DEVELOPMENT OF FLAXSEED FORTIFIED SYNBIOTIC FLAVOURED DAHI AND ITS IMPACT ON CHOLESTEROL-FED MICE MODEL

काशी हिन्दू विश्वविद्यालय

BANARAS HINDU UNIVERSITY

AN INSTITUTION OF NATIONAL IMPORTANCE ESTABLISHED BY AN ACT OF PARLIAMENT

THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

Doctor of Philosophy

in

Food Science and Technology

Supervisor
Dr. Dinesh Chandra Rai
Professor

Submitted by
Manju Tiwari
Enrolment No. 351879
Id. No. PFS-12031

CENTRE OF FOOD SCIENCE AND TECHNOLOGY
INSTITUTE OF AGRICULTURAL SCIENCES
BANARAS HINDU UNIVERSITY
VARANASI – 221 005
INDIA

2016

BHU
capital of knowledge

UNDERTAKING OF THE CANDIDATE

I, **Manju Tiwari,** research scholar under the supervision of **Prof. D. C. Rai,** Professor, Department of Animal Husbandry and Dairying, Institute of Agricultural Sciences, Banaras Hindu University, give undertaking that the thesis entitled **"Development of Flaxseed Fortified Synbiotic Flavoured Dahi and its Impact on Cholesterol-fed Mice Model"** submitted by me for the degree of **Doctor of Philosophy** is a record of first hand research work done by me during the period of study.

I avail myself to responsibility such as an act will be taken on behalf of me, mistakes, errors of fact and misinterpretation are of course entirely my own.

Date: .....
Place: Varanasi **(Manju Tiwari)**

Annexure – E
(See clause XIII.2 (b) (iii))

CANDIDATES'S DECLARATION

I, **Manju Tiwari**, **I.D. No.** PFS-12031, ***Enrolment Number*** 351879, certify that the work embodied in this Ph.D. thesis is my own bonafide work carried out by me under the supervision of **Dr. D. C. Rai,** Professor, Department of Animal Husbandry and Dairying for a period of from **August 2012** to **May 2016** at Centre of Food Science & Technology, Institute of Agricultural Sciences, Banaras Hindu University. The matter embodied in this Ph.D. thesis has not been submitted for the award of any other degree/diploma.

I declare that I have faithfully acknowledged, given credit to and referred to the research workers wherever, their works have been cited in the text and the body of the thesis. I further certify that I have not fully lifted up some other's work, para, text, data, results, etc. reported in the journals, books, magazines, reports, dissertations, thesis, etc., or available at web sites and included them in this Ph.D. thesis and cited as my own work.

Date: ……..
Place: B.H.U., Varanasi

Signature of the Candidate
(Manju Tiwari)

CERTIFICATE FROM THE SUPERVISOR

This is to certify that the above statement made by the candidate is correct to the best of my knowledge.

(Supervisor)
Dr. Dinesh Chandra Rai
(Professor)

(Signature of the Head of the Department with seal)

Annexure –F
(See clause XIII.1 (c) and XIII.2 (b) (iv))

COURSE WORK COMPLETION CERTIFICATE

This is to certify that **Ms. Manju Tiwari, ID. No. PFS-12031,** a bonafide research scholar of the Centre of Food Science and Technology, Institute of Agricultural Sciences, has successfully completed the course work requirement which is a part of her Ph.D. programme.

Date: …….
Place: Varanasi

(Signature of the Head of the Department)

Annexure – F
(See clause XIII.1 (c) and XIII.2 (b) (iv))

COMPREHENSIVE WRITTEN EXAMINATION AS WELL AS COMPREHENSIVE ORAL COMPLETION CERTIFICATE

This is to certify that **Ms. Manju Tiwari, ID. No. PFS-12031,** a bonafide research scholar of the Centre of Food Science and Technology, Institute of Agricultural Sciences, has successfully completed the comprehensive written examination as well as comprehensive oral examination which are a part of her Ph. D. programme.

Date: …….
Place: Varanasi

(Signature of the Head of the Department)

Annexure – F
(See clause XIII.1 (c) and XIII.2 (b) (iv))

PRE-SUBMISSION SEMINAR COMPLETION CERTIFICATE

This is to certify that **Ms. Manju Tiwari, ID. No. PFS-12031,** a bonafide research scholar of the Centre of Food Science and Technology, Institute of Agricultural Sciences, has successfully completed the pre-submission seminar requirement on the topic entitled "**Development of Flaxseed Fortified Synbiotic Flavoured Dahi and its Impact on Cholesterol-fed Mice Model**" held on **11th January 2016** which is a part of her Ph. D. programme.

Date: …….
Place: Varanasi

(Signature of the Head of the Department)

Annexure – G
(See clause XIII.2 (b) (v))

COPYRIGHT TRANSFER CERTIFICATE

Title of the Thesis : Development of Flaxseed Fortified Synbiotic Flavoured *Dahi* and its Impact on Cholesterol-fed Mice Model

Candidate's Name : Manju Tiwari

Copyright Transfer

The undersigned hereby assigns to the Banaras Hindu University all rights under copyright that may exist in and for the above thesis submitted for the award of the Ph.D. degree.

(Signature of the candidate)

Note: However, the author may reproduce or authorize others to reproduce material extracted verbatim from the thesis or derivative of the thesis for author's personal use provided that the source and the University's copyright notice are indicated.

DEVELOPMENT OF FLAXSEED FORTIFIED SYNBIOTIC FLAVOURED DAHI AND ITS IMPACT ON CHOLESTEROL-FED MICE MODEL

काशी हिन्दू विश्वविद्यालय BANARAS HINDU UNIVERSITY

AN INSTITUTION OF NATIONAL IMPORTANCE ESTABLISHED BY AN ACT OF PARLIAMENT

by

Manju Tiwari

Thesis submitted in the partial fulfilment of the requirement for the degree of "**DOCTOR OF PHILOSOPHY IN FOOD SCIENCE AND TECHNOLOGY**", Centre of Food Science and Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

APPROVED BY ADVISORY COMMITTEE

Chairman/Supervisor	:	**Dr. D. C. Rai** Professor Department of Animal Husbandry and Dairying
Members	:	**Dr. R. K. Pandey** Professor and Head Department of Animal Husbandry and Dairying
Members	:	**Dr. V. K. Paswan** Assistant Professor Department of Animal Husbandry and Dairying
Members	:	**Dr. Anand Kumar Singh** Professor Department of Horticulture

EXTERNAL EXAMINER:

ACKNOWLEDGEMENT

With a deep sense of devotion I bow my head with great reverence in the pious feet of ***Bharat Ratna Mahamana Pandit Madan Mohan Malviya ji, Goddess Saraswati, Lord Vishwanath ji*** *and* ***Lord Hanuman ji (Baandh, Allahabad)*** *who provided me an opportunity to study in the holy city Kashi specially in Banaras Hindu University, the dream of* ***Pt. Madan Mohan Malviya ji****, a great patriot, nobleman and patriarch of this university.*

With immense pleasure and profound sense of gratitude, indeed, I take this opportunity to express my heartfelt and sincere thanks to my esteemed supervisor ***Dr. Dinesh Chandra Rai,*** *Professor, Department of Animal Husbandry & Dairying, Banaras Hindu University, for his inspiring guidance and constant encouragement during the course of present investigation. I will forever cherish the affection that he bestowed upon me through my tenure as a student under him which helped me to cope with many difficult situations.*

I owe a special debt of gratitude to ***Prof. R.K. Pandey, Prof. A.K. Chauhan, Dr. V. K Paswan, Prof. Anand Kumar Singh (Horticulture), Dr. Shakuntla , Dr. Arvind, Mr. A.D. Tripathi, Er. Durga Shankar Bunkar, Dr. Amrita Poonia*** *and I am thankful to all the faculty members of Centre of Food Science and Technology, & Department of Animal Husbandry & Dairying, Institute of Agricultural Sciences, B.H.U for their invaluable cooperation and encouragement during my entire course of investigation. They spared their valuable time for me, whenever I needed.*

I feel paucity of words to express my gratefulness and warmest regard to the entire faculty members specially ***Mr. Amresh*** *and* ***Mr. Chandrashekhar*** *and to all non teaching staff members of the Centre of Food Science & Technology, Institute of Agricultural Sciences, B.H.U. for their timely help and co-operation throughout the degree programme.*

I am highly thankful to the company of my friends and my seniors ***Dr. Dipti Rai, Dr. Ajay Maurya, Dr. Dilip Yadav, Mr. Chandrashekhar Singh, Dr. Ashok Yadav, Dr. Rajendra K. Thakur*** *and* ***Dr. Paras Porwal*** *for their moral support, co-operation and priceless suggestions.*

I owe my sincere thanks and deepest debts to my father ***Late Shri Ram Karan Tiwari****, who believed in me and let me persue my dream, whose love and motivation has always helped me to aim high and achieve success in life. I thank him once again because I would have never achieved this level of education without his selfless sacrifices.*

With profound regards in a more personal sense, I owe deepest debts to my mother ***Mrs. Saroj Tiwari*** *who taught me the value of wisdom based on erudition but without enslaved by it and her persistent inspiration, selfless sacrifice, continuous encouragement and blessing gave untiring help and have enabled me to be so today.*

I don't have words to express my emotional feeling, unbounded gratitude towards my sister ***Ms. Anju Tiwari*** *for her immense love, affection, encouragement and blessings which always animated me to rise against all problems and face them with smile and motivated me to achieve success at every step in life. I thank her once again for her moral support.*

Cooperation by the staff members of the department ***Shri Vishwakarma*** *and* ***Shri Anand*** *is also acknowledged.*

I'm also indebted to all the ***mice*** *that were part of my experimental work during my research, may their soul rest in peace.*

Before pen down, I once again confess that I do not know how to acknowledge the help and co-operation of my Supervisor, members of advisory committee, family members and relatives, seniors, juniors & colleagues but above feeling are followed from core of my heart in the shape of words and as gospel truth.

The graces of the God are always blessed to me and gave me patience and power to overcome the difficulties which came my way in accomplishment of this endeavour. I cannot dare to say thanks but only pray to bless me always.

I would like to thank everyone who was involved in completing this work directly or indirectly.

Date:
Place: *Varanasi* ***(Manju Tiwari)***

CONTENTS

ABBREVIATIONS

%	:	Per cent
°C	:	degree Centigrade
µm	:	Micro meter
AC	:	Animal Curd
ADG	:	Average Daily Gain
ALA	:	Alpha-linolenic acid
ANOVA	:	Analysis of Variance
AOAC	:	Association of Official Analytical Chemists
AR	:	Analytical grade
BCG	:	Bromocresol green
BIS	:	Bureau of Indian Standards
BV	:	Biological Value
CA	:	Colour and Appearance
CCD	:	Central Composite Design
CD	:	Critical Difference
CFU	:	Colony Forming Unit
CHD	:	Coronary Heart Diseases
CHO	:	Carbohydrate
cm	:	Centimeters
cP	:	Centi Poise
CD	:	Control *Dahi*
CV	:	Coefficient of Variation
CV	:	Coefficient of Variation
DAM	:	Di Acetyl Monoxime
db	:	Dry Basis
DHA	:	Docosahexanoic acid
DMI	:	Dry Matter Intake
END	:	Enterodiol
ENL	:	Enterolactone
EPA	:	Eicosapentaenoic acid
FAO	:	Food and Agricultural Organisation
FDA	:	Food and Drug Administration
FE	:	Feed Efficiency
FFA	:	Free Fatty Acid
Fig.	:	Figure
FSSAI	:	Food Safety and Standards Authority of India
FOS	:	Fructo-oligosaccharides
g/gm	:	Gram
GLM	:	General Linear Model
GI	:	Gastro intestinal
h/hrs	:	Hours
HDL	:	High Density Lipoprotein
IS	:	Indian Standard
IU/L	:	International Unit per litre

Kg	:	Kilogram
LDL	:	Low Density Lipoprotein
ME	:	Microencapsulation
mg/dl	:	Milli gram per deci litre
min	:	Minutes
ml	:	Millie litre
MRS	:	de Man, Rogosa and Sharpe
MT	:	Metric Tonne
N	:	Normal
NCDC	:	National Collection of Dairy Culture
OA	:	Overall acceptability
PFA	:	Prevention of Food Adulteration
ppm	:	Parts Per Million
PUFA	:	Poly-Unsaturated Fatty Acid
R^2	:	Coefficient of determination
RMSE	:	Root Mean Square Error
RSM	:	Response Surface Methodology
SCFA	:	Short chain fatty acids
SECO	:	Secoisolariciresinol
SDG	:	Secoisolariciresinol diglycoside
S/NS	:	Significant/Non Significant
Sd	:	Standard deviation
sec	:	Second
SEM	:	Stander Error of Mean
SMP	:	Skim Milk Powder
SNF	:	Solid Not Fat
TA	:	Titrable Acidity
TCH	:	Total Carbohydrate
TG	:	Triglycerides
TPC	:	Total Plate Count
TS	:	Total Solids
TSS	:	Total Soluble Solids
TVC	:	Total Viable Count
TVP	:	Textured Vegetable Protein
V/v	:	Volume by Volume
VRBA	:	Violet Red Bile Agar
W/w	:	Weight by weight
WHO	:	World Health Organisation
YMC	:	Yeast and Mould Count
ΔT	:	Change in Temperature

LIST OF TABLES

LIST OF FIGURES

FIGURE NO.	PARTICULARS	PAGE NO.

