose

The flow chart for the preparation of FOS fortified fruit juices is given in Fig 4.1.1. An initial experiment to study the effect of fortification of fruit juice beverages with varying ratios of FOS and sucrose (w/w) (0:100, 25:75, 50:50, 75:25 and 100:0) on the sensory qualities was carried out. Sensory attributes like colour, taste and flavour were evaluated by expert panelists (Table 4.1.1).

It was observed that fruit juice beverages fortified with FOS in the ratio of 75:25 was found to be acceptable and hence was chosen for further studies. The composition of the selected fruit juice beverages fortified with FOS in the ratio of 75:25 is given in table 4.1.2. The fruit juice beverages were formulated to a total soluble solid content of 15 °Brix with a titratable acidity of 0.28-0.30 g/100 ml (citric acid). Fruit juice beverages with only sucrose, with no added FOS was used as control.

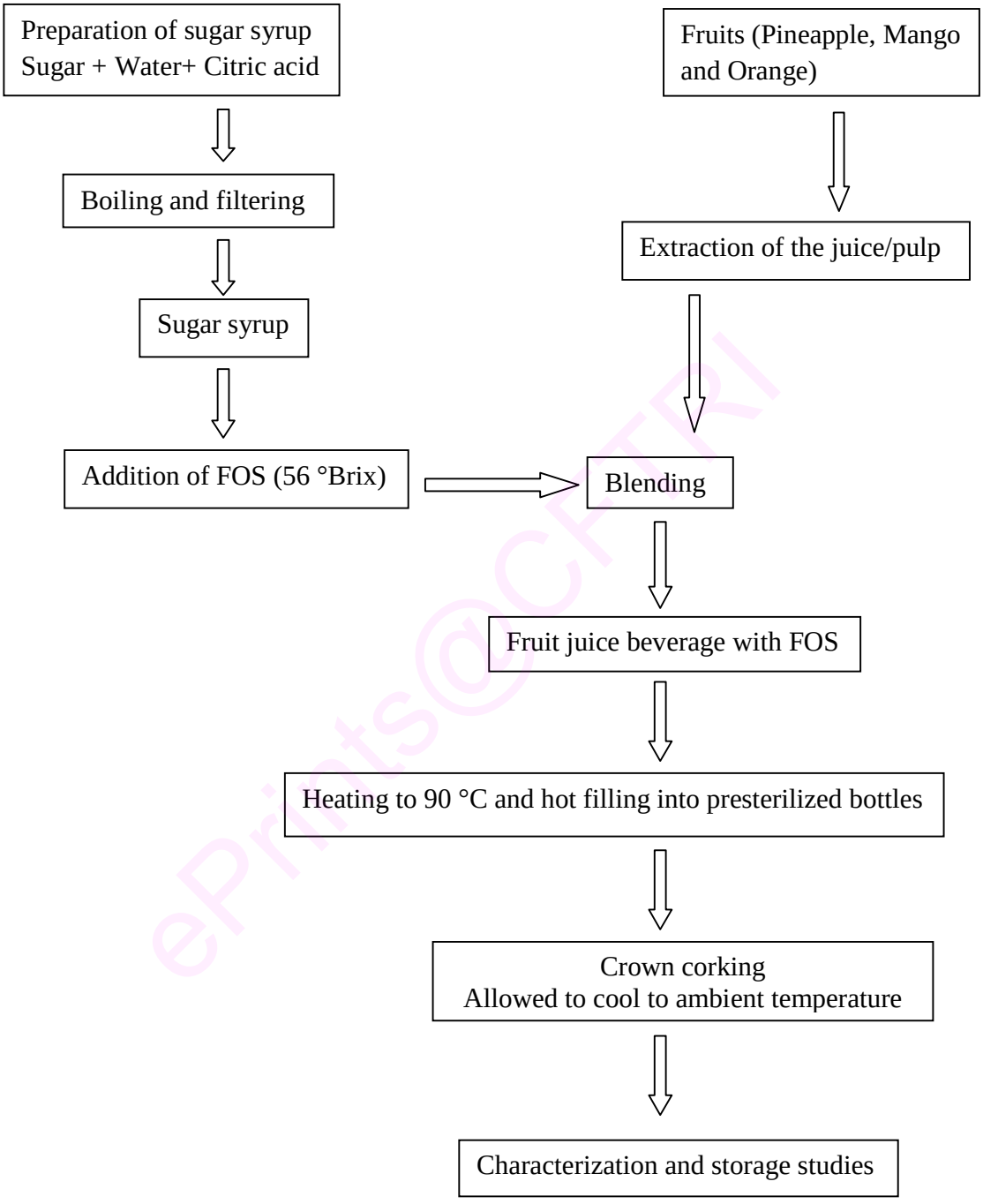

Fig 4.1.1 Flow chart for the preparation of fruit juice beverages

Table 4.1.1 Sensory attributes of fruit juice beverages with varying ratio of FOS and sucrose

FOS: Sucrose (w/w)	Sensory parameters								
	Colour			Taste/Flavor			Overall acceptability*		
	Pineapple	Mango	Orange	Pineapple	Mango	Orange	Pineapple	Mango	Orange
0:100	9.0±0.10	9.0±0.08	9.0±0.09	9.0±0.09	9.0±0.09	9.0±0.10	9.00±0.10	9.00±0.09	9.00±0.10
25:75	8.6±0.11	8.8±0.10	8.5±0.10	8.8±0.13	8.7±0.12	8.6±0.12	8.70±0.12	8.75±0.11	8.75±0.11
50:50	8.0±0.16	8.2±0.11	8.0±0.13	8.5±0.14	8.5±0.15	8.5±0.15	8.25±0.13	8.35±0.13	8.25±0.14
75:25	7.5±0.14	7.8±0.09	7.5±0.11	7.0±0.13	7.2±0.10	7.5±0.10	7.25±0.12	7.50±0.10	7.50±0.10
100:0	5.5±0.10	5.7±0.15	5.5±0.12	5.0±0.15	5.0±0.09	5.5±0.13	5.25±0.11	5.35±0.12	5.50±0.11

* Minimum acceptable score is 7.0

Table 4.1.2 Composition of fruit juice beverages fortified with FOS in the ratio of 75: 25

Components	Pineapple	Mango	Orange
Fruit pulp / Juice (g)	200.00	200.00	200.00
Sucrose (g)*	13.50	3.50	19.50
FOS syrup (g)	200.00	200.00	200.00
Citric acid (g)	1.80	1.80	1.80
Water (g)	584.70	594.70	578.50
Total quantity of fruit juice (g)	1,000.00	1,000.00	1,000.00

* FOS to sucrose ratio is 75:25 (w/w). Addition of sucrose to the fruit juice beverage was based on TSS of fruit juice.

4.1.2.4. Characterization and storage studies of fruit juice beverages

Pasteurized fruit juices were stored at ambient (25±2 °C) and refrigeration (4±2 °C) temperature for 6 months and were analyzed for colour, viscosity, total soluble solids (TSS), titratable acidity (TA), pH changes in the FOS content and sensory attributes at regular intervals of 2 months.

4.1.2.4.1. Colour

The colour of fortified fruit juices was measured in a Hunter Lab colour measuring system (Lab scan XE, Hunter Ass. Lab., Virginia, USA) as described in section 2.2.3.2. Colour values represented are an average of three readings.

4.1.2.4.2. Viscosity

Viscosity was measured using Rheology viscometer at 75 rpm with spindle No.3 (Rheology International Shannon Ltd, Viscometer RI: 3: M, Shannon Ireland).

4.1.2.4.3. TSS and titratable acidity

TSS of the fruit juices fortified with FOS was measured using hand Refractometer (Erma, Japan). The titratable acidity was estimated by AOAC (2006) method and expressed as "g citric acid/100 ml juice".

4.1.2.4.4. pH

The pH of the fruit juices was determined using a control dynamics digital pH meter (Model: APX 175 E/C) at room temperature (25±2 °C).

4.1.2.4.5. FOS Content

FOS was estimated by HPLC method *(Sangeetha et al., 2002)*. A known volume of fruit juice fortified with FOS was centrifuged at 8000 rpm for 20 min. The supernatant was filtered through 0.45 µ membrane filter (Millipore India Pvt. Ltd.) and appropriately diluted with triple distilled water and analyzed by HPLC (SCL-6B, Shimadzu, Japan) as described in section 2.2.3.5.

4.1.2.4.6. Sensory evaluation

Sensory evaluation was carried out as described in the section 2.2.3.8. An internal panel of seven expert members evaluated the products for colour, appearance, taste/flavour, mouth feel and overall acceptability.

4.1.2.4.7. Statistical analysis

Statistical analysis was carried out using a one-way analysis of variance (ANOVA), Origin 6.1 Software. Means were separated using the least significant difference test. Significance was defined at $P<0.05$.

4.1.3. Results and discussion

4.1.3.1. Colour

The *L*a*b** values are shown in Fig 4.1.2, Fig 4.1.3, Fig 4.1.4 and Fig 4.1.5. After 6 months of storage, FOS fortified fruit juice beverages stored at refrigeration temperature, exhibited higher *L** values compared to values of freshly prepared beverages (initial). During storage at ambient temperature, a constant decline of *L** values was observed.

As can be seen from the results, there was no significant difference ($P>0.05$) in the colour of the fruit juices fortified with FOS in comparison with that of control. This has an important bearing on the consumer acceptance of the fortified juices, as colour is one of the primary quality characteristics, which appeals to the consumer. Results also indicate that there was no noticeable change ($P>0.05$) in the colour during storage.

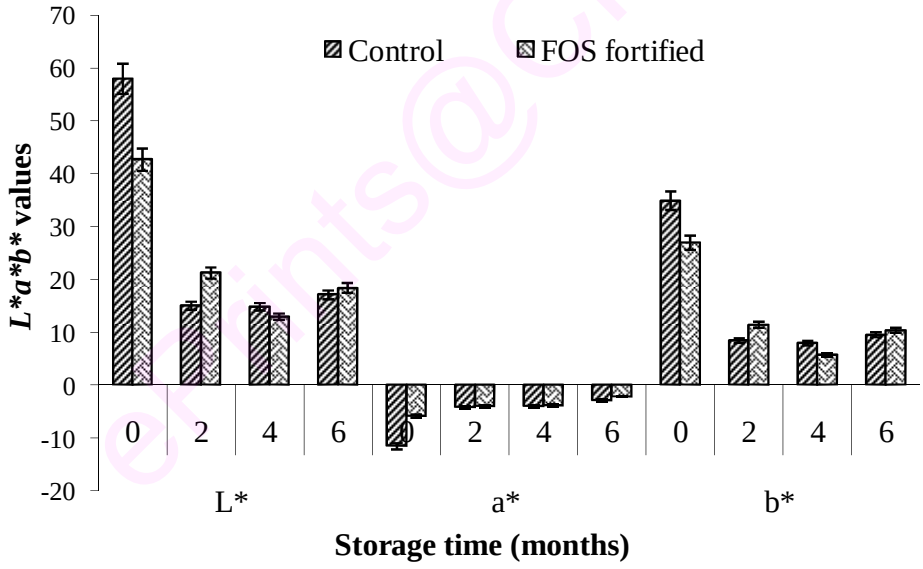

Fig. 4.1.2 Colour (*L*a*b**) values of pineapple juice beverage stored at ambient temperature (25±2 °C)

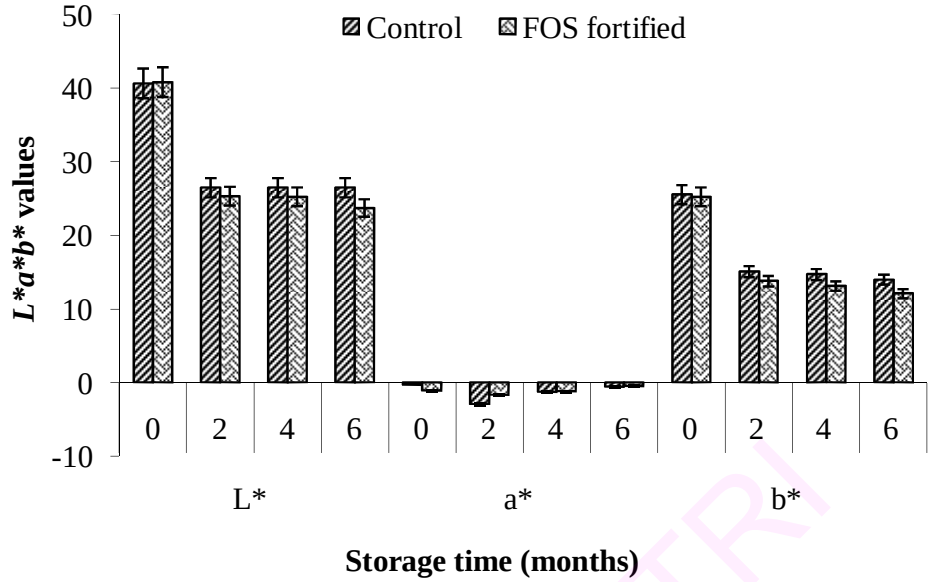

Fig. 4.1.3 Colour (*L*a*b**) values of mango juice beverage stored at ambient temperature (25±2 °C)

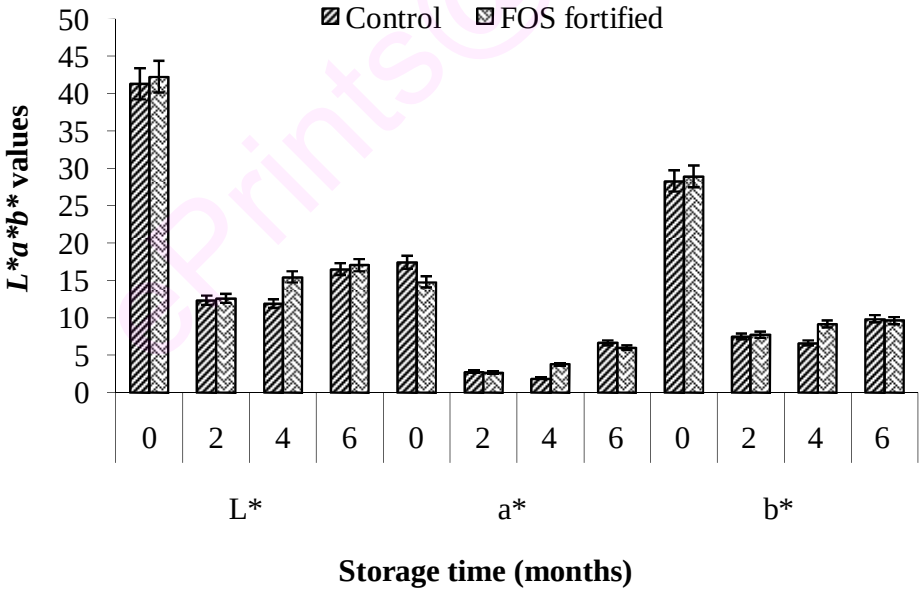

Fig. 4.1.4 Colour (*L*a*b**) values of orange juice beverage stored at ambient temperature (25±2 °C).

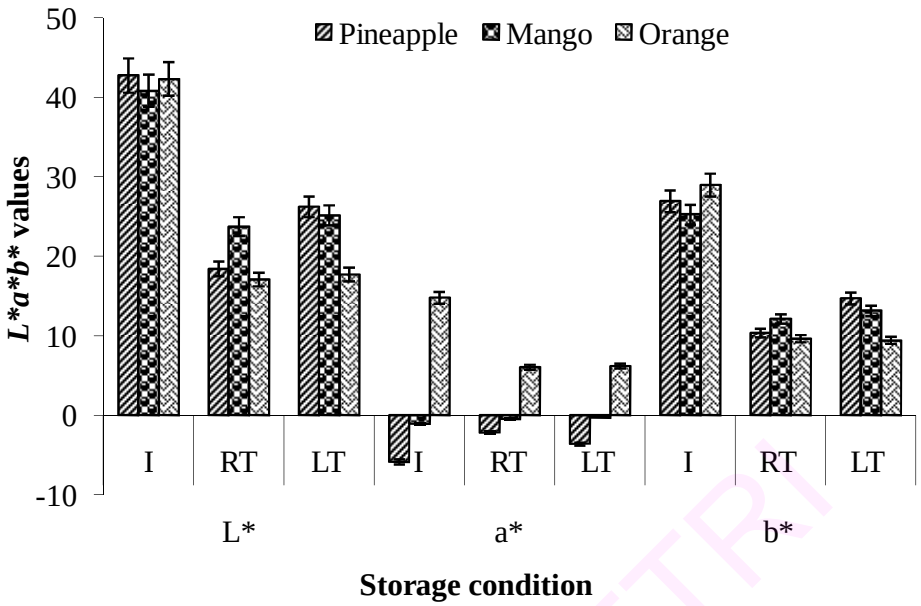

Fig. 4.1.5 Comparison of colour (*L*a*b**) values of fruit juice beverages stored at ambient (25±2 °C) and refrigerated (4±2 °C) temperature.

I: Initial, LT: Low/refrigeration temperature (4±2 °C), RT: Room/ambient temperature (25±2 °C).

4.1.3.2. Viscosity

The viscosity values are presented in Table 4.1.3. The viscosity of pineapple and mango fruit juice beverages fortified with FOS is found to be 25.00 mPas and 23.30 mPas respectively after 6 months of storage at refrigeration temperature. The viscosity of FOS fortified pineapple juice could not be measured after 6 months of storage at ambient temperature. The viscosity of fresh and the stored (4±2 °C and 25±2 °C) orange juice beverages was not in the detectable range.

The viscosity of the juices is an important physical characteristic. It has a bearing on the consumers' appeal and acceptance. A fruit juice beverage generally contains sugars, acids, soluble pectins, proteins, and salts and these form a dispersing phase which determines the viscosity *(Race, 1991)*. The viscosity of pineapple fruit juice beverage, after 6 months of storage at ambient temperature could not be measured, but could be

detected in the pineapple and mango fruit juice beverages stored at refrigerated temperature and there was no significant difference.

Table: 4.1.3 Viscosity of fruit juice beverages stored at ambient (25±2 °C) and refrigerated (4±2 °C) temperature.

Fruits juice beverages	Viscosity (m Pas)		
	Initial	Low/refrigeration temperature (4±2 °C)	Room/ambient temperature (25±2 °C)
PF	25.50	25.00	ND*
MF	24.00	23.30	21.50
OF	ND*	ND*	ND*
PC	25.50	ND*	ND*
MC	25.50	21.80	22.50
OC	ND*	ND*	ND*

PF: FOS fortified Pineapple juice, MF: FOS fortified Mango juice, OF: FOS fortified Orange juice, PC: Pineapple juice with sucrose, MC: Mango juice with sucrose, OC: Orange juice with sucrose (Control)

*ND: Not detectable.

4.1.3.3. Changes in physicochemical characteristics of fruit juice beverages during storage

The changes in physicochemical characteristics of the fruit juice beverages are shown in Fig 4.1.6, Fig 4.1.7 and Fig 4.1.8. As can be seen by ANOVA, the difference in pH (Fig. 4.1.6), TSS (°Brix) (Fig. 4.1.7), and TA (Fig. 4.1.8) were not statistically significant at 5% level between time zero (initial) and 180 days (6 months) of storage at ambient and refrigeration temperature. The pH of all the fruit juice beverages tested ranged between 3.23 and 3.82. TSS of fruit juice beverages varied from 15.00 to 16.20 °Brix. Titratable acidity of fruit juice beverages varied from 0.23 to 0.32 g citric acid/100 ml juice.

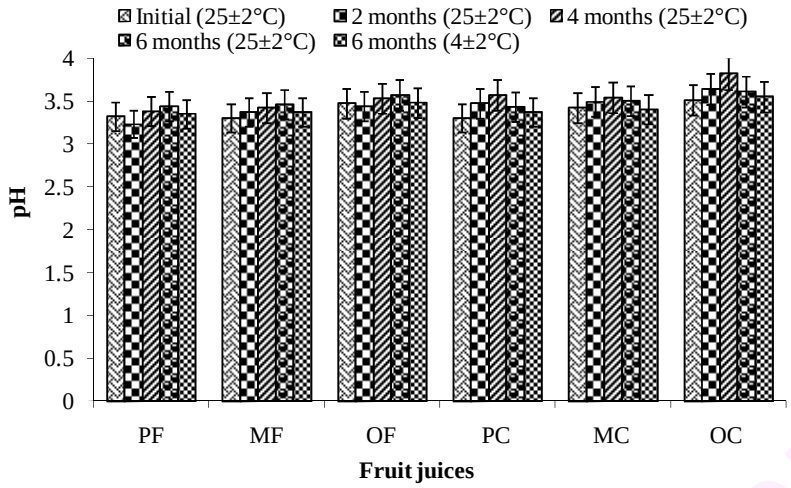

Fig 4.1.6 Changes in the pH of fruit juice beverages during storage

PF: FOS fortified Pineapple juice, MF: FOS fortified Mango juice, OF: FOS fortified Orange juice, PC: Pineapple juice with sucrose, MC: Mango juice with sucrose, OC: Orange juice with sucrose (Control)

The pH of the fruit juice beverages fortified with FOS was in the range of 3.23-3.57 as in the case of sucrose fortified beverages (3.30-3.82). Result shows that during 6 months of storage at ambient and refrigeration temperature, the pH values did not show any significant difference. Similar observations with respect to the changes in pH as a function of storage time and temperature have been made by Kaanane *et al.,* (1988), Martin *et al.,* (1995) and Souci *et al.,* (1987).

TSS (°Brix) of the fruit juice beverage was stable throughout the storage period (4±2 °C and 25±2 °C). The stability of the TSS could be due to the heat treatment (pasteurization) prior to storage. Pasteurization inhibits the growth of microbes especially yeasts which are known to ferment sugar and sugar products such as fruit juice beverages and thus results in sugar reduction and concomitant changes in the TSS (Fig 4.1.7).

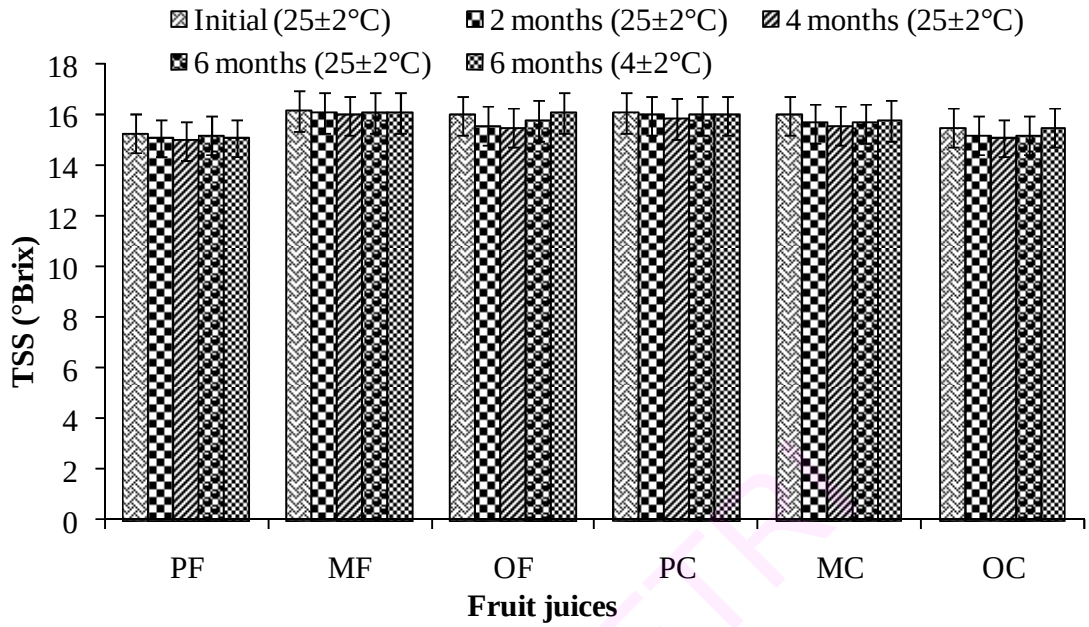

Fig 4.1.7 Changes in the total soluble solids (TSS) of fruit juice beverages during storage PF: FOS fortified Pineapple juice, MF: FOS fortified Mango juice, OF: FOS fortified Orange juice, PC: Pineapple juice with sucrose, MC: Mango juice with sucrose, OC: Orange juice with sucrose (Control)

Organic acids contribute to the particular flavour and palatability of fruit juices. To a large extent, acidity protects against the development of pathogens. Increase in the acidity over storage could be due to the formation of organic acids by the fermentation of sugar by undesired organisms. However, in the present study no significant change in acidity was noticed and the acidity was fairly constant throughout the storage (Fig 4.1.8). This indicates the stability of the fruit juice beverages fortified with FOS over storage. Also, the beverages retained all the desirable sensorial properties, as is the case of fruit juice beverages with sucrose.

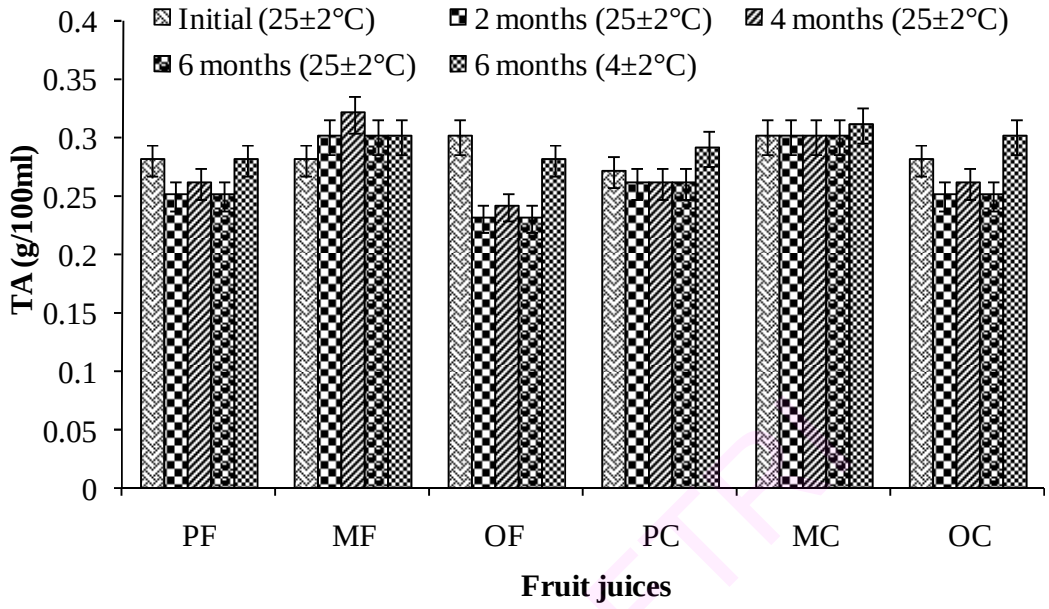

Fig 4.1.8 Changes in the titratable acidity (TA) of fruit juice beverages during storage
PF: FOS fortified Pineapple juice, MF: FOS fortified Mango juice, OF: FOS fortified Orange juice, PC: Pineapple juice with sucrose, MC: Mango juice with sucrose, OC: Orange juice with sucrose (Control)

4.1.3.4. Retention of FOS in the fortified fruit juice beverages

Fig 4.1.9 represents the retention of FOS in the fortified fruit juice beverages as a function of storage time. At the end of the storage (6 months) period, fruit juice beverages stored at refrigeration temperature had retained a good amount of FOS whereas, loss was pronounced in beverages stored at ambient temperature. The fruit juice beverages stored at refrigeration temperature had retained 2.00-2.39 g/100 ml FOS after 6 months of storage whereas retention was found to be only 0.41-0.58 g/100 ml at ambient temperature with 2.69-3.23 g/100 ml and 1.65-2.08 g/100 ml retention recorded during the second and fourth month of storage respectively. Deterioration in the acceptable quality characteristics was noticed only after 4 months storage. Fruit juice beverages in general are fast moving commodity and it generally does not remain unsold for more than 2-4 months. There was a noticeable change in the acceptable quality characteristics only

after 4 months storage at ambient temperature. Thus, the present study clearly indicates that fruit juice beverages can successfully be fortified with FOS with shelf life of 4 months at ambient temperature.

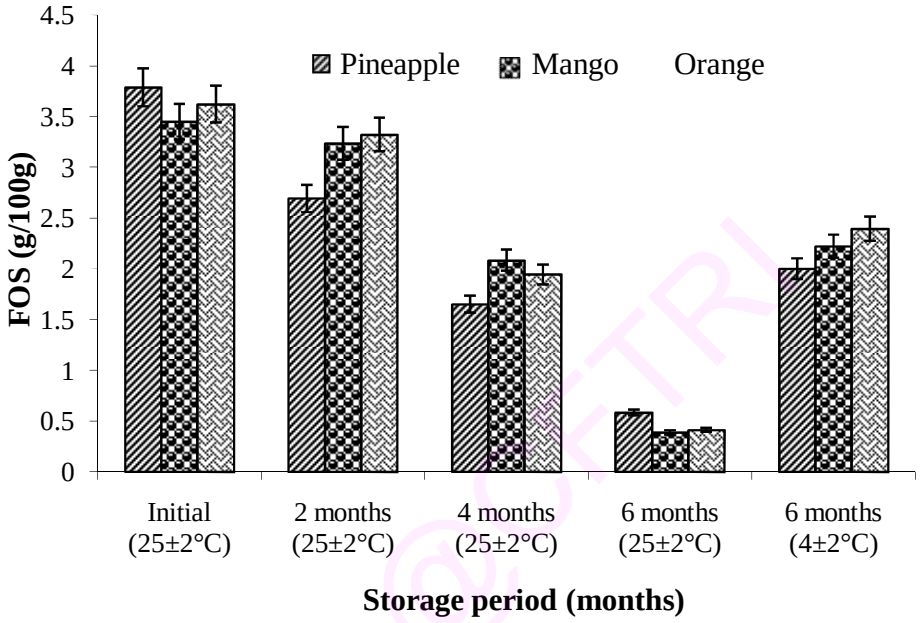

Fig 4.1.9 Changes in the FOS content (g/100g) of fruit juice beverages as function of storage

4.1.3.5. Sensory evaluation

No significant changes were detected in the overall quality of the fruit juice beverages fortified with FOS in comparison with that of sucrose by the panelists after 4 months of storage at ambient temperature. The shelf life of fruit juice beverages was limited to 4-5 months, after which sensory changes attained undesirable levels (deterioration of the typical fruit flavour and taste) (Table 4.1.4).

Sensory qualities of the fruit juice beverages stored at ambient and refrigerated temperature was studied on the basis of the consideration that a minimally acceptable product should be equivalent to rating 5 (colour, consistency, taste, flavour and overall quality) of its sensory quality. Fruit juice beverages, fortified with FOS, were found to be acceptable upto 4 and 6 months based on the evaluation of overall quality.

The changes in the present day consumer's life style have led to a vital change in the marketing trends of food sector. Today's consumer being more health conscious is seeking products with greater health benefits and there is a great demand for 'health foods'. The present study clearly indicates that fruit juice beverages can successfully be fortified with FOS with shelf life of 4 months and 6 months at ambient and refrigeration temperature respectively. There were no undesirable changes in the physicochemical characteristics of the fruit juice beverages fortified with FOS. Overall quality of the fruit juice beverages fortified with FOS and sucrose for 4 months of storage at ambient temperature was acceptable as indicated by sensory analysis. Constant pH, TSS, TA and viscosity of fruit juice beverages clearly indicates that there is no spoilage either due to microbial or enzymatic reaction. The present study has opened up a new avenue for the preparation of a commonly available, hugely popular healthy beverage.

Table 4.1.4 Sensory scores of FOS fortified fruit juice beverages (FOS: Sucrose::75: 25) during storage at ambient (25±2 °C) and refrigerated (4±2 °C) temperature for 6 months

Fruit juice beverages	Colour				Taste/flavor				Overall quality*			
	Storage time (months)											
	Initial	2	4	6	Initial	2	4	6	Initial	2	4	6
Pineapple	9.0±0.05	8.6±0.11	7.5±0.15	6.4±0.11	8.9±0.11	8.0±0.20	7.4±0.2.0	6.3±0.20	9.0±0.05	8.5±0.06	7.6±0.25	6.4±0.11
Mango	9.1±0.05	8.4±0.15	7.4±0.11	6.3±0.20	8.8±0.21	8.1±0.06	7.6±0.25	6.2±0.25	8.7±0.25	8.0±0.20	7.6±0.25	6.3±0.20
Orange	8.8±0.28	8.0±0.20	7.3±0.28	6.4±0.11	8.6±0.11	7.9±0.15	7.5±0.60	6.1±0.25	8.8±0.21	8.1±0.06	7.5±0.15	6.1±0.25

*Minimum acceptable score is 7.0

CHAPTER: 4; SECTION: 4.2

FRUCTOOLIGOSACCHARIDES BASED TRADITIONAL INDIAN ACID COAGULATED MILK SWEET: *GULAB JAMUN*

4.2.1. Introduction

India is home for many of the milk based delicacies. Milk processing started with the objective of better utilization of perishable milk, by preparing a number of shelf stable dairy products like butter, cheese, ghee etc. Thus, the products could be easily transported to a market centre, and also fetch a better price or return. Processed milk products are broadly categorized as fermented milk products, concentrated milk products, acid coagulated milk products, evaporated milk, fat-based milk products and dried milk products.

Gulab jamun occupies a prominent place as a popular delicacy among the acid coagulated milk based sweets throughout Indian subcontinent. *Khoa* (a heat-desiccated intermediary milk product) is a base for preparation of many Indian indigenous sweets. It refers to partially dehydrated whole milk product prepared by the continuous heating of milk in a frying pan, with constant stirring till it reaches a semi-solid consistency. The semi-solid mass is transformed to solid consistency known as *khoa,* with a fat, moisture, and total solid content of 20-23%, 37-44%, and 56-63% respectively. Traditionally, *gulab jamun* is prepared from a mixture of *khoa*, refined wheat flour (maida) and baking powder. The name *gulab jamun* of this sweet preparation is attributed to its resemblance to the popular monsoon fruit, *Jamun* (*Syzygium cumini* L) and to its mild flavour of rose water.

Sugars used in Indian sweetmeats have many functions as preservative, bulking agent, texturizer, humectant, dispersing agent, stabilizer, flavour carrier, browning agent, and decorative agent *(Pai, 2006)*. *Gulab jamun* is one of the popular indigenous dairy products, where sugar is the main ingredient and plays an important role in maintaining the texture and microstructure. Textural quality is primarily a sensory attribute, but usually, instrumental methods are used for quantifying it in foods. Texture profile analysis (TPA) involves compressing a bite-sized piece of food two or more times in a reciprocating motion that simulates the action of the jaw. The resulting force time curve generated is then used to quantify a number of textural parameters that correlate well with results from sensory evaluation *(Bourne and Szczesniak, 2003)*. Understanding the relationship between food texture perception and food structure is of great importance for enterprising manufacturers intending to produce texturally attractive food products

(Wilkinson et al., 2000). The microstructural studies of any food material help us understanding the physical properties of foods such as texture/firmness and sensory attributes. Limited studies have been carried out through scanning electron microscopy (SEM) to understand some of the very important physical properties such as texture/firmness and sensory attributes of foods, in particular of Indian indigenous traditional sweet preparations.

The consumer preference for low calorie sugars is on raise, as they are believed to be effective in reducing weight, management of diabetes, prevention of dental caries and reduction in the risks associated with obesity. Hence consumer's demand for healthy food products has lead to the development and marketing of a large number of functional foods based on sugar substitution in various forms. The discovery of a large number of new sweeteners over the past few decades has led to the development of various sugar free products for people prone to obesity and also for diabetics *(Ozdemir and Sadikoglu, 1986)*. In recent years the acceptance of low calorie food is getting momentum due to increased awareness of their nutritional and health benefits. The sugar has been successfully replaced with non-calorific artificial sweeteners like saccharine, acesulfame-K, cyclamate, sucralose and aspartame. Further to this, use of food ingredients like fructooligosaccharides (FOS) in desserts, ice cream, dry ice cream mix, yoghurt and cheese etc *(Maia et al., 2003)* has been viewed as a healthier alternative.

FOS are naturally occurring sugars that have beneficial effects as food ingredients. They consists of sucrose molecule to which 1, 2 or 3 additional fructose units are added by a *β*-(2-1)-glycosidic linkage to the fructose units of sucrose *(Clevenger et al., 1988)*. The health benefits of these compounds have increased popularity as food ingredients. These oligosaccharides act as prebiotics, since they are not hydrolyzed by the human digestive system, undergo fermentation in the colon and influence the growth of beneficial bacteria in the colon resulting in a healthy gut environment *(Sangeetha et al., 2005)*.

There have been few attempts to prepare some of the commonly available and cherished Indian sweetmeats with alternate sweeteners for e.g., *Burfi* with saccharin, aspartame, acesulfame-K, sucralose *gulab jamun* with sorbitol, aspartame, *shrikhand* with raftilose *(Arora et al., 2008; Arora et al., 2007; Raghvendra and Jha, 2005;*

Chetana et al., 2004). However, there has been no report on the use of FOS for the preparation of *gulab jamun*. The present chapter details mainly on texture, microstructure, and sensory attributes of *gulab jamun* prepared with FOS syrup, sugar syrup and a blend of FOS and sucrose.

4.2.2. Materials and methods

4.2.2.1. Preparation of Syrups

Gulab jamun were sweetened with three different syrups: sugar syrup, FOS syrup and a blend of FOS and sugar syrup. Sugar syrup of 50 °Brix was prepared by dissolving 100 g cane sugar in 100 ml water and boiling for 4-5 min. The TSS of the syrup was recorded using refractometer (Erma, Japan). The detailed protocol for the preparation of FOS syrup is given in the section 2.2.1. The syrup obtained (58 °Brix) was diluted to 50 °Brix using distilled water and further used for the preparation of *gulab jamun*. A blend of FOS and sugar syrup was prepared by mixing FOS syrup (50 °Brix) and sugar syrup (50 °Brix) at a ratio of 1:1 (v/v).

4.2.2.2. Preparation of *gulab Jamun*

Instant jamun mix (100 g: refined wheat flour, skimmed milk powder, vegetable fat, sodium bicarbonate, malic acid, gluten and milk powder) was purchased from the local super market (Mysore, Karnataka, India) and the contents were transferred into a container, to which 35 ml of water was gradually added and the dough was prepared by gentle mixing. It was allowed to stand for about 5 min, rolled into smooth balls of 7 g, deep fat fried at 165±2 °C until the outer crust became brown in color and then transferred to the respective syrups. Fig 4.2.1 shows the photograph of the prepared *gulab jamun* sweetened with (a) FOS, (b) blend and (c) sugar syrup.