Chapter I

INTRODUCTION

In recent years, increasing epidemiological evidence is linking the prevalence of mass non−communicable diseases such as obesity, hypertension, diabetes, hyperlipidemia, and even cancer, to dietary factors. There has been significant increase in death and morbidity arising from coronary heart diseases with subsequent cardiovascular manifestations worldwide. Clinical studies have shown that elevated HDL cholesterol as well as reduction in total cholesterol and LDL-cholesterol using diet or drugs decreases the incidence of coronary heart disease. Hypercholesterol is a risk factor for cardiovascular disease, the leading cause of death in many countries (Law *et al*., 1994). Nowadays, consumers demand foods which not only provide nutrition but have therapeutic effect for prevention of certain non-communicable diseases such as obesity, diabetes, hypercholestorimia and cancer. A functional food can be defined generally as any food which can provide a health benefit to one or more bodily functions beside that of basic nutrition (Hernández Ledesma *et al*., 2011).

Flax (*Linum usitassimum*) belonging to family Lineaceae, is a blue flowering annual herb that produces small flat seeds varying from golden yellow to reddish brown color. Flaxseed possesses crispy texture and nutty taste (Morris, 2007; Rubilar *et al*., 2010). Flaxseed has been the focus of growing interest for the nutritionists and medical researchers due to its potential health benefits associated with its biologically active components—ALA, lignan-Secoisolariciresinol diglycoside (SDG) and dietary fiber (Toure and Xueming, 2010). Flaxseed is establishing importance in the world's food chain as a functional food. Functional food can be defined as the food or food ingredients that may provide physiological benefits and helps in preventing and/or curing of diseases (Alokbi, 2005).

Presently, flaxseed has new prospects as functional food because of consumer's growing interest for food with superb health benefits. Owing to its excellent nutritional profile and potential health benefits, it has become an attractive

ingredient in the diets specially designed for specific health benefits (Oomah, 2001). ALA is one of the essential polyunsaturated fatty acid and reported to exhibit antiinflammatory, anti-thrombotic and anti-arrhythmic properties (Simopoulos, 1999).

Flax is a nutraceutical with many nutritional benefits. Aside from the vitamins, minerals, fiber and protein it contains some primary and secondary metabolites. Flaxseed is a good source of omega-3 fatty acids. Through its high concentration of alpha-linolenic acid, flax provides more omega-3 fatty acids than any other grain (57% of its fatty acids are omega-3s). The flaxseed contains both soluble and insoluble fibers. About one-third of the fiber in flaxseed is soluble and it may help to lower cholesterol and to regulate levels of blood sugar. The remaining two-thirds of the fiber in the flaxseed is insoluble which aids digestion by increasing bulk and preventing constipation (Institute of Medicine, 2002). Secoisolariciresinol diglycoside (SDG) is the major lignan of flaxseed, along with minor contents of matairesinol, pinoresinol, lariciresinol and isolariciresinol (Krajcova *et al*., 2009). SDG is metabolized by bacteria in the colon of humans to synthesize mammalian lignans known as enterodiol (END) and enterolactone (ENL) (Chen *et al*., 2007). SECO, SDG also play an important role in reduction of hypercholesterolemia, atherosclerosis, hypertension and diabetes (Prasad, 2000a, 2004). Daily administration of 100 mg SDG was found to be effective in reducing blood cholesterol and hepatic diseases risk in moderately hypercholesterolemic men (Fukumitsu *et al*., 2010). Flaxssed mucilage can also be used as a good source of prebiotic and dietary fibre (Fodje *et al*., 2009). Many studies have demonstrated that consumption of flaxseed show health benefits such reducing the risk of cardiovascular disease (Bloedon & Szapary, 2004; Hijova *et al*., 2011).

Recently, Fermented foods have emerged into health promoting disease preventive foods which has increased their demand in dairy market. Many research studies have shown that certain foods like oats, dietary flaxseed, (Prasad *et al.,* 1998) legumes, (Lucas *et al.*, 2003) honey, probiotic fermented (Manson *et al*., 1992) dairy product when included in diet can significantly reduce hypercholesterimia. The

reduction of serum cholesterol could be an important health benefit of LAB, as a 1% reduction in serum cholesterol is associated with an estimated reduction of 2 to 3% in the risk of coronary artery disease (Kawase *et al*., 2000).

Dahi, also known as curd is widely consumed dairy product in India prepared by fermentation of milk by lactic acid bacteria (LAB). *Dahi* is fermented by use of mixed starters of mesophilic lactococci and flavour-inducing metabolite is diacetyl which gives it unique flavor. *Dahi* can be used as potential vehicle for delivery of nutraceuticals by fortifying with many probiotic organisms (Yadav *et al.,* 2007) cereals, fruits and honey (Ghadge *et al.,* 2008) to combat chronic and non-communicable diseases like hypertension. The occurrence of various bioactive peptides in fermented milks, e.g. yoghurt, sour milk and "*Dahi*," has been reported in many studies. *Dahi* is known to contain ACE inhibitory bioactive peptide Ser-Lys-Val-Tyr-Pro (Ashar and Chand, 2004). Plain *Dahi* can be made probiotic dairy product by incorporation of probiotic bacteria such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. The efficiency of added probiotic bacteria depends on the dose level and their viability throughout during storage, product shelf-life and their survival in gut environment (Kailasapathy and Chin, 2000). Dairy products containing omega-3, phytosterols, isoflavones, conjugated linoleic acid, minerals, and vitamins also have a prominent role in the development of functional foods (Ozer and Kirmaci, 2010). When enriched with omega-3 FA, *dahi*/yogurt has even greater health promoting properties. *Dahi* / Yoghurts come in a variety of textures (e.g. liquid, set, and smooth), fat contents (e.g. luxury, low-liquid, virtually fat-free) and flavors (e.g. natural, fruit, cereal), can be consumed as a snack or part of a meal, as a sweet or savoury food, and are available all year round. This versatility, together with their acceptance as a healthy and nutritious food, has led to their widespread popularity across all population subgroups (McKinley, 2005).

Incorporation of new ingredients may affect the delicate gel structure of yoghurt or *dahi* like product as well as survivability of probiotic bacteria in product to produce positive health impact to the comsumer. To protect probiotic bacteria from environmental stresses and enhancing the viable count, microencapsulation of bacteria along with prebiotics have emerged as promising technique (Gibson and

Roberfroid, 1995; Kailasapathy, 2002; Crittenden *et al*., 2005). But there is limited knowledge about the metabolism and kinetics of the added probiotic microcapsules in food products during processing and storage so as to provide sufficient viability of the probiotics in the products, without any negative influence on the sensory acceptance of the products (Gruz *et al*., 2009) which requires to be studied extensively.