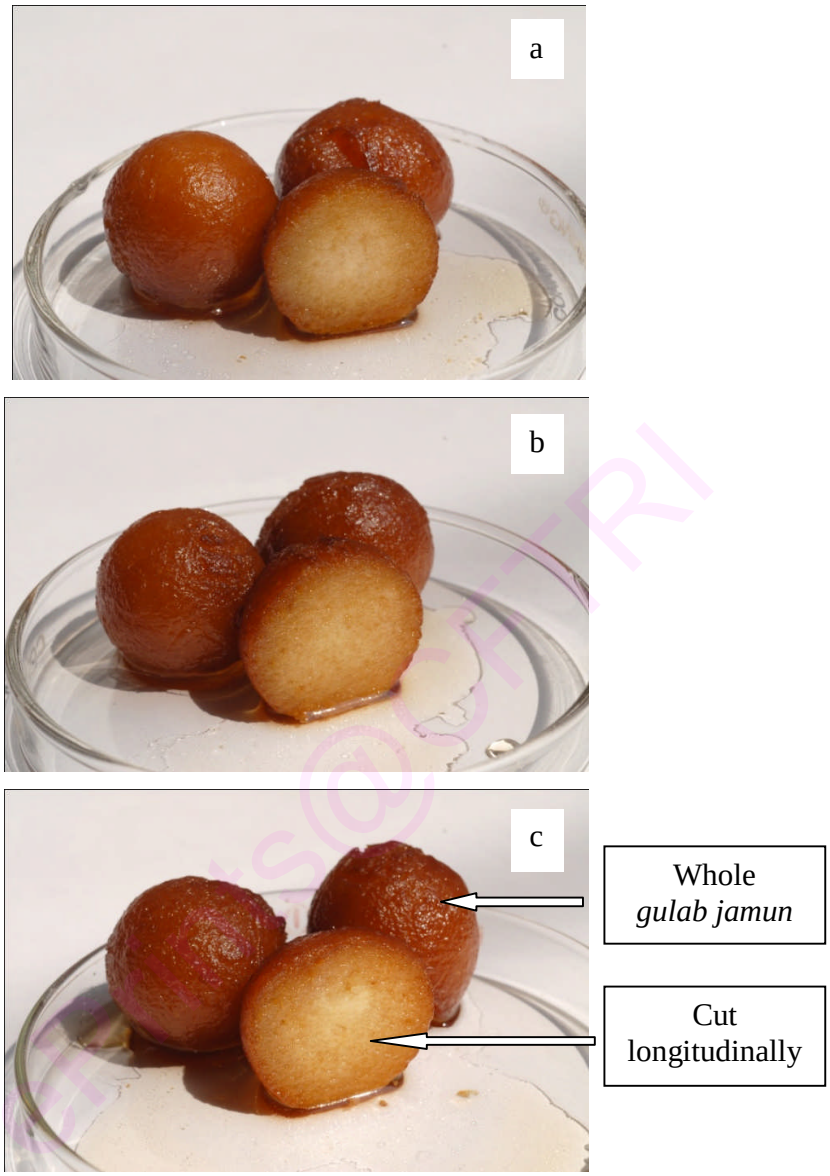

Fig 4.2.1 *Gulab jamun* sweetened with (a) FOS, (b) blend of FOS and sucrose, and (c) sugar syrup

The moisture content, water activity, colour, texture, sensory, FOS content and microbiological attributes of the *gulab jamun* were estimated immediately after the preparation and also at the end of 2nd and 4th day of storage at ambient temperature (25±2 °C). The structural analysis was carried out only on the initial day.

4.2.2.3. Moisture content and water activity (a_w)

The moisture content and a_w were determined as detailed in section 2.2.3.1.

4.2.2.4. Colour

The colour was measured as per the protocol described in section 2.2.3.2.

4.2.2.5. Texture Profile Analysis (TPA)

The texture of *gulab jamun* was characterized in terms of hardness, adhesiveness, springiness, cohesiveness, gumminess and chewiness. TPA tests were performed using LLOYDS universal testing machine (UTM), model 4103 using 80 mm probe *(Krishnamurthy and Kantha, 2003)*. The force distance curve was obtained for a dual bite compression cycle employing a crosshead speed of 50 mm/min. The values presented are an average of three readings. The TPA data were subjected to statistical analysis. Mean and standard deviation were individually calculated for scores obtained for all quality attributes of the product.

4.2.2.6. Analysis of FOS and blend of FOS and sugar syrup

The detailed protocol for the quantification of FOS in the syrup is given in section 2.2.3.5.

4.2.2.7. FOS content of the *gulab Jamun*

Gulab jamuns sweetened with FOS syrup and a blend of FOS and sugar syrup were taken (10 g) and macerated with triple distilled water (10-20 ml) in a mortar and pestle. The extract was centrifuged at 8000 rpm for 20 min and the supernatant was filtered through 0.22 µ membrane filter (Millipore Pvt. Ltd., India). The appropriately

diluted samples were analyzed using HPLC (SCL-6B, Shimadzu, Japan) (section 2.2.3.5) *(Sangeetha et al., 2002)*.

4.2.2.8. Sensory Analysis

4.2.2.8.1. Physical set up

The laboratory consisted of individual booths equipped with fluorescent lights to provide daylight illumination without shadows on the table of each booth, temperature was maintained at 22±2 °C, and free from noise and odor.

4.2.2.8.2. The panel

The panel consisted of 15 members of both sexes aged 25 to 55, were selected on the basis of their sensitivity to the basic color, taste, odor and texture as well as their experience as panelists for the sensory analysis of various products.

4.2.2.8.3. Scaling method

Quantitative descriptive analysis (QDA) of intensity scaling was followed *(Stone and Sidel, 1998)*. The score card consisted of 15 cm QDA scale wherein 1.25 cm was anchored as low and 13.75 cm as high. A panel of 15 people was asked to mark the perceived intensity of the attribute by drawing a vertical line on the scale and writing the code numbers. *Gulab jamuns* were served in porcelain bowls with 3 digit codes in a randomized order, one at a time. The selected members were familiarized with texture terminology and texture profiling through model systems in 6 sessions. Panelists were asked to describe terms, as they found applicable and an open discussion was held to finalize the descriptor as per the well known guidelines given by Dravnieks (1985) and Jowitt (1974). The common descriptors selected by at least one third of the panel were utilized for the development of score card. They were further trained with the actual product to be evaluated by including some extreme variations of the product in the sessions. Thus, the scale variations were looked into to understand each one of the attributes by the panel-in-training. One of the products in every group was always duplicated for the purposes of using it as a built in control. The difference between the

coded controls was used for monitoring the panel performance. The data were analyzed and used to generate sensory profile by plotting sample mean scores over attributes *(Ravi et al., 2002)*.

4.2.2.9. Ultrastructure by Scanning Electron Microscope (SEM)

Structural analyses of *gulab jamun* sweetened with FOS, sucrose and a blend of FOS and sugar syrup were carried out using SEM (Leo 435 VP, Leo Electron Microscopy Ltd. (Zeiss), Cambridge, U.K). The detailed protocol for the preparation of samples is given in section 2.2.3.6. Gold-coated samples were examined at 100x and 500x magnifications.

4.2.2.10. Storage studies

Gulab jamuns sweetened with FOS, sucrose and a blend of FOS and sugar syrup were stored at ambient temperature (25±2 °C) in glass bowls covered with glass lid. The shelf stability in terms of microbial load was determined initially, on 2^{nd} and 4^{th} day of storage by standard plate count method *(APHA, 1967)*. A known volume of serially diluted samples were plated on plate count agar (PCA), potato dextrose agar (PDA) and McConkey agar (MCA). The PDA plates were incubated at 25 °C for 48 h. The PCA and MCA plates were incubated at 37 °C for 24 h. The colonies were counted and expressed as cfu/g of samples.

4.2.2.11. Statistical analysis

Means were established using one-way analysis of variance (ANOVA). Statistical software (Origin 6.1) was used to test the least significant differences ($P<0.05$) at 5%. Correlation between sensory and instrumental texture parameters were established using Pearson's product moment correlation 'r' values ($P<0.05$)

4.2.3. Results and discussion

4.2.3.1. Moisture content and water activity (a_w) of *gulab jamun*

Moisture content and a_w are important quality parameters of food which has a bearing on food safety, stability, quality and physical properties *(Lewicki, 2004)*. Both a_w and moisture content have a significant bearing on the texture of finished products and its properties *(Cornillon and Salim, 2002)*. The moisture content of all the *gulab jamuns* was found to be 34.38% - 36.89% initially, and was 31.76% - 33.39% at the end of storage period. The loss of moisture content of *gulab jamun* sweetened with FOS, a blend and sugar syrup over a period of 4 days of storage was found to be 2.03%, 2.62% and 3.13% respectively. The a_w of *gulab jamuns* sweetened with FOS, a blend and sugar syrup was 0.90, 0.91 and 0.92 respectively on 4^{th} day of storage. It was observed that the moisture loss in FOS sweetened *gulab jamun* was low when compared with that of blend and sucrose sweetened *gulab jamun*. Thus the a_w was found to be maintained throughout the storage in FOS sweetened *gulab jamun.*

4.2.3.2. Colour

Fig 4.2.2, Fig 4.2.3 and Fig 4.2.4 show the *L*a*b** values of *gulab jamun* sweetened with FOS, blend and sucrose respectively. *L** value represents the changes in lightness of *gulab jamun*. All the samples exhibited decreased *L** values, may be due to the temperature (165±2 °C) of the frying medium, which is also related to Maillard reaction and caramelization *(Carabasa and Ibarz, 2000)*. Lightness value of *gulab jamun* depends also on many factors such as composition of products, the extent of non-enzymatic browning, nature of sugar syrups used etc. On the other hand the observed minor difference in lightness values is probably due to the colour of sugar syrups used. The colour of milk and milk products undergoing Maillard browning is characterized by two primary hues *viz.,* red and yellow. The brownness of *gulab jamun* under present study is due to the combination of redness (positive value of *a**) and yellowness (positive *b**). There were no observable changes in the *a** and *b** values of all the samples throughout the storage.

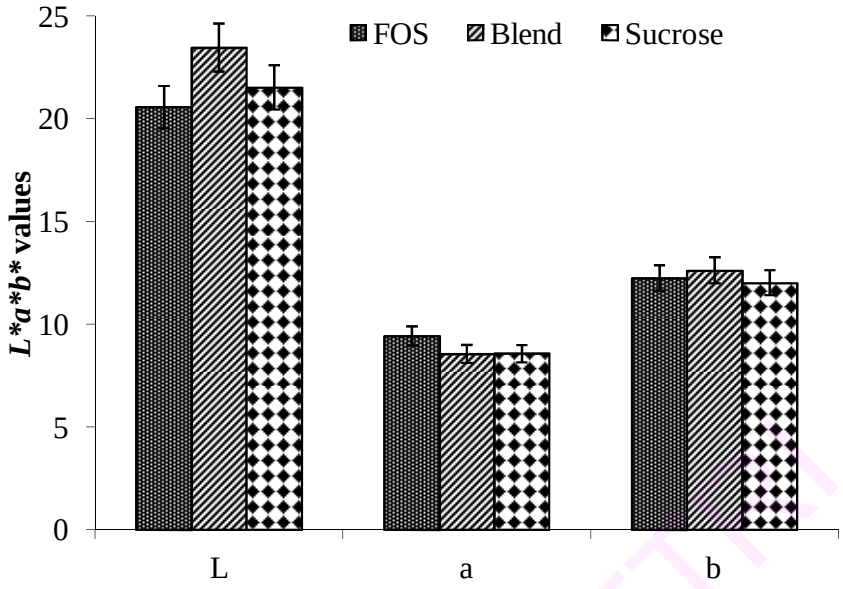

Fig 4.2.2 Colour (*L*a*b** values) of freshly prepared *gulab jamun* sweetened with FOS, blend of FOS-sucrose and sucrose

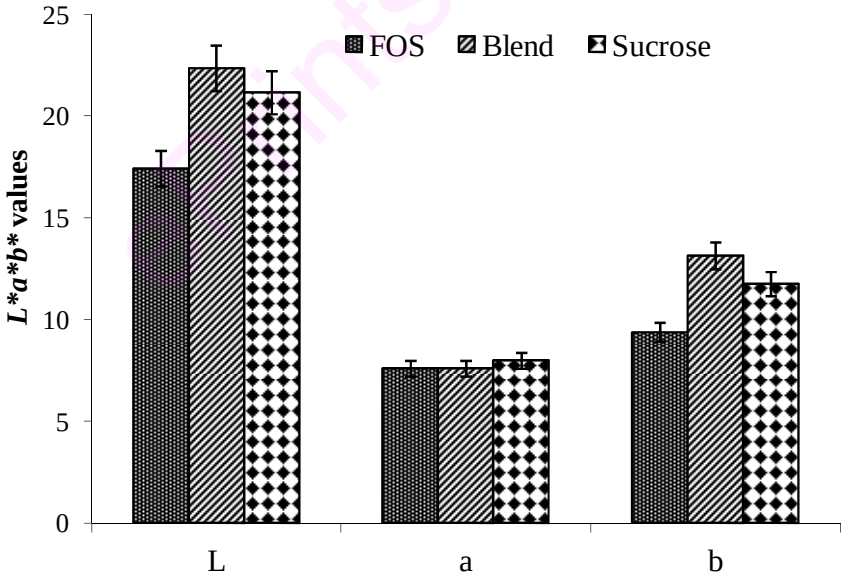

Fig 4.2.3 Colour (*L*a*b** values) of *gulab jamun* sweetened with FOS, blend of FOS-sucrose and sucrose, at the end of 2nd day of storage at ambient temperature (25±2 °C)

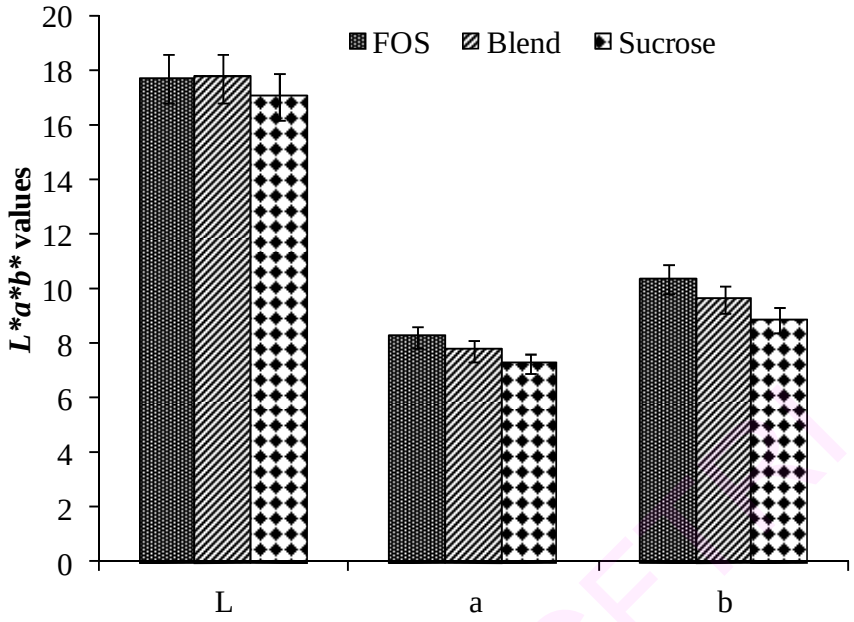

Fig 4.2.4 Colour (*L*a*b** values) of *gulab jamun* sweetened with FOS, blend of FOS-sucrose and sucrose, at the end of 4th day of storage at ambient temperature (25±2 °C)

4.2.3.3. Texture Profile Analysis (TPA)

The most recurrent parameters among food texture attributes are hardness, adhesiveness, springiness, cohesiveness gumminess and chewiness. Texture is widely recognized as an important quality attribute for product acceptability affecting consumer perception. TPA is a "two-bite" test, which includes the first and second compression cycles. The first and second compression cycles indicate the force versus time data during the first and second compression of the product by the instrument probe. Representative values for most important texture attributes of *gulab jamun* sweetened with FOS, blend and sucrose are given in Table 4.2.1.

Hardness is defined as the peak force of the first compression of the product and is determined as the force required to compress a substance between molar teeth or between tongue and palate. The hardness values were slightly higher (P<0.05) in case of FOS and a blend of FOS and sucrose sweetened *gulab jamuns* than that of *jamun* sweetened with sucrose throughout the storage period (Table 4.2.1) as indicated by

ANOVA. This could be due to the presence of higher oligosaccharides in FOS sweetened *gulab jamun*.

Adhesiveness is also referred as stickiness. It is defined as the negative force area for the end of the first bite, representing the work necessary to pull the plunger away from the food sample. Adhesiveness of FOS sweetened *gulab jamun* was higher as compared to that of *gulab jamun* sweetened with a blend and sucrose throughout the storage. The increase in adhesion in *gulab jamun* may be due to the maintenance of free moisture by oligosaccharide components during storage. FOS is highly hygroscopic in nature and as the degree of polymerization increases, the water holding capacity also increases *(Prapulla et al., 2000)*.

The distance that the food recovered in its height during the time that elapsed between the end of the first bite and the start of the second bite is defined as springiness *(Bourne, 1978)*. A slight decrease in springiness values of all the samples was observed on 2^{nd} and 4^{th} day of storage period. Cohesiveness is determined as the degree to which a substance is compressed between the teeth before it breaks. There were no significant difference ($P>0.05$) in the cohesiveness values of the conventionally prepared (sucrose) *gulab jamun* and those with FOS and a blend.

Gumminess is the energy required to disintegrate a food to a state ready for swallowing. FOS sweetened *gulab jamun* had slightly higher values for gumminess as compared to *jamun* sweetened with a blend and sucrose during storage. Chewiness is the length of time required to masticate the sample at constant rate of force application to reduce it to a consistency suitable for swallowing. No significant difference ($P>0.05$) was observed in the chewiness values of FOS, a blend and sucrose sweetened *gulab jamun*. The texture of the *gulab jamun* depends on the consistency of dough, frying temperature etc. *(Madhura et al., 2009)*. The type and concentration of syrup used also greatly affects the texture of the final product.

Table 4.2.1 Texture profile analysis of *gulab jamun* sweetened with FOS, blend ((FOS and sucrose) and sucrose

Quality Parameters	**Storage days**	**FOS**	**Blend**	**Sucrose**
Hardness	0	8.97±0.63	6.35±0.83	6.00±1.51
(N)	2	9.10±0.15	6.10±0.42	5.97±0.48
	4	10.90±0.43	8.90±0.29	6.41±0.26
Adhesiveness	0	-0.50±0.59	-0.24±0.06	-0.27±0.60
(N)	2	0.69±0.01	0.39±0.01	0.50±0.03
	4	0.73±0.02	0.50±0.01	0.48±0.01
Springiness	0	3.14±0.60	3.35±0.17	3.12±0.09
(mm)	2	2.19±0.17	2.70±0.01	2.49±0.19
	4	2.32±0.12	2.66±0.15	2.67±0.05
Cohesiveness	0	0.16±0.01	0.19±0.01	0.20±0.01
(ratio)	2	0.18±0.01	0.20±0.01	0.17±0.01
	4	0.18±0.01	0.18±0.01	0.18±0.01
Gumminess	0	1.49±0.17	1.23±0.19	1.18±0.01
(N)	2	1.92±0.05	1.12±0.10	1.03±0.02
	4	1.80±0.10	2.32±0.09	1.18±0.02
Chewiness	0	4.68±0.49	4.17±0.49	3.69±0.10
(N mm)	2	4.20±0.12	4.20±0.12	3.78±0.18
	4	4.81±0.06	4.84±0.24	3.79±0.05

Means ± Standard error mean (n=3)

4.2.3.4. Ultrastructure by Scanning Electron Microscope (SEM)

The microstructural studies have been used by food scientists for studying the internal structure of a wide variety of food products since 1950. In recent years, scanning electron microscopy (SEM) has gained considerable importance in the study of surface topology and to develop correlations between the structure and the physical appearance of various food materials. According to Bourne (2002), texture of foods is derived from their microstructure.

The microstructure of the *gulab jamun* sweetened with FOS, a blend and sucrose is shown in the Fig 4.2.5. A well-defined compact network was found in *gulab jamun* sweetened with FOS (Fig 4.2.5a and Fig 4.2.5b) and a blend of FOS and sucrose (Fig 4.2.5c and Fig 4.2.5d). Whereas loosely arranged structures with air pockets were observed in case of sucrose sweetened *gulab jamun* (Fig 4.2.5e and Fig 4.2.5f). This indicates slight hardness of *gulab jamun* sweetened with FOS and a blend when compared with sucrose sweetened *gulab jamun*. Lack of a well defined 3-D microstructure in *burfi* and *kalakand* prepared using low calorie sweeteners as against that of sucrose has been reported *(Arora et al., 2008; Arora et al., 2007)* which is related to decreased hardness in the texture. In the present study, it was observed that the texture and the microstructure of *gulab jamuns* sweetened with FOS and blend of FOS and sucrose was similar to that of *burfi* and *kalakand* prepared with sucrose. This indicates FOS maintains the structure of *gulab jamun* because of its water binding ability as in case of sucrose and it provides enhanced stability. Based on the SEM study along with results obtained from texture analysis, it can be inferred that sucrose can be replaced partially or fully with FOS to prepare *gulab jamun* and has the potential to be highly acceptable by the consumer.

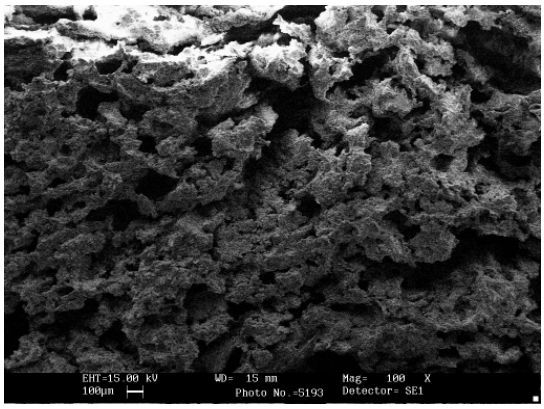

Fig 4.2.5(a); *Gulab jamun* with FOS (100x)

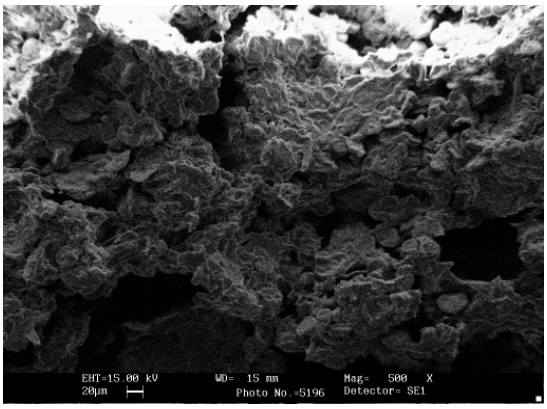

Fig 4.2.5(b); *Gulab jamun* with FOS (500x)

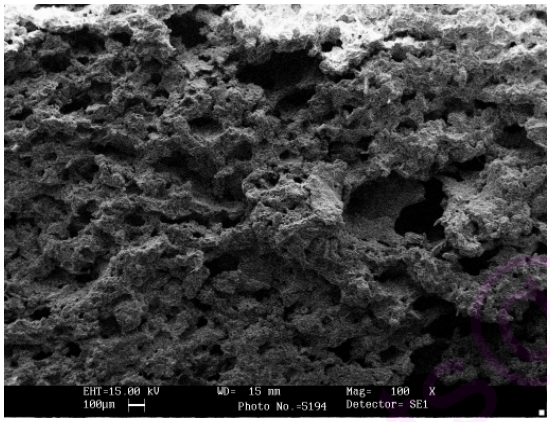

Fig 4.2.5(c); *Gulab jamun* with F+S (100x)

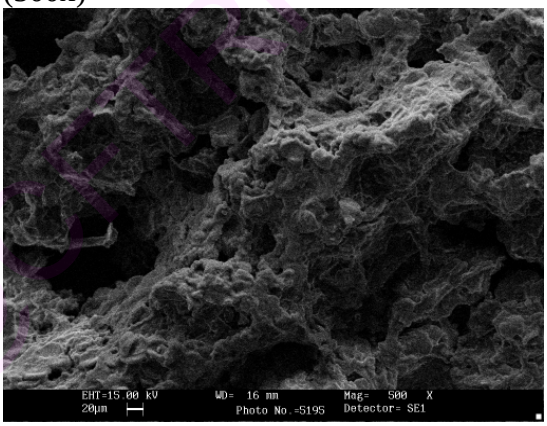

Fig 4.2.5(d); *Gulab jamun* with F+S (500x)

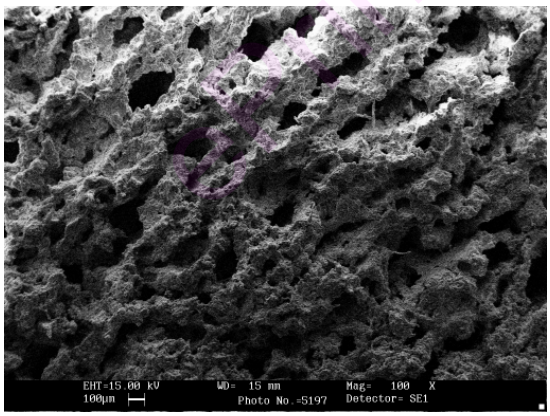

Fig 4.2.5(e); *Gulab jamun* with sucrose (100x)

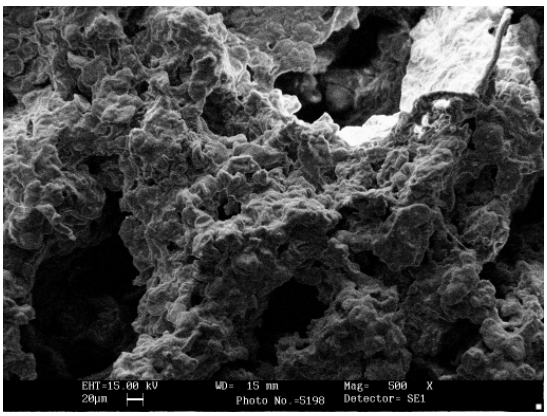

Fig 4.2.5(f); *Gulab jamun* with sucrose (500x)

Fig 4.2.5 Scanning electron micrographs of *gulab jamun* sweetened with FOS, a blend (F+S) and sucrose

4.2.3.5. FOS content

The FOS content of the *gulab jamun* sweetened with FOS and a blend of FOS and sucrose is shown in the Fig 4.2.6. The freshly prepared FOS sweetened *gulab jamun* showed 6.03 g of glucose, 8.70 g of sucrose and 17.60 g of FOS in 100 g of *jamun*. On storage at room temperature (4th day), the FOS content slightly decreased. Freshly prepared *gulab jamun* sweetened with a blend of FOS and sucrose showed 2.40 g of glucose, 15.60 g of sucrose and 9.20 g of FOS in 100 g of *jamun*. The FOS content was found to be 8.30 g and 7.80 g/100g of *jamun* on 2nd and 4th day of storage at ambient temperature (25±2 °C) respectively. Sucrose content of freshly prepared *gulab jamun* sweetened with only sucrose was found to be 30.50 g, followed by a slight decrease on storage.

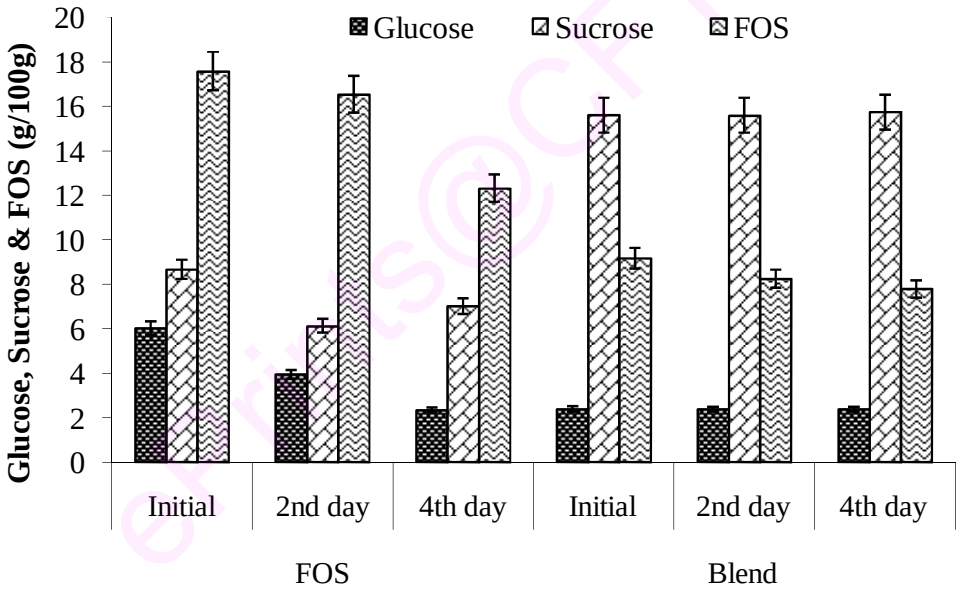

Fig 4.2.6 FOS content of *gulab jamun* sweetened with FOS and blend

The results indicate that, the sucrose can be replaced with FOS, a healthier food ingredient in the preparation of traditional Indian sweets. Studies have indicated that FOS can also be used in the preparation of fruit juice beverages *(Renuka et al., 2009)* and it can be an alternative non-nutritive sweetener without any adverse effect on various diabetic related metabolic parameters *(Mabel et al., 2008)*.

4.2.3.6. Sensory quality

The sensory attributes of *gulab jamun* prepared using three different syrups were evaluated. There was no significant differences (P>0.05) in brownness, softness, chewiness, mealiness, milkyness and juiciness of the *gulab jamun* sweetened either with FOS or blend, when compared with that of sucrose sweetened *gulab jamun* (Fig 4.2.7).

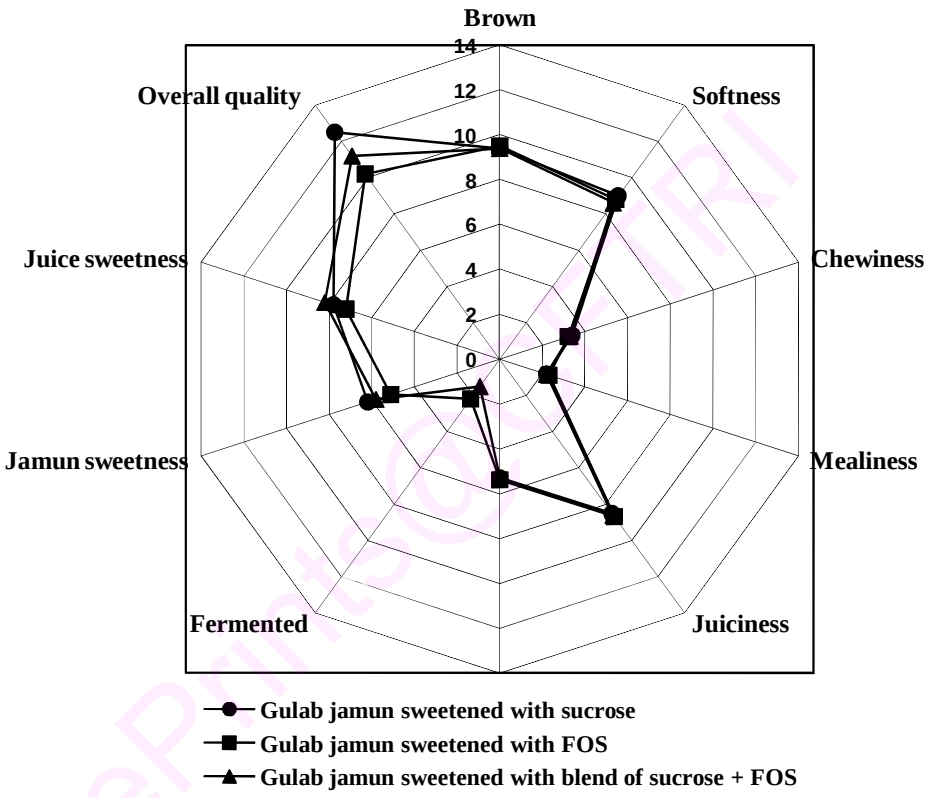

Fig 4.2.7 Profilogram of *gulab jamun* sweetened with FOS, blend and sucrose

In terms of sweetness perception, the panelist rated FOS sweetened *gulab jamun* towards slightly lower sweetness in comparison with those of blend and sucrose sweetened *gulab jamun*. However, all the *jamuns* sweetened with FOS, blend and sugar syrup showed score of more than 10 (on a score range of 0-15), which is generally regarded as highly acceptable, indicating the suitability and also the acceptance of FOS for the preparation of *gulab jamun*. In addition, FOS sweetened *gulab jamun* will have an

added advantage over that of sucrose sweetened *gulab jamun* from the point of low calorie, non digestibility and other well known health benefits.

4.2.3.7. Correlation between sensory and instrumental texture parameters

Relationship between sensory and instrumental textural parameters was established using "r" values. Results have indicated sensory brown is highly correlated negatively with overall quality (OQ) (-0.83) and sensory softness with instrumental springiness (-0.90). Juiciness was correlated negatively with cohesiveness (-0.96). On the other hand, OQ was mostly influenced by instrumental hardness values (-0.88). Instrumental gumminess, cohesiveness and chewiness were correlated with OQ with 0.94, -0.90, and -1.0 respectively.

4.2.3.8. Shelf life of *gulab jamun*

Gulab jamun sweetened with FOS, blend and sucrose was found to be stable at ambient temperature (25±2 °C) for 4 days. There were no coliforms and fungal colonies observed on MCA and PDA plates respectively. This is indicative of the microbiological quality of the prepared *gulab jamuns*. The number of total viable count on plate count agar (PCA) plates per gram of *gulab jamun* sweetened with FOS, blend and sucrose were 19×10^4, 22×10^4 and 23×10^4 respectively.

From this study, it can be concluded that FOS syrup can be an effective replacement either fully or partially for the preparation of *gulab jamun* without much changes in the physicochemical characteristics and sensory attributes of the same. The present investigations have given all the positive indications for the use of FOS in the preparation of *gulab jamun*. The change in life style has been the causative agent for the increase in the number of diabetics all over the world. With this present scenario it would be most welcoming to have healthier alternatives to sucrose.

CHAPTER: 5

ISOLATION AND CHARACTERIZATION OF POTENT PROBIOTICS FROM FRUITS AND VEGETABLES

5.1. Introduction

The isolation and screening of microorganisms from natural sources have always been the most powerful means for obtaining useful and genetically stable strains for industrially important products. Lactic acid bacteria (LAB) are the first and largest group of microorganisms to be regarded as probiotics *(Mombelli and Gismondo, 2000)* and are important in the food and dairy industries because the lactic acid and other organic acids produced by these bacteria act as natural preservatives as well as flavour enhancers. Their probiotic effect is accredited to their production of metabolic byproducts such as acid, hydrogen peroxide, and bacteriocins (*e.g.* lactocidin and acidophilin) that manifest antibiotic properties and inhibit the growth of a wide spectrum of pathogens such as *Escherichia coli, Klebsiella, Enterobacter, Pseudomonas, Salmonella, Serratia* and *Becteroides (Krasaekoopt et al., 2003; Chen and Yao, 2002).* LAB finds increasing acceptance as probiotics which aid in stimulation of immune responses, reduction of serum cholesterol, and alleviation of lactose intolerance, enhancement of resistance against pathogens and prevention of traveler's diarrhea *(Reid, 2006).*

A particular microorganism has to fulfill a number of specific properties for it to be regarded as a probiotic strain. These properties are dependent on its specific purpose and on the location on which the specific property has to be expressed. Considerable beneficial properties/functions offered by the probiotics have resulted in a boom in the market volume of probiotics *(Brashears et al., 2003; Hamilton-Miller, 2003).*

Taxonomy of LAB genera has also undergone considerable changes since the time of Orla-Jensen (1919), resulting in the genera listed in Table 5.1. Such taxonomic knowledge of a strain may, therefore, give an indication of the strain's origin, habitat, and physiology, and have important consequences for the selection of novel strains for application in food fermentation or for use as a probiotic. The first step in an organism or product being referred to as a probiotic is to identify and characterize the organism to genus and species level. For identification of LAB, phenotypic methods have been most commonly used *(Corsetti et al., 2001).* More recently, genetic techniques, such as 16S rDNA sequencing have been developed which allows a more consistent and accurate identification of individual strains *(Buddhiman et al., 2008).* New tools for classification and identification of LAB are underway *(Sascha and Magdalena, 2010).*

Traditional phenotypic characterization of probiotic bacteria is rather tedious and not always reliable since certain species cannot be distinguished by these methods. Molecular techniques have emerged as a replacement or complement to traditional phenotypic tests for comparing strains or species of probiotic bacteria. Amongst these methodologies, sequence analysis of the 16S rRNA gene *(Kullen et al., 2000; Ward et al., 1990)* and 16-23S internally transcribed spacer regions *(Tannock et al., 1999)* have proven to be useful tools for identification. DNA pulsed-field gel electrophoresis (PFGE) is also an efficient means for differentiating strains *(O'Riordan and Fitzgerald 1997).* LAB is of essential importance as starter cultures in fermented food industries *(Robert, 2008; Guasch-Jane et al., 2005)*. Various metabolic and enzymatic activities of LAB lead to production of volatile substances, which contribute to flavor, aroma and texture developments *(Soukoulis et al., 2007; Kleerebezemab et al., 2000)*. The ability of LAB to produce prebiotics and stimulation of the immune system *(Kalliomäki et al., 2001)* render this group of microorganisms most essential in dairy industry.

Table 5.1 Examples of probiotic microorganisms

***Lactobacillus* species**
L. acidophilus, L. amylovorus, L. casei, L. crispatus, L. delbrueckii sub sp. *bulgaricus, L. gallinarum, L. gasseri, L. johnsonii, L. paracasei, L. plantarum, L. reuteri, L. rhamnosus*
***Bifidobacterium* species**
B. adolescentis, B. animalis, B. bifidum, B. breve, B. infantis, B. lactis, B. longum
Other lactic acid bacteria
Enterococcus faecalis, Enterococcus faecium, Lactococcus lactis, Leuconstoc mesenteroides, Pediococcus acidilactici, Sporolactobacillus inulinus, Streptococcus thermophilus
Non-lactic acid bacteria
Bacillus cereus var. Toyoi, *Escherichia coli* strain nissle, *Propionibacterium freudenreichii, Saccharomyces cerevisiae, Saccharomyces boulardii*

*Source: Holzapfel *et al.,* 1998

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LAB possessing the generally regarded as safe (GRAS) status find application in the production of food and feed. Food fermentation has been shown to have not only preservative effects and ability to modify the physico-chemical properties of various foods, but also the capability to provide significant impact on the nutritional quality and functional performance of the raw material *(Knorr, 1998)*. This offers a possibility to explore the use of probiotics as functional starters for the manufacture of fermented foods. Functional starters are defined as cultures that possess at least one inherent, functional property, aimed at improving the quality of the end product *(De Vuyst, 2000)*. Certain LAB strains are characterized by their ability to transform lactose and improve the digestibility of fermented dairy products *(Weinberg et al., 2007)* as well as their preservation *(Abdelbasset and Djamila, 2008)*.

The increasing application of probiotics in food products underscores the need to properly identify and distinguish these beneficial bacteria among the originally presented microbial population. Moreover, certain probiotic activities are strain-specific and thus, identification of probiotics to the strain level is most essential. Therefore, isolation, characterization and identification of the microorganisms with a prospective selection of probiotic LAB are important to support the technical process and to obtain a food product with a desired quality.

Probiotics are a feasible solution against gastrointestinal infections. An important number of bacterial strains are used for this purpose (*Gibson et al., 2000*). The genus *Enterococcus* could be an alternative in the search for probiotics. In some studies *E. faecium* showed antagonistic effect against some bacteria, and it is of great relevance to look for other species, from the same genus, with probiotic characteristics (*Audisio et al., 2000; Audisio et al., 1999; Laukova et al., 1998*). The main aim of this chapter was to characterize the diverse phenotypes of *Enterococcus sps* isolated from fruits and vegetables.