Plain *dahi*/yoghurt is predominantly sour, therefore fruit, flavourings and sweeteners have been added either to improve the flavour balance (Kagan, 1985) or to mask partially the other bland flavour of ingredients added to it. Sucrose and corn syrup have been the traditional and most commonly used sweeteners in the dairy industry. Although honey has been added as a flavouring agent in fermented dairy products, honey yoghurt combinations are relatively uncommon (Brown and Kosikowski, 1970; Roumyan *et al.,* 1996). Honey is a natural invert sugar dissolved in water (14–20%) with minor amounts of organic acids, along with traces of minerals and vitamins that may serve as sources of dietary antioxidants (Gheldof and Engeseth, 2002; Gheldof *et al.,* 2002). Honey is derived from the nectar of flowering plants which the honey bee collects. The source of honey determines many of the attributes of honey such as aroma, flavour, colour and composition (White, 1980). Honey is primarily composed of the monosaccharides glucose and fructose, which can be found in amounts of between 55 and 75%. A complex mixture of minor carbohydrates (10−25%), mainly disaccharides and trisaccharides, is also present. Moreover, the presence of four tetrasaccharides, one pentasaccharide, and one hexasaccharide has been detected in New Zealand honeydew honeys. Since antiquity, honey has been considered to be an important source of energy, being used in medical therapies and as a valuable food ingredient. Certain components of honey can provide antioxidant activities, seen as beneficial for human health, and various studies have revealed the inhibitory properties against certain pathogens. Shamala *et al*., (2000) carried out in vitro and in vivo studies in the small and large intestines of rats and proposed that honey enhanced the growth of lactic acid bacteria. Moreover, honey has been shown to support lactic acid production in skim milk fermented with lactic acid bacteria in a manner similar to that of other sweeteners such as sucrose and fructose. More recently, Kajiwara *et al*., (2002) showed that the growth, in pure culture, of

commercial strains of bifidobacteria was enhanced by honey in a manner similar to that of other commercial prebiotic oligosaccharides (fructooligosaccharide (FOS), galactooligosaccharides (GOS), and inulin).

Bioactive compounds found in the mangoes, among other plants and herbs have been shown to have possible health benefits with antioxidative, anticarcinogenic, antiatherosclerotic, antimutagenic, and angiogenesis inhibitory activities (Cao and Cao, 1999). Interestingly, mangoes are known to contain large amounts of phenolic antioxidants other than the well-known vitamins C, E, and carotenoids. Mangiferin, gallic acids (m-digallic and m-trigallic acids), gallotannins, quercetin, isoquercetin, ellagic acid, and β-glucogallin are among the polyphenolic compounds already identified in the mango pulp (Schieber *et al*., 2000). Mango juice/pulp when incorporated in dairy products enhances its vitamin A, C, and mineral contents. Mango has strong sweet flavor which can easily mask other undesireable flavors.

Therefore, the incorporation of flaxseed (for omega fatty acid and SDG), honey (as sweetner and prebiotic) and mango (for antioxidant properties and masking flavour of flaxseed) into *dahi* may result into a flavoured fermented product with advanced health promoting properties, hence might play an important role in the reduction of the risk of hypercholestremia and other chronic diseases.

Keeping in view of the above facts the present investigation entitled "**Development of Flaxseed Fortified Synbiotic Flavoured *Dahi* and its Impact on Cholesterol-Fed Mice Model"** has been conducted at Centre of Food Science and Technology with the following objectives:

Objectives

- To optimize flaxseed fortified synbiotic flavoured *dahi* with optimum levels of flaxseed powder, mango pulp and honey.
- To study the physico-chemical, functional properties and acidification kinetics of flaxseed fortified synbiotic flavoured *dahi.*

- To study the post acidification changes during shelf life of flaxseed fortified synbiotic flavoured *dahi*
- To study the impact of flaxseed fortified synbiotic flavoured *dahi* on hypercholestromic mice model**.**

Hypothesis

- Flaxseed and antihypertensive peptides of *dahi* may lower cholesterol in blood and other organs of mice. Honey may enhance the growth of probiotic bacteria while microencapsulation of probiotic bacteria may ensure the high survivability of probiotic bacteria in acidic environment of product during cold storage. Mango may improve the acceptability and therapeutic value of flaxseed fortified synbiotic flavoured *dahi*.

Chapter II

REVIEW OF LITERATURE

2.1 Flax

Flax (*Linum usitassimum*) belonging to family Lineaceae, is a blue flowering annual herb that produces small flat seeds varying from golden yellow to reddish brown color. Flaxseed possesses crispy texture and nutty taste (Morris, 2007; Rubilar *et al.,* 2010). Flaxseed is also known as linseed and these terms are used interchangeably. Flaxseed is often used to describe flax when consumed by humans while linseed denotes when it is used specifically for industrial applications (Morris, 2007). Almost all parts of linseed plant are utilized for various purposes.

Currently, it is cultivated in more than 50 countries, predominantly in the Northern hemisphere. The important flaxseed growing countries include India, China, United States, and Ethiopia (Singh *et al.,* 2011a). India ranks first among the leading flaxseed producing countries in terms of acreage accounting 23.8 % of the total and third in production contributing to 10.2 % of the world's production (Singh *et al.*, 2011a). In India flaxseed is mainly cultivated in Madhya Pradesh, Maharashtra, Chattisgarh and Bihar. It is interesting to know that flaxseed was native of India and was a staple food crop. In India, flaxseed is still being consumed as food and as well as for medicinal purposes (Shakir and Madhusudan, 2007). It enjoys a good status among oilseeds because of its versatile uses. It has emerged as an attractive nutritional food because of its exceptionally high content of alpha-linolenic acid (ALA), dietary fiber, high quality protein and phytoestrogens. Flaxseeds contain about 55 % ALA, 28– 30 % protein and 35 % fiber (Rubilar *et al.*, 2010; Rabetafika *et al.*, 2011).

Flaxseed has been the focus of growing interest for the nutritionists and medical researchers due to its potential health benefits associated with its biologically active components—ALA, lignan-Secoisolariciresinol diglycoside (SDG) and dietary fiber (Toure and Xueming, 2010). Flaxseed is establishing importance in the world's

food chain as a functional food. Functional food can be defined as the food or food ingredients that may provide physiological benefits and helps in preventing and/or curing of diseases (Alokbi, 2005).