5.2. Materials and Methods

5.2.1. Isolation of probiotic LAB from fruits and vegetables

LAB were isolated from fresh fruits and vegetables (banana, sapota, star fruit, beans, brinjal, carrot, cucumber, drum stick, radish, tomato) procured from local market (Mysore, Karnataka). Fruits and vegetables were cut into small pieces and suspended (10 g) in 90 ml sterile saline (0.85% NaCl) and incubated at 37 ^{o}C under static micro aerobic conditions for 3-4 days. Fermented samples were drawn aseptically each day and were serially diluted. The appropriate dilutions were plated on de Man Rogosa Sharpe (MRS) agar, incubated for 24-48 h at 37 ^{o}C. The plates were examined for well isolated colonies.

Based on the difference in morphological characteristics of the colony on MRS agar, individual colonies were randomly picked and inoculated in to MRS broth. Purity of the isolates was established by taking a loopful of the culture from the broth and streaking on MRS agar plates and also by microscopic observation. The pure colonies were used for further studies.

5.2.2. Identification of the selected isolates

5.2.2.1. Morphology by Gram staining

Gram staining is an empirical method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative) based on the chemical and physical properties of their cell walls *(BSOPTP 39 - Staining Procedures)*. The bacterium isolated from fruits and vegetables were Gram stained and observed under the microscope. Those bacteria that hold on to primary dye iodine complex and remain violet are Gram positive and those which get decolorized and subsequently take up counter stain (pink/red) are Gram negative.

5.2.2.2. Catalase test

The test was carried out by placing a drop of 3% (v/v) hydrogen peroxide (H_2O_2) (NICE chemicals, Mumbai) on a clean microscopic slide containing a smear of 24 h old culture and it was allowed to react for 30 sec *(Cappuccino and Sherman, 2004)*. The

bubbles or froth formed from the organism was recorded as catalase-positive and catalase negative for the absence of bubbles.

Out of 40 isolates screened, 17 were found to be gram positive and catalase negative, which are the characteristic feature of LAB. Probiotic properties of these 17 LAB were evaluated by *in vitro* methods.

5.2.3. *In vitro* evaluation of probiotic properties of selected LAB

5.2.3.1. Acid tolerance

Tolerance to low pH was tested for 17 isolates as described by Yeong *et al.*, (2002). The isolates were activated thrice in MRS broth at 37 ^{o}C for 12-15 h each time and the active culture with an optical density of 0.280 at 600 nm was inoculated (10% v/v) to MRS broth modified with 0.1N HCl to pH values of 2.0, 2.5 and 3.0 and incubated at 37 °C for 4 h. The viable cells count from 0 h to 4 h were determined by plating serial decimal dilutions (10^{-2} to 10^{-6}) of samples in saline (0.85% NaCl) on MRS agar, incubated at 37 °C for 24-48 h. The survival rate was calculated using the formula as, the percentage of colonies grown on MRS agar compared to the initial bacterial concentration (*Fuller, 1989*).

$$\% \text{ Survival} = \frac{\text{log number of viable cells survived (cfu/ml)}}{\text{log number of initial viable cells inoculated (cfu/ml)}} \times 100 \quad \text{---------- (1)}$$

5.2.3.2. Bile tolerance

For the bile tolerance test, 17 LAB isolates were cultured and treated similarly to the MRS broth as described in the section 5.2.3.1, except that the acid was replaced by 0.3%, 0.6%, and 1.0% (w/v) bile salt respectively. Samples were incubated under the same conditions and viable cell counts from 0-4 h were determined as described above (section 5.2.3.1) *(Yeong et al., 2002)*.

5.2.3.3. Viability of selected LAB isolates in synthetic gastric juice (SGJ)

The transit tolerance through simulated gastric juice was determined according to the method of Cotter *et al.,* (2001). The LAB isolates showed >75% survivability at pH 2.0 and 1.0% bile salt were further tested for survivability in SGJ (8.3 g of protease peptone, 3.5 g of glucose, 2.05 g of NaCl, 0.6 g of KH_2PO_4, 0.11 g of $CaCl_2$, 0.37 g of KCl, 0.05 g of bile, 0.1 g of lysozyme and 13.3 mg of pepsin; composition is per litre). The pH of the prepared medium was adjusted to pH 2.5 with 1N HCl. The media was filter sterilized *(Pedersen et al., 2004)* using 0.22 µm membrane filter (Millipore, India). The isolates were activated thrice in MRS broth at 37 ^oC for 12-15 h each time and the active culture (1 ml) with an optical density of 0.280 at 600 nm was inoculated (10% v/v) to SSJ (9 ml). The 0-4 h sample of each isolates was drawn (1 ml), serially diluted with sterile saline (10^{-2} to 10^{-6}) and plated on MRS agar plates, incubated at 37 ^oC for 24 h. The viable cell counts were determined as detailed in the section 5.2.3.1.

5.2.3.4. Cell surface hydrophobicity

The bacterial adhesion to hydrocarbons (BATH) test was performed to assess the adherent ability of the 6 LAB isolates for their cell surface hydrophobicity, using a hydrocarbon- xylene as described by Canzi *et al.,* (2005) with some modifications. 10 ml of 12 h grown LAB cells were harvested by centrifugation at 6500 rpm for 5 min at 4 °C, washed twice with phosphate buffer saline (PBS: 140 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.2) and re-suspended in the same buffer. The cell suspension was adjusted approximately to the value of 1.0 at absorbance of 600 nm. Briefly 0.6 ml of hydrocarbon was added to 3 ml of bacterial suspension and mixed thoroughly using vortex for 2-3 min and the two phases were allowed to stand for 1 h at 37 °C for phase separation. The bottom aqueous phase was removed carefully and its absorbance at 600 nm was measured. The decrease in optical density (OD) correlates with the hydrophobicity of the strain. The decrease in absorbance of the aqueous phase correlates with the measure of the cell surface hydrophobicity (H %), calculated using the formula,

Cell surface hydrophobicity $H\% = [(A_o-A)/A_o] X100$ ---------- (2)

Where, A_o and A are the absorbance before and after extraction with xylene respectively.

5.2.3.5. Antibacterial test

The antagonistic activity of the LAB isolates (Cu8 and R32) resistant to SGJ and showed good adherent ability to mucosal surface was determined against well known food borne pathogens (*Micrcoccus*, *Staphylococcus aureus* FR1722, *Escherichia coli* MTCC118, *Listeria murrayi* FB 69, *Aeromonas hydrophila* B445, *Salmonella paratyphi* FB254, *Salmonella typhii* FB231, *Yersinia enterocolotica* MTCC859, *Listeria innocua* FB 21, *Listeria monocytogenus* Scott A, and *Bacillus cereus* F4433) by agar well diffusion method as described by Jacobsen *et al*., (1999). The isolates grown in MRS broth, with an optical density of 0.280 was centrifuged at 8000 rpm at 4 ^{o}C for 15 min. The culture filtrate (CF) was neutralized with 0.1 N NaOH, and 50 µl of neutralized CF poured to MRS agar wells for checking its antagonistic activity against these food borne pathogens. One ml of the indicator pathogens in nutrient broth was mixed with 7 ml of soft agar (0.7%) and overlayed immediately over the MRS agar on which the CF were inoculated. The plates were incubated aerobically at 37 ^{o}C for 24 h and the diameter of zone of inhibition was measured in millimeter (mm). The zone of inhibition around the wells indicates positive (+) for antibacterial activity while the absence of zone indicates a negative (-) result.

5.2.3.6. Antibiotics susceptibility test

Antibiotic resistance of selected LAB isolates (Cu8 and R32) was assessed by paper disc method using antibiotic discs (HiMedia Pvt. Ltd., Mumbai, India) *(Reddy et al., 2007)*. The antibiotic discs were placed on MRS agar plates previously seeded with active culture. The plates were incubated at 37 °C for 24-48 h, after which zones of inhibition were examined. The diameter of the zone of inhibition was measured and expressed in mm. The susceptibility of the strains was expressed as described by Charteris *et al.*, (1998b).

5.2.3.7. Carbohydrate utilization test

The selected LAB isolates (Cu8 and R32) were characterized according to their ability to ferment a range of different carbohydrates using KB009 HiCarbohydrate^{TM} kit (HiMedia, Mumbai, India). The different carbohydrates tested include Lactose, Xylose,

Maltose, Fructose, Dextrose, Galactose, Raffinose, Trehalose, Mellibiose, Sucrose, L-Arabinose, Mannose, Inulin, Sodium gluconate, Glycerol, Salicin, Glucosamine, Dulcitol, Inositol, Sorbitol, Mannitol, Adonitol, α-methyl D-glucoside, Ribose, Rhamnose, Cellobiose, Melezitose, α methyl D- mannoside, Xylitol, ONPG (ortho- nitro phenol β-galactopyranoside), Esculin, D-Arabinose, Citrate, Malonate, and Sorbose. The active culture was centrifuged at 8000 rpm at 4 ^{o}C for 10-15 min. Supernatant was discarded and the cell pellet was added with sterile saline and again centrifuged. The sterile saline was added to the cell pellets obtained after centrifugation, mixed well (vertexed) to get uniform suspension and used for inoculating the kit. The kit was opened aseptically in laminar air flow chamber. Inoculum (50 µl) was transferred to each well of KB kit by surface inoculation method. Lid was replaced carefully and the kit was incubated at 37 ^{o}C for 24-48 h. Interpretation of the result was based on the KB009 HiCarbohydrate^{TM} kit interpretation chart.

5.2.3.8. Thermotolerance, growth at high salt concentration and sodium azide test

The LAB isolates (Cu8 and R32) were aseptically inoculated to sterile MRS broth tubes and subjected to heat treatment at temperatures 60 ^{o}C and 70 ^{o}C for 30 min. Heat treated Cu8 and R32 tubes were incubated at 37 ^{o}C for 24 h and growth was observed visually (turbidity).

Requisite quantity of MRS broth tubes with the addition of sodium chloride (NaCl; 10%) and sodium azide [0.04% (w/v)] was prepared. The active cultures of each isolates (Cu8 and R32) were aseptically inoculated to the sterile MRS broth with added salts. The tubes containing 10% NaCl and 0.04% sodium azide were subjected to 50 ^{o}C and 37 ^{o}C for 24 h respectively. The change in colour of the broth, incubated at 37 ^{o}C for 24 h indicates positive for sodium azide test and the turbidity on the 10% NaCl tubes incubated at 50 ^{o}C indicates positive for the test.

5.2.4. Genotypic identification

5.2.4.1. Isolation of chromosomal DNA

The total genomic DNA of the LAB isolates was extracted from active cultures grown in MRS broth as described by Mora *et al.,* (2000) with slight modification. The cells were harvested by centrifuging at 8000 rpm for 10 min (4 °C), followed by washing the cell pellet thrice with 0.4 ml TE buffer [Tris 10mM (pH 8.0), EDTA 1mM (pH 8.0)] containing 15 µl of lysozyme (0.45 mg lysozyme/ml) and incubated at 37 °C for 1 h. The 20% SDS solution (15 µl) and proteinase K (15 µl) were added to the digested cells and incubated at 55 ^{o}C for 15 min. The mixture was extracted with Tris saturated phenol (250 µl) followed by phase separation by centrifugation at 8000 rpm for 15 min (4 °C). The aqueous phase was removed, extracted with chloroform and precipitated with 15 µl sodium acetate (3 M, pH 4.8) and 400 µl ethanol (95%) by incubating at -20 °C for 30-60 min. The precipitate was harvested by centrifugation at 10,000 rpm for 20 min. The pellet was washed with 70% ethanol and centrifuged at 10000 rpm for 15 min (4 °C), air dried and resuspended in 50 µl TE buffer. The quality of isolated DNA was checked by electrophoresis using 0.8% agarose gel in 1X TAE buffer.

5.2.4.2. Analysis of chromosomal DNA by agarose gel electrophoresis

Agarose gel electrophoresis is a method used to separate DNA or RNA molecules by their size and charge. The standard λ DNA marker (3 µl) (MBI Fermentas, Lithuania) and the DNA samples of isolates (5 µl) were mixed with loading dye and loaded in subsequent wells in an agarose gel (0.8% agarose, dissolved in 1X TE buffer) placed in the electrophoresis tank filled with 1X TAE buffer. Electrophoresis was carried out at 90 volts for about 45 minutes or until the DNA samples containing the dye travel 1/2 to 3/4 of the way down the gel. The gel was removed from the tank and stained by placing in a solution of 0.5 µg/ml ethidium bromide for 10-15 min. The gel was de-stained in distilled water for 10 min, examined on a UV transilluminator (Photodyne, USA) and photographed using gel documentation system (Gel Doc EQ, Biorad, USA).

5.2.4.3. Polymerase Chain Reaction (PCR)

PCR was carried out to amplify the targeted 1.4 kb 16S rRNA gene. The PCR reaction mixture has the following composition.

Components used in PCR reaction mixture:

Nuclease free water	:	14.0 µl
10x reaction buffer (100 mM Tris, pH 9.0, 500 mM KCl, 15 mM $MgCl_2$ and 0.1% gelatin)	:	2.5 µl
dNTP mixture (10 mM)	:	5.0 µl
Taq DNA polymerase	:	0.5 µl
Primer	:	1.0 µl
Template DNA/Genomic DNA	:	2.0 µl
Total volume	:	25.0 µl

Primer sequence: (Bangalore genie)

Forward sequence- 5^1GAG TTT GAT CCT GGC TCA GG 3^1

Reverse Sequence- 3^1TCA TCT GTC CCA CCT TCG GC5^1

The master mixture was prepared by mixing all the components except template DNA and sterile water. This master mixture was taken in PCR tubes containing template DNA and sterile water. The contents of the tubes were mixed by brief spin in a microcentrifuge. The tubes were then placed in thermocycler GeneAmp PCR System 9700 (Perkin-Elmer, USA) and the reaction parameters were as follows.

Initial denaturation:	94 °C for 4 min	
Denaturation:	94 °C for 1 min	34 cycles
Annealing:	50 °C for 1 min	
Extension:	72 °C for 1 min	
Final extension:	72 °C for 10 min	

5.2.4.4. Analysis of PCR products by agarose gel electrophoresis

The PCR products were analyzed by using 1.5% agarose gel electrophoresis. The amplified PCR product (10 µl) was mixed with 2 µl of loading dye and loaded in subsequent wells in an agarose gel (1.5% agarose, dissolved in 1X TE buffer containing ethidium bromide solution 10 µg/ml) placed in the electrophoresis tank filled with 1X TAE buffer.

Electrophoresis was carried out at 70 volts for 60 min or until the samples containing the dye travels to 3/4 of the way down the gel. The gel was visualized under UV light and photographed using gel documentation system (Gel Doc EQ, Biorad). The size was confirmed by comparing with 3 Kb ladders which was used as a molecular size standard.

5.2.4.5. Purification of PCR product

The PCR product to be ligated in vector of selected isolates was gel-purified by using Sigma GenElute PCR clean up kit. The amplicon from the gel was excised with a sterile sharp scalpel and taken in a sterile pre-weighed eppendorfs tube. To one volume of gel fragment, three volume of solubilization buffer was added (100 mg/300 µl). The mixture was incubated at 50 ^{o}C for 10 min until the gel gets completely dissolved. The contents in the tube were mixed often for every 2-3 min. Once the gel is completely dissolved the color of the mixture turns yellow.

The contents of the tubes were mixed, spinned and the solubilization fluid was then transferred into the Qiangen's elution column. The column was centrifuged at 10,000 rpm for 2 min. The flow through was discarded and 750 µl of PE wash buffer was added to the column which retains the DNA fragment and it was incubated at room temperature for 10 min. The column was again centrifuged at 10,000 rpm for 2 min and the flow through was discarded. The PCR product was eluted by adding 20 µl of deionized sterile water and stored at -20 ^{o}C.

5.2.4.6. Confirmation of purified PCR product

The PCR product (3 µl) was run on 0.8% agarose gel electrophoresis to confirm its concentration and purity.

5.2.4.7. 16S rDNA cloning and transformation

5.2.4.7.1. Cloning 16S rDNA into vector

After purification, 16S rDNA products were cloned into the pGEM-T easy vector according to the manufacturer's recommendations (Promega Corporation). Ligation mixture consisted of pGEM-T Easy Vectors (1µl), 2x rapid ligation buffer (5 µl), PCR product (3 µl), DNA ligase (1µl) (3 Weiss units/µl) and deionized water to a final volume of 10 µl. The resulting ligation mixture was incubated at 4°C, overnight and after incubation was transformed in *E. coli* DH5 α cells.

5.2.4.7.2. Preparation of competent cells using $CaCl_2$

Competent cell preparation was carried out as described by Sambrook and Russell (2001). A single colony of *E. coli* DH5α cells from a plate, freshly grown for 16-20 h at 37 °C was picked and inoculated into 2 ml of LB broth in test tube, incubated at 37 °C with shaking. The 250 ml conical flask containing 50 ml of LB broth was seeded with 0.2 ml of overnight grown culture and was incubated at 37 ^{o}C at 225 rpm. The culture was allowed to grow till the OD_{600} reached 0.40-0.50. The cells were transferred aseptically to sterile polypropylene tube and the culture was cooled in ice for 10-15 min. The cells were harvested by centrifugation at 6000 rpm for 10 min at 4 °C. The media was decanted from the cell pellet. The cell pellet was re-suspended in 10 ml of ice-cold 0.1 M $CaCl_2$ and stored in ice for at least half an hour. The cells were harvested by centrifugation at 6000 rpm for 10 min at 4 °C. The supernatant from the cell pellet was decanted and the tubes were kept in an inverted position for 1 minute to drain the last traces of fluid. The cell pellet was re-suspended in 2.0 ml of ice-cold 0.1 M $CaCl_2$ and the competent cells were stored at 4 °C overnight.

5.2.4.7.3. Transformation of competent cells

Transformation of *E. coli* was carried out by $CaCl_2$ method *(Sambrook* and *Russell, 2001)*. The competent cells (200 µl) were taken in sterile micro-centrifuge tubes, 10 µl of ligation mixture was added to each tube and the contents of the tubes were mixed by swirling gently followed by keeping the tubes in ice for 30 min. The competent cells

that received standard super coiled plasmid DNA and the competent cells that received no plasmid DNA were included as control samples. The cells were given a heat shock treatment by placing the tubes in water bath (45 ^{o}C) for 90 sec. The tubes were immediately transferred to ice and the cells were allowed to cool for 1-2 min. LB broth of 800 µl was added to each tube and the culture was incubated at 37 ^{o}C for 12 h at 180 rpm. The tubes were centrifuged at 6000 rpm for 5 min to concentrate the cells. The concentrated cells were plated in appropriate selection media.

5.2.4.7.4. Selection of transformants/recombinants

The transformants were selected on LB agar plates. The transformation mixture (200 µl) was spread on LB agar plates containing 100 µg/ml ampicillin, 0.5 mM IPTG and 80 µg/ml X-Gal. The control mixture, *E. coli* competent cells transformed with pGEM-T easy vector were spread on to LB plates containing ampicillin, X-Gal and IPTG. The plates were incubated overnight at 37 °C. After incubation the clones that contain PCR products produce white colonies were selected.

5.2.4.8. Analysis of transformants/recombinants

The transformants were checked for the presence of plasmid by isolating plasmid DNA and performing 0.8% agarose gel electrophoresis. Restriction digestion experiments were set up to check for insert release from recombinants.

5.2.4.8.1. Isolation of plasmid DNA from the transformed colonies

The plasmids were isolated from the transformed *E. coli* cells by alkali lysis method *(Birnboim and Doly, 1979).* Single colonies of appropriate strain were inoculated in 2 ml of LB broth containing required antibiotic and grown overnight in a shaker incubator at 37 °C and 180 rpm. The overnight culture (1.5 ml) was transferred to a 1.5 ml micro centrifuge tube and the cells were harvested by centrifugation at 10000 rpm for 2 min. After discarding the supernatant, 100 µl of solution I (50 mM glucose, 25mM Tris HCl, pH 8.0; 10 mM EDTA, pH 8.0) was added and mixed thoroughly until no visible clumps of cells were observed. The samples were kept on ice for 5 min. About 200 µl of freshly prepared solution II (1% SDS, 0.2N NaOH) was added to the tube and mixed

gently by inverting the tubes several times until the lysed cell suspension became clear. The samples were kept on ice for 5 min, 150 µl of ice-cold solution III (5 M potassium acetate, glacial acetic acid) was added, and tubes were inverted gently. The tubes were centrifuged at 10000 rpm for 10 min.

The supernatant was transferred to a fresh tube and equal volume of phenol-chloroform was added and mixed thoroughly. Centrifugation at 10000 rpm for 10 min separated the two phases. The upper aqueous phase was transferred to a fresh tube and equal volume of chloroform was added. The tubes were centrifuged at 10000 rpm for 10 min. The upper aqueous phase was transferred to a fresh tube and 2 volumes of absolute ethanol were added. The tubes were kept at -20°C for 1 h to overnight for precipitation. The tubes were centrifuged at 10000 rpm for 10 min and the supernatant was discarded. The pellet was washed with 300 µl of 70% ethanol and the air-dried pellet was dissolved in 20 µl of TE buffer. Samples were tested by carrying out agarose gel (0.8%) electrophoresis along with control plasmid. The isolated plasmid was used for the further PCR and restriction analysis to confirm the presence of the insert.

5.2.4.8.2. Double restriction digestion of recombinant plasmids for insert release

Restriction enzymes such as *Eco RI and Pst I* (MBI Fermentas, Lithuania) were used for double digestion experiment. The following constituents were added to a micro centrifuge tube in the order stated:

Constituents	**Volume (µl)**
Nuclease-free water	13.0
Restriction enzyme 10 x buffer	2.0
Plasmid DNA	3.0
Restriction enzyme 1 (*Eco RI)*	1.0
Restriction enzyme 2 *(Pst I)*	1.0
Final volume	20.0

The contents of the tube were mixed gently and the tube was centrifuged briefly at 10000 rpm to collect the contents at the bottom of the tube. The reactions were incubated at 37 °C for 4-8 h. The samples were analyzed by agarose gel (1.5%) electrophoresis.

5.2.4.9. Sequence analysis

DNA sequencing was carried out using M13F and M13R primers by dideoxy chain termination method *(Sanger et al., 1977)*. The reaction was carried out in an automatic DNA sequencer (ABI prism, Applied Biosystems, USA) with fluorescent dideoxy chain terminators at Bangalore Genie, Bangalore, India. A database search was performed in GenBank database release 155 using BLAST programme (National Centre for Biotechnology Information, Maryland, and USA).

5.3. Results and discussion

LAB comprise the natural microflora of the animal and human intestine. When present in sufficient numbers, these bacteria are able to create a healthy equilibrium between beneficial and potentially harmful microflora in the gut, preventing intestinal infection. Therefore, these bacteria have been widely used as a probiotic or as standard bacterium to screen novel probiotics for humans and animals.

5.3.1. Prevalence of LAB

Out of 40 isolates from fruits and vegetables, 17 isolates were positive for LAB as confirmed by Gram staining and catalase test (Table 5.2).

Table 5.2 Prevalence of lactic acid bacteria in fruits and vegetables

Fruits/ vegetables	Total number of isolates	Total number of isolates positive for gram staining	Total number of isolates negative for catalase test	Total number and (%) of LAB isolates
Banana	4	2	2	2 (50.00)
Brinjal	5	2	1	1 (20.00)
Carrot	6	3	5	3 (50.00)
Cucumber	4	4	4	4 (100.00)
Drumstick	3	1	1	1 (33.33)
Pear	5	0	1	0
Radish	4	4	4	4 (100.00)
Star fruit	5	2	2	1 (25.00)
Tomato	4	2	2	1 (20.00)
Total	**40**	**20**	**22**	**17 (42.50)**

Radish and cucumber showed the highest prevalence of LAB (100%), but no LAB were isolated from pears. Banana, brinjal, carrot, cucumber, drumstick, star fruit and tomato showed 20-50% prevalence of LAB. Moreover, the measured prevalence of LAB differed significantly among sources and fermentation time. The 17 isolates were coded as B2, S3, Br4, C5, R7, Cu8, B11, R15, D16, Cu17, T18, R24, Cu26, R32, C33, Cu34, and C37.

5.3.2. Acid and bile salt tolerance

To determine the selection criteria for probiotic properties of the isolated LAB, their stability on acid and bile, should first be assessed *(Klein et al., 1998; Salminen et al., 1998)*. One of the most important properties for a probiotic to provide health benefits is that it must be able to overcome physical and chemical barriers such as acid and bile in the gastrointestinal tract *(Gibson et al., 2000)*. Therefore, the 17 LAB isolates (B2, S3, Br4, C5, R7, Cu8, B11, R15, D16, Cu17, T18, R24, Cu26, R32, C33, Cu34, and C37)

were subjected to the effective properties, as prerequisites for probiotics, *i.e.,* tolerance to low pH (2, 2.5 and 3) and high bile salt concentration (0.3%, 0.6%, and 1.0%), so that the LAB isolated from different sources would be more likely to colonize in the human intestine. For a strain to be considered as probiotic, its resistance to acid pH and tolerance to bile salt are most important *(García-Galaz et al., 2004; Prasad et al., 1998).* Fig 5.1 and Fig 5.2 shows the percentage resistance of the LAB isolates to low pH and high bile salt concentration respectively.

All strains showed tolerance to pH 2.0 and 2.5 for 2 h despite variations in the degree of viability. Cu8, R15, Cu17, Cu26, R32, and Cu34 were the most acid-tolerant strains, with more than 90% survivability after incubation for 2 h at pH 2.0, while B2, S3, Br4, C5, R7, B11, D16, T18, R24, C33, and C37 were the most acid-sensitive strains, with only 80% survivability after the 2 h incubation. In general, there was a greater reduction in total colony forming units for the strains of B2, S3, br4, D16 compared with those of Cu8, R15, Cu17, Cu26, R32, and Cu34 for the first hour of incubation. However, the isolate of Cu8, R15, Cu17, Cu26, R32, and Cu34 showed greater acid tolerance over the entire incubation period (Fig 5.1), and their counts decreased by $\geq$90% to $\geq$80% at pH 2.0. These results were compared with *L. salivarius* CFR 2158, a reference culture from Central Food Technological Research Institute (CFTRI) culture collection center, which showed >70% survivability at pH 2.0 and 90% at pH 2.5 for 4 h of incubation *(Reddy and Prapulla, 2007).*

As seen from result, out of 17 LAB isolates, 6 were found to be resistant at pH 2.0 and 2.5. In general, most of the strains tested in this study showed a good resistance to low pH. Therefore, it has been assumed that isolated strains may survive passage through the digestive system that has specific condition such as the low pH of the stomach. This phenomenon has previously been observed in a number of probiotic bacteria where a substantial decrease in the viability of strains was often observed at pH 2.0 or below *(Hood and Zottola, 1988)*. However, as reported by Prasad *et al.,* (1998), the probiotic strains are likely to be buffered by food or other carrier matrix molecules following consumption and are thus not likely to be exposed to the extremes of pH in the stomach.

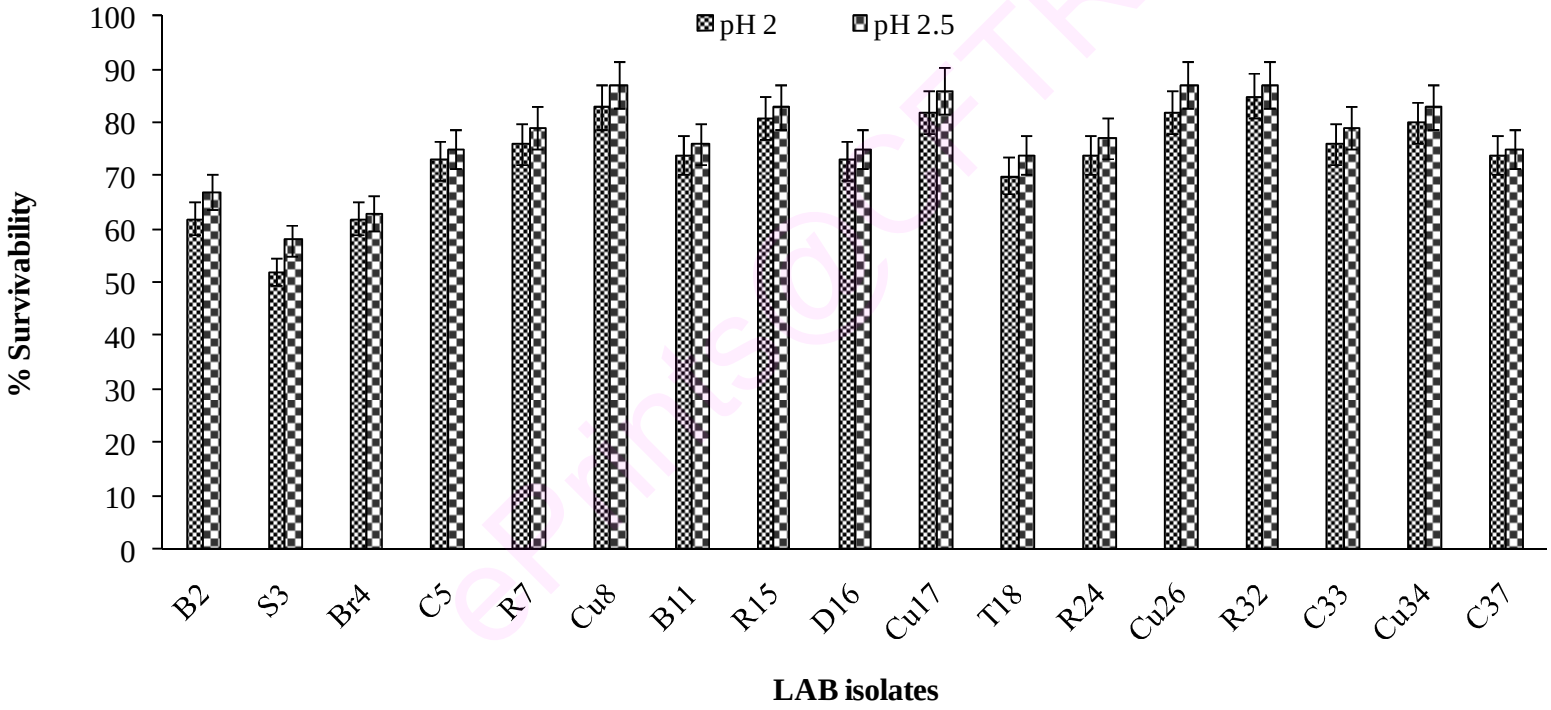

Fig 5.1 Survivability of the LAB isolates to pH 2.0 and 2.5 for 4 h incubation at 37 ^{o}C

Bile tolerance is one of the properties required for lactobacilli to survive in the small intestine, enabling them to play a role in the physiological function of this organ *(Kaur et al., 2002; Reid and Friendship, 2002; Saarela et al., 2000).* Selection of bile tolerant strains at bile concentrations between 0.3 and 1.0% w/v in growth media is typical. Fig 5.2 shows the survival rates (%) of the tested LAB isolates simulating bile tolerance of bacteria in the human proximal intestinal conditions. Similar to the results of acid tolerance study, the Cu8, R15, Cu17, and R32 exhibited higher viability (≥70%) than B2, S3, Br4, C5, R7, B11, D16, T18, R24, Cu26, C33, Cu34 and C37 (55-65%) under the same treatment conditions (P>0.05). Cu8 and R32 had the highest survival rates (76% and 71% respectively) of all the tested LAB strains for the same bile concentrations and treatment periods. The extended treatment time (4 h) resulted in significant lowering of survival rates (P<0.05) for each LAB at the same bile salt concentrations. Although it is found that the growth rates of all 17 LAB isolates were slightly suppressed by bile, all showed growth, even in 1% bile salt (Fig 5.2). A bile concentration of 1% is considered greater than that normally observed in animal intestines. According to Gilliland *et al.*, (1984) 0.3% bile is considered to be a crucial concentration to evaluate a bile tolerant probiotic LAB. Among 6 acid tolerant strains (Cu8, R15, Cu17, Cu26, R32, and Cu34), 4 strains (Cu8, R15, Cu17 and R32) were found to survive at the tested bile salt concentrations (0.3, 0.6 and 1%) for 12 h, whereas the survival of the isolate Cu26 and Cu34 were found to decrease with an increase in bile salt concentration.

The gastric acid and bile salts are two critical factors affecting the survival of LAB which are ingested and transit through the gastrointestinal tract (GIT). In the *in vitro* studies, hydrogen chloride (HCl) and bile salts are usually used to imitate the unfavorable conditions that exist in the GIT. To be useful probiotic candidates, LAB must possess the properties of acid and bile tolerance to help them survive and grow in the conditions found in the gut. In the present study, Cu8, and R32 displayed higher tolerance towards acid and bile salts than other isolates (Fig 5.1 and Fig 5.2). However, the reference strain, *L. salivarius* CFR 2158, is reported to be the highest survivability in all acid and bile conditions tested *(Reddy and Prapulla, 2007)*. In general, each tested strain showed a significant decrease in survival rate, with increased bile concentrations and decreased pH values.

Gilliland *et al.,* (1984) reported that bile tolerant lactobacilli occur in high numbers in the upper section of the intestine. Although the bile concentrations in the gastro-intestinal tract vary, the mean intestinal bile concentration is believed to be 0.3% w/v *(Gilliland et al., 1984).* This phenomenon was also observed by Begley *et al.,* (2005) and they reported that hydrolysis of bile salts by bile tolerant bacteria results in increased usage of cholesterol to synthesize new bile salts and this resulted in lowering of serum cholesterol levels. However, it is worth mentioning that too high bile salt hydrolase activity in the intestinal lumen can result in low availability of conjugated bile salts needed for lipid digestion *(Kim* and *Lee, 2005)*. Klaenhammer (1982) reported that probiotic bacteria vary considerably in their level of bile tolerance. Also, they explained the mechanism of tolerance is not understood and the minimum acceptable level of bile tolerance for a candidate probiotic remains unknown. Until now, the lethality of bile on microorganisms in the human small bowel was thought to be low, even negligible, because of *in vitro* data that showed conjugated bile salts, which constitute the majority of bile salts present in the small bowel, were less bactericidal than deconjugated bile salts.

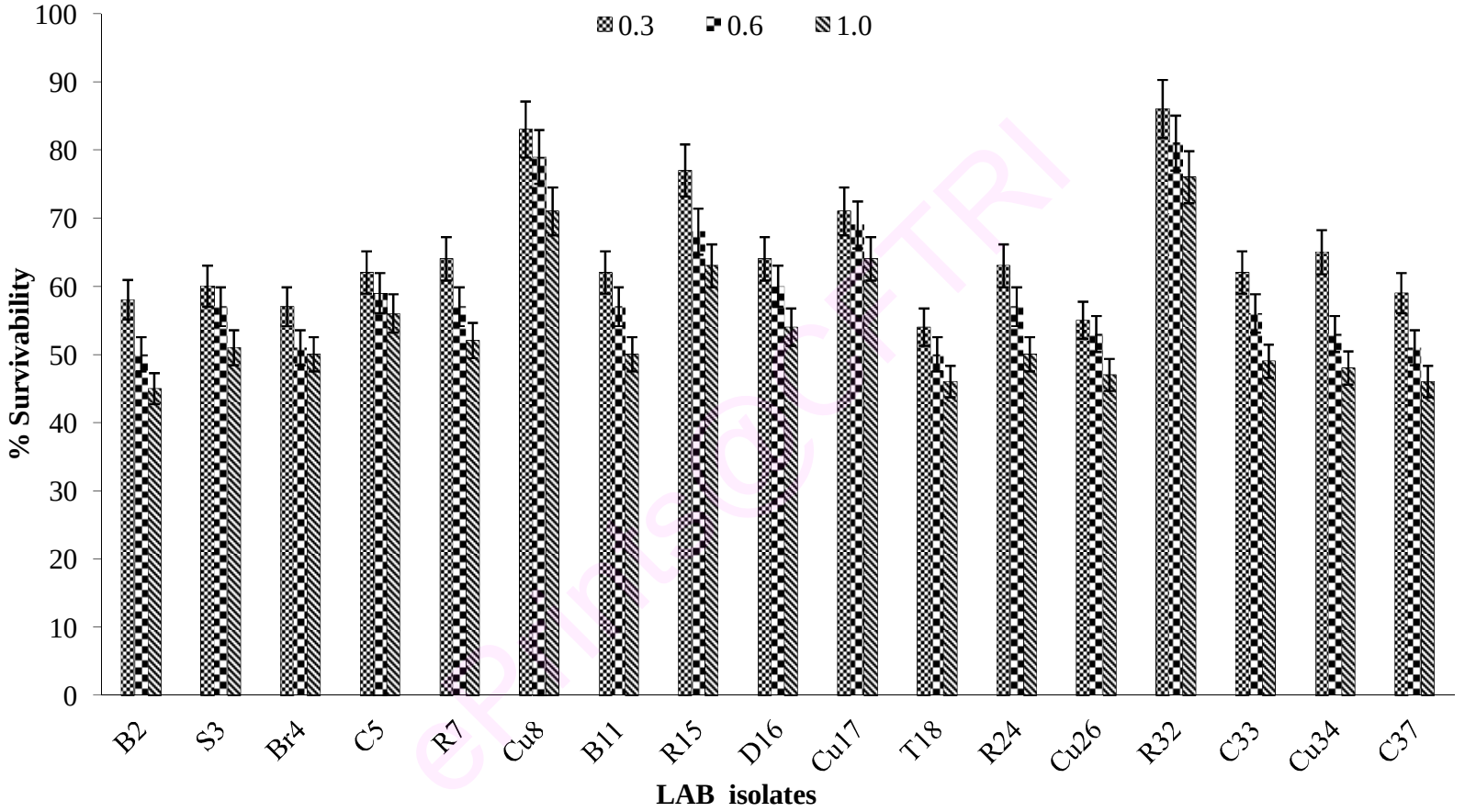

Fig 5.2 Survivability of the LAB isolates to bile salt (0.3, 0.6 and 1.0%) for 4 h incubation at 37 ^{o}C

5.3.3. Viability of selected LAB isolates in synthetic gastric juice (SGJ)

In order to analyze the effects of various components of simulated gastric juice on viability of lactobacilli, 6 LAB isolates, which showed more than 80% survivability at low pH and more than 70% survivability at high bile salt concentration was selected. The effects of SGJ on viability of LAB isolates are presented in Fig 5.3.

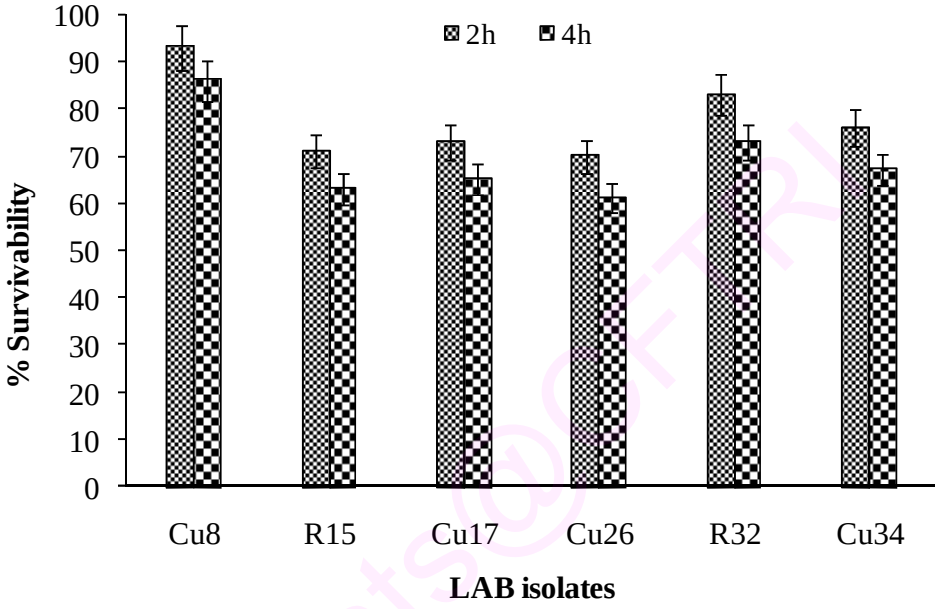

Fig 5.3 Survivability of the LAB isolates to synthetic gastric juice

When the synthetic gastric juice of pH 2.5 was used, the Cu8 and R32 showed the highest survival rate (86% and 73%) over 4 h of exposure to synthetic gastric juice. While, the poorest survivor was R15, the concentration of Cu17, Cu26 and Cu34 were also declined to 65% after 4 h of exposure (Fig 5.3). Therefore, even under starving condition, it is considered that pH of stomach is around 2.0 and the required time to release is about 1.5 h. The isolates Cu8 and R32 could pass through gastrointestinal tract after ingestion with food products.

5.3.4. Cell surface hydrophobicity

One of the important criteria for the LAB isolate to consider it as probiotic is its ability to adhere to mucosal surface of the human gastro intestinal tract. The beneficial

effect of LAB isolates has been attributed to their ability to colonize human and animal gastrointestinal tracts *(Dharmawan et al., 2006)*. Several factors are involved in the adhesion of probiotic LAB, one is the hydrophobicity of the bacterial cell surface. BATH is a method for determining the hydrophobic surface characteristics of bacterial cells. The strains affinity for water is evaluated by thoroughly mixing a culture and hydrocarbon suspension and then evaluating the decrease in optical density (OD) of the culture phase. The decrease in OD correlates with the hydrophobicity of the LAB isolates. Reddy and Prapulla (2007) reported that the *L. salivarius* CFR 2158 was strongly adhesive to HT-29 intestinal epithelial cell line. Taking this as a reference point, LAB isolates (Cu8 and R32) were compared with *L. salivarius* CFR 2158. The most hydrophobic LAB isolates were found to be Cu8 (69.27%); R32 (65.09%) and Cu8 (91.40%); R32 (61.62%) when xylene and hexane were used as the hydrocarbon. The least hydrophobic (<30%) LAB isolates were found to be R15, Cu17, Cu26 and Cu34. Therefore, the isolate Cu8 and R32 were selected to study other probiotic properties.

It has been reported by Wadstrom *et al.,* (1987) that, compared to hydrophilic strains, hydrophobic lactobacilli adhered better to intestinal epithelial cells. As bacterial cells alter their membrane fluidity under various environmental conditions, growth conditions may have a profound effect on the fatty acid composition of their lipids and subsequently on the hydrophobicity and adhesion ability of bacterial strains. *Lactobacillus* can adhere to the epithelium in the intestinal tract and may thus prevent the adherence of pathogenic microorganisms. Adherence indicates the capacity of the strain to colonize the mucosa. Moreover, the attachment to mucosa prolongs the time for probiotics to influence the gastrointestinal immune system and microbiota of the host. Thus the ability to adhere to intestinal surfaces is considered to be an indicator of the efficacy of the probiotic strain.

5.3.5. Antibacterial test

One of the major criteria for probiotic LAB is its inhibitory effect on the growth of pathogenic bacteria *(Lin et al., 2007)*. Bacterial antagonism provides information about inhibitory properties against some pathogens. Probiotic bacteria produce inhibitory substances as organic acids, peroxide and bacteriocins. Table 5.3 indicates the wide

spectrum inhibitory effects of the isolates Cu8 and R32 against well known food borne pathogenic organisms (*Micrococcus*, *S. typhi* FB231, *Y. enterocolitica* MTCC 859, *E. coli* MTCC118, *L. murreyi* FB 69, *L. innocua* FB 21, *L. monocytogenus* Scott A, *S. aureus* FR1722, *B.cereus* F4433, *S. paratyphi* FB254, *A. hydrophila* B445). The inhibition zone was found to be in the range of 6-10 mm (+) and 10 mm and above (++).

The inhibitory effect of LAB might be due to the production of H_2O_2, organic acids, bacteriocins, reuterins, etc. *(Jacobsen et al., 1999)*. An *in vitro* study proposed that lactic acid production by *L. acidophilus* is responsible for the inhibition of gastrointestinal pathogens *(Lin et al., 2007)*. An agar well diffusion assay was performed to find out the nature of the antibacterial compound for the culture filtrate of the Cu8 and R32. On studying the antibacterial activity, both the isolates showed a strong antibacterial effect (++) on *Micrococcus*, *S. typhii* FB231, *E. coli* MTCC118, *L. monocytogenus* Scott A, *S. aureus* FR1722 and *A. hydrophila* B445, and these native isolates were unable to inhibit the growth of *B.cereus* F4433 and *S. paratyphi* FB254. The LAB isolate R32 showed strong inhibition on *L. murreyi* FB 69, where Cu8 showed moderate effect. The Cu8 showed strong antibacterial effect on *L. monocytogenus* Scott A, while R32 showed no effect (Table 5.3).

Table 5.3 Antibacterial effect of the LAB isolates against food borne pathogens

Food borne pathogens	LAB isolates	
	Cu8	**R32**
Micrococcus	++	++
S. typhii FB231	++	++
Y. enterocolitica MTCC 859	+	+
E. coli MTCC118	++	++
L. murreyi FB 69	+	++
L. innocua FB 21	+	+
L. monocytogenus Scott A	++	-
S. aureus FR1722	++	++
B.cereus F4433	-	-
S. paratyphi FB254	-	-
A. hydrophila B445	++	++

++ Above 10mm zone of inhibition, + 6-10 mm, - No zone of inhibition

5.3.6. Antibiotics susceptibility test

The use of antibiotics is very common in order to treat any diseases or infections. One of the problems with this treatment procedure is the development of bacterial resistance. For example, in 1979 less of 1% of *Salmonella* strains isolated from pigs were resistant to penicillin. In 1996, *Salmonella* strains resistant to penicillin increased to 34% *(CSPI News, 1999).* Development of resistance to drugs induced by inadequate use implies a constant search of new antibiotics and this search increases costs of production *(Trieu-Cuot and Poyart, 1999; Mc Kellar, 1998)*. It is very important to search for an alternative to the use of antibiotics. A kind of functional food is one which is supplemented with probiotics.

The selected acid and bile tolerant isolates (Cu8 and R32) were tested for antibiotic susceptibility *(Charteris et al., 1998b)*. The isolate Cu8 was moderately susceptible to Erythromycin and Co-trimoxazole whereas, R32 showed resistant to these

antibiotics and a moderate susceptibility to Tetracycline. Both the LAB isolates were resistant to most of the antibiotics (Penicillin-G, Kanamycin, Metronidazole, Vancomycine, Streptomycin, Rifampicin and Polymyxin-B) and a moderate susceptibility to Ampicillin and Chloramphenicol (Table 5.4). The results implicated that LAB isolates which have shown resistance to specific antibiotics can be consumed by the patient at the time of antibiotic treatment. In the treatment of urogenital infections and traveler's diarrhea, better results can be obtained when concurrent therapy is made with probiotic LAB during antibiotic treatment but safety of the applied strain should be considered.

Antibiotics cause diarrhea due to an imbalance of intestinal bacterial flora in 20% of patients treated. In double-blind placebo-controlled randomized studies, probiotics such as *Saccharomyces boulardii (McFarland et al., 1995; Surawicz et al., 1989), Lactobacillus rhamnosus* GG strain *(Pant et al., 1996), Bifidobacterium longum (Colombel, 1987),* and *Enterococcus faecium* SF 68 strain *(Buydens and Debeucklaere, 1996)* significantly decreased the incidence of diarrhea in healthy subjects and patients treated with antibiotics. Meta-analysis of the effects of probiotics on antibiotic induced diarrhea in nine double-blind placebo-controlled studies has been performed, and the results clarified the significance of the actions of probiotics such as *S. boulardii* and *Lactobacillus (D'Souza et al., 2002).*

Table 5.4 Antibiotic susceptibility for LAB isolates

Antibiotics		Interpretative zone diameter (mm)	
Type	**Disc Conc. (µg)**	**Cu8**	**R32**
Penicillin-G	10	10 (R)	14 (R)
Kanamycin	30	00 (R)	00 (R)
Metronidazole	5	00 (R)	00 (R)
Vancomycine	30	00 (R)	00 (R)
Ampicillin	10	14 (MS)	15 (MS)
Chloramphenicol	30	15 (MS)	15 (MS)
Streptomycin	10	00 (R)	00 (R)
Co-trimoxazole	25	11 (MS)	00 (R)
Tetracycline	30	14 (R)	15 (MS)
Erythromycin	15	14 (MS)	00 (R)
Rifampicin	5	15 (R)	12 (R)
Polymyxin-B	300	00 (R)	00 (R)

(R)-Resistance; (MS)-Moderate susceptible; (S)-Susceptible, in accordance to performance of standards for antimicrobial disk susceptibility test.

5.3.7. Carbohydrate utilization test

Carbohydrate utilization by LAB isolates (Cu8 and R32) was carried. Change in the color of wells from red to yellow indicates positive results for carbohydrate utilization and no change in color indicates negative result (Fig 5.5a and Fig 5.5b).

Fig 5.5 (a) Carbohydrate fermentation test of the probiotic LAB isolate Cu8 (wells numbered from 1-35 correspond to carbohydrates mentioned in Table 5.5 in the same order and well number 36 represents control)

Fig 5.5 (b) Carbohydrate fermentation test of the probiotic LAB isolate R32 (wells numbered from 1-35 correspond to carbohydrates mentioned in Table 5.5 in the same order and well number 36 represents control)

In ONPG test, media color change from colorless to yellow indicates positive results and absence of color change indicates negative results. A color change from cream to black indicate Esculin hydrolysis (positive result) and if remains cream, indicates negative results. Change in medium color from yellowish green to blue shows positive results for citrate utilization. In malonate utilization test, color of the medium changes from light green to blue indicates positive results. Both the isolates Cu8 and R32 produced acid from maltose, fructose, dextrose, trehalose, sucrose, mannose, inulin, cellobiose, and hydrolyzed the esculin. Isolate Cu8 was able to ferment galactose, salicin,

mannitol, α-Methyl-D-glucoside, melezitose and D-arabinose. In contrast, isolate R32 was able to ferment lactose (Table 5.5).

Table 5.5 Carbohydrate fermentation and organic substrates utilization profile

Sl. No.	**Substrate**	**Cu8**	**R32**	**Sl. No.**	**Substrate**	**Cu8**	**R32**
1	Lactose	-	+	19	Sorbitol	-	-
2	Xylose	-	-	20	Mannitol	+	-
3	Maltose	+	+	21	Adonitol	-	-
4	Fructose	+	+	22	Arabitol	-	-
5	Dextrose	+	+	23	Erythritol	-	-
6	Galactose	+	-	24	α-Methyl-D-glucoside	+	-
7	Raffinose	-	-	25	Rhamnose	-	-
8	Trehalose	+	+	26	Cellobiose	+	+
9	Melibiose	-	-	27	Melezitose	+	-
10	Sucrose	+	+	28	α-Methyl-D-mannoside	-	-
11	L-arabinose	-	-	29	Xylitol	-	-
12	Mannose	+	+	30	ONPG	-	-
13	Inulin	+	+	31	Esculin hydrolysis	+	+
14	Sodium gluconate	-	-	32	D-Arabinose	+	-
15	Glycerol	-	-	33	Citrate utilization	-	-
16	Salicin	+	-	34	Malonate utilization	-	-
17	Dulcitol	-	-	35	Sorbose	-	-
18	Inositol	-	-	36	Control		

(+): substrate fermented; (-): substrate not fermented.

5.3.8. Genotypic Identification

5.3.8.1. Extraction of genomic DNA from probiotic LAB and PCR amplification of 16S rRNA gene

The use of molecular biology methods for identification of specific LAB strains in food products is very convenient. The genomic DNA of two probiotic LAB (Cu8 and R32) was extracted in duplicates as per the method of Mora *et al.*, (2000) with little modification and used as a template for PCR amplification. The quality of extracted genomic DNA of probiotic LAB was analyzed by agarose (0.8%) gel electrophoresis (Fig 5.6).

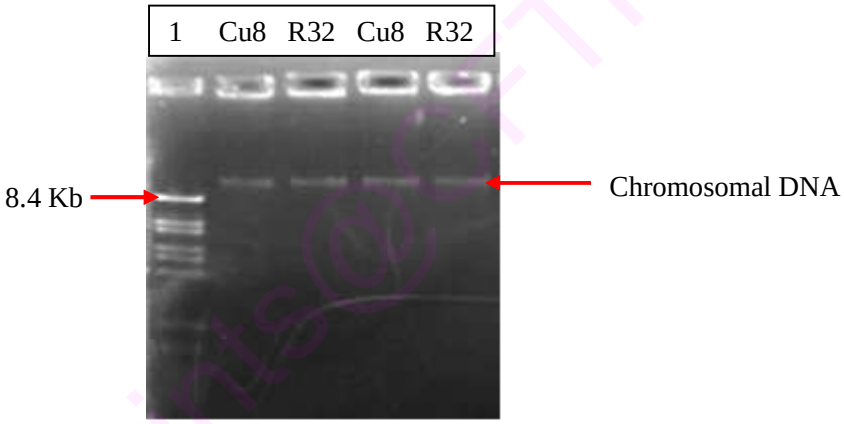

Fig 5.6 Agarose gel (0.8%) showing the distinct bands of chromosomal DNA of the isolates, Cu8 and R32

Lane 1 indicates the λ DNA marker

16S ribosomal RNA (rRNA) gene of LAB isolates was amplified using BSF, forward primer and BSR, reverse primer with thermo cycling conditions as described by Halami *et al.,* (2008). The PCR was performed following the conditions mentioned in Table 5.6, in the Thermocycler Gene Amp PCR system 9700 (Applied Biosystem, USA). The primer annealing temperature of 51 ^{o}C was not suitable for the amplification of 16S rRNA of the native isolate Cu8 and hence annealing was carried out at 56 ^{o}C.

Approximate size of the amplicon was obtained from agarose gel electrophoresis wherein the size of the amplicon was checked against a λ DNA marker.

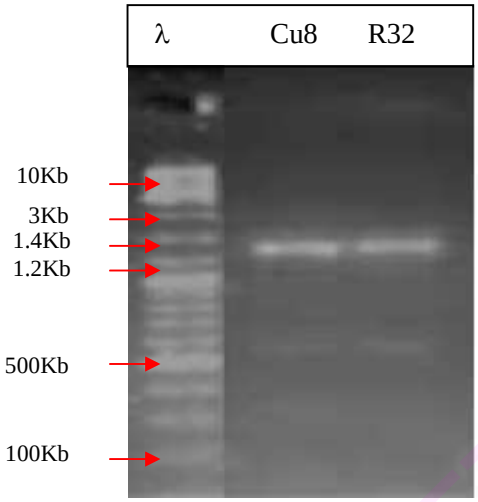

Fig 5.7 Agarose gel (1.5%) showing PCR amplification of 1.4 kb 16S rDNA gene fragment

Lane Cu8 and R32 shows the band that indicates the 16S rRNA gene amplified at 1.4kb region. Lane 1 indicates the 10 kb DNA marker

From the above photograph (Fig 5.7), it was confirmed that the target gene was amplified and the specificity of the primer along with the absence of non specific amplification. The PCR amplified products, ≈ 1.4 kb to be ligated with T- tail vector was gel-purified purified by using Sigma QIAQuick gel extraction kit.

Table 5.6 PCR reaction parameters

PCR reaction parameter	**Cu8**	**R32**
Initial denaturation	95 ^{o}C/ 3 min	95 ^{o}C/ 3 min
Denaturation .	94 ^{o}C/ 40 sec	94 ^{o}C/ 40 sec
Annealing	**56 ^{o}C/ 60 sec**	**51 ^{o}C/ 60 sec**
Extension	72 ^{o}C/ 20 sec	72 ^{o}C/ 20 sec
Final extension	72 ^{o}C/ 15 min	72 ^{o}C/ 15 min

5.3.8.2. Cloning of 16S rRNA gene and sequence analysis of the selected LAB

After purification, the purified PCR products were cloned into the pGEM-T easy vector according to the manufacturer's recommendations (Promega Corporation). The cloned vector was transformed in *E. coli* DH5 α cells. The recombinants were selected based on blue/white colonies and confirmed for the presence of 16S rDNA products in the recombinant *E. coli* DH5 α cells by isolation of plasmid DNA and analysis on gel (Fig 5.8) and also with Restriction analysis.

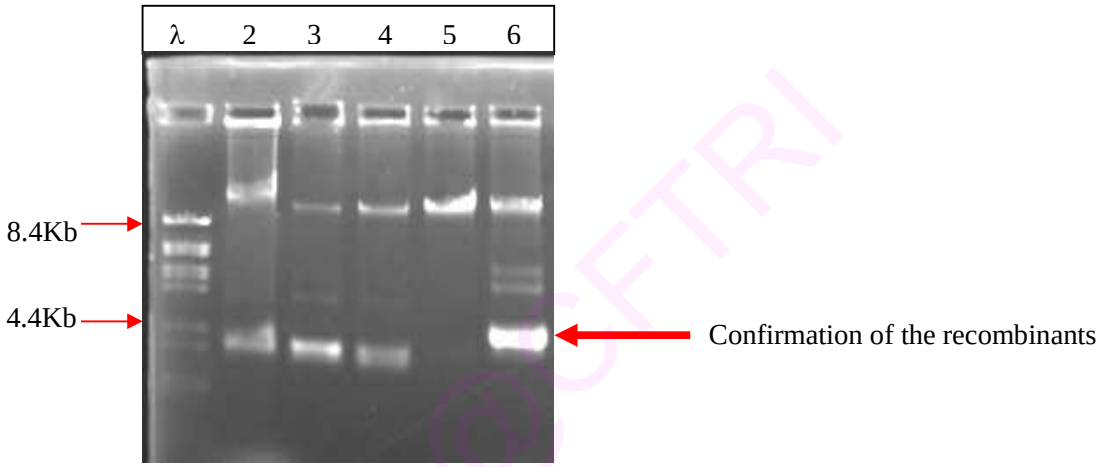

Fig 5.8 Analysis of plasmid DNA isolated from recombinant and non recombinant *E. coli* DH5 α cells

Lane 1 shows λ marker. Lane 2, 3, 4 and 5 are the vector. Lane 6 shows confirmation of recombinants.

A double digestion using *Eco*RI/ N*ot*I shows an insert release of a 1.4 Kb fragment. The recombinant plasmids was subjected for sequencing using M13 forward and reverse primers at the sequencing facility of Genie, Bangalore, India. The nucleotide sequence was analyzed in GenBank database release 155 using Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI, USA) database, for homogenous gene search.

The genotypic identification of the selected probiotic LAB from fruits and vegetables was studied using a total DNA extraction, PCR amplification of 16S rDNA, cloning the 16S rDNA into pGEM-T easy vector and sequence analysis. Identification of

the selected isolates was performed by partial sequencing of approximately 700 bp of 16S rDNA gene. The gene sequences of amplified 16S rRNA gene fragment obtained from representative of each groups were aligned with all sequences present in the GenBank database and resulted in the final identification of the selected LAB isolates. Sequence analysis of PCR products revealed that, for both the isolates there was 1% or no differences with the most closely matched sequence in the databank. According to the reports on the correlation between the DNA-DNA homology and 16S rDNA sequence homology *(Guha and Jaffe, 1996; Kobayashi and Ritmann, 1982)*, strains reveal DNA-DNA homology higher than 70% and show sequence homology higher than 99.5% when strains were identified. Consequently, the two isolates were assigned to the genera *Enterococcus* on the basis of morphological, cultural, biochemical, and physiological characteristics and 16S rRNA gene sequences. They were identified and tentatively named as *Enterococcus hyrae* (Cu8) and *Enterococcus faecium* (R32). These isolates were grouped at species level on the basis of key phenotypic characteristics and 16S rRNA gene sequences.

5.3.8.3. Sequence

The sequence of 589 and 460 bp of rRNA gene obtained for the LAB isolates Cu8 (*Enterococcus hyrae*) and R32 (*Enterococcus faecium*) after sequencing using M13F and M13R primers is shown in the Fig 5.9. The sequence of rRNA gene from Cu8 and R32 were homologous to an extent of 99% with that of other *Enterococcus hirae* and *Enterococcus faecium* strains respectively (Table 5.7 and Table 5.8). Multiple sequence alignment of Cu8 and R32 was carried out using 'Multalin' software. The method used is as described by Corpet (1988). The multiple sequence alignment is shown in the Fig 5.10 and Fig 5.11 respectively. Strain numbers have been assigned for the LAB isolates *Enterococcus hirae* and *Enterococcus faecium* as CFR 3001 and CFR 3002 respectively by culture collection centre of CFTRI, Mysore, Karnataka, India.

LAB isolate Cu8 (*Enterococcus hyrae*)

GAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAA
GTCGAACGCTTCTTTTTCCACCGGAGCTTGCTCCACCGGAAAAAGAGGAGTG
GCGAACGGGTGAGTAACACGTGGGTAACCTGCCCATCAGAAGGGGATAACA
CTTGGAAACAGGTGCTAATACCGTATAACAATCGAAACCGCATGGTTTTGAT
TTGAAAGGCGCTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCT
AGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCATAGCCGACCTGAGAG
GGTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCA
GCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGCCGCGT
GAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGG
ATGAGAGTAACTGTTCATCCCTTGACGGTATCTAACCAGAAAGCCACGGCTA
ACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATT
TATTGGGCGTAAAGCGAG

LAB isolate R32 (*Enterococcus faecium*)

CATGCAAGTCGTACGCTTCTTTTTCCACCGGAGCTTGCTCCACCGGAAAAAGA
AGAGTGGCGAACGGGTGAGTAACACGTGGGTAACCTGCCCATCAGAAGGGG
ATAACACTTGGAAACAGGTGCTAATACCGTATAACAATCGAAACCGCATGGT
TTTGATTTGAAAGGCGCTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCAT
TAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCCACGATGCATAGCCGACCT
GAGAGGGTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGG
GAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAAC
GCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCCTGTTGTTAGAGAA
GAACAAGGATGAGAGTAACTGTTCATCCCTTGACGGTATCTAACC

Fig 5.9 16s rRNA sequence of the probiotic LAB isolates (Cu8 and R32) after DNA sequence analyzer

Table 5.7 Sequences producing significant alignments with that of Cu8 isolate

Accession No.	Description	Max score	Total score	Query coverage	E value	Max Identity
GU727814.1	*Enterococcus hirae* strain T3 16S ribosomal RNA gene, partial sequence	1088	1088	100%	0.0	100%
AB362598.1	*Enterococcus hirae* gene for 16S rRNA, partial sequence, strain: NRIC 0109	1083	1083	100%	0.0	99%
AB362597.1	*Enterococcus hirae* gene for 16S rRNA, partial sequence, strain: NRIC 0108	1083	1083	100%	0.0	99%
AB362596.1	*Enterococcus hirae* gene for 16S rRNA, partial sequence, strain: NRIC 0107	1083	1083	100%	0.0	99%
AB362593.1	*Enterococcus hirae* gene for 16S rRNA, partial sequence, strain: NRIC 0104	1083	1083	100%	0.0	99%
AB362591.1	*Enterococcus hirae* gene for 16S rRNA, partial sequence, strain: NRIC 0102	1083	1083	100%	0.0	99%

Table 5.8 Sequences producing significant alignments with that of R32 isolate

Accession No.	Description	Max score	Total score	Query coverage	E value	Max identity
HM638426.1	*Enterococcus faecium* strain Vm3 16S ribosomal RNA gene, partial sequence	1090	1090	87%	0.0	99%
HM218167.1	*Enterococcus faecium* strain NM31-5 16S ribosomal RNA gene, partial sequence	1090	1090	87%	0.0	99%
HM218160.1	*Enterococcus faecium* strain NM30-4 16S ribosomal RNA gene, partial sequence	1090	1090	87%	0.0	99%
HM218152.1	*Enterococcus faecium* strain NM29-3 16S ribosomal RNA gene, partial sequence	1090	1090	87%	0.0	99%
FJ378892.1	*Enterococcus faecium* strain B-30 16S ribosomal RNA gene, partial sequence	1090	1090	87%	0.0	99%

```
           1                                                                                                  80
       Cu8 GAGTTTGATC CTGGCTCAGG ACGAACGCTG GCGGCGTGCC TAATACATGC AAGTCGAACG CTTCTTTTTC CACCGGAGCT
GU727814.1 GAGTTTGATC CTGGCTCAGG ACGAACGCTG GCGGCGTGCC TAATACATGC AAGTCGAACG CTTCTTTTTC CACCGGAGCT
AB362597.1 GAGTTTGATC CTGGCTCAGG ACGAACGCTG GCGGCGTGCC TAATACATGC AAGTCGAACG CTTCTTTTTC CACCGGAGCT
 Consensus GAGTTTGATC CTGGCTCAGG ACGAACGCTG GCGGCGTGCC TAATACATGC AAGTCGAACG CTTCTTTTTC CACCGGAGCT

           81                                                                                                 160
       Cu8 TGCTCCACCG GAAAAAGAGG AGTGGCGAAC GGGTGAGTAA CACGTGGGTA ACCTGCCCAT CAGAAGGGGA TAACACTTGG
GU727814.1 TGCTCCACCG GAAAAAGAGG AGTGGCGAAC GGGTGAGTAA CACGTGGGTA ACCTGCCCAT CAGAAGGGGA TAACACTTGG
AB362597.1 TGCTCCACCG GAAAAAGAGG AGTGGCGAAC GGGTGAGTAA CACGTGGGTA ACCTGCCCAT CAGAAGGGGA TAACACTTGG
 Consensus TGCTCCACCG GAAAAAGAGG AGTGGCGAAC GGGTGAGTAA CACGTGGGTA ACCTGCCCAT CAGAAGGGGA TAACACTTGG

           161                                                                                                240
       Cu8 AAACAGGTGC TAATACCGTA TAACAATCGA AACCGCATGG TTTTGATTTG AAAGGCGCTT TCGGGTGTCG CTGATGGATG
GU727814.1 AAACAGGTGC TAATACCGTA TAACAATCGA AACCGCATGG TTTTGATTTG AAAGGCGCTT TCGGGTGTCG CTGATGGATG
AB362597.1 AAACAGGTGC TAATACCGTA TAACAATCGA AACCGCATGG TTTTGATTTG AAAGGCGCTT TCGGGTGTCG CTGATGGATG
 Consensus AAACAGGTGC TAATACCGTA TAACAATCGA AACCGCATGG TTTTGATTTG AAAGGCGCTT TCGGGTGTCG CTGATGGATG

           241                                                                                                320
       Cu8 GACCCGCGGT GCATTAGCTA GTTGGTGAGG TAACGGCTCA CCAAGGCGAC GATGCATAGC CGACCTGAGA GGGTGATCGG
GU727814.1 GACCCGCGGT GCATTAGCTA GTTGGTGAGG TAACGGCTCA CCAAGGCGAC GATGCATAGC CGACCTGAGA GGGTGATCGG
AB362597.1 GACCCGCGGT GCATTAGCTA GTTGGTGAGG TAACGGCTCA CCAAGGCGAC GATGCATAGC CGACCTGAGA GGGTGATCGG
 Consensus GACCCGCGGT GCATTAGCTA GTTGGTGAGG TAACGGCTCA CCAAGGCGAC GATGCATAGC CGACCTGAGA GGGTGATCGG

           321                                                                                                400
       Cu8 CCACATTGGG ACTGAGACAC GGCCCAAACT CCTACGGGAG GCAGCAGTAG GGAATCTTCG GCAATGGACG AAAGTCTGAC
GU727814.1 CCACATTGGG ACTGAGACAC GGCCCAAACT CCTACGGGAG GCAGCAGTAG GGAATCTTCG GCAATGGACG AAAGTCTGAC
AB362597.1 CCACATTGGG ACTGAGACAC GGCCCAAACT CCTACGGGAG GCAGCAGTAG GGAATCTTCG GCAATGGACG AAAGTCTGAC
 Consensus CCACATTGGG ACTGAGACAC GGCCCAAACT CCTACGGGAG GCAGCAGTAG GGAATCTTCG GCAATGGACG AAAGTCTGAC

           401                                                                                                480
       Cu8 CGAGCAACGC CGCGTGAGTG AAGAAGGTTT TCGGATCGTA AAACTCTGTT GTTAGAGAAG AACAAGGATG AGAGTAACTG
GU727814.1 CGAGCAACGC CGCGTGAGTG AAGAAGGTTT TCGGATCGTA AAACTCTGTT GTTAGAGAAG AACAAGGATG AGAGTAACTG
AB362597.1 CGAGCAACGC CGCGTGAGTG AAGAAGGTTT TCGGATCGTA AAACTCTGTT GTTAGAGAAG AACAAGGATG AGAGTAACTG
 Consensus CGAGCAACGC CGCGTGAGTG AAGAAGGTTT TCGGATCGTA AAACTCTGTT GTTAGAGAAG AACAAGGATG AGAGTAACTG

           481                                                                                                560
       Cu8 TTCATCCCTT GACGGTATCT AACCAGAAAG CCACGGCTAA CTACGTGCCA GCAGCCGCGG TAATACGTAG GTGGCAAGCG
GU727814.1 TTCATCCCTT GACGGTATCT AACCAGAAAG CCACGGCTAA CTACGTGCCA GCAGCCGCGG TAATACGTAG GTGGCAAGCG
AB362597.1 TTCATCCCTT GACGGTATCT AACCAGAAAG CCACGGCTAA CTACGTGCCA GCAGCCGCGG TAATACGTAG GTGGCAAGCG
 Consensus TTCATCCCTT GACGGTATCT AACCAGAAAG CCACGGCTAA CTACGTGCCA GCAGCCGCGG TAATACGTAG GTGGCAAGCG

           561                  589
       Cu8 TTGTCCGGAT TTATTGGGCG TAAAGCGAG
GU727814.1 TTGTCCGGAT TTATTGGGCG TAAAGCGAG
AB362597.1 TTGTCCGGAT TTATTGGGCG TAAAGCGAG
 Consensus TTGTCCGGAT TTATTGGGCG TAAAGCGAG
```

Fig 5.10 Multiple sequence alignment of rRNA gene of Cu8 with other *Enterococcus sp*. Accession Numbers: GU727814.1 (*Enterococcus hirae* strain T3 16S ribosomal RNA gene) and AB362598.1 (*Enterococcus hirae* gene for 16S rRNA, partial sequence, strain: NRIC 0109)

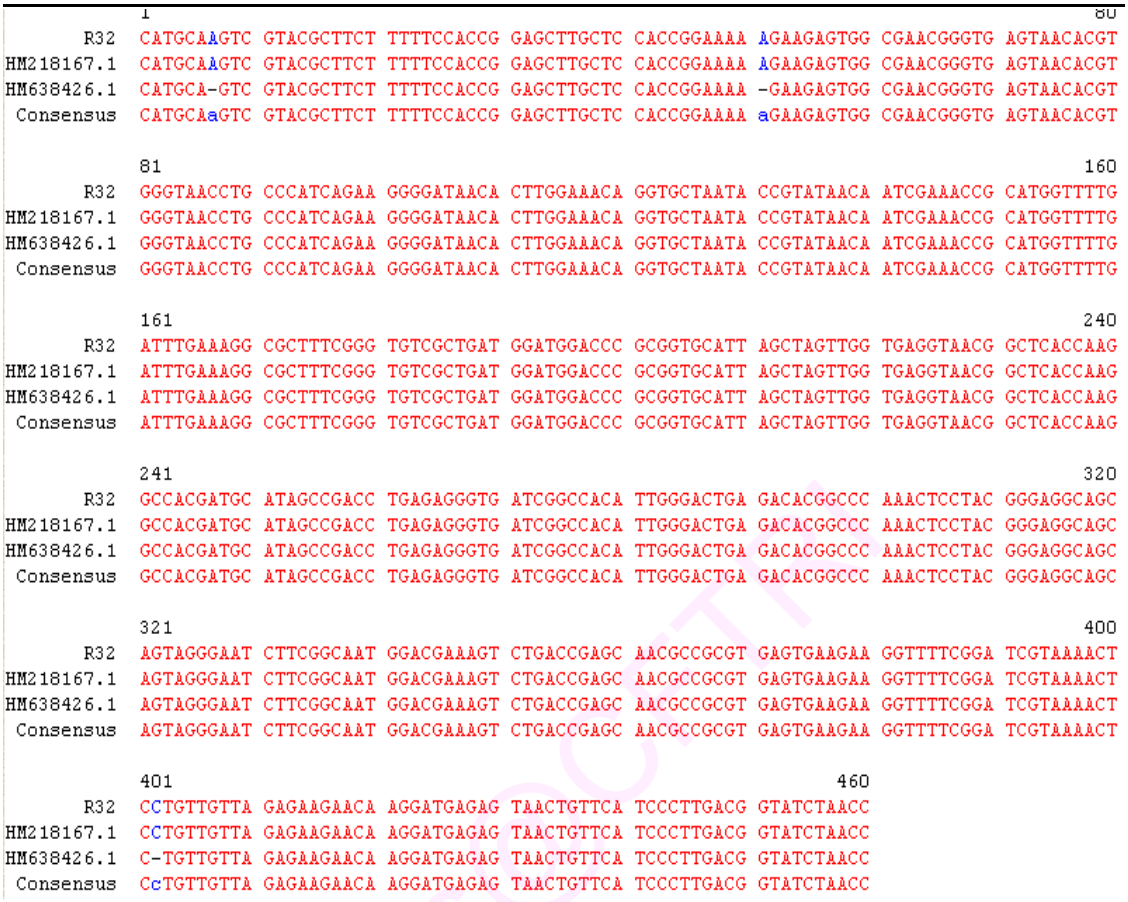

Fig 5.11 Multiple sequence alignment of rRNA gene of R32 with other *Enterococcus sp*. Accession Numbers: HM218167.1 (*Enterococcus faecium* strain NM31-5 16S ribosomal RNA gene) and HM638426.1 (*Enterococcus faecium* strain Vm3 16S ribosomal RNA gene)

5.3.8.4. Thermotolerance, high salt (NaCl) concentration and sodium azide test for *Enterococcus*

The preliminary, confirmatory tests for the identified *Enterococcus hirae* and *Enterococcus faecium* was carried based on the genotypic characterization. Both *Enterococcus hirae* and *Enterococcus faecium* were tolerant to high temperatures (60 ^{o}C and 70 ^{o}C) and high NaCl (10%) concentration and they showed excellent growth (turbidity), which clearly indicated that, both the isolates belong to the genera *Enterococcus*.

The selective media, sodium azide in MRS broth, usually form the basis for the estimation of *Enterococcus* growth was carried. Both *E. hirae* and *E. faecium* showed positive for the tests, indicated the ability of the isolates to ferment 0.04% sodium azide and turns media into red colour. Enterococci are characterized by growth at high temperature, high NaCl and in the presence of sodium azide. The results of carbohydrates, especially maltose and lactose fermentation, thermotolerance, high NaCl concentration and growth at sodium azide test obtained for both the strains clearly confirmed that they belong to *Enterococcus* group and were identified as *E. hirae* and *E. faecium* (section 5.3.8.3).

CHAPTER: 6; SECTION: 6.1

SYNBIOTIC FUNCTIONAL STAR FRUIT WITH FRUCTOOLIGOSACCHARIDES AND SELECTED PROBIOTICS

6.1.1. Introduction

In the recent years, much emphasis is on the 'optimized' nutrition that offers maximum life expectancy and quality living. Changing trends in population demography, consumer affluence, increased education, and improved healthcare have been the driving force behind the rapidly emerging health conscious consumers and thus, the 'functional food' is recognized as the number one global food biotechnology industry *(Dillard and German, 2000; Belem, 1999; Childs, 1999)*. The term functional food was first introduced in Japan in the mid 1980s and is defined as 'any food or food ingredient that provides a health benefit beyond the traditional nutrients it contains' *(Mazza, 1998)*. A food can be said to be functional if it contains a component (which may or may not be a nutrient) that affects one or a limited number of functions in the body in a targeted way so as to have positive effects on health *(Bellisle et al., 1998)* or if it has a physiological or psychological effect beyond the traditional nutritional effect *(Clydesdale, 1997)*. As the market for functional foods continues to expand, research in the development of food products containing prebiotic and probiotic bacteria will also continue to grow *(Christian et al., 2010; Boylston et al., 2004)*. Foods enriched with physiologically active compounds such as probiotics, prebiotics, vitamins, minerals and dietary fibers are considered as functional foods. These foods exhibit beneficial effects in the maintenance of host health and prevention of diseases.

It is well known that probiotic bacteria have beneficial effects on human health and well being. Some of these effects include improvement in gut health, lowering of blood cholesterol and enhancement of body's natural defense mechanisms. Additional health benefits include stimulation of the immune system, vitamin synthesis, anti carcinogenesis and antibacterial activities. The consumer acceptance and survival of added probiotics are the two important criteria to determine efficacy and success of products enriched with probiotics *(Heenan et al., 2004)*. The use of probiotics in food and pharmaceutical industries is mainly driven by the increasing consumer health awareness. Incorporation of probiotics in pharmaceutical products and aquaculture feeds has been of considerable industry interest *(Calo-Mata et al., 2008)*. Scientific evidences suggest that the consumption of probiotic bacteria at high levels (10^9-10^{11} cfu/day) can decrease the

incidence, duration and severity of some intestinal illness *(Boylston et al., 2004; Sanders, 1999)*.

The prebiotic approach advocates the administration of non-viable entities. Dietary carbohydrates such as non-digestible oligosaccharides are the most promising prebiotics, because of their selective metabolism. Current trend in marketing the functional foods is to combine probiotic with prebiotics. Products containing these two components are generally termed 'synbiotics' *(Holzapfel and Schillinger, 2002)*. Synbiotics are used in dairy *(Alegro et al., 2007)* and non dairy products such as cereal bars *(Ouwehand et al., 2004)*, fruit and berry juices *(Tharmaraj et al., 2004)* and confectionary *(Mattila-Sandholm et al., 2002)*. A few probiotic preparations are exclusively consumed as powders, tablets, ice creams, kefir, and fermented dairy products such as yogurt, cheddar cheese, freeze-dried or spray dried preparations *(Golker, 1993; Champagne et al., 1991)*, but are not generally a part of fruit and/or vegetable preparations. Synbiotic products have not been extensively studied to date *(Bielecka et al., 2002)*. Roberfroid (2002) suggested that synbiotic products can improve the survival of probiotics when they pass through the upper part of the gastrointestinal tract and produce greater effects in the large bowel.

The star fruit *(Averrhoa carambola)* is a member of the oxalis family. The fruit acquired its name from the five pointed star shape when cut across the middle of the fruit. The fruit is native to Sri Lanka and popular throughout Southeast Asia, India, southern China, New Zealand, and Australia. Preservation of highly seasonal crops can prevent the huge wastage and make them available in the off season at remunerative prices. A study has been carried out with an objective of value addition to star fruit, an underutilized fruit which is a native to tropical lowlands of India.