Presently, flaxseed has new prospects as functional food because of consumer's growing interest for food with superb health benefits. Owing to its excellent nutritional profile and potential health benefits, it has become an attractive ingredient in the diets specially designed for specific health benefits (Oomah, 2001). ALA is one of the essential polyunsaturated fatty acid and reported to exhibit antiinflammatory, anti-thrombotic and anti-arrhythmic properties (Simopoulos, 1999).

Nutritionists all over the world suggest incorporation of omega 3 fatty acid sources in the diet. Flaxseed serves as the best omega 3 fatty acid source to the non-fish eaters. Edible flaxseed products include the whole flaxseed, ground meal and extracted oil or mucilage. These products have been proposed as nutritional additives in the preparation of a number of dietary items such as baked cereal products, dairy products, ready to eat cereals, fiber bars, salad toppings, meat extenders, bread, muffins and spaghetti (Singh *et al.*, 2011a). In-spite of the multiple clinical evidences of flaxseeds, people are still unaware about its nutritional as well as therapeutic benefits.

2.1.1 Nutritional composition

Among the functional foods, flaxseed has emerged as a potential functional food being good source of alpha-linolenic acid, lignans, high quality protein, soluble fiber and phenolic compounds (Oomah, 2001). The composition of flaxseed is presented in Table 2.1 (Morris, 2007; Gopalan *et al.,* 2004). Chemical composition of flaxseed depends upon growing environment, genetics and processing conditions (Morris, 2007). The lipid content of flaxseed varies from 37 to 45 g/100 g of the seed as reported by various scientists (Payne, 2000; Morris, 2007). Cotyledons are the major oil storage tissues, containing 75 % of the seed oil (Rubilar *et al.,* 2010; Singh *et al.*, 2011b).

Table 2.1 Nutritional composition of flaxseed

Nutrients	Amount per 100 g of edible flaxseed
Moisture (g)	6.5
Protein (N×6.25) (g)	20.3
Fat (g)	37.1
Minerals (g)	2.4
Crude fiber (g)	4.8
Total dietary fiber (g)	24.5
Carbohydrates (g)	28.9
Energy (kcal)	530.0
Potassium	750.0
Calcium (mg)	170.0
Phosphorous (mg)	370.0
Iron (mg)	2.7
Vitamin A (µg)	30.0
Vitamin E (mg)	0.6
Thiamine (B1) (mg)	0.23
Riboflavin (B2) (mg)	0.07
Niacin (mg)	1.0
Pyridoxine (mg)	0.61
Pantothenic acid	0.57
Biotin (µg)	0.6
Folic acid (µg)	112

Source: Morris, 2007; Gopalan *et al* .2004

Flaxseed oil constitutes 98 % triacylglycerol, phospholipids and 0.1 % free fatty acids (Mueller *et al.,* 2010). On an average it contains 21 % protein. Majority of the protein is concentrated in the cotyledons (Rabetafika *et al.,* 2011). Major protein fractions are globulin (26–58 %) and albumin (20–42 %). Nutritional value and amino acid profile of flaxseeds are comparable to that of soya proteins (Madhusudan and Singh, 1985). Flaxseed protein is rich in arginine, aspartic acid and glutamic acid, while lysine is limiting (Singh *et al.,* 2011a; Chung *et al.,* 2005). High cysteine and methionine contents improve the antioxidant levels, thus helps in reducing risk of cancer (Oomah, 2001). The processing conditions, dehusking and defatting affect the protein content. The defatted and dehusked meals have high protein content (Oomah,

and Mazza, 1997, 1998) Flaxseed proteins exhibit antifungal properties against *Alternaria solani, Candida albicans* and *Aspergillus flavus* (Xu *et al.,* 2008). Flaxseed is the richest source of phytoestrogens (lignans).The amount of secoisolariciresinol diglycoside (SDG) varies from 77 to 209 mg SDG/tbsp. of whole flaxseed (Morris, 2007; Toure and Xueming, 2010). Flaxseed contains very low level of carbohydrates (1 g/100 g) and thus contributing very little to total carbohydrates intake (Morris, 2007).

Flaxseeds contain a good amount of phenolic compounds. These phenolic compounds are well known for anticancer and anti-oxidative properties. Basically, flaxseeds have three different types of phenolic compounds–phenolic acids, flavonoids and lignans. Major phenolic acids present in defatted flaxseed are ferulic acid (10.9 mg/g), chlorogenic acid (7.5 mg/g), gallic acid (2.8 mg/g). Other phenolic acids include p-coumaric acid glucosides, hydroxycinnamic acid glucosides and 4-hydroxybenzoic acid that are present in low quantities (Beejmohun *et al.,* 2007; Mazza, 2008). Flavone C and Flavone O-glycosides are the major flavonoids found in flaxseeds (Mazza, 2008). It serves as a good source of minerals especially, phosphorous (650 mg/100 g), magnesium (350–431 mg/100 g), calcium (236–250 mg/100 g) and has very low amount of sodium (27 mg/100 g) (Morris, 2007). It contains highest amount of potassium 5600–9200 mg/kg among various foods and high potassium intake is inversely related to blood platelet aggregation, free radicals in blood and stroke incidence (Carter, 1993). Flaxseed contains small amounts of water-soluble and fat-soluble vitamins. Vitamin E is present as γ-tocopherol, amounting to 39.5 mg/100 g. γ-tocopherol is an antioxidant providing protection to cell proteins and fat from oxidation; promotes sodium excretion in urine, which may help in lowering of blood pressure and heart disease risks and Alzheimer disease (Morris *et al.*, 2005; Morris, 2007).

2.1.2 Anti-nutrients

Flaxseeds contain anti-nutrients that may have adverse influence on the health and well-being of human population. Cyanogenic glycosides are the major anti-nutrients and are fractionated into linustatin (213–352 mg/100 g), neolinustatin (91–203 mg/100 g), linmarin (32 mg/100 g). The content of these three glycosides depend

upon cultivar, location etc. (Oomah *et al.,* 1992). Fiber type linseed has a higher percentage of glycosides than the seed type, and ripe seed contains less glycoside than the immature seed. Whole flaxseed contains 250–550 mg/100 g cyanogenic glycoside (Singh *et al.*, 2011a, b). In the intestine, cyanogenic glycosides release hydrogen cyanide, a potent respiratory inhibitor, by intestinal β-glycosidase that produces thiocyanates. Thiocyanates interfere with iodine uptake by thyroid gland and long term exposure aggravates iodine-deficiency disorders, goiter and cretinism. Cyanogenic glycosides are heat labile and easily destroyed by processing methods namely autoclaving, microwave roasting, pelleting and by certain detoxifying enzymes such as β-glycosidases, releasing hydrogen cyanide which can be evaporated by using steam (Cunnane *et al.,* 1993; Yamashita *et al.,* 2007). Phytic acid, another anti-nutrient present in flaxseed, ranges from 23 to 33 g/kg of the flaxseed meal (Oomah *et al.,* 1996 a, b). Phytic acid interferes with the absorption of calcium, zinc, magnesium, copper and iron. It is a strong chelator, forming protein and mineral-phytic acid complexes and thus reducing their bioavailability (Akande *et al.,* 2010). Clinical studies reveal that flaxseed fed rats had no effect on their Zn status (Ratnayake *et al.,* 1992). Ganorkar and Jain, (2013) have also reviewed that flaxseed antinutrients have lesser impact on human health as compared to that of soyabean and canola. Linatine (antipyrodoxidine factor) has been identified as a vitamin B6 antagonist in case of chicks. While in humans, flaxseeds are not found to be associated with vitamin B6 deficiency (Dieken, 1992; Ratnayake *et al.,* 1992). Trypsin inhibitors are also reported in flaxseed, though activity is insignificant as compared to soybean and canola seeds (Bhatty, 1993).