The present chapter details the incorporation of fructooligosaccharides (FOS) and probiotics (*Lactobacillus salivarius* CFR 2158 and *Enterococcus hirae* CFR 3001) to star fruits using vacuum osmotic dehydration (VOD) aiming to produce both prebiotic and synbiotic enriched star fruits.

6.1.2. Materials and methods

6.1.2.1. Preparation of FOS syrup

The detailed protocol for the preparation of FOS syrup is given in the section 2.2.1.

6.1.2.2. Production of probiotics (*Lactobacillus salivarius* CFR 2158 and *Enterococcus hirae* CFR 3001)

E. hirae CFR 3001 was isolated from cucumber and a reference culture of *L. salivarius* CFR 2158 was obtained from Central Food Technological Research Institute culture collection center (CFR). Both the isolates were grown in de Man, Rogosa and Sharpe (MRS) broth for 24-48 h at 37 °C. The cells were harvested by centrifuging at 6000 rpm for 15 min at 4 °C (Remi cooling centrifuge, C-30, Mumbai, India) *(Homayouni et al., 2008)*. The cells were stored at 4 °C and were used for further studies.

6.1.2.3. Sample preparation

Star fruits (Bangalore, India) were cleaned and cut into slices. They were water blanched and immediately immersed either in 70 °Brix FOS or in 70 °Brix FOS syrup containing 1% probiotic cell biomass (>300x10^{16} cfu/ml) (w/v). The fruit to FOS ratio was maintained at 1:5 (w/v). Experiments were carried out under vacuum as described in section 2.2.2.

6.1.2.4. Physicochemical characterization of prebiotic and synbiotic star fruits

Moisture content and a_w, colour, texture, FOS content and sensory attributes of prebiotic and synbiotic star fruits over a period of 6 months storage were carried out as detailed in section 2.2.3.1, 2.2.3.2, 2.2.3.3, 2.2.3.5 and 2.2.3.8 respectively.

6.1.2.5. Scanning electron microscopy (SEM) of synbiotic star fruits

A comparative study on the structural changes before and after VOD of star fruits has been carried out using SEM (Leo 435 VP, Leo Electron Microscopy Ltd. (Zeiss), Cambridge, U.K). The sample fixation was carried out as described in section 2.2.3.6.

The specimens were mounted on aluminium studs with double sticky tape and were gold coated (3 A° thickness) for 2 min at 20 mA current using Polaron E5100 SEM coating system. Gold-coated samples were examined at different magnifications (500x, 10,000x and 20,000x).

6.1.2.6. Viability of probiotics

Survivability of probiotics (initially, 3rd month and 6th month) in synbiotic star fruits was determined by viable cell count method. A 5 g of sample was macerated with 0.9% saline (20 ml) and a known volume of serially diluted sample was plated on MRS agar. The plates were incubated at 37 °C for 24-48 h, and the colonies were counted and expressed as cfu/g of samples. The experiment was performed in triplicate and the averages of results are reported.

6.1.2.7. Microbiological analysis

Microbial load of prebiotic (FOS) and synbiotic (*L. salivarius* CFR 2158 and *E. hirae* CFR 3001) enriched star fruits was determined initially and at 2 months intervals up to 6 months of storage as described in section 2.2.3.7.

6.1.2.8. Statistical analysis

Statistical analysis was carried out using a one-way analysis of variance (ANOVA), Origin 6.1 Software. Means were separated using the least significant difference test. Significance was defined at $P<0.05$.

6.1.3. Results and discussion

6.1.3.1. Moisture content and water activity

Fig 6.1.1 shows the moisture content of prebiotic and synbiotic star fruits during 6 months of storage at ambient temperature (25±2 ^{o}C). No significant changes ($P>0.5$) in the moisture content were observed for prebiotic and synbiotic star fruits during storage, indicated that the addition of probiotics (1%) did not have any adverse effect on the moisture content of the final product. The moisture content of the prebiotic and synbiotic

star fruit was around 12% as against that 89% of fresh star fruit. The loss in water content might be due to the increase in osmotic pressure gradient *(Azoubel and Murr, 2004)* during vacuum treatment. The replacement of native liquid or air in the pores of the star fruits is achieved by an external hypertonic solution (FOS) during VOD. Impregnated star fruits were dried at 40 °C for 24 h in order to retain the desirable attributes of fruits, higher survivability of probiotics and stability of the product. Drying at 40 °C helps in water holding sites of the fruit tissues to come close to one another to form a metastable state and thus, helps in reducing the extent of shrinkage. Consequently, the water binding capacity remains unchanged *(Klewick et al., 2009)*. However, drying at higher temperature could result in greater shrinkage of fruits. There was only 1% decrease in the moisture content of prebiotic and synbiotic star fruit during the storage period of six months, which is insignificant. The water activity of prebiotic and synbiotic star fruits was found to be 0.65 and 0.64 respectively. With the decrease in moisture content, the water activity of the final product was also decreased, resulting in stable prebiotic and synbiotic enriched star fruit.

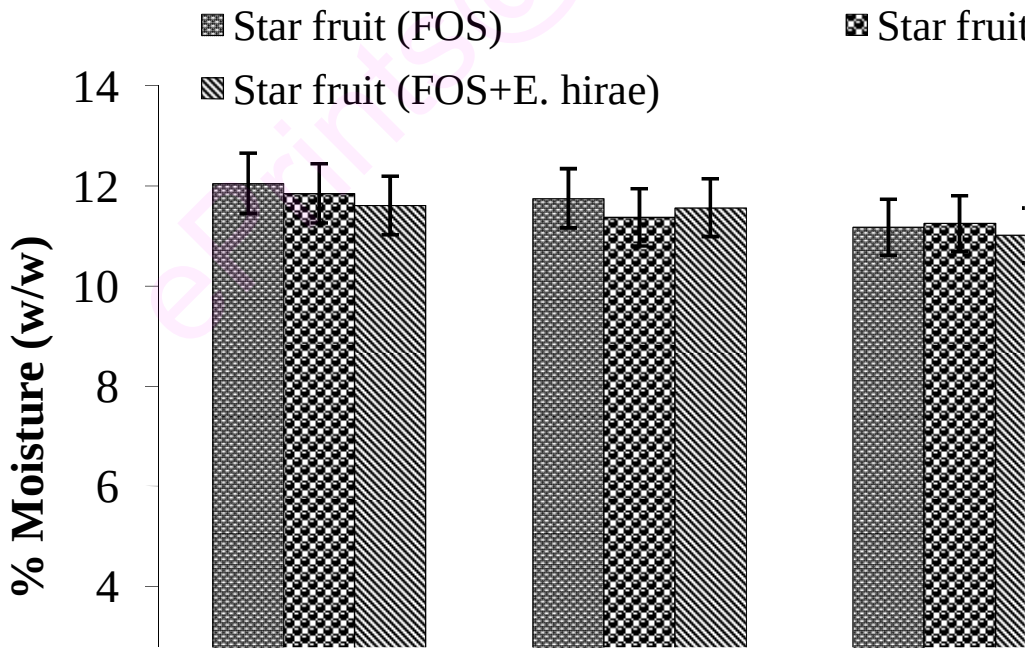

Fig 6.1.1 Moisture content of prebiotic and synbiotic star fruits

6.1.3.2. Effect of impregnation of FOS and probiotics on colour and texture of star fruit

Lightness (*L**) and chroma (*a** and *b**) values are the measure of retension of colour in any processed product. Fig 6.1.2 shows the variation in *L**, *a**, and *b** parameters of star fruits observed after the VOD and compared with the values of fresh star fruits. Higher luminosity values (*L**=51.55) were observed in fresh star fruits, while prebiotic star fruits showed a decrease in *L** parameter (35.82). Similarly synbiotic star fruits (*L. salivarius* CFR 2158 and *E. hirae* CFR 3001) also showed decreased *L** values (34.35 and 35.21 respectively). This indicated that the synbiotic star fruits did not show any significant difference ($P>0.05$) when compared with that of prebiotic enriched star fruit.

The *L***a***b** values of synbiotic star fruits did not show any significant difference ($P>0.05$) up to 4 months of storage at ambient temperature (25±2 ^{o}C), however a very slight decrease was observed at the end of storage period (6 months). This is evident from the observation, that the original *L** *a** and *b** co-ordinates of prebiotic and synbiotic star fruits were maintained as indicated by absence of browning up to 4 months of storage (Fig 6.1.2) at ambient temperature (25±2 ^{o}C). These results are in accordance with the observations of Krokida *et al.*, (2000) on apple and banana, and of Tan *et al.*, (2001) on pineapple. The positive *b** value indicated the retention of yellow tinge in prebiotic and synbiotic star fruits. The impregnation of FOS in fruits would have caused a relative stability of color parameters (*L***a***b**) during storage (6 months). Moreover, the impregnation of star fruit with *E. hirae* CFR 3001 did not result in any significant colour change as in the case of impregnation with a reference culture *L. salivarius* CFR 2158.

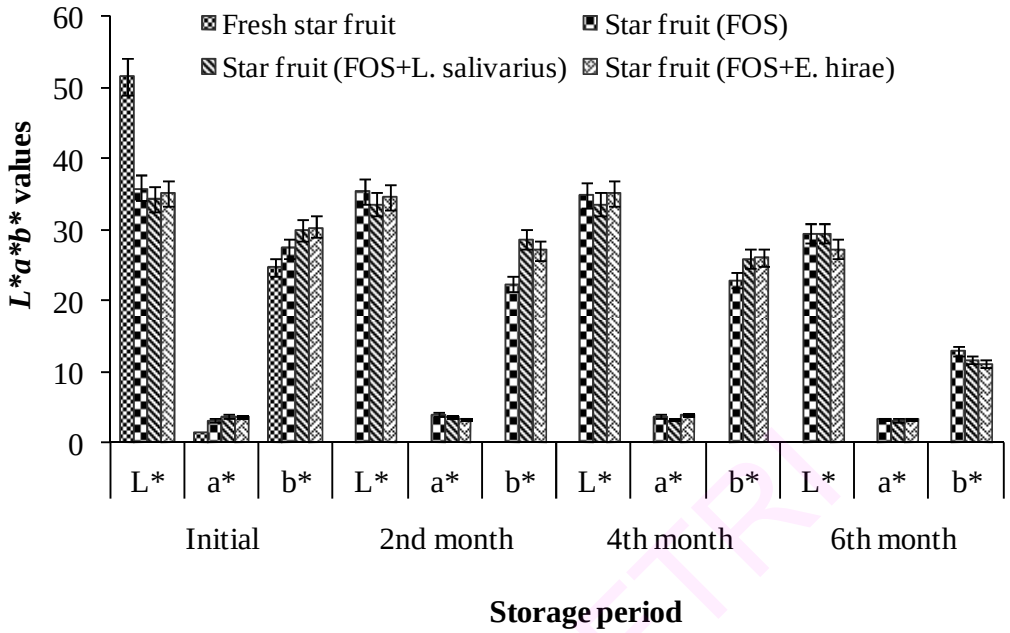

Fig 6.1.2 Colour values of fresh, prebiotic and synbiotic enriched star fruits

Texture makes a significant contribution to the overall food quality, contributing more or less equally with both flavor and appearance *(Bourne, 2002)*. Textural behavior is also related to the structure of foods *(Ramana and Taylor, 1994)*. A marginal increase in shear values of prebiotic and synbiotic star fruits, in comparison with that of fresh fruit was noticed during storage at ambient temperature (Fig 6.1.3). These results were consistent with the work of Tregunno and Goff, (1996), who reported that the presence of sugars increased firmness of thawed apple samples. Monsalve-Gonzalez *et al.*, (1993) reported that firmness correlated significantly with sugar uptake. Torreggiani and Bertolo (2001) observed that cells protected by sugars exhibited less damage to the middle lamella and less severe shrinking of the cell content.

The results showed that the VOD star fruits were more resistant to deformation than the fresh star fruit, indicating that the VOD process increased the elasticity of the star fruit, though it was sensorially perceptible *(Pereira et al., 2003)*. Results also indicated the clear influence of both probiotics (*E. hirae* CFR 3001 and *L. salivarius* CFR 2158) and prebiotic (FOS) ingredient on firmness and juiciness of the synbiotic star fruits. Therefore, during storage period, the texture of star fruit remained constant

(P>0.05) for all the treatments (prebiotic and synbiotic) used. It can be concluded that VOD will result in dehydrated products with firm texture that can withstand further handling and processing. Together with texture improvement, the penetration of solutes combined with dehydration effect could modify the fruit composition and improve pigment, colour, vitamin and aroma retention during storage.

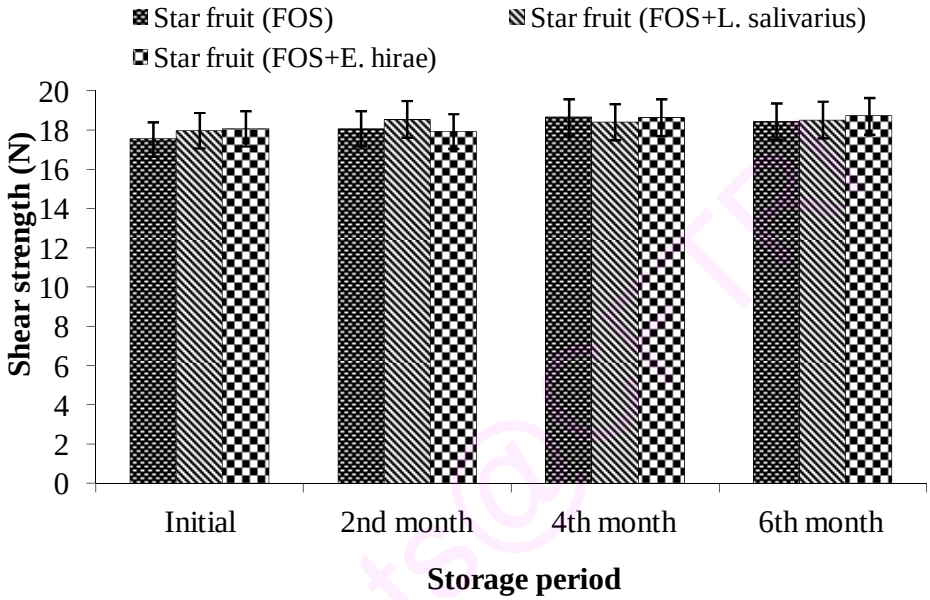

Fig 6.1.3 Texture of prebiotic and probiotic impregnated star fruits

6.1.3.3. FOS content

The FOS content of prebiotic and synbiotic star fruit is shown in Fig 6.1.4. There was no significant changes (P>0.05) in the FOS content of the fruits and was maintained throughout the storage period (6 months). The penetration of FOS syrup into the tissue of star fruit is resulted from the HDM during VOD. The thinner the fruit slices, greater the water losses, which is replaced by the external solution. It was observed that the uptake of FOS by star fruit was significantly higher. This may be due to the greater surface area of slices resulting in increased exposure to the impregnation solution.

Christian *et al.*, (2010) reported that about 100 g of synbiotic fresh-cut apple slices can supply 2-3 g of prebiotics. In the present study, the FOS content was in the range of 17.80-20.24 g/100g of star fruit (initial day). The star fruits stored at ambient

temperature (25±2 ^{o}C) had retained 16.09-18.62 g/100g FOS after 6 months of storage (Fig 6.1.4), which is high enough to claim the product as 'prebiotic star fruit'. Interestingly, the FOS content did not show any significant change (P>0.05) over a period of 6 months of storage at ambient temperature (25±2 ^{o}C). The storage study was, however, limited to 6 months considering the viability of probiotics in the star fruits, since the main focus of the present study was on synbiotic star fruit. The FOS syrup used along with probiotics (*L. salivarius* CFR 2158 and *E. hirae* CFR 3001) probably resulted in certain compositional changes leading to improved quality of the final product.

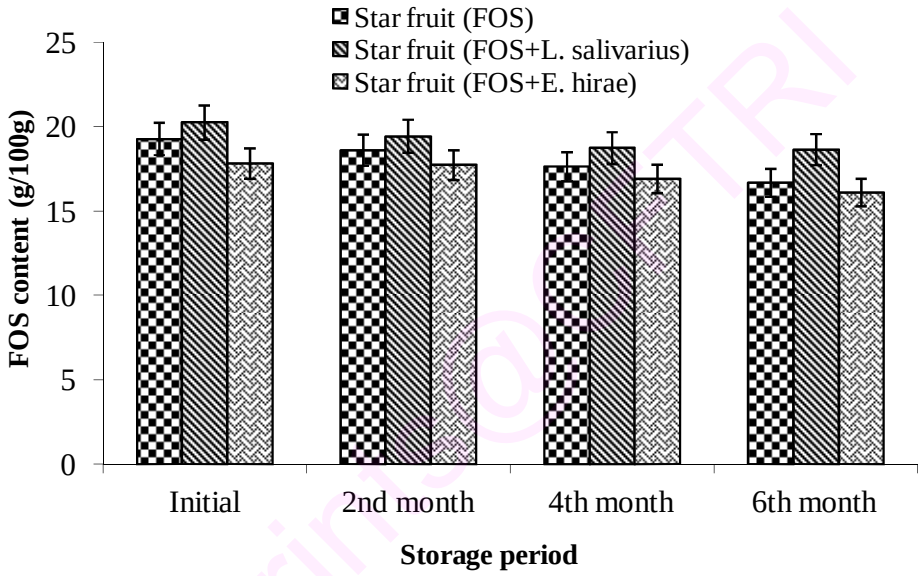

Fig 6.1.4 Retention of FOS content in prebiotic (FOS) and synbiotic fruits

6.1.3.4. Scanning electron microscopy (SEM) of fresh and synbiotic star fruit

Fruits and vegetables offer enough opportunities for the development of new functional products without disrupting their desired cellular structure. Fresh star fruit tissue has a well-organized structure consisting of cell and intercellular spaces (Fig 6.1.5a). VOD resulted in successful uptake of FOS, the base material used as the carrier of probiotics. Thus, the intercellular spaces were filled with *L. salivarius* CFR 2158 (Fig 6.1.5b) and *E. hirae* CFR 3001 (Fig 6.1.5c). The size of the intercellular spaces (210-310 µm) *(Lapsley et al., 1992)* facilitates lodging of probiotics. It was observed that the cell concentration per gram of product varied with the type of organism used. The probiotics

found in the intercellular spaces is probably due to the flow of FOS syrup, originated by pressure gradients created during the VOD.

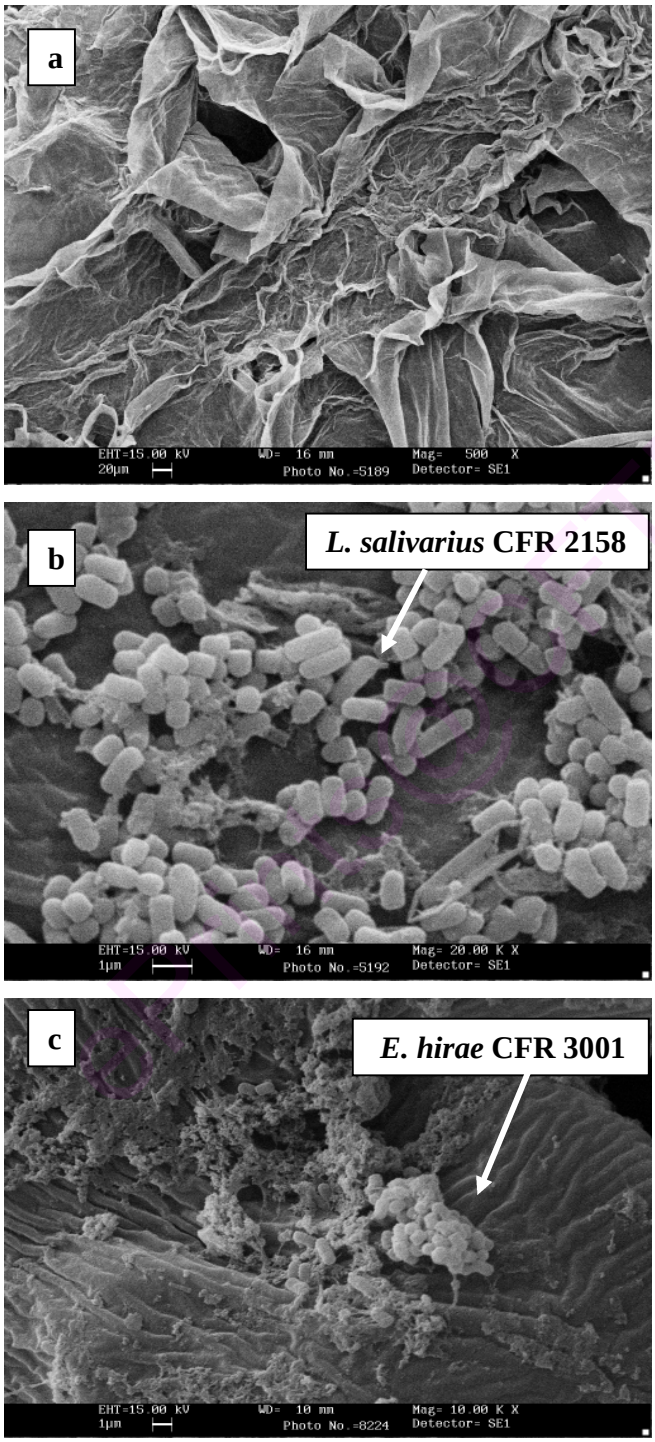

Fig 6.1.5 SEM photographs of fresh (a) and synbiotic [FOS+*L. salivarius* CFR 2158 (b) and FOS+*E. hirae* CFR 3001 (c)] star fruits

6.1.3.5. Viability of probiotics

The viability and stability of probiotics have been both a marketing and technological challenge for industrial producers. It is important to have a significant number of viable probiotics present in the probiotic/synbiotic products for maximum health benefits *(Shah, 2001)*. The viable cell count of *L. salivarius* CFR 2158 and *E. hirae* CFR 3001 was found to be $178x10^6$ cfu/g and $138x10^6$ cfu/g respectively in synbiotic star fruits up to 4 months of storage at ambient temperature (25±2 ^oC). There was slight decrease in the survivability ($34x10^6$ cfu/g and $26x10^6$ cfu/g) of probiotics (*L. salivarius* CFR 2158 and *E. hirae* CFR 3001 respectively) at the end of the storage period (6 months) and this could be significant enough to consider the proposed process as adequate to develop synbiotic functional foods. Vinderola *et al.,* (2002) have indicated the suitability of Argentinean fresco cheese as a food carrier for probiotic cultures and cheese may be used as a vehicle for probiotic bacteria. On a similar line, the fruit used in the present study acts as a vehicle for probiotic bacteria. According to Boylston *et al.,* (2004) the recommended levels of probiotic microorganism in food at the time of consumption in order to have a beneficial effect on consumer health is 10^6 cfu/g. This criterion is fulfilled in the synbiotic star fruits prepared in the present study.

Previously, Rybka and Kailaspathy (1995) reported that the consumption of probiotic yogurt should be more than 100 g/day containing 10^6 cfu/ml. The final product exhibiting high viability can offer beneficial synbiotic properties to the consumer. The product is likely to be affected by harsh conditions of gastric acid and bile salts before it reaches the gastro intestinal tract, which is the site of action. Reddy *et al.,* (2009) observed a higher percentage survivability of *L. salivarius* CFR 2158 at pH 2.5 and 0.3% bile salt concentration. Similarly, *E. hirae* CFR 3001 also showed greater percentage survivability at pH 2.5 and 0.3% bile salt (section 5.3.2). High survivability observed has a bearing from the point of commercialization and marketing of such symbiotic foods with nutraceutical value. Products containing probiotics and prebiotics could improve the survival of the bacteria, crossing the upper part of the gastrointestinal tract, thus enhancing their effects in the large bowel *(Roberfroid, 2002)*. Moreover, probiotic and prebiotic effects might be additive or even synergistic. These synbiotic foods represent an exciting market opportunity for the food and dairy industries.

Star fruits enriched with FOS along with *E. hirae* CFR 3001 and *L. salivarius* CFR 2158 have shown good synbiotic properties and the survivability of probiotics in the impregnated fruits was found to be highly significant. This is sufficient for a probiotic effect, and is comparable to counts of probiotic bacteria in commercially available products.

6.1.3.6. Microbiological analysis

Star fruits enriched with prebiotic FOS along with probiotics *L. salivarius* CFR 2158 and *E. hirae* CFR 3001 were found to be stable at ambient temperature (25±2 ^{o}C) throughout the storage period (6 months). There were no bacterial counts, yeast/molds and coliform detected in prebiotic and synbiotic star fruits up to 4 months of storage. However, few colonies of coliform (17 to 24x10^{2} cfu/100g) were observed at the end of storage period (6 months). The storage study was limited to 6 months considering the growth of coliforms. Observed numbers of yeasts and moulds were lower than bacteria. Tournas (2005) obtained similar results with samples of fresh and minimally-processed vegetables.

6.1.3.7. Sensory evaluation

Sensory attributes are the key factor in determining the shelf life of many food products. The sensory scores for various attributes of prebiotic and synbiotic star fruits are given in Table 6.1.1. As can be seen from the results the overall acceptability of prebiotic and synbiotic star fruits did not show any significant difference (P>0.05) and were found to be highly acceptable by the panelist. In synbiotic fruits, it is important that the prebiotic component should impart good sensory properties by improving mouth feel and texture of the product. Product quality and sensory properties of the final product were highly acceptable indicating that the FOS with 1% probiotic cells resulted in a star fruits with desirable sensory attributes.

The synbiotic star fruits exhibited all desirable sensory properties as in the case of prebiotic star fruit. In general, substitution with prebiotic ingredients has a greater influence on texture and aroma, whereas substitution with probiotics has a greater effect on flavor and aroma. A number of authors reported that probiotic microorganisms

affected the flavour of food product to which they were added. Bernardi *et al.,* (2004) observed that an acerola flavoured ice cream with fermented milk containing *B. longum* and *B. lactis* had a moderate acceptance, whereas ice-cream not supplemented with these microorganisms was well accepted by consumers.

In the present study, star fruit is shown to be an excellent vehicle for the incorporation of probiotics like *E. hirae* CFR 3001 and *L. salivarius* CFR 2158 and the prebiotic, FOS. The prebiotic ingredient is incorporated into the product matrix, conferring and enforcing already existent bonding between different components of the food, resulting in a palatable texture. Metabolism of the probiotic culture can result in the production of components that may contribute negatively to the aroma and taste of the product. In addition to this, it is also reported that *Bifidobacterium* spp. produce acetic acid as a product of their metabolism, which resulted in a vinegary flavor in the product, prejudicing the performance in sensory assessments *(Tamime et al., 1995)*. In the present study, however, the addition of 1% probiotics (*E. hirae* CFR 3001 and *L. salivarius* CFR 2158) did not adversely affect the sensory attributes of the final product.

Table 6.1.1 Sensory attributes of prebiotic and synbiotic fruits

Star fruits	Sensory parameters				
	Colour	**Aroma/ Flavour**	**Firmness**	**Juiciness**	**Overall acceptability**
Prebiotic star fruit (FOS)	8.00±0.25	8.00±0.16	7.00±0.22	7.00±0.25	8.00±0.25
Synbiotic star fruit (FOS+ *L. salivarius* CFR 2158)	8.00±0.25	8.00±0.25	8.00±0.16	7.00±0.25	8.00±0.25
Synbiotic star fruit (FOS+ *E. hirae* CFR 3001)	8.00±0.22	8.00±0.26	8.00±0.16	7.00±0.25	8.00±0.22

*Minimum acceptable score is 7.00

From the results obtained it was concluded that the probiotic *E. hirae* CFR 3001 was highly comparable with the reference culture, *L. salivarius* CFR 2158 for all the properties studied. The star fruits enriched with prebiotic FOS along with probiotic *E. hirae* CFR 3001 and *L. salivarius* CFR 2158 was in turn comparable with prebiotic enriched star fruits without affecting the sensory and other properties. It is suggested that novel synbiotic fruits can be prepared using prebiotics in combination with probiotics.

CHAPTER: 6; SECTION: 6.2

SHRIKHAND: A FERMENTED MILK BASED DESSERT ENRICHED WITH FRUCTOOLIGOSACCHARIDES AND PROBIOTIC *Enterococcus faecium* CFR 3002

6.2.1. Introduction

In the last couple of decades, the growing concern about health and life quality has encouraged people to exercise regularly, eat healthy food and decrease the consumption of food rich in sugar and salt. In addition, there has been an increase in the demand by consumers for food with functional properties. With increased consumer interest in reducing sugar intake, food products made with sweeteners other than sugar have become more popular. The discovery of a great number of sweeteners has triggered the development of new sugar free products, particularly for diabetics, people on special diets and/or for the obese *(Ozdemir and Sadikoglu, 1998)*. Artificial sweeteners are alternative substances to table sugars, which give food a sweet taste (*Ministério da Saude, 1997*), and are used to replace sucrose partially or totally. During the past few decades, low-calorie artificial sweeteners, such as aspartame, saccharin, acesulfame and sucralose, have become sugar alternatives to replace sucrose, and have been widely used in dairy products *(Calorie Control Council, 2002)*.

India has emerged as the largest milk producing country in the world (*Lee, 2010*). A considerable portion of milk in India is utilized for the manufacture of indigenous milk products *viz*. clarified butter (ghee), heat desiccated milk (khoa), acid coagulated milk (chhana), Indian cheese (paneer) and varieties of sweets. Heat and acid coagulation, heat desiccation, separation, fermentation etc are the different processing steps involved in the preparation of above products (*Patil, 2005*). Indian fermented milk products utilize only 7% of total milk produced (*Aneja et al., 2002*) and mainly includes three products dahi (curd), *shrikhand* (sweetened concentrated curd) and lassi (stirred curd), which may be considered as equivalent to western yoghurt, quarg and stirred yogurt, respectively.

Among various fermented milk products, the market for 'yogurt' has increased considerably (*Saavedra et al., 2004*), owing to yogurt's nutritional and therapeutic benefits (*Sarkar and Misra, 2002a*). Today, organized dairies make only 1 to 2% of the yoghurt consumed in India, but demand for industrially produced yoghurt is currently growing at a pace of 20% per year. International Market Analysis Research and Consulting (IMARC) Group, one of the world's leading research and advisory firms, finds in its new report "Indian Dairy Market Report and Forecast 2011-2016" that the growth of the organized dairy sector is expected to increase rapidly in the next five years

reaching values worth US$ 29 Billion by 2016. The reports expect that by 2016, around 26% of the dairy market will be organized compared to 19% in 2010. This report provides an analytical and statistical insight into the Indian dairy market.

Recently, attempts have been made to incorporate certain probiotic lactic acid bacteria (LAB) with the objective of enhancing the dietetic properties of yoghurt. LABs are of essential importance for their role in most industries of fermented foods as starter cultures. Use of LAB in dairy industry has resulted in different cultured milk products with improved preservation accompanied with characteristic flavor, aroma and texture, non-identical from the original food *(Kleerebezemab et al., 2000; Saloff-Coste, 1994).* There have been few reports on the use of certain probiotic LAB along with the starter cultures in the preparation of traditional milk products. These include *L. delbrueckii* subsp. *bulgaricus, S. thermophilus, B. bifidum (Geetha et al., 2003)* and *L. delbrueckii* subsp. *bulgaricus, S. thermophilus, L. dextranicum (Patil et al., 2006, Suryawanshi et al., 2006)* and *L. casei, L. lactis* subsp. *diacetylactis (Sinha et al., 2005).*

Enterococci are ubiquitous LAB that occurs frequently in large numbers in dairy products and other fermented foods and are part of the normal intestinal microbiota. *E. faecalis*, *E. faecium* and *E. durans* are the most frequently found species in dairy products *(Foulquie-Moreno et al., 2006)*. They play an important role in cheese making and contribute to the development of the sensory characteristics of many varieties of cheese *(Manolopoulou et al., 2003)*.

All fermented milk products contain live LAB, unless they are pasteurized after fermentation. The addition of probiotics to these products is a natural way of enhancing the functionality of such products. The most common functional dairy products are with probiotic bacteria, quite frequently enriched with prebiotics *(Saxelin et al., 2003)*. Given the commercial success and growth of yoghurt as a healthy choice among consumer, it is considered a good vehicle for probiotics *(Bran-non, 2006)*. Yoghurt, the oldest Indian fermented milk product may be either sour or sweet, consumed directly as a part of diet. It is also used as a base material for the preparation of certain fermented Indian dairy products such as *shrikhand,* a popular Indian dessert belonging to the group of fermented and coagulated milk products.

The current chapter details the studies on preparation, physicochemical, microbiological and sensory attributes of synbiotic *shrikhand* with fructooligosaccharides (FOS) and probiotic *Enterococcus faecium* CFR 3002.

6.2.2. Materials and methods

6.2.2.1. Preparation of FOS powder

The FOS syrup of 58 °Brix was diluted to 30 °Brix using distilled water. Maltodextrin [10% w/v)] and tri-calcium phosphate [0.5% (w/v)] were added to the diluted FOS syrup and it was spray dried (BE 1216; Bowen engineering, Inc., Somerville, New Jersey, USA) at an inlet temperature of 130-140 ^{o}C. An outlet air temperature of 90-95 ^{o}C was maintained at a liquid flow rate of 60-70 ml/min, to obtain fine FOS powder (57%).

6.2.2.2. Propagation of yoghurt starter cultures (*Lactobacillus bulgaricus* CFR 2028 and *Streptococcus thermophilus* ATCC 19258) and probiotic *Enterococcus faecium CFR 3002*

L. bulgaricus and *S. thermophilus* were acquired from Central Food Technological Research Institute culture collection center (CFR) and American Type Culture Collection (ATCC) respectively and *E. faecium* CFR 3002 was isolated from radish. These strains were activated by transferring it [10% (v/v)] to MRS broth, followed by incubation at 37 °C for 24 h. Thereafter, the cultures were transferred [10% (v/v)] to sterile reconstituted skim milk medium (3 ml of peptone broth and 6 ml of skim milk), incubated at 37 °C for 15-18 h and used for further studies.

6.2.2.3. Preparation of *shrikhand*

Cow milk (1L, 3% fat Nandini milk; Packed and marketed by Mysore-Chamarajanagar Dist., Co-operative milk producers societies union Ltd., Mysore, Karnataka, India) was heated to 85 °C for 30 min, and then cooled to 40±2 °C. *S. thermophilus* (1% v/v) and *L. bulgaricus* (1% v/v) in combination with probiotic *E. faecium* CFR 3002 (2.0 % v/v) were inoculated to the milk and incubated for 10-12 h at

40±2 °C using incubator (Alpha Scientific Co. Ranla Plaza, Bangalore, India). The pH of the set curd at the end of 12 h was 4.6 to 4.8 with a corresponding acidity of 0.46 to 0.52% lactic acid. Lactic fermented curd was suspended in muslin cloth for 6 to 8 h to separate out whey and the resultant solid mass known as 'chakka' was subsequently used for the preparation of *shrikhand* with FOS and/or sucrose. The chakka was divided into three equal portions. Each portion was thoroughly mixed with FOS (40%, w/w), sucrose (cane sugar) (40%, w/w) *(Patel and Chakraborty, 1982)*, and a blend of FOS (20%, w/w) and sucrose (20%, w/w) till attains a semi-soft consistency and sweetish sour taste, typical of *shrikhand*. No coloring and flavoring ingredients were added to the prepared product. Fig 6.2.1 shows the photograph of prepared *shrikhand* with FOS (a), blend (b) and sucrose (c).

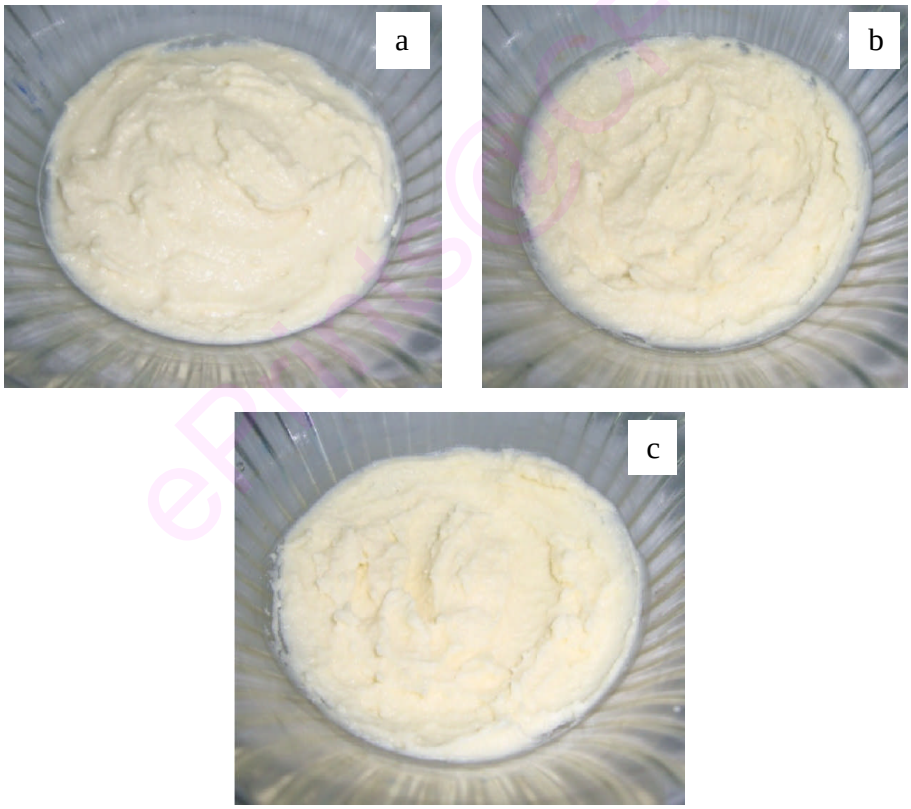

Fig 6.2.1 *Shrikhand* with (a) FOS, (b) blend of FOS and sucrose, and (c) sucrose

The moisture content, water activity, colour, rheology, and FOS content of *shrikhand* were determined. The viability of probiotics and other relevant microbiological and sensory attributes were evaluated during two months of storage at refrigerated temperature (4±2 °C). The structural analysis was also carried out.

6.2.2.4. Moisture content and water activity (a_w)

The moisture content and a_w were determined as detailed in section 2.2.3.1.

6.2.2.5. Colour

The colour was measured as per the protocol described in section 2.2.3.2.

6.2.2.6. Rheology

Strain controlled Rheometer (SR5, Rheometric Scientific) was used to measure rheology of *shrikhand*. The tests were carried out on 25 mm Teflon plates with a gap of 1.0 mm between the plates. A thin coat of low viscosity silicon oil was applied to the free surface of the sample to prevent moisture evaporation *(Kulkarni et al., 2006)*. Gap setting between the samples was performed slowly so as to avoid high shear rates during sample loading and the same protocol was followed every time. Dynamic strain sweep and frequency sweep experiments were performed at a temperature of 25 ^{o}C. Temperature was controlled using a forced convection oven. The rheological data were analyzed with the supporting software 1SR3:RS1 Orchestrator**.** The samples were first subjected to a frequency sweep test using a frequency ramp from 0.1 to 10 Hz at a constant strain of 0.5% (determined from an amplitude sweep at 1 Hz) to ascertain the viscoelastic properties. The storage and loss module and phase shift were recorded for all the samples.