2.1.3 Flaxseed as functional food

Flaxseed is considered as functional food owing to the presence of three main bioactive components—alpha-linolenic acid, lignans and dietary fiber.

2.1.3.1 Alpha-linolenic acid

Alpha-linolenic acid is the main functional component of flaxseed. It serves as an exclusive source of omega-3 fatty acid in the vegetarian diets (Riediger *et al.,* 2009). Flaxseed oil is rich in polyunsaturated fatty acid (73 % of total fatty acid),

moderate in monounsaturated fat (18 %) and low amount of saturated fat (9 %) (Cunnane *et al.,* 1993; Dubois *et al.,* 2007). It is rich in both the essential fatty acids—alpha-linolenic acid (ALA), and linolenic acid (LA). Fatty acids are termed as essential because both they are required by the body but body cannot synthesize them, therefore need to be supplied in the diet. Human body lacks the enzymes which are required for the synthesis of these essential fatty acids (de Lorgeril *et al.,* 2001).

2.1.3.1(a) Metabolism

Omega-3 fatty acid is known as essential fatty acid because humans cannot introduce a double bond beyond the ninth carbon from carboxyl end of fatty acid. The metabolism of essential fatty acids is depicted in Fig. 1. ALA serves as the precursor for the synthesis of polyunsaturated fatty acids—EPA (Eicosapentaenoic acid) and DHA (Docosahexanoic acid). During the transformation of ALA into EPA and DHA, a series of fatty acids belonging to n-3 PUFA family are also synthesized via desaturation and elongation reactions in the presence of specific desaturases and elongases. Similarly, linolenic acid is also synthesized using similar enzymatic reactions. It has been reported that the conversion of ALA to EPA and DHA is not very efficient in humans and animals and there exist competition between both the fatty acids for the same enzymes. Lower order animals are known to have such enzymatic activities which are capable of converting n-6 fatty acid to n-3 fatty acids, while mammals lack such activities. But recent research findings indicated that mice are the only mammals possessing the enzymes capable of converting n-6 fatty acid to n-3 fatty acid (Kang, 2007). Long chain PUFAs, EPA and DHA are further metabolized by the enzymes cyclooxygenase and lipoxygenase to eicosanoids, prostaglandins, leukotrienes. Among these eicosanoids, E2 series prostaglandins, leukotrienes B4 derived from linoleic acid are the key metabolites which are responsible for many inflammatory diseases like cardiovascular diseases and arthritis, while eicosanoids and E3 series prostaglandins derived from linolenic acid have anti-inflammatory responses (Funk, 2001; Barcelo-Coblijn and Murphy, 2009; Kaur *et al.,* 2012).

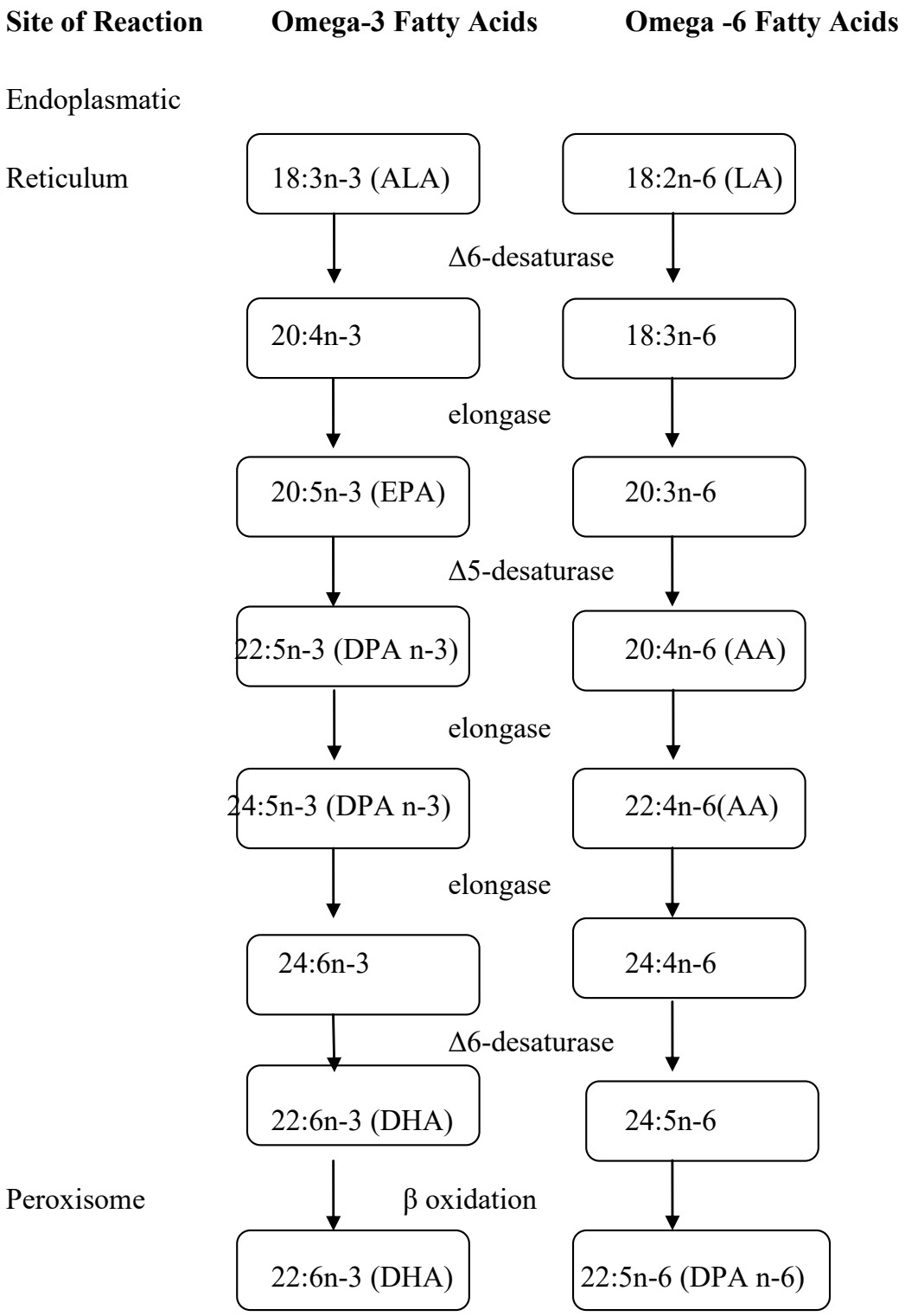


Figure 2.1 The metabolic pathway of omega-3 (n-3) and omega-6 (n-6) fatty acids. Adapted from Voss *et al*., (1991).