6.2.2.7. FOS content of *shrikhand*

Shrikhand prepared with FOS, blend of FOS and sucrose, and sucrose along with probiotic *E. faecium* CFR 3002 were taken (5 g), mixed with triple distilled water (5-10 ml) with rigorous stirring, and centrifuged at 8000 rpm for 20 min. The aqueous phase was filtered using 0.22 µ filter membranes (Millipore Pvt. Ltd, India). Appropriately

diluted samples were analyzed by HPLC (SCL-6B, Shimadzu, Japan) as explained in section 2.2.3.5 (*Sangeetha et al., 2002*).

6.2.2.8. Ultrastructure by Scanning Electron Microscope (SEM)

Structural analysis of *shrikhand* enriched with FOS, blend, and sucrose along with *E. faecium* CFR 3002 were carried out using SEM [Leo 435 VP, Leo Electron Microscopy Ltd. (Zeiss), Cambridge, U.K]. The *shrikhand* smear was prepared on a cover slip and was fixed using glutaraldehyde (2% in 0.1 M phosphate buffer; pH 7.2 to 7.4) at 4 °C, for 24 h. The samples were dehydrated as explained under the section 2.2.3.6. Gold-coated samples were examined at 20000x magnification.

6.2.2.9. Viability of probiotics

The survivability of total probiotics in *shrikhand* [freshly prepared (0 day) and stored at 4±2 ^{o}C] was carried out at 20 day intervals following viable cell count method as described in section 6.1.2.6.

6.2.2.10. Storage studies

Shrikhand samples sweetened with FOS, blend, and sucrose along with *E. faecium* CFR 3002 were placed in glass bowls covered with glass lid and were stored at 4±2 °C. The shelf stability was determined initially and at 20 days intervals up to 60 days of storage by standard plate count method as described in section 2.2.3.7.

6.2.2.11. Sensory Analysis

Sensory attribute of *shrikhand* sweetened with FOS, blend, and sucrose along with *E. faecium* CFR 3002 was carried out using the protocol explained in section 2.2.3.8.

6.2.2.12. Statistical analysis

Statistical analysis was carried out using a one-way analysis of variance (ANOVA), Origin 6.1 Software. Means were separated using the least significant difference test. Significance was defined at $P<0.05$.

6.2.3. Results and discussion

6.2.3.1. Moisture content and water activity (a_w)

Moisture content of *shrikhand* enriched with FOS, blend and sucrose along with probiotic *E. faecium* CFR 3002 was 45.05%, 45.13%, and 44.88% respectively and the corresponding a_w was 0.88, 0.89, and 0.89. The FOS sweetened *shrikhand* did not show any significant difference in moisture content, when compared with sucrose sweetened *shrikhand*. There were no significant differences ($P>0.05$) in the moisture content and a_w of all the samples over a period of 60 days storage. Thus, the result indicated that the addition of FOS and probiotic *E. faecium* CFR 3002 had not resulted in any undesirable changes in the physicochemical characteristics of the *shrikhand.*

6.2.3.2. Colour

The *L* a* b** values (Fig 6.2.2) of *shrikhand* enriched with prebiotic FOS, blend and sucrose along with probiotic *E. faecium* CFR 3002 were measured immediately after its preparation (0 day), and after storage for 20, 40 and 60 days at refrigerated temperature (4±2 °C). As can be seen from the Fig., the *L**and *b** values of *shrikhand* sweetened with FOS and blend of FOS and sucrose decreased gradually as the storage increased to 60 days. Where as *a** value of *shrikhand* did not show any significant difference ($P>0.05$) during this period. Similar trend was observed in the case of sucrose sweetened *shrikhand*, during the same storage period. The change in *L** value of FOS, blend and sucrose sweetened *shrikhand* ranged from 71.10 to 65.79, 71.55 to 67.15 and 73.10 to 66.44 respectively and the change in *b** value ranged from 10.92 to 7.37, 11.50 to 7.88 and 11.37 to 7.82 respectively over 60 days storage. *L** value is an estimation of whiteness *(Owens et al., 2001)*. Consumers have the highest appeal for products with appealing visual properties and the perception of products whiteness has been reported to have the most positive influence on increasing consumer appeal *(Hutchings et al., 1994)*. The decrease in *L** value is most likely attributable to the decreased size of casein micelles.

The lower *L** and *b** values observed in the *shrikhand* could be due to the higher levels of whey protein in the yoghurt. The replacement of sucrose either fully or partially with prebiotic FOS along with *E. faecium* CFR 3002 did not affect the colour of the final product. The ANOVA showed that the effect of FOS and *E. faecium* CFR 3002 on the colour of *shrikhand* sweetened with FOS and blend of FOS and sucrose was insignificant (P>0.05) when compared with that of sucrose sweetened *shrikhand*.

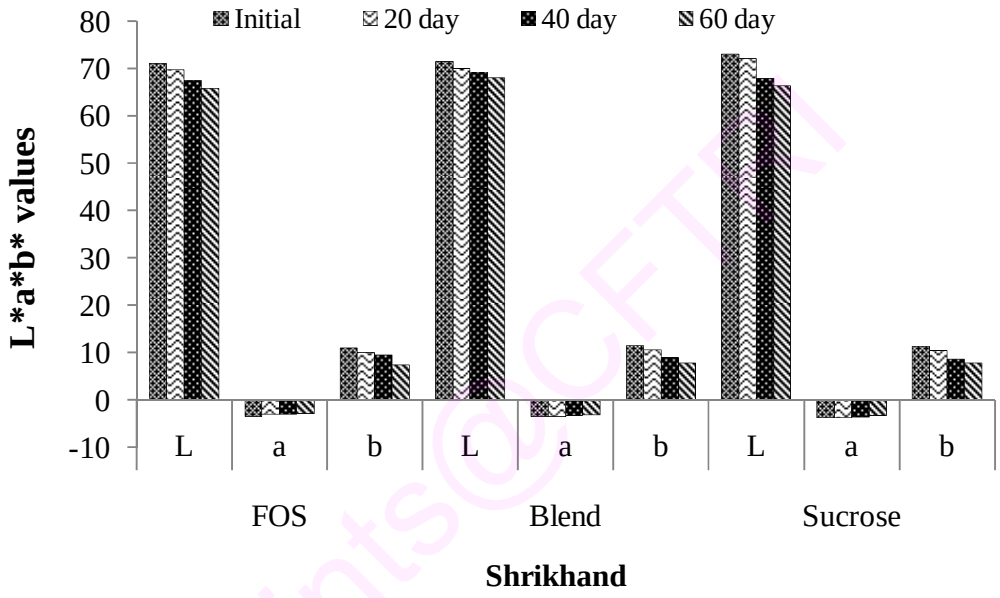

Fig 6.2.2 Colour of *shrikhand* enriched with prebiotic FOS, blend and sucrose and probiotic *E. faecium* CFR 3002

6.2.3.3. Rheology

Rheological properties of foods, such as fermented dairy products, are important in the design of flow processes, quality control, storage, processing and in predicting the texture of foods (*Shaker et al., 2000*). Rheology and structure of a product evaluated by instrumental methods give relevant information on its textural properties, even though sensory and instrumental data are not always easily correlated (*Sodini et al., 2004*). Food material exhibits both solid and liquid characteristics, and rheology can identify the properties of such food (*Tunick, 2000*). Rheology can be used as a sensitive tool to quantify some of the attributes of *shrikhand* such as its texture and consistency. The

literature on the rheology of *shrikhand* is relatively scarce and more work with different sweetener/prebiotics is required to understand the effect of rheology on the texture and consistancy of *shrikhand.* The flow behaviour of its precursor yogurt has been widely studied and reviewed *(O'Donnell and Butler, 2002a; O'Donnell and Butler 2002b; van Marle et al., 1999)*. Rheometers working in dynamic mode are used to calculate storage and loss modulus that describe the elastic and viscous properties of the gelling system, respectively (*Sodini et al., 2004*).

Elasticity, viscosity and stress are some of the rheological properties, which are generally studied. These properties are studied in terms of different modulous like storage (G´), loss (G´´), complex (G*) modulii and phase angle; tan delta (δ). To study the viscoelastic behaviour of *shrikhand* prepared with FOS, blend and sucrose, the sweep of G´ and G´´ modulii with respect to dynamic frequency sweep (DFS) was carried out.

The DFS shows that the value of storage modulus (G´) is greater than loss modulus (G´´) over a measurable frequency range in all the *shrikhand* samples. In case of FOS enriched *shrikhand* the value of storage and loss moduli is slightly on higher side when compared to *shrikhand* sweetened with that of blend and sucrose. However, not much difference was found in magnitude of elastic and viscous modulii during frequency sweep experiments. *Shrikhand* prepared with FOS, blend and sucrose along with *E. faecium* CFR 3002 seem to be consistent along with the frequency (Fig 6.2.3). It has been reported that the *shrikhand* exhibits a combination of several rheological properties such as weak gel-like visco-elasticity, an apparent yield stress and long structural recovery time scales *(Kulkarni et al., 2005)*. From the result obtained, it can be concluded that the FOS enriched *shrikhand* exhibited better gel like consistency, as in case of blend and sucrose without affecting other properties like color, microstructure etc.

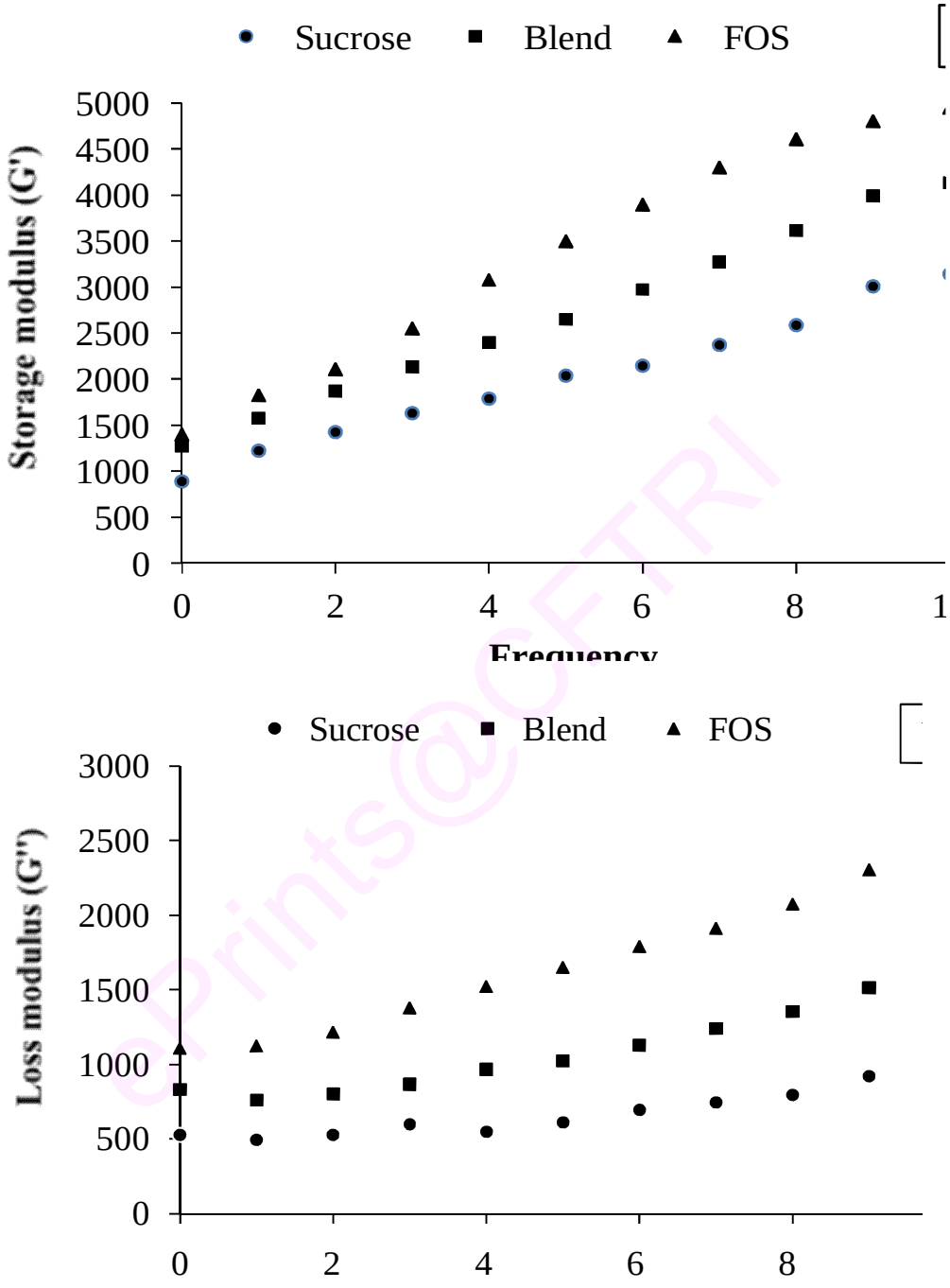

Fig 6.2.3 Rheology [(a) storage and (b) loss modulus] of *shrikhand* enriched with prebiotic FOS, blend and sucrose along with probiotic *E. faecium* CFR 3002

6.2.3.4. FOS content

Fig 6.2.4 shows the FOS content of *shrikhand* enriched with FOS and blend of FOS and sucrose along with probiotic *E. faecium* CFR 3002. The initial (0 day) FOS content of *shrikhand* enriched with FOS and blend of FOS and sucrose was found to be 10.32 g/100g and 5.55 g/100g respectively. It was found to be decreased to 6.16 g/100g and 1.76 g/100g at the end of the storage period (60 days) at 4±2 ^{o}C. The popularity of dairy products prepared with prebiotics and probiotics continues to increase, as consumers desire appetizing foods that will fulfill their health needs. The incorporation of prebiotics can increase the mouth feel attributes of the product while providing an adequate sweetness level *(Guggisberg et al., 2009)*. *Shrikhand* enriched with prebiotic FOS can therefore drive preference and present additional health benefits, beyond traditional ingredients. The result shows that, the FOS could be a good replacer to sucrose in the preparation of any milk based products. It is used in a wide range of foods *(Renuka et al., 2010)* and beverages *(Renuka et al., 2009)*, as it doesn't interact with any commonly used food ingredients.

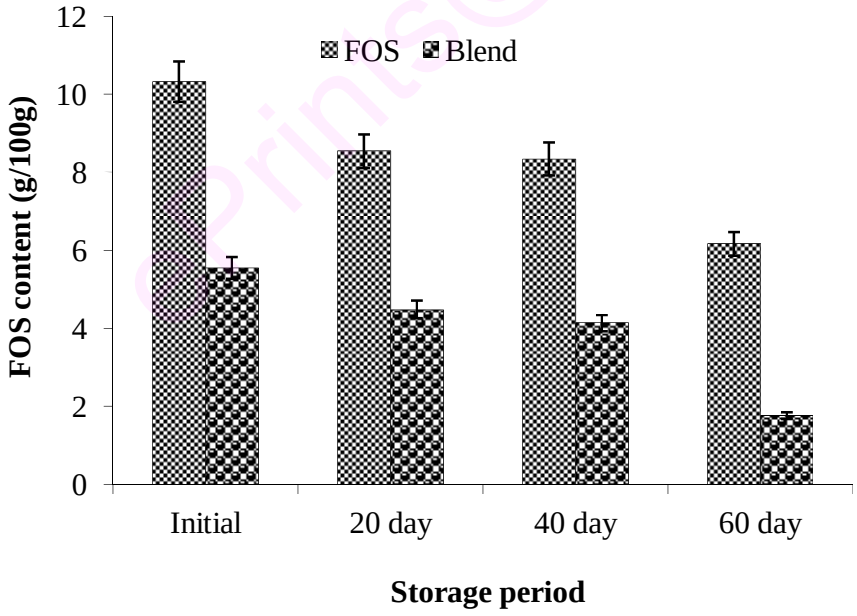

Fig 6.2.4 FOS content of *shrikhand* enriched with FOS and blend of FOS and sucrose

6.2.3.5. Ultrastructure by Scanning Electron Microscope (SEM)

The composition of *shrikhand* and the processing conditions determine its microstructure, which in turn decides the texture and consistency of the product. Microstructure of *shrikhand* enriched with FOS, a blend of FOS and sucrose, and sucrose along with probiotic *E. faecium* CFR 3002 are presented in Fig 6.2.5 (a), (b) and (c) respectively. It is well recognized that the structure of foods greatly affects their various properties including texture, functionality and appearance. Microstructure has a major impact on the texture and other physical properties of acid milk gels. Various factors such as heating of milk, total solids content, pH, lactic acid bacteria and thickening agents have been reported to affect the microstructure of milk based products *(Kalab et al., 1983)*.

The formation of the microstructure and its firmness as affected by the type of culture, extent of syneresis, and thermal treatment has been studied by many researchers *(Haque et al., 2001; Lucey, 2001; Lucey et al., 1999)*. It would be interesting to see the effect of a prebiotic FOS, compared to sucrose, on *shrikhand* gel network formation. SEM observation revealed that in the presence of either FOS or a blend of FOS and sucrose, the *shrikhand* matrix was predominantly casein micelles arranged in double longitudinal polymers [Fig 6.2.5 (a) and (b)] along with clusters in some spots. Where as in case of *shrikhand* sweetened with sucrose, casein micelles associated in the form of clusters [Fig 6.2.5 (c)], causing the matrix to form small void spaces.

The present study showed that there was slight difference in the microstructure of *shrikhand* enriched with FOS, when compared with that of sucrose sweetened *shrikhand*. Even though, there observed a slight difference by SEM analysis, the texture of *shrikhand* was palatable and acceptable by the panelist.

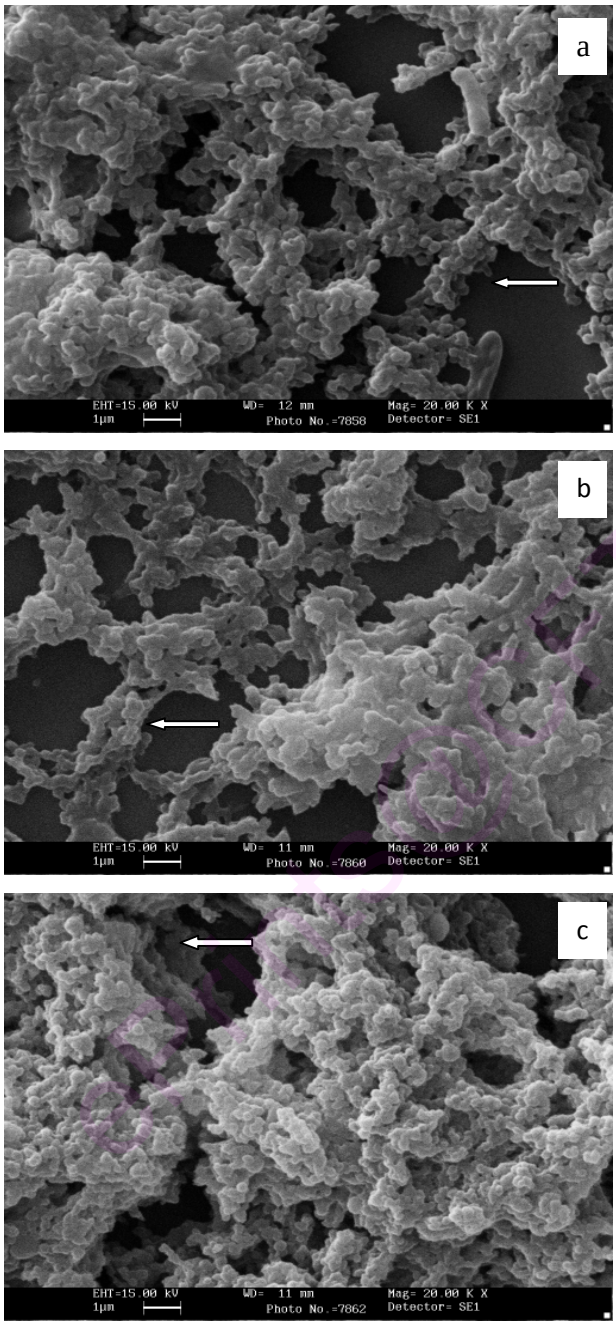

Fig 6.2.5 Scanning electron micrograph (SEM) of *shrikhand* enriched with (a) FOS, (b) a blend of FOS-sucrose, and (c) Sucrose

6.2.3.6. Viability of total probiotics

Milk based products generally have a shelf life of <40 days and viability of probiotic bacteria in products over long shelf life at refrigerated temperature is reported to be poor *(Dave and Shah, 1997)*. Several researchers have reported poor survival of probiotics in milk based product during refrigerated storage *(Gilliland and Speck, 1997)*. The survival of total probiotics in *shrikhand* enriched with FOS, blend of FOS and sucrose and sucrose, along with probiotic *E. faecium* CFR 3002 are given in Table 6.2.1. No change (P>0.05) in the colony counts of total probiotics was observed for 20 days of storage at 4±2 ^{o}C. There was slight decrease in the viable count of probiotics from 40 days and at the end of storage period (60 days) and the population of all the probiotics was ≥140 x 10^6 cfu/g. This is well above the levels suggested as providing therapeutic benefits *(Boylston et al., 2004)*. All the three types of *shrikhand* showed similar trends with slight decrease in viable probiotics which confirms that the probiotic cultures remained viable in the product until the end of storage (60 days) which is satisfactory for the *shrikhand* to be claimed as probiotic *shrikhand.* Desai *et al.,* (2004) and Ozer *et al.,* (2005) have reported improvement in the survival of probiotics in yoghurt in the presence of inulin. Similarly, the presence of FOS could also have contributed to the survival of the probiotics during storage.

Table 6.2.1 Total probiotics* of *shrikhand* enriched with prebiotic FOS, blend of FOS and sucrose and sucrose along with probiotic *E. faecium* CFR 3002 stored at refrigerated temperature (4±2 ^{o}C)

Storage period (days)	Total probiotics (cfu/100g)		
	FOS	Blend	Sucrose
0	>300x10^6	>300x10^6	>300x10^6
20	>300x10^6	>300x10^6	>300x10^6
40	281x10^6	274x10^6	256x10^6
60	172x10^6	148x10^6	141x10^6

6.2.3.7. Sensory analysis

The results of sensory attributes of *shrikhand* are presented in Table 6.2.2. Analysis of variance explicit that *shrikhand* enriched with prebiotic FOS and probiotic *E. faecium* did not show any significant changes (P>0.05) with respect to sensory attributes like color, taste, texture, flavor, mouth-feel and overall acceptability. No significant differences (P>0.05) were observed between the overall quality scores of *shrikhand* enriched with FOS, blend and sucrose, though the scores of sucrose sweetened *shrikhand* was slightly on higher side. The result indicated that the *shrikhand* containing FOS in replacement of sucrose is sensorially acceptable and microbiologically safe with tendency to have extended shelf-life. *Shrikhand* enriched with FOS and blend have obtained fairly good score when compared with sucrose sweetened *shrikhand*. Sensory scores showed that blend was preferred by the panelists because it gave the desired taste to the *shrikhand*, yet FOS enriched *shrikhand* was also acceptable by them. The results

concerning with the score for colour, texture and mouth feel of *shrikhand* disclosed a very similar scores between the samples. Overall acceptability was determined on the basis of quality scores obtained from the evaluation of color, taste, flavor, texture and mouth feel of the *shrikhand*. This makes FOS not only promising sugar substitute but alternative sweetener with real practical applicability in this type of products. The products with prebiotics and probiotics can therefore drive preference and present additional health benefits, beyond what traditional ingredients such as sweeteners.

Table 6.2.2 Sensory attributes of *shrikhand* enriched with prebiotic FOS, blend of FOS and sucrose and sucrose along with probiotic *E. faecium* CFR 3002 stored at refrigerated temperature (4±2 ^{o}C)

Shrikhand	Sensory parameters					
	Colour	**Texture**	**Mouth feel**	**Flavour**	**Taste**	**Overall quality**
FOS	9±0.05	9±0.15	8±0.10	9±0.05	7.5±0.15	8±0.10
Blend	9±0.10	9±0.10	8±0.20	9±0.15	8±0.20	8±0.15
Sucrose	9±0.10	9±0.12	9±0.16	9±0.10	9±0.05	9±0.05

* Minimum acceptable score is 7.0

6.2.3.8. Storage studies

Shrikhand has a long shelf-life compared with other cultured milk products and has been reported to be stable for >30 days *(Sarkar et al., 1996b)*. The higher shelf-life of *shrikhand* than other milk based products may be due to higher acidity, reduced water content and the addition of sugar *(Sharma and Zariwala, 1980)*. The analysis of freshly prepared *shrikhand* showed no growth (plate count, yeast/molds and coliform) at zero day. However, the number of colony forming unit (CFU) increased (20 to 25x10^2 cfu/100g) at the end of storage period (60 days). The presence of low number of CFU in

the *shrikhand* prepared with replacement of FOS is associated with rather rare ability of microorganisms to metabolize FOS compared with the microbial utilization of sucrose *(Winkelhausen and Kuzmanova, 1998)*. In view of these observations, the synbiotic *shrikhand* enriched with FOS along with probiotic are not only microbiologically safe but their shelf-life could be much longer too.

CHAPTER: 7

SUMMARY AND CONCLUSION

7.1. Summary and Conclusion

The incidence of acute and chronic gut disorders continues to rise, with many diseases being untreatable. The functional food industry's perception of the importance of gut microbiology in human health and nutrition has led to a major increase in the development of probiotic and prebiotic based products**.** In this context, the thesis titled ***'Biotechnological approaches for the preparation of fructooligosaccharides (FOS) based prebiotic and probiotic foods'*** presents the details of the study carried to enrich fruits and vegetables and a few selected Indian traditional foods with FOS and probiotic lactic acid bacteria (LAB). The important observations made in this study are summarized as follows.

- A few fruits and vegetables (apple, banana, jack fruit, papaya, sapota, star fruit, ashgourd, pumpkin and carrot) were enriched with FOS, a low calorie prebiotic, using vacuum osmotic dehydration (VOD) process. The study clearly indicated that FOS could be a healthier alternative to the sugar solution normally used for the preparation of osmotically dehydrated fruits and vegetables.

- The FOS content in the final product (FOS enriched fruits and vegetables) was found to be 8.02-17.21 g/100g product. The results also implied that the effect of process parameters such as temperature, pressure, vacuum treatment time and atmospheric restoration period could be varied in order to get a desired FOS content.

- The following conditions- vacuum pressure of 550 mm Hg, temperature of 45 ^{o}C, vacuum treatment time of 45 min and atmospheric restoration period of 16.44 min- were found to be optimum to maximize uptake of FOS in banana using VOD. These optimized conditions resulted in an FOS uptake of 9.64 g/100g banana and the products exhibited a shear strength value of 13.42 N.

- Response surface methodology (RSM) and graphical optimization methods were effective in ascertaining the optimum processing conditions for VOD of banana slices. Analysis of variances showed that the effects of all the process variables were statistically significant for FOS uptake. Second order polynomial models were obtained for predicting FOS uptake and shear strength of banana slices. This methodology could, therefore, be successfully employed to study the importance of individual, cumulative and interactive effects of the test variables in vacuum osmotic processes.

- The pH, total soluble solids, titratable acidity, colour and viscosity of selected fruit juice beverages (pineapple, mango and orange juice) fortified with FOS did not change significantly ($P>0.05$) during 6 months storage at ambient (25±2 °C) and refrigeration temperature (4 °C). The initial FOS contents of pineapple, mango and orange juices were 3.79, 3.45, and 3.62 g/100ml respectively. The fruit juice beverages stored at refrigeration temperature showed 2.00-2.39 g/100ml FOS after six months of storage. However, fruit juice beverages stored at ambient temperature (25±2 °C) showed a gradual reduction in FOS content after the second, fourth and sixth months (2.69-3.32, 1.65-2.08 and 0.38-0.58 g/100ml) respectively.

- There were no undesirable changes in the physicochemical characteristics of the fruit juice beverages fortified with FOS. The sensory analysis showed that the fruit juice beverages fortified with FOS were acceptable up to 4 and 6 months storage at ambient and refrigeration temperature respectively.

- *Gulab jamun*, a traditional Indian sweet, was prepared by replacing sucrose with FOS and FOS-sucrose blend. The FOS content was found to be 17.58 and 9.18 g/100g respectively. The effect of FOS enrichment on colour, texture, sensory and microbiological attributes was evaluated. FOS and FOS-sucrose blend did not affect colour values and sensory scores of *gulab jamun*,

adversely which slightly decreased during storage for 4 days at ambient temperature (25±2 °C).

- The results also indicated the possibility of using FOS as an effective alternative either fully or partially for the preparation of *gulab jamun*. In addition, FOS sweetened *gulab jamun* would have an added advantage over that of sucrose based *jamun* from the point of low calorie, non digestibility and other well known health benefits of FOS.

- LAB isolates (17) from fruits and vegetables were tested for their probiotic features such as their ability to withstand low pH, high bile salt concentration, and cell surface hydrophobicity. The selected LAB isolates were able to survive (>80% and 70%) at low pH (2.5) and relatively high bile (1.0%) concentration.

- Out of 17 LAB isolates, 2 isolates (Cu8 and R32) were categorized as potent probiotics based on the studied probiotic properties and were assigned to the genera *Enterococcus* on the basis of morphological, biochemical, physiological characteristics and 16S rRNA gene sequencing. The 2 isolates, Cu8 and R32, were identified and designated as *Enterococcus hirae* CFR 3001 and *Enterococcus faecium* CFR 3002.

- A synbiotic star fruit enriched with prebiotics (FOS) and probiotics (*L. salivarius* CFR 2158 and *E. hirae* CFR 3001) was prepared. The FOS content of the prepared synbiotic star fruits was 17.80-20.24 g/100g (initial day). The star fruits stored at ambient temperature (25±2 ^{o}C) had retained 16.09-18.62 g/100g FOS after 6 months of storage. Impregnation of FOS syrup along with *L. salivarius* CFR 2158 and *E. hirae* CFR 3001 into structural matrix of fresh star fruit tissue resulted in synbiotic star fruits with probiotics ranging 138-178x10^{6} cfu/g up to 4 months of storage at ambient temperature (25±2 ^{o}C).

- The survivability ($34x10^6$ cfu/g and $26x10^6$ cfu/g) of probiotics (*L. salivarius* CFR 2158 and *E. hirae* CFR 3001 respectively) at the end of the storage period (6 months) in synbiotic star fruit is well within the recommended/desired concentration (10^6 cfu/g probiotics) in food at the time of consumption, sufficient enough to exert the beneficial probiotic effect on the host. The final product exhibited desirable textural and sensory attributes. These synbiotic functional star fruits can be successfully marketed for the improvement of health through the promotion of a proper balance of gut microflora.

- The study also intended to evaluate the effects of FOS on the physicochemical, microbiological and sensory characteristics of *shrikhand* enriched with probiotic *E. faecium* CFR 3002. The result indicated that the *shrikhand* containing FOS in replacement of sucrose is sensorially acceptable and microbiologically safe. The initial FOS content of *shrikhand* enriched with FOS and blend of FOS and sucrose was found to be 10.32 g/100g and 5.55 g/100g respectively. It was found to be decreased to 6.16 g/100g and 1.76 g/100g during storage period of 60 days at 4±2 ^oC.

- The survival of total probiotics in *shrikhand* enriched with FOS, blend of FOS and sucrose and sucrose did not change significantly ($P>0.05$) up to 20 days of storage at 4±2 ^oC. The population of all the probiotics was ≥140 x 10^6 cfu/g from 40 days and at the end of storage period (60 days) which is satisfactory for the *shrikhand* to be claimed as probiotic *shrikhand.* The result suggested that the FOS could be a healthier alternative to sucrose in the preparation of certain milk based products.

The research work carried out encompasses the evaluation of FOS as a novel osmotic agent for impregnation of fruits and vegetables using VOD process, followed by the optimization of process parameters using banana as a case study. Preparation and characterization of FOS based fruit juice beverages and *gulab jamun,* isolation and

characterization of potent probiotic cultures from fruits and vegetables and their use in the production of synbiotic start fruits and synbiotic s*hrikhand* are the other studies presented in the thesis.

7.2. Future perspectives

The beneficial properties of prebiotics and probiotics offer a new dimension for the development of functional fruits and vegetable foods. Opportunities exist in exploring the improved knowledge of the synbiotic relationships between colonic microbiota, prebiotics and whole body physiopathology. Fruits and vegetable juices are logical matrices for the addition of prebiotic and probiotic compounds, not only because beverage market is ever growing but also it is rich source of desirable healthier ingredient of our diet. A likely expansion of the fruits and vegetable juice market which contain either one (pre/probiotic) or both (synbiotics) of these bioactive ingredients is envisaged. However, adding prebiotics and probiotics to fruits and vegetable foods offers many technological challenges and there is much place for future research.

Improved or new methods to enhance the stability during storage are certainly desirable. Furthermore, little is known of the functionality of these products. In particular, can health claims obtained with probiotics and prebiotics be transferred to fruits and vegetables which carry the same bioactive principles? Consumers increasingly wish to ingest bioactive ingredients through foods rather than any other route. Therefore, the health community would surely benefit from using fruits and vegetable products as matrix bases to carry their active components to patients. Another alternative matrix for the incorporation of prebiotics and probiotics is the traditional Indian foods, especially fermented diary products. The results from the present study using *gulab jamun* and s*hrikhand*, the two most popular traditional Indian delicacies, expand the scope of similar traditional foods as better carriers of functional food ingredients.

The emergence of prebiotics and probiotics as important elements of a healthy diet has been accompanied by sophisticated technological developments in the food industry. Predictions are that the demand for nutraceuticals and functional food additives will continue to grow, stimulated by new products and an increasing number of health conscious consumers. Functional foods are definitely here to stay. However, to what

extent products enriched with prebiotics and probiotics will contribute to sustained growth in this sector, depends on the results of research in validating the perceptions of the health benefits of these bioactive ingredients and the efforts of the food industry in formulating and promoting products which carry these benefits to the consumer.

BIBLIOGRAPHY

- Aachary AA & Prapulla SG. Value addition to spent osmotic sugar solution (SOS) by enzymatic conversion to fructooligosaccharides (FOS), a low calorie prebiotic. *Innovative Food Science and Emerging Technologies*. 2009, 10 (2): 284-288.
- Abdelbasset M & Djamila K. Antimicrobial activity of autochthonous lactic acid bacteria isolated from Algerian traditional fermented milk "Raïb". *African Journal of Biotechnology*, 2008, 7: 2908-2914.
- Ade-Omowaye BIO, Rastogi NK, Angersbach A, & Knorr D. Osmotic dehydration behavior of red paprika (*Capsicum annuum* L.). *Journal of Food Science*, 2002, 67: 1790-1796.
- Alander M, Matto J, Kneifel W, Johansson ML, Kogler B, Crittenden R, Mattila-Sandholm T & Saarela M. Effect of galacto-oligosaccharide supplementation on human faecal microflora and on survival and persistence of *Bifidobacterium lactis* Bb-12 in the gastrointestinal tract. *International Dairy Journal*, 2001, 11: 817-825.
- Albors A, Salvatori D, Andres A, Chiralt A & Fito P. Structure changes profiles in apple tissue during mass transfer phenomena. *In Proceedings of the Meeting on Minimally Processing of Foods and Process Optimization*, 1996. Copernicus Program Warsaw.
- Alegro LCA, Alegro JHA, Cardarelli HR, Chiu MC & Saad SMI. Potentially probiotic and synbiotic chocolate mousse. *LWT-Food Science and Technology*, 2007, 40: 669-675
- Alzamora SM, Salvatori D, Tapia MS, Lopez-Malo A, Welti-Chanes J & Fito P. Novel functional foods from vegetable matrices impregnated with biologically active compounds. *Journal of Food Engineering*, 2005, 67: 205-214.
- Alzamora SM, Gerschenson LN, Vidales S & Nieto A. Structural changes in the minimal processing of fruits: Some effects of blanching and sugar impregnation. 1997. In Fito P, Ortega-Rodriguez E & Barbosa-Canovas GV (Ed.). *Food Engineering*, 2000, 117-140. New York: Chapman & Hall.

- Alzamora SM, Tapia MS, Leunda A, Guerrero SN, Rojas AM, Gerschenson LN & Parada-Arias E. Minimal preservation of fruits: A cited project. In Lozano JE, Anon C, Parada-Arias E & Barbosa-Canovas GV (Ed.). *Trends in food engineering,* 2000, 205-225. *Pennsylvania*: *Technomic Publishing Company*. Chapter 16.
- Alzamora SM & Gerschenson LN. Effect of water activity depression on textural characteristics of minimally processed fruits. In Barbosa-Canovas GV, Lombardo S, Narsimhan G & Okos MR. New frontiers in food engineering. *Proceedings of the Fifth Conference of Food Engineering*, 1997, 72-75. New York: AICHE.
- Andres I, Salvatori D, Chiralt A & Fito P. Vacuum impregnation viability of some fruits and vegetables. 2001. In Fito P, Chiralt A, Barat JM, Spiess WEL, Behsnilian D, Barat JM, Spiess WEL & Behsnilian D (Ed.). Osmotic dehydration and vacuum impregnation. Behsnilian.
- Aneja RP, Mathur BN, Chandan RC & Benerjee AK. Technology of Indian milk products; *Dairy India Publication*, New Delhi, 2002.
- Anikó-Matusek, Bálint Czukor, Péter Merész & Ferenc Örsi. Comparison of diffusion of fructo-oligosaccharide components during vacuum impregnation and osmotic dehydration. *European Food Research and Technology*, 2008, 227 (2): 417-423.
- AOAC. Official methods of analysis. International volume I and II, 18th Edition, Revision 1. 2006.
- AOAC, Association of Official Analytical Chemists. *Official methods of analysis*. 1980, 14th Ed. Washington, DC.
- APHA (American Public Health Association). Standard methods for the examination of dairy products, 1967, D.C. 79, 12th Ed. Washington.
- Argaiz A, Lopez-Malo A, Palou E & Welti J. Osmotic dehydration of papaya with corn syrup solids. *Drying Technology*, 1994, 12: 1709-1725.
- Arora S, Gawande H, Narendra K, Yarrakula S, Singh VP, Sharma V, Wadhwa BK, Tomer SK & Sharma GS. Textural and structural properties of *kalakand*

made with artificial sweeteners-saccharin, acesulfame-k, sucralose and aspartame. *Journal of Food Science and Technology*, 2008, 45 (3): 263-266.