Therefore, it is advised that human beings should consume a diet that contains a balanced ratio of omega-3 and omega-6 essential fatty acids. The two groups of essential fatty acids compete with each other for placement within cell membranes. If the intercellular environment has a higher proportion of one type of fatty acid as compared to the other, it is likely that the predominant fatty acid will be incorporated into cell membrane, resulting in adverse effects in the fluidity of the cell membrane affecting cellular functions and overall health of the cell. If there is an equal proportion of both the essential fatty acid in the intercellular environment, there is selective preference for omega-3 fatty acid. Both these fatty acids have opposing, yet necessary, influences over physiological functions (Lunn and Theobald, 2006; Kaur *et al.,* 2012).

EPA and DHA can be converted endogenously into different metabolites known as resolvins, neuroprotectins and protectins. The resolvins act as an effective antiinflammatory mediator. In particular, they function to limit the extent of inflammation by blocking the actions of prostanoids, and also by helping to clear site of inflammation from breakdown products of inflammatory process. Resolvins and protectins promote resolution in oral, lung, kidney, skin, gastrointestinal and various other inflammations to maintain homeostasis by activating specific mechanisms. DHA is converted into neuroprotectins which exhibit neuroprotective effects (Simpolous, 2011; Macmohan and Godson, 2004). In order to maintain good health, it is therefore important that both fatty acids should be present in a balanced ratio.

2.1.3.1(b) Ratio of omega-6 to omega-3 fatty acid

The flaxseed contains highest amount of linolenic acid followed by soybeans and mustard oil, while sunflower and safflower oils contain large amount of linoleic acid which may leads to various diseases. Over the past 100 to 150 years, the consumption of vegetable oils from corn, sunflower seeds, safflower seeds, cottonseeds and soybeans has greatly increased, which resulted in drastic imbalance of the essential fatty acids. Today, the ratio of omega-6 to omega-3 fatty acid is shifted to 20–30:1 in western diets and the situation is even worse in case of Indian diets where this ratio attains a high value of 38–50:1 which reveals that more of

omega-6 fatty acids are incorporated into the cell membrane (Simpolous, 2004; Pella *et al.,* 2003). Therefore, the cellular functions support more of the pro-inflammatory processes than anti-inflammatory processes. Simple dietary choices, which favour foods containing omega-3 fatty acids, can ameliorate this imbalance. The recommended ratio of omega-6 to omega-3 fatty acids may be in the range of 4:1 to 10:1, and omega-6 and omega-3 fatty acid intakes should account for at least 3 and 0.5 % of total energy intake, respectively (de Lorgeril *et al.,* 2001; Tolkachev and Zhuchenko, 2000; WHO, 2003). These variations in the ratio of fatty acids may be considered as a deciding factor for the development of therapeutic doses for prevention of colorectal cancer, asthma and rheumatoid arthritis (Arend and Dayer, 1995).

2.1.3.1(c) Health benefits

A large number of clinical studies have recognized the tremendous potential of n-3 polyunsaturated fatty acids against inflammatory mediators like prostaglandins E2, leukotriene B4,TNF-α, interleukin, and cytokines. These clinical studies revealed that n-3 polyunsaturated fatty acids are helpful in prevention of coronary heart diseases, atherosclerosis, rheumatoid arthritis and asthma (Kremer, 2000). Flaxseed oil supplementation for about 4 weeks resulted in protecting the mice against Streptococcus pneumonia infection (Saini *et al.,* 2010). Flaxseed and its oil reduces the growth of tumors at the later stage of carcinogenesis; whereas, mammalian lignin precursor exert the greatest inhibitory effect on the growth of new tumors (Thompson *et al.,* 1996). The role of flaxseed oil in tumors prevention is attributed to its high alpha-linolenic acid. The fatty acid composition of the tumors revealed higher incorporation of alpha-linolenic acid which in turn resulted in suppression of the growth of the tumor cells (Gonzalez *et al.,* 1991; Thompson, 1996; Gabor and Abraham, 1986).

Flaxseed possesses antioxidant and hepatoprotective properties. Several studies advocated the cholesterol lowering benefits of flaxseed meal (Cunnane *et al.,*1993; Ridges *et al.,* 2001; Bhathena *et al.,*2003). A study by Shakir and Madhusudan, (2007) on hypercholesterolemic rats fed on flaxseed chutney

supplemented diet (15%) revealed significant reduction in LDL cholesterol and total serum cholesterol and no change in HDL cholesterol. In CCl4 intoxicated rats, lipid peroxidation products were neutralized by flaxseed lignans. Several clinical studies showed that EPA and DHA play a major role in reducing depression symptoms. During depression or stress pro inflammatory cytokines such as TNF-α, interferon gamma etc. are produced. Increased of n-6 fatty acid to n-3 fatty acid ratio may lead to the production of proinflammatory cytokines which causes depression and mood swings in elderly persons (Maes *et al.,* 1996; Locke and Stoll, 2001). The α-linolenic acid in flaxseed can reduce the risk of cardiovascular disease (CVD) osteoporosis, rheumatoid arthritis, and cancer (Adlercreutz, 2007; Spence *et al.*, 2003; Clark *et al.*, 2000; Prasad *et al.*, 1998). Omega-3 fatty acids present in flaxseed help in reducing blood triglycerides, blood pressure, platelet reactivity, neutrophil activity, and increase blood HDL cholesterol thereby helping in lowering CVD risk (Gorder *et al.*, 1986; Cunnane *et al.*, 1993; Li *et al.*, 1999).