- Arora S, Singh VP, Yarrakula S, Gawande H, Narendra K, Sharma V, Wadhwa BK, Tomer SK & Sharma GS. Textural and microstructural properties of *burfi* made with various sweeteners. *Journal of Texture Studies*, 2007, 38: 684-697.
- Asahara T, Nomoto K, Shimizu K, Watanuki M & Tanaka R. Increased resistance of mice to *Salmonella enterica* serovar Typhimurium infection by synbiotic administration of bifidobacteria and transgalactosylated oligosaccharides. *Journal of Applied Microbiology*, 2001, 91: 985-996.
- Audisio MC, Oliver G & Apella MC. Protective effect of Enterococcus faecium J96, a potential probiotic strain, on chicks infected with *Salmonella* Pullorum. *Journal of Food Protection*, 2000, 63 (10): 1333-1337.
- Audisio MC, Oliver G & Apella MC. Antagonistic effect of *Enterococcus faecium* J96 against human and poultry pathogenic *Salmonella* spp. *Journal of Food Protection*, 1999, 62 (7): 751-755.
- Azoubel PM & Murr FEX. Mass transfer kinetics of osmotic dehydration of cherry tomato. *Journal of Food Engineering*, 2004, 61: 291-295.
- Bamba T, Kanauchi O, Andoh A & Fujiyama Y. A new prebiotic from germinated barley for nutraceutical treatment of ulcerative colitis. *Journal of Gastroenterology and Hepatology*, 2002, 17: 818-824.
- Barat JM, Talens P, Barrera C, Chiralt A & Fito P. Pineapple candying at mild temperature by applying vacuum impregnation. *Journal of Food Science*, 2002, 67 (8): 3046-3052.
- Barat JM, Chiralt A & Fito P. Effect of osmotic solution concentration, temperature and vacuum impregnation pretreatment on osmotic dehydration kinetics of apple slices. *Food Science and Technology International*, 2001, 7: 451-456.
- Barat JM, Chiralt A & Fito P. Equilibrium in cellular food osmotic solution systems as related to structure. *Journal of Food Science*, 1998, 63: 836-840.

- Barbosa-Canovas GV & Vega-Mercado H. Osmotic dehydration. Dehydration of foods. Florence: *International Thomson Publishing*, 1996, 265-288.
- Begley MC, Gahan GM & Hill C. The interaction between bacteria and bile. *FEMS Microbiology Reviews*, 2005, 29: 625-651.
- Belem MAF. Application of biotechnology in the product development of nutraceuticals in canada. *Trends in Food Science Technology*, 1999, 10: 101-106.
- Bellisle F, Diplock AT & Hornstra G. Functional food science in Europe. *British Journal of Nutrition*, 1998, 80: 3-4.
- Benno Y, Sawada K & Mitsuoka T. The intestinal microflora of infants: composition of fecal flora in breastfed and bottle-fed infants. *Microbiology and Immunology*, 1984, 28: 975-986.
- Bernardi S, Bodini RB & Favaro-Trindade CS. Avaliac-a osensorial de sorvete de acerola com propriedades probioticas. *In Proceedings of the 19th Congresso brasileiro de ciencia e tecnologia de alimentos.* Campinas: Sociedade Brasileira de Cie ncia e *Tecnologia de Alimentos*, 2004, 138.
- Berner LA & O'Donnell JA. Functional foods and health claims legislation: applications to dairy foods. *International Dairy Journal,* 1998, 8: 355-362.
- Betalleluz-Pallardel N, Betoret D Campos & Fito P. Prebiotic fortification of apple by vacuum impregnation. *International Conferences on Food Innovation*, 2010, 25-29 October.
- Betoret N, Puente L, Diaz MJ, Pagan MJ, Garcia MJ, Gras ML, Martinez-Monzo J & Fito P. Development of probiotic enriched dried fruits by vacuum impregnation. *Journal of Food Engineering*, 2003, 56 (2-3): 273-277.
- Biazus JPM, Souza AG, Santana JCC, Souza RR & Tambourgi EB. Optimization of drying process of *Zea mays* malt to use as alternative source of amylolytics enzymes. *Brazilian Archive of Biology and Technology*, 2005, 48 (6): 185-190.
- Bielecka M, Biedrzycka E & Majkowska A. Selection of probiotics and prebiotics for synbiotics and confirmation of their in vivo effectiveness. *Food Research International*, 2002, 35: 125-131.

- Birnboim HC & Doly J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Research*, 1979, 7 (6): 1513-1523.
- Biswal RN & Bozorgmehr K. Mass transfer in mixed solute osmotic dehydration of apple rings. *ASAE Transactions*, 1992, 35: 257-262.
- Biswal RN & Maguer LM. Mass transfer in plant materials in aqueous solutions of ethanol and sodium chloride: Equilibrium data. *Journal of Food Process Engineering*, 1989, 1: 149-153.
- Bolin HR, Huxsoll CC, Jackson R & Ng KC. Effect of osmotic agents and concentration on fruit quality. *Journal of Food Science*, 1983, 48: 202-205.
- Bongirwar DR & Sreenivasan A. Studies on osmotic dehydration of banana. *Journal of Food Science and Technology*, 1977, 14: 104-112.
- Borges AL. et al. O Cultivo da Banana. Cruz das Almas. EMBRAPA-CNPMF. Circular Técnica. EMBRAPA-CNPMF, 1997, 27: 109.
- Bouhnik Y, Vahedi K, Achour L, Attar A, Salfati J, Pochart P, Marteau P, Flourie B, Bornet F & Rambaud JC. Short-chain fructooligosaccharide administration dose-dependently increases fecal bifidobacteria in healthy humans. *Journal of Nutrition*, 1999, 129: 113-116.
- Bourne MC & Szczesniak AS. Sensory evaluation-texture. *In Encyclopedia of Food Sciences and Nutrition*, 2003, 5167-5174. (Caballero B, Trugo L & Finglas P. (Ed.). *Academic Press*, Amsterdam, `Natherlands.
- Bourne MC. In Food Texture and Viscosity: Concept and Measurement Taylor SL. (Ed.). *Academic Press*, San Diego CA, 2002, 1-32.
- Bourne MC. Texture profile analysis. *Food Technology*, 1978, 32 (7): 62-72.
- Boylston TD, Vinderola CG, Ghoddusi HB & Reinheimer JA. Incorporation of bifidobacteria into cheeses: Challenges and rewards. *International Dairy Journal*, 2004, 14: 375-387.
- Bran-non CA. Prebiotics as “good carbs’. *Today’s Dietitian*, 2006, 8 (8): 12-21.
- Brashears MM, Jaroni D & Trimble D. Isolation, selection, and characterization of lactic acid bacteria for a competitive exclusion product to reduce shedding of

Escherichia coli O157:H7 in cattle. *Journal of Food Protection*, 2003, 66: 355-363.

- Breithaupt DE. Determination of folic acid by ion-pair RP-HPLC in vitamin-fortified fruit juices after solid-phase extraction. *Food Chemistry*, 2001, 74: 521-525.
- BSOPTP 39 - Staining Procedures. Issued by Standards Unit, Evaluations and Standards Laboratory Centre for Infections.
- Buddhiman T, Jyoti PT, Ulrich S, Charles MAPF, Michael G & Wilhelm HH. Phenotypic and genotypic identification of lactic acid bacteria isolated from ethnic fermented bamboo tender shoots of North East India. *International Journal of Food Microbiology*, 2008. 121 (1): 35-40.
- Buydens P & Debeucklaere S. Efficacy of SF 68 in the treatment of acute diarrhea: a placebo controlled trial. *Scandinavian Journal of Gastroenterology*, 1996, 31: 887-891.
- Calo-Mata P, Arlindo S, Boehme K, de Miguel T, Pascoal A & Barros-Velazquez J. Current applications and future trends of lactic acid bacteria and their bacteriocins for the biopreservation of aquatic food products. *Food and Bioprocess Technology*, 2008, 1: 43-63.
- Calorie Control Council, Low-calorie sweeteners. *http://www.caloriecontrol.org/lowcalqa.html*, Accessed on 25th February, 2002.
- Canzi EI, Guglielmetti S, Mora D, Tamagnani I & Parini C. Conditions affecting cell surface properties of human intestinal bifidobactoria. *Antonie van Leeuwen*, 2005, 88: 207-219.
- Cappuccino JG & Sherman N. Microbiology: A Laboratory manual. 6th Ed. *Pearson Education Publication*, 2004, 528.
- Carabasa GM & Ibarz RA. Kinetics of colour development in aqueous glucose system at high temperatures. *Journal of Food Engineering*, 2000, 44: 181-189.
- Cargill. Cargill beverage concepts will address consumer demands for health, taste and texture at IFT 2008. *http://www.cargill.com/news-center/news-releases/2008/NA3007612.jsp*. 2009, accessed July 20, 2009.

- Cháfer M, González-martínez C, Ortolá MD, Chiralt A & Fito P. Kinetics of osmotic dehydration in orange and mandarin peels. *Journal of Food Process Engineering*, 2001, 24 (4): 273-289.
- Chafer M, Ortola MD, Chiralt A & Fito P. Orange peel products obtained by osmotic dehydration. In: Fito P, Chiralt PA, Barat JM, Spiess WEL, Beshnilian D (Ed.). Osmotic dehydration and Vacuum Impregnation. Applications in Food Industries. *Lancaster: Technomic Publishing Company*, 2001, 93-106.
- Carpita NC, Sabularse D, Montezinos D & Delmer DP. Determination of pore size of cell walls of living plant cells. *Science*, 1979, 205: 1144-1147.
- Champagne CP, Gardner N, Brochu E & Beaulieu Y. The freeze-drying of lactic acid bacteria: A review. Canadian Institute, *Food Science Technology Journal*, 1991, 24: 118–128.
- Charteris WP, Kelly PM, Morelli L & Collins JK. Antibiotic susceptibility of potentially probiotic lactobacillus species. *Journal of Food Protection*, 1998b, 61: 1636-1643.
- Chen BH & Yao YQ. Beneficial microbe composition, new protective material for the microbes, method to prepare the same and uses thereof. US Patent 6368591, 2002.
- Chen HC, Chang CC, Mau WJ & Yen LS. Evaluation of N-acetylchitooligosaccharides as the main carbon sources for the growth of intestinal bacteria. *FEMS Microbiology Letters*, 2002, 209: 53-56.
- Chen HL, Lu YH, Lin JJ & Ko LY. Effects of isomalto-oligosaccharides on bowel functions and indicators of nutritional status in constipated elderly men. *Journal of American College of Nutrition*, 2001, 20: 44-49.
- Chethana R, Manohar B & Yella Reddy SR. Process optimization of gulab jamun, an Indian traditional sweet, using sugar substitutes. *European Food Research Technology*, 2004, 219: 386-392.
- Childs NM. Nutraceutical industry trends. *Journal of Nutraceuticals, Functional and Medical Foods*, 1999, 2: 73-85.

- Chiralt A & Fito P. Transport mechanisms in osmotic dehydration: The role of the structure. *Food Science and Technology International*, 2003, 9 (3): 179-186
- Chiralt A, Fito P, Andres A, Barat JM, Martinez-Monzo J & Martinez-Navarrete N. Vacuum impregnation: A tool in minimally processing of foods. In Oliveira FAR & Oliveira JC (Ed.). Processing of foods: Quality optimization and process assessment. Boca Raton, FL: *CRC Press LLC*, 1999, 341-356.
- Chiralt A, Fito P, Barat JM, Andres A, Gonzalez-Martinez C, Escriche I & Camacho MM. Use of vacuum impregnation in food salting process. *Journal of Food Engineering*, 2001, 49 (2-3): 141-151.
- Christian Rößle, Nigel Brunton, Ronan T. Gormley, Paul R. Ross & Francis Butler. Development of potentially synbiotic fresh-cut apple slices. *Journal of Functional Foods*, 2010, 2 (4): 245-254.
- Clevenger MA, Turnbull D, Inone H, Enomoto M, Allen JA, Henderson L & Jones E. Toxicological evaluation of neosugar: genotoxicity, cariogenicity and chronic toxicity. *Journal of American College of Toxicology*, 1988, 7: 643-667.
- Clydesdale F. A proposal for the establishment of scientific criteria for health claims for functional foods. *Nutrition Review*, 1997, 55: 413-422.
- Cochran WG & Cox GM. Some methods for the study of response surfaces. In Experimental Designs. 2nd Ed. New York: John Wiley and Sons, Inc. 1957, 127-131.
- Collignan A & Raoult-Wack AL. Dewatering and salting of cod by immersion in concentrated sugar/salt solutions. *Lebensmittel-Wissenschaft und-Technologie*, 1994, 27: 259-264.
- Colombel JF, Cortot A, Newt C & Romond C. Yoghurt with Bifidobacterium longum reduces erythromycin induced gastrointestinal effects. *The Lancet*, 1987, 2 (8549): 43.
- Contreras C, Martin ME, Martinez-Navarrete N & Chiralt A. Effect of vacuum impregnation and microwave application on structural changes which occurred during air-drying of apple. *Lebensmittel Wissenschaft und Technologie*, 2005, 38: 471-477.

- Cornell JA. Experiments with mixtures: designs, models and the analysis of mixture data. 3rd Ed. New York. 2002, 21-99.
- Cornillon P & Salim LC. Characterization of water mobility and distribution in low and intermediate moisture food systems. *Magnetic Resonance Imaging*, 2002, 18: 335-341.
- Corpet F. Multiple sequence alignment with hierarchical clustering. *Nucleic Acids Research*, 1988, 16: 10881-10890.
- Corsetti A, Lavermicocca P, Morea M, Baruzzi F, Tosti N & Gobbetti M. Phenotypic and molecular identification and clustering of lactic acid bacteria and yeasts from wheat (species *Triticum durum* and *Triticum aestivum*) sourdoughs of Southern Italy. *International Journal of Food Microbiology*, 2001, 64 (1-2): 95-104.
- Corzo O & Gomez ER. Optimization of osmotic dehydration of cantaloupe using desired function methodology. *Journal of Food Engineering*, 2004, 64: 213-219.
- Cotter PD, Gahan CG & Hill C. A glutamate decarboxylase system protects *Listeria monocytogenes* in gastric fluid. *Molecular Microbiology*, 2001, 40: 465-475.
- Cristhiane C. Ferrari, Javier R. Arballo, Rodolfo H. Mascheroni, Miriam D. Hubinger. Modelling of mass transfer and texture evaluation during osmotic dehydration of melon under vacuum. *International Journal of Food Science and Technology*, 2011, 46 (2): 436-443.
- Crittenden RG & Playne MJ. Production, properties and applications of food-grade oligosaccharides. *Trends Food Science and Technology*, 1996, 7: 353-360.
- CSPI News. FDA should ban the use of certain antibiotics to fatten farm animals. *http://www.cspinet.org/new/antibiotics.htm*, 1999.
- Cuervo RB, Guilarte A, Juarez & Martınez J. Production of fructooligosaccharides by β-fructofuranosidase from *Aspergillus* sp. 27H. *Journal of Chemical Technology and Biotechnology.*, 2004, 79: 268-272.

- D'Souza AL, Rajkumar C, Cooke J & Bulpitt CJ. Probiotics in prevention of antibiotic-associated diarrhoea: meta-analysis. *British Medical Journal,* 2002, 324: 1361-1367.
- Dave RI & Shah NP. Viability of yogurt and probiotic bacteria in yogurts made from commercial starter cultures. *International Dairy Journal*, 1997, 7: 31-41.
- De Boever P, Wouters R & Verstraete W. Combined use of Lactobacillus reuteri and soygerm powder as food supplement. *Letters in Applied Microbiology*, 2001, 33: 420-424.
- De Vuyst L. Technology aspects related to the application of functional starter cultures. *Food Technology and Biotechnology*, 2000, 38: 105-112.
- Deka BC & Choudhury S. Post-harvest treatments for shelf life extension of banana under different storages. *Acta Horticulturae*, 2006, 723: 948-950.
- Desai AR, Powell IB & Shah NP. Survival and activity of probiotic lactobacilli in skim milk containing prebiotics. *Journal of Food Science*, 2004, 69 (3): 57-60.
- Deshpande RP, Chinnan MS & McWatters KH. Optimization of a chocolate-flavored, peanut-soy beverage using response surface methodology (RSM) as applied to consumer acceptability data. *LWT- Food Science and Technology*, 2008, 41: 1485-1492.
- Dharmawan J, Surono IS & Kun LY. Adhesion properties of indigenous Dadih lactic acid bacteria on human intestinal mucosal surface. *Asian-Australian Journal of Animal Science,* 2006, 19: 751-755.
- Dillard CJ & German JB. Phytochemicals: Nutraceuticals and human health. *Journal of Science of Food and Agriculture,* 2000, 80: 1744-1756.
- Dimer C & Gibson GR. An overview of probiotics, prebiotics and synbiotics in the functional food concept: perspectives and future strategies. *International Dairy Journal,* 1998, 8: 473-479.
- Diplock AT, Aggett PJ, Ashwell M, Bornet F, Fern EB & Roberfroid MB. Scientific concepts of functional foods in Europe: consensus document. *British Journal of Nutrition,* 1999, 81: 11-27.

- Dravnieks A. Atlas of odour character profiles compiled by ASTM data series. 1985, D.S 61, Philadelphia, USA.
- Durieux A, Fougnies C, Jacobs H & Simon JP. Metabolism of chicory fructooligosaccharides by bifidobacteria. *Biotechnology Letters*, 2001, 23: 1523-1527.
- EFFCA Guidelines for Probiotics in Food and Dietary Supplements. European Food and Feed Culture Association. *http://bbs.bio668.com/simple/index.php?t35919.html*. 2008, accessed on January 15, 2009.
- El-Aouar AA, Azoubel PM, Barbosa Jr JL & Murr FE. Influence of the osmotic agent on the osmotic dehydration of papaya (*Carica papaya* L.). *Journal of Food Engineering*, 2006, 75: 267-274.
- Engelen L, de Wijk RA, Prinz JF, Janssen AM., van der Bilt A, Weenen H, et al. A comparison of the effects of added saliva, α-amylase and water on texture perception in semi solids. *Physiology and Behaviour*, 2003, 78: 805-811.
- FAO. FAO Technical meetings on prebiotics: Food Quality and Standards Service (AGNS). Rome: Food and Agricultural Organization, *www.fao.org/ag/agn/agns/index_en.stm*, 2007, 1-11,
- FAO/WHO. Guidelines for evaluation of probiotics in food. Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food. April 30 and May 1, 2002, London, Ontario, Canada.
- FAO/WHO. Evaluation of health and nutritional properties of powder milk with live lactic acid bacteria. Report from FAO/WHO Expert Consultation. 1-4 October, 2001, Cordoba, Argentina.
- FAO: FAO Corporate Document Repository, Market for tropical fruits in India. CCP: BA/TF 01/11.
- Fern E. Marketing of functional foods: a point of view of the industry. International developments in science & health claims. *ILSI International symposium on functional foods in Europe*, 2007.

- Ferrando M & Spiess WEL. Cellular response of plant tissue during the osmotic treatment with sucrose, maltose and trehalose solutions. *Journal of Food Engineering*, 2001, 49: 115-127.
- Ferreira GB, Evangelista AF, Severo Junior JB, Souza RR, Santana JC, Tambourgi EB & Jordão E. Partitioning optimization of proteins from *Zea mays* malt in ATPS PEG 6000/$CaCl_2$. *Brazilian Archives of Biology and Technology*, 2007, 50 (3): 557-564.
- Fichtati J, Van De Voort FR & Khury AI. Multiresponse optimization of acid casein production. *Journal of Food Process Engineering*, 1990, 12 (4): 247-258.
- Fito P, Chiralt A, Barat JM & Martinez-Monzo J. Mass transfer and deformation-relaxation phenomena in plant tissues. In Welti-Chanes J, Barbosa GV & Aguilera JM (Ed.). Engineering and food for the 21st century Boca Raton, FL: *CRC Press*, 2002, 235-252.
- Fito P, Chiralt A, Barat JM, Andres A, Martinez-Monzo J & Martinez-Navarrete N. Vacuum impregnation for development of new dehydrated products. *Journal of Food Engineering*, 2001, 49: 297-302.
- Fito P, Chiralt A, Betoret N, Gras M, Chafer M, Martinez-Monzo J, Andres A & Vidal D. Vacuum impregnation and osmotic dehydration in matrix engineering application in functional fresh food development. *Journal of Food Engineering*, 2001a, 49 (2-3): 175-183.
- Fito P, Chiralt A, Barat JM, Andres A, Martinez-Monzo J & Martinez-Navarrete N. Vacuum impregnation for development of new dehydrated products. *Journal of Food Engineering*, 2001b, 49: 297-302.
- Fito P, Chiralt A, Barat JM & Martinez-Monzo J. Vacuum impregnation in fruit processing. In Lozano JE, Anon C, Parada-Arias E & Barbosa-Canovas GV (Ed.). Trends in food engineering. Pennsylvania: *Technomic Publishing Company*, 2000, 149-164.
- Fito P & Chiralt A. Vacuum impregnation of plant tissues. In Alzamora SM, Tapia MS & Lopez-Malo A (Ed.). Minimally processed fruits and vegetables:

Fundamental aspects and applications Gaithersburg: *Aspen Publication*, 2000, 189-204.

- Fito P & Chiralt A. An approach to the modeling of solid food-liquid operations: Application to osmotic dehydration. 1997. In Fito P, Ortega E & Barvosa G (Ed.). *Food Engineering*, 2000, 231-252. New York: Chapman & Hall.
- Fito P, Andres A, Pastor R & Chiralt A. Modelling of vacuum osmotic dehydration of foods. In Singh P & Oliveira F (Ed.). Process optimization and minimal processing of foods. Boca Raton: *CRC Press*, 1994, 107-121.
- Fito P & Pastor R. Non-diffusional mechanisms occurring during vacuum osmotic dehydration. *Journal of Food Engineering*, 1994, 21 (4): 513-519.
- Fito P. Modeling of vacuum osmotic dehydration of food. *Journal of Food Engineering*, 1994, 22: 313-328.
- Floch MH, Binder HJ, Filburn B & Gershengoren W. The effect of bile acids on intestinal microflora. *American Journal of Clinical Nutrition*, 1972, 25: 1418-1492
- Flores Gutierrez., A.A. Manejo Postcosecha de Frutas y Hortalizas en Venezuela. Experiencias y Recomendaciones. 2nd Ed. UNELLEZ, San Carlos, Cojedes, Venezuela, 2000, 86-102.
- Floros J & Chinnan M. Seven factor response surface optimization of a double-stage lye (NaOH) peeling process of pimiento peppers. *Journal of Food Science*, 1988, 53: 631-638.
- Food and Drug Administration. Guidance for industry on complementary and alternative medicine products and their regulation by the Food and Drug Administration. Draft Guidance. U.S. Department of Health and Human Services. December 2006.
- Foulquie Moreno MR, Sarantinopoulos P, Tsakalidou E & De Vuyst L. The role and application of *Enterococci* in food and health. *International Journal of Food Microbiology*, 2006, 106: 1-24.
- Froseth BR & Creedon SW. Inulin infused fruit and method of preparation. United States Patent Publication. US 2005, 0064082 A1.

- Fuller R. Probiotics in man and animals. *Journal of Applied Bacteriology*, 1989, 66: 365-378.
- Gabas AL, Menegalli FC, Ferrari F & Talis-Romero J. Influence of drying conditions on the rheological properties of prunes. *Drying Technology*, 2002. 20: 1485-1502.
- García-Galaz A, Perez-Morales R, Diaz-Cinco M & Acedo-Felix E. Resistance of Enterococcus strains isolated from pigs to gastrointestinal tract and antagonistic effect against *Escherichia coli* K88. *Revista Latinamericana de Microbiologia*, 2004, 46 (1-2): 5-11.
- Garrote R, Coutaz V, Luna J, Silva E & Bertone R. Optimizing processing conditions of chemical peeling of potatoes using response surface methodology. *Journal of Food Science*, 1993, 58: 821-826.
- Garrote RL, Silva ER & Bertone RA. Osmotic concentration at 5 ^{o}C and 25 ^{o}C of pear and apple cubes and strawberry halves. *Lebensmittel-Wissenschaft und Technologie*, 1992, 25: 133-138.
- Garrote RL & Bertone RA. Osmotic concentration at low temperature of frozen strawberry halves. Effect of glycerol, glucose and sucrose solutions on exudates loss and thawing. *Lebensmittel-Wissenschaft und Technologie*, 1989, 22: 264-267.
- Geetha VV, Sarma KS, Reddy VP, Reddy YK, Moorthy PRS & Kumar S. Physico-chemical properties and sensory attributes of probiotic *shrikhand. Indian Journal of Dairy and Biosciences,* 2003, 14: 58–60.
- Giangiacomo R, Torreggiani D & Abbo E. Osmotic dehydration of fruit: Sugar exchange between fruit and extracting syrup. *Journal of Food Processing and Preservation*, 1987, 2: 265-284.
- Gibson GR. From probiotics to prebiotics and a healthy digestive system. *Journal of Food Science*, 2004, 69: 141-143.
- Gibson G, Heber D, Meydani S, Postaire E & Sanders ME. Functional Dairy Products. John Libbey Eurotext, France. 2000.

- Gibson GR, Rastall RA & Roberfroid MB. Prebiotics. In Colonic Microbiota, Nutrition and Health (Ed.). Gibson GR & Roberfroid MB. Doordrecht: *Kluwer Academic Press*. 1999, 101-124.
- Gibson GR & Roberfroid MB. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *Journal of Nutrition*, 1995, 125: 1401-1412.
- Gilliland SE, Staley TE & Bush LJ. Importance of bile tolerance of *Lactobacillus acidophilus* used as a dietary adjunct. *Journal of Dairy Science*, 1984, 67: 3045-3051.
- Gilliland SE & Speck ML. Instability of *Lactobacillus acidophilus* in yogurt. *Journal of Dairy Science*, 1977, 60: 1394-1398.
- Gionchetti P, Rizzello F, Venturi A & Campieri M. Probiotics in infective diarrhoea and inflammatory bowel diseases. *Journal of Gastroenterology and Hepatology*, 2000, 15: 489-493.
- Giovani BM Carvalho, Daniel P Silva, Júlio C Santos, Hélcio J Izário Filho, António A Vicente, José A Teixeira, Maria das Graças A Felipe & João B Almeida e Silva. Total Soluble Solids from Banana: Evaluation and Optimization of Extraction Parameters. *Applied Biochemistry and Biotechnology*, 2009, 153, 34-43.
- Giraldo G, Vázquez R, Martín-Esparza ME & Chiralt A. Rehydration kinetics and soluble solids lixiviation of candied mango fruit as affected by sucrose concentration. *Journal of Food Engineering*, 2006, 77 (4): 825-834.
- Giraldo G, Talens P, Fito P & Chiralt A. Influence of sucrose solution concentration on kinetics and yield during osmotic dehydration of mango. *Journal of Food Engineering*, 2003, 58: 33-43.
- Gmeiner M, Kneifel W, Kulbe KD, Wouters R, De Boever P, Nollet L & Verstraete W. Influence of a synbiotic mixture consisting of Lactobacillus acidophilus 74-2 and a fructooligosaccharide preparation on the microbial ecology sustained in a simulation of the human intestinal microbial ecosystem (SHIME reactor). *Applied Microbiology and Biotechnology*, 2000, 53: 219-223.

- Golker C. Final recovery steps: Lyophilisation, spray-drying. In Rehm HJ, Reed G, Stephanopou G. los (Ed.). *Biotechnology*, 1993, 3 (2): 695-714. Weinheim: VCH.
- Grajek W, Olejnik A & Sip A. Probiotics, prebiotics and antioxidants as functional foods. *Acta Biochimica polonica*, 2005, 52 (3): 665-671
- Gras ML, Vidal D, Betoret N, Chiralt A & Fito P. Calcium fortification of vegetables by vacuum impregnation Interactions with cellular matrix. *Journal of Food Engineering*, 2003, 56: 279-284.
- Guasch-Jane M, Andres-Lacueva C, Jauregui O & Lamuela- Raventos R. First evidence of white wine in ancient Egypt from Tutankhamun's tomb. *Journal of Archaeology Science*, 2005, 33: 1075-1080.
- Guggisberg D, Cuthbert-Steven J, Piccinali P, Bütikofer U & Eberhard P. Rheological, microstructural and sensory characterization of low-fat and whole milk set yoghurt as influenced by inulin addition. *International Dairy Journal*, 2009, 19: 107-115.
- Guha S & Jaffe PR. Biodegradation kinetics of phenanthrene partitioned into the micellar phase of nonionic surfactants. *Environmental Science and Technology*, 1996, 30: 605-611.
- Guillou AA & Floros J Multi-response optimization minimizes salt in natural cucumber fermentation and storage. *Journal of Food Science*, 1993, 56: 1381-1389.
- Gurmail S Mudahar R, Toledo T & Joseph J. A response surface methodology approach to optimize potato dehydration process. *Journal of Food Processing and Preservation*, 1990, 14: 93-106.
- Halami PM. Production of polyhydroxyalkanoate from starch by the native isolate *Bacillus cereus* CFR06. *World Journal of Microbiology and Biotechnology*, 2008, 24: 805-812.
- Hamilton-Miller JM. The role of probiotics in the treatment of and prevention of Helicobacter pylori infection. *International Journal of Antimicrobial Agents*, 2003, 22: 360-366.

- Haminiuk CWI, Sierakowski MR, Branco IG, Maciel GM & Masson ML. Rheological study of ternary mixtures and pectic gels of red fruit pulps. *Journal of Food Science and Technology*, 2007, 42: 629-639.
- Haque A, Richardson RK & Morris ER. Effect of fermentation temperature on the rheology of set and stirred yogurt. *Food Hydrocolloids*, 2001, 15: 593-602.
- Hawkes J & Flink JM. Osmotic dehydration of fruit slices prior to freeze-dehydration. *Journal of Food Processing and Preservation*, 1978, 2: 265-284.
- Heenan CM, Adams MC, Hosken RW & Fleet GH. Survival and sensory acceptability of probiotic microorganisms in a non fermented frozen vegetarian dessert. *LW T- Food Science and Technology*, 2004, 37: 461-466.
- Higuti IH, Silva PA, Papp J, Okiyama VMO, Andrade EA, Marcondes AA & Nascimento AJ. Colorimetric determination of α and β-cyclodextrins and studies on optimization of CGTase production from *B. firmus* using factorial designs. *Brazilian Archives of Biology and Technology*, 2004, 47 (6): 837-841.
- Hindu Business Line. Online edition of India's National Newspaper. Sunday, April 03, 2011.
- Holzapfel WH & Schillinger U. Introduction to pre and probiotics. *Food Research International*, 2002, 35: 109-116.
- Holzapfel WH, Haberer P, Snel J, Schillinger U & Huis in't Veld JHJ. Over view of gut flora and probiotics. *International Journal of Food Microbiology*, 1998, 41: 85-101.
- Homayouni A, Azizi A, Ehsani MR, Yarmand MS & Razavi SH. Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. *Food Chemistry*, 2008, 111: 50-55.
- Hood SK & Zottola EA. Effect of low pH on the ability of *Lactobacillus acidophilus* to survive and adhere human intestine cells. *Journal of Food Science*, 1988, 53: 1514-1516.
- Hoover MW & Miller NC. Factors influencing impregnation of apple slices and development on a continuous process. *Journal of Food Science*, 1975, 40: 698-700.

- Hutchings JB. Food colour and appearance. Blackie Academic, 1994.
- India 2011- A Reference Manual, compiled by Research, Reference and Training Division, Publication Division, Ministry of Information and Broadcasting, Government of India, 55th Ed. 2011.
- Islam MN & Flink JM. Dehydration of potato: II. Osmotic concentration and its effect on air drying behavior. *Journal of Food Technology*, 1982, 17: 387-403.
- Isolauri E, Salminen S & Ouwehand AC. Probiotics. *Best Practice and Research in Clinical Gastroenterology,* 2004, 18: 299-313.
- Izumi T, Piskula MK & Osawa S. Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *Journal of Nutrition*, 2000, 130: 1695-1699.
- Jacobsen C, Rosenfeldt V, Hayford E, Moller L, Michaelsen F, Paerregaard A, Sandstrom B, Tvede M & Jakobsen M. Screening of probiotic activities of forty-seven strains of Lactobacillus spp. by in vitro techniques and evaluation of the colonization ability of five selected strains in humans. *Applied Environmental Microbiology*, 1999, 65: 4949-4956.
- Jowitt R. The terminology of food texture. *Journal of Texture Studies,* 1974, 5: 351-358.
- Justfood. Global market review of functional foods-forecasts to 2012. *http://www.alacrastore.com/acm/2023* sample.pdf. 2006, 84, Accessed Oct 12, 2009.
- Kaanane A, Kane D & Labuza TP. Time and temperature effect on stability of Moroccan processed orange juice during storage. *Journal of Food Science*, 1988, 53: 1470-1473 & 1489.
- Kalab M, Allan-Wojtas P & Phipps-Todd BE. Development of microstructure in set-style nonfat yoghurt – A review. *Food Microstructure*, 1983, 2 (1): 51-66.
- Kalliomäki M, Salminen S, ArvilommiH, Kero P, Koskinen P & Isolauri E Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *The Lancet*, 2001, 357: 1076-1079.

- Kanauchi O, Mitsuyama K, Araki Y & Andoh A. Modification of intestinal flora in the treatment of inflammatory bowel disease. *Current Pharmaceutical Design*, 2003, 9: 333-346.
- Kanauchi O, Hitomi Y, Agata K, Nakamura T & Fushiki T. Germinated barley foodstuff improves constipation induced by loperamide in rats. *Bioscience, Biotechnology and Biochemistry*, 1998a, 62: 1788-1790.
- Kanauchi O, Nakamura T, Agata K, Mitsuyama K & Iwanaga T. Effects of germinated barley foodstuff on dextran sulfate sodium-induced colitis in rats. *Journal of Gastroenterology*, 1998b, 33: 179-188.
- Katapodis P, Kalogeris E, Kekos D, Macris BJ & Christakopoulos P. Biosynthesis of fructo-oligosaccharides by *Sporotrichum thermophile* during submerged batch cultivation in high sucrose media. *Applied Microbiology and Biotechnology*, 2004, 63: 378-382.
- Kaur IP, Chopra K & Saini A. Probiotics: potential pharmaceutical applications. *European Journal of Pharmaceutical Sciences*, 2002, 15: 1-9.
- Kaymak-Ertekin F & Sultanoglu M. Modeling of mass transfer during osmotic dehydration of apples. *Journal of Food Engineering*, 2000, 46: 243-250.
- Kim GB & Lee BH. Biochemical and molecular insights into bile salt hydrolase in the gastrointestinal microflora: a review. *Asian-Australian Journal of Animal Science*, 2005, 18: 1505-1512.
- Klaenhammer TR & Kullen MJ. Selection and design of probiotics. *International Journal of Food Microbiology*, 1999, 50: 45-57.
- Klaenhammer TR. Microbiological considerations in selection and preparation of *Lactobacillus* strains for use as dietary adjuncts. *Journal of Dairy Science*, 1982, 65: 1339-1345.
- Kleerebezemab M, Hols P & Hugenholtz J. Lactic acid bacteria as a cell factory: rerouting of carbon metabolism in *Lactococcus lactis* by metabolic engineering. *Enzyme and Microbial Technology*, 2000, 26: 840-848.

- Kleessen B, Sykura B, Zunft HJ & Blaut M. Effects of inulin and lactose on fecal microflora, microbial activity and bowel habit in elderly constipated persons. *American Journal of Clinical Nutrition*, 1997, 65: 1397-1402.
- Klein GA, Pack & Reuter G. Antibiotic resistance patterns of enterococci and occurrence of vancomycin-resistant entero-cocci in raw minced beef and pork in Germany. *Applied Environmental Microbiology*, 1998, 64: 1825-1830.
- Klewicki R, Konopacka D, Uczciwek M, Irzyniec Z, Piasecka E & Bonazzi C. Sorption isotherms for osmo-convectively-dried and osmo-freezedried apple, sour cherry, and blackcurrant. *Journal of Horticultural Science & Biotechnology*, 2009, ISAFRUIT Special Issue, 75-79.
- Knorr D. Technology aspects related to microorganisms in functional foods. *Trends in Food Science and Technology*, 1998, 9: 295-306.
- Kobayashi H & Ritmann BE. Microbial removal of hazardous organic compounds. *Environmental Science and Technology*, 1982, 16: 170-183.
- KPMG. Processed Food and Agribusiness. 2007, 1-55.
- Krasaekoopt W, Bhandari B & Deeth H. Evaluation of encapsulation techniques of probiotics for yoghurt. *International Dairy Journal*, 2003, 13: 3-13.
- Krishnamurthy S, Kantha HJ. Food colour measurement: Instrumentation and Techniques. *Journal of Instrument Society of India*, 2005, 35 (2): 227-238.
- Krishnamurthy S & Kantha HJ. Applications of universal testing machine (UTM) for food texture analysis. *Journal of Instrument Society of India*, 2003, 33 (1): 60-73.
- Krokida MK, Karathanos VT & Maroulis ZB. Effect of osmotic dehydration on viscoelastic properties of apple and banana. *Drying Technology*, 2000, 18: 951-966.
- Kulkarni C, Belsare N & Lele A. Studies on Shrikhand Rheology. *Journal of Food Engineering*, 2006,74 (2): 169-177.
- Kullen MJ, Sanozky-Dawes RB, Crowell DC & Klaenhammer TR. Use of the DNA sequence of variable regions of the 16S rRNA gene for rapid and accurate

identification of bacteria in the *Lactobacillus acidophilus* complex. *Journal of Applied Microbiology*, 2000, 89: 511-516.

- Lapsley KG, Escher FE & Hoehn E. The cellular structure of selected apple varieties. *Food Structure*, 1992, 11: 339-349.
- Laukova A, Czikkova S, Vaslikova Z, Juris P & Marekova M. Ocurrence of bacteriocin production among environmental enterococci. *Letters in Applied Microbiology*, 1998, 27: 178-182.
- Lazarides HN & Mavroudis NE. Freeze/thaw effects on mass transfer rates during osmotic dehydration. *Journal of Food Science*, 1995, 60: 826-828, 857.
- Lee K. Industry News: Progressive Dairymen. 2010.
- Lehmann U, Jacobasch G & Schmiedl D. Characterization of resistant starch type III from banana (*Musa acuminata*). *Journal of Agricultural and Food Chemistry*, 2002, 50: 5236-5240.
- Lenart A & Lewicki PP. Osmotic dehydration of carrots at high temperature. In Spiess WEL & Schubert H. Engineering and food preservation processes and related techniques London: *Elsevier*, 1990, 2: 731-740.
- Lenart A & Flink JM. Osmotic concentration of potato: I. Criteria for the end-point of osmosis process. *Journal of Food Technology*, 1984, 19: 45-63.
- Lerici CL, Pinnavaia G, Dalla Rosa M & Bartolucci L. Osmotic dehydration of fruit: Influence of osmotic agents on drying behavior and product quality. *Journal of Food Science*, 1985, 50: 1217-1219.
- Leunda MA, Guerrero SN & Alzamora SM. Color and chlorophyll content changes of minimally processed kiwifruit. *Journal of Food Processing and Preservation*, 2000, 24: 17-38.
- Lewicki P. Water as the determinant of food engineering properties. A review. *Journal of Food Engineering*, 2004, 61: 483-495.
- Lim CC, Ferguson LR & Tannock GW. Dietary fibres as "prebiotics": implications for colorectal cancer. *Molecular Nutrition and Food Research*, 2005, 49: 609-619.

- Lin W, Yu B, Jang S & Tsen H. Different probiotic properties for *Lactobacillus fermentum* strains isolated from swine and poultry. *Anaerobe*, 2007, 13: 107-113.
- Lucey JA. The relationship between rheological parameters and whey separation in milk gels. *Food Hydrocolloids*, 2001, 15: 603-608.
- Lucey JA, Munro PA & Singh H. Effect of heat treatment and whey protein addition on the rheological properties and structure of acid skim milk gels. *International Dairy Journal*, 1999, 9: 275-279.
- Luckow T, Sheehan V, Fitzgerald G & Delahunty C. Exposure, health information and flavour-masking strategies for improving the sensory quality of probiotic juice. *Appetite*, 2006, 47: 315-323.
- Mabel MJ, Sangeetha PT, Kalpana Platel, Srinivasan K & Prapulla SG. Physiological characterization of fructooligosaccharides and evaluation of their suitability as a potential sweetener for diabetics. *Carbohydrate Research*, 2008, 343: 56-66.
- Macfarlane GT, Steed H & Macfarlane S. 2008. Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. *Journal of Applied Microbiology*, 2008, 104: 305-344.
- Madhura U, Joshi, Anirban Sarkar, Rekha S, Singhal & Aniruddha B. Pandit. Optimizing the formulation and processing conditions of *gulab jamun*: a statistical design. *International Journal of Food Properties*, 2009, 12: 162-175.
- Maguina G, Tapia MS, Briceno AG, Rodriguez C, Sanchez D, Rao V & Lopez-Malo A. Incorporation of Bifidobacterium spp. En una matriz porosa de fruta por el mecanismo hidrodinamica (PF-08). In *Actas del 2^{o} Congreso Espanol de Ingenieria de Alimentos*. Lleida, Espana: Universitat de Lleida. 2002, 1-4.
- Maia GA, Ritter UG, Figueiredo RWD, Oliveira GSFD, Gaspar JC, Monterio JCS, De Digueiredo RW & De Oliveira GSF. Preparation and evaluation of low caloric drink based on acerola. *Revista Ciencia Agronomica*, 2003, 34: 233-240.
- Manning TS & Gibson GR. Prebiotics. *Best Practice and Research in Clinical Gastroenterology*, 2004, 18: 287-298.

- Manolopoulou E, Sarantinopoulos P, Zoidou E, Aktypis A, Moschopoulou E, Kandarakis, IG & Anifantakis EM Evolution of microbial populations during traditional Feta cheese manufacture and ripening. *International Journal of Food Microbiology*, 2003, 82: 153-161.
- Mark-Herbert C. Innovation of a new product category - Functional foods. *Technovation*, 2004, 24: 713-719.
- Martin JJ, Solanes E, Bota E & Sancho J. Chemical and organoleptic changes in pasteurized orange juice. *Alimentaria*, 1995, 261: 59-63.
- Martinez-Monzo J, Martinez-Navarrete N, Chiralt A & Fito P. Machanical and structural change in apple (var. Granny Smith) due to vacuum impregnation with cryoprotectants. *Journal of Food Science*, 1998, 63: 499-503.
- Mastrocola D, Serverini C, Lerici CR & Sensidoni A. Disidratazione per via osmotica della carota. *Industrie Alimentari*, 1987, 26: 133-138.
- Mattila-Sandholm T, Myllarinen P, Crittenden R, Mogensen G, Fonden R & Saarela M. Technological challenges for future probiotic foods. *International Dairy Journal*, 2002, 12: 173-182.
- Mavroudis NE, Gekas V & Sjoholm I. Osmotic dehydration of apples-effects of agitation and raw material characteristics. *Journal of Food Engineering*, 1998, 35: 191-209.
- Mazza G. Functional foods: Biochemical and processing aspects. Lancaster PA: *Technolomic Publishing Company*, 1998, 437.
- McFarland LV, Surawicz CM, Greenberg RN, Elmer GW, Moyer KA, Melcher SA, Bowen KE & Cox JL. Prevention of beta-lactam- associated diarrhea by *Saccharomyces boulardii* compared with placebo. *American Journal of Gastroenterology*, 1995, 90: 439-448.
- McKellar QA. Antimicrobial resistance: a veterinary perspective. *British Medical Journal*, 1998, 317: 610-611.
- Menrad K. Market and marketing of functional food in Europe. *Journal of Food Engineering*, 2003, 56: 181-188.

- Mettler E & Seibel W. Effects of emulsifiers and hydrocolloids on whole wheat bread quality: A response surface methodology study. *Cereal Chemistry*, 1993, 70 (4): 373-377.
- Ministério da Saúde. (1997) *Regulamento Técnico: Aditivos Alimentares– Definições, Classificação e Emprego*. Ministério da Saúde, Secretaria da Vigilância Sanitária, Portaria no. 540 de 27 de Outubro de 1997, Brazil.
- Mitsuoka T. Bifidobacteria and their role in human health. *Journal of Industrial Microbiology*, 1990, 6: 263-268.
- Mombelli B & Gismondo MR. The use of probiotics in medical practice. *International Journal of Antimicrobial Agents*, 2000, 16: 531-536.
- Monsalve Gonzalez A, Barbosa-Canovas GV & Cavalieri RP. Mass transfer and textural changes during processing of apples by combined methods. *Journal of Food Science*, 1993, 58: 1118-1124.
- Moore N, Chao C, Yang LP, Storm H, Oliva-Hemker M & Saavedra JM. Effects of fructooligosaccharides-supplemented infant cereal: double-blind, randomized trial. *British Journal of Nutrition*, 2003, 90: 581-587.
- Mora D, Fortina MG, Parini C, Daffonchio D & Manachini PL. Genomic sub-populations within the species *Pediococcus acidilactici* detected multilocus typing analysis: relationships between pediocin AcH/PA-1 producing and non-producing strains. *Microbiology*, 2000, 146: 2027-2038.
- Moreira R & Sereno AM. Evaluation of mass transfer coefficients and volumetric shrinkage during osmotic dehydration of apple using sucrose solutions in static and non-static conditions. *Journal of Food Engineering*, 2003, 57: 25-31.
- Moreno J, Chiralt A, Escriche I & Serra JA. Effect of blanching/osmotic combined methods on quality and stability of minimally processed strawberries. *Food Research International*, 2000, 33, 609-616.
- Moro G, Minoli I, Mosca M, Fanaro S, Jelinek J, Stahl B & Boehm G. Dosage-related bifidogenic effects of galacto- and fructooligosaccharides in formula-fed term infants. *Journal of Pediatric Gastroenterology and Nutrition*, 2002, 34: 291-295.

- Mujica-Paz H, Valdez-Fragoso A, Lopez-Malo A, Paloub E & Welti-Chanes J. Impregnation and osmotic dehydration of some fruits: Effect of the vacuum pressure and syrup concentration. *Journal of Food Engineering*, 2003, 57: 305-314.
- Mujica-Paz H, Valdez-Fragoso A, Lopez-Malo A, Palou E & Welti-Chanes J. Impregnation properties of some fruits at vacuum pressure. *Journal of Food Engineering*, 2002, 56: 307-314.
- Muntada V, Gerschenson LN, Alzamora SM & Castro MA. Solute infusion effects on texture of minimally processed kiwifruit. *Journal of Food Science*, 1998, 63: 616-620.
- Murphy O. Non polyol low digestible carbohydrates: food applications and functional benefits. *British Journal of Nutrition*, 2001, 85: 47-53.
- Myers RH & Montgomery DC. Response surface methodology, process and product optimization using designed experiments, 2nd Ed. New York: John Wiley and Sons, 1995.
- Myers RH. Response surface methodology. DOE Book by Boston: Allyn and Bacon. 1971.
- O'Donnell HJ & Butler F. Time-dependent viscosity of stirred yogurt. Part-I: Couette flow. *Journal of Food Engineering*, 2002a, 51: 249-254.
- O'Donnell HJ & Butler F. Time-dependent viscosity of stirred yogurt. Part-II: tube flow. *Journal of Food Engineering*, 2002b, 51: 255-261.
- Olano-Martin E, Gibson GR & Rastall RA. Comparison of the in vitro bifidogenic properties of pectins and pectic-oligosaccharides. *Journal of Applied Microbiology*, 2002, 93: 505-511.
- Olano-Martin E, Mountzouris KC, Gibson GR & Rastall RA. In vitro fermentability of dextran, oligodextran and maltodextrin by human gut bacteria. *British Journal of Nutrition*, 2000, 83: 247-255.
- Ong DE & Chytil F. In: Aurbach, GD., (Ed.). Vitamins and hormones. *Academic press*, New York. 1983, 105-112.

- O'Riordan K & Fitzgerald GF. Determination of genetic diversity within the genus *Bifidobacterium* and estimation of chromosomal size. *FEMS Microbiology Letters*, 1997, 156: 259-264.
- Orla-Jensen S. The lactic acid bacteria. Copenhagen: *Anhr Fred Host and Son*, 1919.
- Ouwehand AC, Kurvinen T, & Rissanen. Use of probiotic *Bifidobacterium* in a dry food matrix, an *in vivo* study. *International Journal of Food Microbiology*, 2004, 95: 103-106.
- Ouwehand AC, Kirjavainen PV, Shortt C, Salminen S. Probiotics: mechanisms and established effects. *International Dairy Journal*, 1999, 9: 43-52.
- Owens SL, Brewer JL & Rankin SA. Influence of bacterial cell population and pH on the color of nonfat milk. *Lebensmittel-Wissenschaft Technology*, 2001, 34: 329-333.
- Ozdemir M & Sadikoglu H. Characterization of rheological properties of systems containing sugar substitutes and carrageenan. *International Journal of Food Science and Technology*, 1998, 33 (5): 439-444.
- Ozen BF, Dock LL, Ozdemir M & Floros JD. Processing factors affecting the osmotic dehydration of diced green peppers. *International Journal of Food Science and Technology*, 2002, 37: 497-502.
- Ozer D, Akin S & Ozer B. Effect of inulin and lactulose on survival of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium bifidum* BB-02 in acidophilus-bifidus yogurt. *Food Science and Technology International*, 2005, 11: 19-24.
- Pai JS. Traditional Indian foods: Physico-chemical aspects. In *PFNDAI Bulletin*, December 2006. Badami MC, Holla KS, Padgaonkar SV, Sakhavalkar JA & Singh SD. (Ed.). *http://www.pfndai.com/Dec06.pdf*, 2006, 3-6.
- Panagiotou NM, Karathanos VT & Maroulis ZB. Effect of osmotic agent on osmotic dehydration of fruits. *Drying Technology*, 2007, 25: 175-189.

- Pant AR, Graham SM, Allen SJ, Harikul S, Sabchareon A, Cuevas L & Hart CA. Lactobacillus GG and acute diarrhoea in young children in the tropics. *Journal of Tropical Pediatrics*, 1996, 42: 162-165.
- Paolo Boffetta, Elisabeth Couto, Janine Wichmann, et al. Fruit and vegetable intake and overall cancer risk in the European prospective investigation into cancer and nutrition (EPIC). *Published by Oxford University Press*, 2010, 102 (8): 529-537
- Pascal G. Prebiotics and food safety. *In: Handbook of Prebiotics.* Gibson GR & Roberfroid MB. (Ed.). *CRC Press*, Taylor & Francis Group, Boca Raton, USA, 2008, 449-470.
- Patel D, Dufour Y & Domigan N. Functional food and nutraceutical registration process in Japan and China. Similarities and differences. *Journal of Pharmacy and Pharmaceutical Science*, 2008, 11 (4): 1-11.
- Patel RS & Chakraborty BK. Process alterations in Shrikhand Technology. Ph.D. Thesis, submitted to Kurukshetra University. 1982.
- Patil AM, Khedkar CD & Kalyankar SD. Studies on antimicrobial properties of probiotic *dahi. XXXV Dairy Industry Confererence*, November 23-25, Kolkata, India, DPP No. 46, 2006.
- Patil AM, Desosarkar SS, Kalyankar SD & Khedkar CD. Is a probiotic *dahi* safe for human consumption? *XXXIV Dairy Industry Confererence*, November 23-25, Bangalore, India, DP No. 2005, 49.
- Peanagiotou NM, Karathanos VT & Maroulis ZB. Mass transfer modeling of the osmotic dehydration of some fruits. *International Journal of Food Science and Technology*, 1998, 33: 267-284.
- Pedersen C, Jonsson H, Lindberg JE & Roos S. Microbiological characterization of wet wheat distillers' grain, with focus on isolation of lactobacilli with potential as probiotics. *Applied Environmental Microbiology*, 2004, 70: 1522-1527.
- Peiro-Mena R, Camacho MM & Martinez-navarrete N. Compositional and physicochemical changes associated to successive osmodehydration cycles of

pineapple (*Ananas comosus*). *Journal of Food Engineering*, 2007, 79 (3): 842-849.

- Pereira RB, Singh H, Munro PA & Luckman KS. Sensory and instrumental characteristics of acid milk gels. *International Dairy Journal*, 2003, 13: 655-667.
- Perrin S, Grill JP & Schneider F. Effects of fructooligosaccharides and their monomeric components on bile salt resistance in three species of bifidobacteria. *Journal of Applied Microbiology*, 2000, 88: 968-974.
- Pisulewski P & Kostogrys RB. Functional properties of foods of animal origin and the methods of their assessment. *Polish Journal of Food and Nutrition Sciences*, 2003, 12/53 (1): 65-73.
- Piva A, Panciroli A, Meola E & Formigoni A. Lactitol enhances short-chain fatty acid and gas production by swine cecal microflora to a greater extent when fermenting low rather than high fiber diets. *Journal of Nutrition*, 1996, 126: 280-289.
- Ponappa T, Scheerens JC & Miller AR. Vacuum infiltration of polyamines increases firmness of strawberry slices under various storage conditions. *Journal of Food Science*, 1993, 58: 361-364.
- Prado FC, Parada JL, Pandey A & Soccol CR.Trends in non-dairy probiotic beverages. *Food Research International*, 2008, 41: 111-123.
- Prapulla SG, Sangeetha PT & Ramesh MN. A process for the preparation of prebiotic bread spread, 2002, 344/DEL.
- Prapulla SG, Subhaprada V & Karanth NG. Microbial production of oligosaccharides. *Advanced in Applied Microbiology*, 2000, 47: 299-343.
- Prasad J, Gill H, Smart J & Gopal PK. Selection and characterization of *Lactobacillus* and *Bifidobacterium* strains for use as probiotics. *International Dairy Journal*, 1998, 8: 993-1002.
- Qi H, Sharma SK & Lemaguer M. Modelling multicomponent mass transfer plant material in contact with aqueous solution of sucrose and sodium chloride during osmotic dehydration. *International Journal of Food Properties*, 1999, 2: 39-54.

- Quintero-Ramos A, De La Vega C, Hernandez E & Anzaldua-Morales A. Effect of the conditions on osmotic treatment on the quality of dried apple dices. In Barbosa-Canovas GV, & Okos MR. (Ed.). New York: AIChE. *Food Dehydration*, 1993, 108-113.
- Race SW. Improved product quality through viscosity measurement. *Food Technology*, 1991, 45: 86-88.
- Raghavendra Singh & Jha YK. Effect of sugar replacers on sensory attributes, biochemical changes and shelf-life of shrikhand. *Journal of Food Science and Technology*, 2005, 42 (2): 199-202.
- Ramana SV & Taylor AJ. Effect of various agents on rheological properties of carrot cells and protoplasts, *Journal of Science of Food Agriculture*, 1994, 64: 519-525.
- Ramesh MN, Ramalakshmi K, Sangeetha PT & Prapulla SG. Beverage concentrate based on fructooligosaccharides. Indian Patent Office, Gov. of India, 2004, 457/ DEL.
- Ranganna S. Plant pigments. In: Analysis and quality control for fruit and vegetable products. 2003, 84-86.
- Rao V, Tapia MS & Millan F. Mass balance in porous foods impregnation. *Journal of Food Science*, 2001, 66: 1332-1336.
- Raoult-Wack AL. Recent advances in the osmotic dehydration of foods. *Trends in Food Science and Technology*, 1994, 5: 46-51.
- Rastall RA and Gibson GR. Prebiotic oligosaccharides: evaluation of biological activities and potential future developments. In: Tannock W (editor), Probiotics and prebiotics: where are we going? England: *Caister Academic Press*, 2002, 107-148.
- Rastogi NK, Angersbach A & Knorr D. Evaluation of mass transfer mechanisms during osmotic treatment of plant materials. *Journal of Food Science*, 2000, 65: 838-841.
- Rastogi NK & Niranjan K. Enhanced mass transfer during osmotic dehydration of high pressure treated pineapple. *Journal of Food Science*, 1998, 63: 508-511.

- Rastogi NK, Raghavarao KS & Niranjan K. Mass transfer during osmotic dehydration of banana: Fickian diffusion in cylindrical configuration. *Journal of Food Engineering*, 1997, 31: 423-432.
- Rastogi NK & Raghavarao KSMS. Kinetics of osmotic dehydration under vacuum. *Lebensmittel Wissenschaft und Technologie*, 1996, 29: 669-672.
- Rastogi NK & Raghavarao KSMS. Effect of osmotic dehydration under vacuum. *Lebensmittel Wissenschaft und Technologie*, 1994, 27: 564-567.
- Ravi R, Maya Prakash & Rajalakshi D. Relationship between sensory and texture measurement of jamun and positioning of jamun samples. *Journal of Sensory Studies*, 2002, 17: 29-41.
- Reddy KBPK, Madhu AN & Prapulla SG. Comparative survival and evaluation of functional probiotic properties of spray-dried lactic acid bacteria. *International Journal of Dairy Technology*, 2009, 62 (2): 240-248.
- Reddy KBPK & Prapulla SG. *In vitro* evaluation of lactic acid bacteria as potent probiotics for nutraceuticals applications. *International Journal of Probiotics and Prebiotics*, 2007, 2 (2-3): 75-84.
- Reddy KBPK, Raghavendra P, Girishkumar B, Misra MC & Prapulla SG. Screening of probiotic properties of lactic acid bacteria isolated from *Kanjika*, an ayurvedic fermented product: An *in vitro* evaluation. *Journal of General and Applied Microbiology*, 2007, 53: 207-213.
- Reid G. The extra intestinal effects of probiotics. In: Gibson GR, (Ed.). Interventions for Improving Human Health. London: Wiley and Sons Ltd., 2006, 201-211.
- Reid G & Friendship R. Alternatives to antibiotic use: probiotics for the gut. *Animal Biotechnology*, 2002, 13: 97-112.
- Reid G. Regulatory and clinical aspects of dairy probiotics. Background paper for FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food including Powder Milk and Live Lactic Acid Bacteria. Cordoba, Argentina. October 1-4, 2001.

- Renuka B, Prakash M & Prapulla SG. Fructooligosaccharides based low calorie gulab jamun: Studies on the texture, microstructure and sensory attributes. *Journal of Texture Studies*, 2010, 41 (4): 594-610.
- Renuka B, Kulkarni SG, Vijayanand P & Prapulla SG. Fructooligosaccharide fortification of selected fruit juice beverages: Effect on the quality characteristics. *LWT - Food Science and Technology*, 2009, 42 (5): 1031-1033.
- Research and Markets. Functional foods market assessment 2007, 2008. Available from: *http://www.researchandmarkets.com/reports/*. Accessed Jun 27, 2008.
- Roberfroid MB. Functional foods: Concepts and application to inulin and oligofructose. *Journal of Nutrition*, 2002, 84: 139-143.
- Robert S. Ecology of fermented foods. *Human Ecology Review*, 2008, 15: 25-31.
- Rodriguez MI. Estudio de la penetracion de microorganismos en frutas mediante el modelo Hidrodinamico (HDM). Thesis. Instituto de Ciencia y Tecnologia de Alimentos, Universidad Central de Venezuela. 1998.
- Rosa MD & Giroux F. Osmotic treatments (OT) and problems related to the solution mangement. *Journal of Food Engineering*, 2001, 49: 223-236.
- RTS Resource Ltd. Food and ingredients market research and analysis. England: RTS Resource Ltd., 2008, *www.rts.resource.com/*
- Rybka S, Kailaspathy K, The survival of culture bacteria in fresh and freeze dried AB yogurts. *Australian Journal of Dairy Technology*, 1995, 50 (2): 51-57.
- Rycroft CE, Jones MR, Gibson GR & Rastall RA. A comparative *in vitro* evaluation of the fermentation properties of prebiotic oligosaccharides, *Journal of Applied Microbiology*, 2001, 91: 878-887.
- Rycroft CE, Jones MR, Gibson GR & Rastall RA. Fermentation properties of gentio-oligosaccharides. *Letters in Applied Microbiology*, 2001, 32: 156-161.
- Saarela M, Mogensen G, Fonden R, Matto J & Mattila-Sandholm T. Probiotic bacteria: safety, functional and technological properties. *Journal of Biotechnology*, 2000, 84: 197-215.

- Saavedra JM, Abi-Hanna A, Moore N & Yolken R.H. Long-term consumption of infant formulas containing live probiotic bacteria: tolerance and safety. *American Journal of Clinical Nutrition,* 2004, 79: 261-67.
- Sabater-Molina M, Larque E, Torrella F & Zamora S. Dietary fructooligosaccharides and potential benefits on health. *Journal of Physiology and Biochemistry,* 2009, 65 (3): 315-328.
- Sacchetti G, Gianotti A & Dalla Rosa M. Sucrose-salt combined effect on mass transfer kinetics and product acceptability. Study on apple osmotic treatment. *Journal of Food Engineering*, 2001, 47: 163-173.
- Saldanha LG. US Food and Drug Administration regulations governing label claims for food products, including probiotics. *Clinical Infectious Disease*, 2008, 46 (2): 119-121.
- Salminen S, Wright A, Morelli L, Marteau L, Brassart P & de Vos DWM. Demonstration of safety of probiotics a review. *International Journal of Food Microbiology*. 1998, 44: 93-106.
- Saloff-Coste CJ. Lactic acid bacteria. *Danone World Newsletter*, 1994, 5: 2.
- Sambrook J & Russell D. Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory,Cold Spring Harbor, New York. United States of America 3rd Ed. 2001.
- Sanders ME. Probiotics: considerations for human health. *Nutrition Review*, 2003, 61: 91-99.
- Sanders ME & Huis in't Veld J. Bringing a probiotic-containing functional food to the market: microbiological, product, regulatory and labeling issues. *Antonie van Leeuwenhoek*, 1999, 76: 293-315.
- Sanders ME. Prebiotics. *Food Technology,* 1999, 53 (11): 67-77.
- Sanders ME. Overview of functional foods: emphasis on probiotic bacteria. *International Dairy Journal*, 1998, 8: 341-347.
- Sangeetha PT, Ramesh MN & Prapulla SG. A process for the preparation of honey like product, 2003, 92/DEL.

- Sangeetha PT, Ramesh MN & Prapulla SG. Recent trends in the microbial production, analysis and application of Fructooligosaccharides. *Trends Food Science and Technology*, 2005, 16 (10): 442-457.
- Sangeetha PT, Ramesh MN & Prapulla SG. Fructooligosaccharide production using fructosyl transferase obtained from recycling culture of *Aspergillus oryzae* CFR202. *Process Biochemistry*, 2005a, 40 (3-4): 1085-1088.
- Sangeetha PT, Ramesh MN & Prapulla SG. Recent trends in microbial production, analysis and application of Fructooligosaccharides. *Food Science and Technology*, 2005b, 16 (10): 442-457.
- Sangeetha PT, Ramesh MN & Prapulla SG. Microbial production of Fructooligosaccharides. *Asian Journal of Microbiology Biotechnology and Environmental Science*, 2003, 5 (3): 313-318.
- Sangeetha PT, Ramesh MN & Prapulla SG. Influence of media components and reaction parameters on the production of Fructosyl transferase and fructooligosaccharides. *Sciences des aliments*. 2002, 22: 277-287.
- Sanger F, Nicklen S & Chase AR. DNA Sequencing with Chain Terminating Inhibitors. *Proceedings in National Academy of Science*, USA. 1977, 74 (12): 5463-5468.
- Sarkar S & Misra AK. Yogurt: Nutritional and therapeutic significance. *Indian Journal of Microbiology*, 2002a, 42: 275-287.
- Sarkar S, Kuila RK & Misra AK. Effect of incorporation of Gelodan™ SB 253 (Stabilizer cum preservative) and nisin on the microbiological quality of *shrikhand. Indian Journal of Dairy Science*, 1996b, 49: 176-184.
- SAS. SAS user's guide: Statistics. 1993, SAS Institute, Cary, USA.
- Sascha S & Magdalena K. Mass spectrometry tools for the classification and identification of bacteria. *Nature Reviews Microbiology*, 2010, 8: 74-82.
- Saxelin M, Korpela R & Mayra-Makinen A. Introduction: classifying functional dairy products. In Mattila-Sandholm T, & Saarela M. (Ed.). Functional Dairy Products, Cambridge, England: *Woodhead Publishing Limited*. 2003, 1-16.

- Sereno AM, Moreira R & Martinez E. Mass transfer coefficients during osmotic dehydration of apple in single and combined aqueous solutions of sugar and salt. *Journal of Food Engineering*, 2001, 47: 43-49.
- Shah NP. Functional cultures and health benefits. *International Dairy Journal*, 2007, 17: 1262-1277.
- Shah NP. Functional foods from probiotics and prebiotics. *Food Technology,* 2001, 55 (11): 46-53.
- Shaker RR, Jumah RY & Abu-Jdayil B. Rheological properties of plain yogurt during coagulation: impact of fat content and preheat treatment of milk. *Journal of Food Engineering*, 2000, 44: 175-180.
- Sharma VP & Zariwala IT. Deterioration of *shrikhand* during storage. *Indian Journal of Dairy Science*, 1980, 33: 223-231.
- Sheehan VM, Sleator RD, Hill C & Fitzgerald GF. Improving gastric transit, gastrointestinal persistence and therapeutic efficacy of the probiotic strain *Bifidobacterium breve* UCC2003. *Microbiology,* 2007, 153: 3563-3571.
- Shi XQ & Fito P. Vacuum osmotic dehydration of fruits. *Drying Technology*, 1993, 11: 1429-1442.
- Shi XQ, Chiralt A, Fito P, Serra J, Escoin C & Gasque L. Application of osmotic dehydration technology on jam processing. *Drying Technology*, 1996, 14: 841-847.
- Side C. Overview on marketing functional foods in Europe. *Functional Food Network General Meeting*, 2006.
- Sidel JL & Stone H. The role of sensory evaluation in the food industry. *Food Quality and Preference*, 1993, 4: 65-73.
- Silvi S, Rumney CJ, Cresci A & Rowland IR. Resistant starch modifies gut microflora and microbial metabolism in human flora-associated rats inoculated with feces from Italian and UK donors. *Journal of Applied Microbiology*, 1999, 86: 521-530.
- Singh HP. Research and development in banana and plantain in India-a pathway for enhancing yield potential. *Indian Horticulture*, 2007, 52: 3-8.

- Sinha PR, Yadav H & Jain S. Preparation of low calorie probiotic *lassi. XXXIV Dairy Industry Conference,* November 23-25, Bangalore, India, DP No. 06, 2005.
- Siro I, Kapolna E, Kapolna B & Lugasi A. functional food, Product development, marketing and consumer acceptance- A review. *Appetite*, 2008, 51: 456-467.
- Sodini I, Remeuf F, Haddad S & Corrieu G. The relative effect of milk base, starter, and process on yogurt texture: A review. *Critical Reviews of Food Science and Nutrition*, 2004, 44: 113-137.
- Souci SW, Fachmann W & Kraut H. Food composition and nutrition tables 1986/1987. *Wissenschaftliche verlagsgesellschaft GmbH*, Stuttgart, Germany. 1986.
- Soukoulis C, Panagiotidis P, Koureli R & Tzia C. Industrial yogurt manufacture: monitoring of fermentation process and improvement of final product quality. *Journal of Dairy Science*, 2007, 90: 2641-2654.
- Sousa R, Salvatori D, Andres A & Fito P. Analysis of vacuum impregnation of banana (*Musa acuminata* cv. *Giant Cavendish*). *Food Science and Technology International*, 1998, 4: 127-131.
- Stack A. Quality juice processing. *Fruit Processing*, 1995, 6-10.
- Stanton C, Ross RP, Fitzgerald GF & Van Sinderen D. Fermented functional foods based on probiotics and their biogenic metabolites. *Current Options in Biotechnology*, 2005, 16: 198-203.
- Surawicz CM, Elmer GW, Speelman P, McFarland LV, Chinn J & van Belle G. Prevention of antibiotic associated diarrhea by *Saccharomyces boulardii.* A prospective study. *Gastroenterology*, 1989, 96: 981-988.
- Suryawanshi SU, Khedkar CD & Kalyankar SD. Studies on effect of storage temperature on viable counts of probiotic organisms in *shrikhand. XXXV Dairy Industry Conference,* November 23-25, Collate, India, DPP No. 43, 2006.
- Szczesniak AS. Texture: is it still an overlooked food attribute?, *Food Technology*, 1990, 44 (9): 86-95.
- Szilagyi A. Review article: lactose - a potential prebiotic. *Alimentary Pharmacology and Therapeutics*, 2002, 16: 1591-1602.

- Tamime AY, Marshall VM & Robinson RK. Microbiological and technological aspect of milks fermented by bifibacteria. *Journal of Dairy Science*, 1995, 62: 151-187.
- Tan M, Chua KJ, Mujumdar AS & Chou SK. Effect of osmotic pre-treatment and infrared radiation on drying rate and color changes during drying of potato and pineapple. *Drying Technology*, 2001, 19 (9): 2193-2207.
- Tannock GW, Tilsala-Timisjarvi A, Rodtong S, Ng J, Munro K & Alatossava T. Identification of *Lactobacillus* isolates from the gastrointestinal tract, silage, and yoghurt by 16S-23S rRNA gene intergenic spacer region sequence comparisons. *Applied Environmental Microbiology*, 1999, 65: 4264-4267.
- Tharmaraj N, Shah NP. Survival of *Lactobacillus acidophilus, Lactobacillus paracasei* subsp. *Paracasei, Lactobacillus rhamnosus, Bifidobacterium animalis* and *Propionibacterium* in cheese based dips and the suitability of dips as effective carriers of probiotic bacteria. *International Dairy Journal*, 2004, 14: 1055-1066.
- Torreggiani D & Bertolo G. Osmotic pre-treatments in fruit processing: chemical, physical and structural effects. *Journal of Food Engineering*, 2001, 49: 247-253.
- Torreggiani D. Osmotic dehydration in fruit and vegetable processing. *Food Research International*, 1993, 26: 59-68.
- Torreggiani D. Technological aspects of osmotic dehydration in foods. In Barbosa-Canovas GV & Welti-Chanes J (Ed.). Food preservation by moisture control. Fundamentals and applications. Lancaster*: Technomic Publisher Company*, 1995, 281-304.
- Tournas VH. Moulds and yeasts in fresh and minimally processed vegetables, and sprouts. *International Journal of Food Microbiology*, 2005, 99: 71-77.
- Tregunno NB & Goff HD. Osmodehydrofreezing of apples: Structural and textural effects. *Food Research International*, 1996, 29: 471- 479.
- Trieu-Cuot P & Poyart C. Visita con guía al centro del arsenal bacteriano. *Mundo Científico*. 1999, 197: 46-50.

- Tsukahara T, Koyama H, Okada M & Ushida K. Stimulation of butyrate production by gluconic acid in batch culture of pig cecal digesta and identification of butyrateproducing bacteria. *Journal of Nutrition*, 2002, 132: 2229-2234.
- Tunick MH. Rheology of dairy foods that gel, stretch and fracture. *Journal of Dairy Science*, 2000, 83: 1892-1898.
- Tuohy KM, Ziemer CJ, Klinder A, Knobel Y, Pool-Zobel BL & Gibson GR. A human volunteer study to determine the prebiotic effects of lactulose powder on human colonic microbiota. *Microbial Ecology in Health and Disease*, 2002, 14: 165-173.
- USDA National Nutrient Database for Standard Reference. 2003.
- Valdez-Fragoso A, Mujica-Paz H, Giroux F & Welti-Chanes J. Reuse of sucrose syrup in pilot-scale osmotic dehydration of apple cubes. *Journal of Food Process Engineering*, 2002, 25: 125-140.
- Valero D, Martinez-Romero D, Serrano M & Riquelme F. Influence of postharvest treatment with putrescine and calcium on endogenous polyamines, firmness, and abscissic acid in lemon (Citrus lemon L. Burm Cv. Verna). *Journal of Agriculture Food Chemistry*, 1998a, 46: 2102-2109.
- Valero D, Martinez-Romero D, Serrano M & Riquelme F. Postharvest gibberellins and heat treatment effects on polyamines, abscisic acid and firmness in lemons. *Journal of Food Science*, 1998b, 63 (4): 611-615.
- Van Laere KM, Hartemink R, Beldman G, Pitson S, Dijkema C, Schols HA & Voragen AG. Transglycosidase activity of Bifidobacterium adolescentis DSM 20083 alpha-galactosidase. *Applied Microbiology and Biotechnology*, 1999, 52: 681-688.
- van Loo J. Prebiotics: a nutritional concept gaining momentum in modern nutrition. *Food Science and Technology Bulletin: Functional Foods*, 2005, 2 (8): 83-100.
- van Loo J, Coussement P, De Leenheer L, Hoebregs H & Smits G. On the presence of inulin and oligofructose as natural ingredients in the Western diet. *Critical Reviews in Food Science and Nutrition*, 1995, 35: 525-552.

- van Marle ME, van den Ende D, de Kruif CG and Mellema J. Steady-shear viscosity of stirred yogurts with varying ropiness. *Journal of Rheology*, 1999, 43: 1643-1662.
- Verbeke W. Consumer acceptance of functional foods: Sociodemographic cognitive and attitudinal determinants. *Food Quality and Preference*, 2005, 16 (1): 45-57.
- Vijayanand P, Chand N & Eipeson WE. Optimization of osmotic dehydration of cauliflower. *Journal of Food Processing and Preservation*, 1995, 19: 229-242.
- Vinderola CG, Costa GA, Regenhardt S & Reinheimer JA. Influence of compounds associated with fermented dairy products on the growth of lactic acid starter and probiotic bacteria. *International Dairy Journal*, 2002, 12: 579-589.
- Wadstrom T, Anderson K, Sydow M, Axelsson L, Lindgren S & Gullmar B. Surface properties of lactobacilli isolated from the small intestine of pigs. *Journal of Applied Bacteriology*, 1987, 62: 513-520.
- Wanasundara PK, Amarowicz RA, Pegg RB & Shand PJ. Preparation and characterization of hydrolyzed proteins from defibrinated bovine plasma. *Journal of Food Science*, 2002, 67: 623-630.
- Wang X, Brown IL, Khaled D, Mahoney MC, Evans AJ & Conway PL. Manipulation of colonic bacteria and volatile fatty acid production by dietary high amylose maize (amylomaize) starch granules. *Journal of Applied Microbiology*, 2002, 93: 390-397.
- Warczok J, Ferrando M, Lopez F, Pihlajamaoki A & Guoell C. Reconcentration of spent solutions from osmotic dehydration using direct osmosis in two configurations. *Journal of Food Engineering*, 2007, 80 (1): 317-326
- Ward DM, Weller R & Bateson MM. 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. *Nature*, 1990, 345: 63-65.
- Weinberg Z, Shatz O, Chen Y, Yosef E, Nikbahat M, Ben-Ghedalia D & Miron J. Effect of lactic acid bacteria inoculants on *in vitro* digestibility of wheat and corn silages. *Journal of Dairy Science*, 2007, 90: 4754-4762.

- White LA, Newman MC, Cromwell GL & Lindemann MD. Brewers dried yeast as a source of mannan oligosaccharides for weanling pigs. *Journal of Animal Science*, 2002, 80: 2619-2628.
- Wills RBH & Sirivatanapa S. Evaluation of postharvest infiltration of calcium to delay the ripening of avocados. *Australian Journal of Experimental Agriculture*, 1988, 28: 801-804.
- Wills RBH & Tirmazi SIH. Effect of calcium and other minerals on ripening of tomatoes. *Australian Journal of Plant Physiology*, 1979, 6: 221-227.
- Winkelhausen E & Kuzmanova S. Microbial conversion of D-xylose to xylitol. *Journal of Fermentation and Bioengineering*, 1998, 86: 1-14.
- Xie J & Zhao Y. Nutritional enrichment of fresh apple (Royal Gala) by vacuum impregnation. *International Journal of Food Science and Nutrition*, 2003a, 54: 387-398.
- Yang DC & Maguer ML. Osmotic dehydration of strawberries in a batch recirculation system. *Journal of Food Quality*, 1992, 15: 387-397.
- Yeong S, Ji-Young Lee P, Young-Suk K & Dong-Hwa S. Isolation and characterization of lactic acid bacteria from feces of newborn baby and from dongchimi. *Journal of Agricultural Food Chemistry*, 2002, 50: 2531-2536.
- Yin H, Chen Z, Gu Z & Han Y. Optimization of natural fermentative medium for selenium-enriched yeast by D-optimal mixture design. *LWT - Food Science and Technology*, 2009, 42: 327-331.
- Ylimaki G, Hawrysh RT & Thomson ABR. Application of response surface methodology to the development of rice flour yeast breads: Objective measurements. *Journal of Food Science*, 1988, 53: 1800-1805.
- Yun JW. Fructooligosaccharides occurrence, preparation and application. *Enzyyme Microbial Technology*, 1996, 19: 107-117.
- Zhao Y & Xie J. Practical applications of vacuum impregnation in fruit and vegetable processing. *Trends in Food Science and Technology*, 2004, 15: 434-451.

PAPERS:

Published

1. Fructooligosaccharide fortification of selected fruit juice beverages: Effect on the quality characteristics. **Renuka B**, Kulkarni SG, Vijayanand P and Prapulla SG. *LWT - Food Science and Technology*, 2009, 42 (5):1031-1033. ***(Impact Factor: 2.29)***.
2. Fructooligosaccharides based low calorie gulab jamun: Studies on the texture, microstructure and sensory attributes. **Renuka B**, Prakash M and Prapulla SG. *Journal of Texture Studies*, 2010, 41(4): 594-610. ***(Impact Factor: 0.59)***.

Communicated

1. Fructooligosaccharides: A novel osmotic agent for impregnation of fruits and vegetables**. Renuka B** and Prapulla SG
2. Novel synbiotic functional foods: Fruits and vegetables impregnated with fructooligosaccharides and *Lactobacillus salivarius*, *Pediococcus acidilactici* and *Enterococcus hirae*. **Renuka B** and Prapulla SG

Under preparation

1. Reutilization of spent fructooligosaccharides syrup for successive vacuum osmotic dehydration cycles of star fruit
2. An integrated process for the production of fructooligosaccharides and osmotic dehydration of fruits and vegetables
3. Vacuum osmotic dehydration of banana using fructooligosaccharides: Optimization of process parameters using a statistical experimental design
4. Isolation and characterization of potent probiotics from fruits and vegetables
5. Fermented milk based dessert, *Shrikhand* enriched with selected probiotic *Enterococcus faecium* and prebiotic fructooligosaccharides
6. Fructooligosaccharides based edible film.

PAPERS PRESENTED IN SYMPOSIUM:

1. **Renuka B** and Prapulla SG. Novel functional foods from fruits and vegetables impregnated with probiotics and prebiotics, 19th Indian Convention of Food Scientists and Technologists (ICFOST) on Health Foods, Indian Institute of Technology (IIT), Kharagpur, Kolkata, India, 31st December 2007 to 2nd January 2008.
2. **Renuka B** and Prapulla SG. Development of prebiotic enriched fruits and vegetables, 18th Indian Convention of Food Scientists and Technologists (ICFOST), ANGRAU, Rajendra Nagar, Hyderabad, India, 16th & 17th November 2006.
3. **Renuka B**, Aachary AA and Prapulla SG. An integrated process for the production of Fructooligosaccharides and development of prebiotic enriched fruits and vegetables, National Symposium on New Horizons in Fermentation and Food Biotechnology- Organized by Department of Biotechnology, Punjabi University, Patiala (Punjab), India, 21st to 22nd March 2006.