Bloedon *et al.,* (2008) found that flaxseed reduced lipoprotein a (Lp[a]) by a net of 14%, and reduced the homeostatic model assessment of insulin-resistance index (HOMA-IR) by 23.7% compared to wheat in 10 weeks. In men, flaxseed reduced HDLC concentrations by a net of 16% and 9% in 5 and 10 weeks, respectively. Ground flaxseed (50 g/day) consumed over four weeks increased average daily ALPA plasma levels by about 10 times in healthy adults. It also resulted in the reduction of serum total cholesterol 6–9% and low-density lipoprotein cholesterol 9–18% (Cunnane *et al.*, 1995). In humans flaxseed lowers serum total cholesterol and low-density lipoprotein cholesterol; however, it has no effect on serum high-density lipoprotein cholesterol and triglycerides. The findings of the study suggest that the hypocholesterolemic effect of flaxseed probably resides in the non–oily part and not in the α-linolenic acid. Reductions in hypercholesterolemic atherosclerosis by flaxseed, flaxseed with very low alpha-linolenic acid, and SDG were 46%, 69%, and 73%, respectively. The anti-atherogenic effect of SDG could be due to its antioxidant activity and also its lipid-lowering effect (Jenkins *et al.*, 1999; Prasad, 2000b). The study carried out by Ridges *et al.,* (2001) indicates that the

regular inclusion of foods containing soy and linseed in the diets may improve plasma lipids in hypercholesterolemic postmenopausal women.

2.1.3.2 Lignans

Lignans are phytoestrogens, which are abundantly available in fiber rich plants, cereals (wheat, barley, and oats), legumes (bean, lentil, soybean), vegetables (broccoli, garlic, asparagus, carrots) fruits, berries, tea and alcoholic beverages. Flaxseed contains about 75–800 times more lignans than cereal grains, legumes, fruits and vegetables (Hosseinian and Beta, 2009). Secoisolariciresinol diglycoside (SDG) is the major lignan of flaxseed, alongwith minor contents of matairesinol, pinoresinol, lariciresinol and isolariciresinol (Krajcova *et al.,* 2009). SDG ranges from 11.7 to 24.1 mg/g in defatted flour and 6.1 to 13.3 mg/g in whole flaxseed flour (Johnsson *et al.,* 2000). Lignans are the diphenolic compounds synthesised by the coupling of two coniferyl alcohol residues existing in cell wall of higher plants (Toure and Xueming, 2010; Westcott and Muir, 2003). Secoisolariciresinol (SECO) is produced by acid hydrolysis of secoisolariciresinol diglycoside.

2.1.3.2(a) Metabolism

SDG is metabolized by bacteria in the colon of humans to synthesize mammalian lignans known as enterodiol (END) and enterolactone (ENL) (Chen *et al.,* 2007). In human body, the lignans are acted upon by the gastrointestinal microflora to release SECO, non-sugar moiety of SDG. Further hydroxylation and demethylation by the microflora, lead to the production of mammalian lignan-enterodiol (END), which is then oxidized to give enterolactone (ENL) (Morris, 2007; Toure and Xueming, 2010). Bacteroides as well as Clostridia have been identified to release the glucosyl moieties from SDG to yield SECO (Clavel *et al.*, 2006; Struijs *et al.,* 2009). *Pepto streptococcus productus, Eubacterium callanderi, Eubacterium limosum* and Bacteroides methyl trophicumare found to be responsible for carrying out demethylation reactions, while dehydroxylation reaction are carried out by *Eubacterium lentum* (Wang *et al.*, 2000; Clavel *et al.,* 2006). The dehydrogenation of END into ENL has been carried out by several *Clostridia* and *Ruminococcus* sp.

(Clavel *et al.,* 2007; Jin *et al.,* 2007). The END and ENL, so formed can be excreted in faeces or are absorbed by the human colon and enter the circulation.

2.1.3.2(b)Health benefits

Epidemiological studies indicate that phytoestrogens rich diets reduce the risk of various hormone dependent cancers, heart diseases and osteoporosis (Krajcova *et al.,* 2009; Toure and Xueming, 2010). Research studies also demonstrate the ability of SDG to scavenge hydroxyl free radicals and shown that it is a potent antioxidant. Human body produces free radicals during the oxidation of fats, proteins and carbohydrates. Free radicals damages tissues, membrane lipids, nucleic acids, proteins which may cause cancer, lung diseases, neurological diseases, premature aging and diabetes (Prasad, 1997; Toure and Xueming, 2010). Anticancer activity of lignans is attributed to its ability to scavenge hydroxyl free radicals (Prasad, 1997; Hu *et al*., 2007; Sok *et al.,* 2009). SECO, SDG also play an important role in reduction of hypercholesterolemia, atherosclerosis, hypertension and diabetes (Prasad, 2000a, 2004). Daily administration of 100 mg SDG was found to be effective in reducing blood cholesterol and hepatic diseases risk in moderately hypercholesterolemic men (Fukumitsu *et al.,* 2010). The antioxidant capacity of flaxseed lignan (SDG) is related to the suppression of the oxidant conditions of the reactive species of oxygen. Secoisolariciresinol diglycoside and its aglycone secoisolariciresinol display a very high antioxidant capacity and act as protectors against damage to DNA and liposomes – especially in the epithelial cells of the colon exposed to these compounds – during the metabolism of colon bacteria which transform them into mammal lignans (Hu *et al.*, 2007). Flaxseed lignans behaviour depends on biological levels of estrogen hormone. At normal levels of estrogen, it exhibit antagonistic activity, but in postmenopausal phase when estrogen level is low, flaxseed lignans acts as weak estrogen (Sok *et al.,* 2009; Toure and Xueming, 2010; Saini *et al.,* 2010). The mammalian lignans stimulate the synthesis of sex hormone binding globulin, which binds sex hormones and reduce their circulation in blood stream, and decrease their biological activity and thus reducing the risk of developing cancer (Thompson *et al.*, 1996). Flax lignans are reported to have antioxidant property which presumably is the

main reason of the anticancer activity (Prasad, 1997). The lower incidences of prostate and breast cancers in Asian men and women compared to European men and women has been speculated to be due to the higher consumption of diets rich in fruits and vegetables. (Adlercreutz, 1990; Morton *et al.,* 1997). Various clinical studies imply that lignans prevent breast cancer by balancing the hormonal mechanisms. The lignans inhibit the aromatase activity in adipose tissue resulting in the circulation of estrogen (Sturgeon *et al.,* 2008; Adlercreutz *et al.,* 1993). In postmenopausal women, lignans act as weak estrogens, while at normal estrogen levels, lignans act as estrogen antagonists (Wang *et al.,* 1994; Hutchins and Slavin, 2003). Dietary flaxseed moderately lowers the serum levels of steroid sex hormones which are implicated in development of breast cancer in obese postmenopausal women (Sturgeon *et al.,* 2008).

2.1.3.3 Dietary fiber

Flaxseeds serve as a good source of both soluble and insoluble dietary fiber. Flaxseed holds a unique place among the oilseeds due to presence of mucilage located in outer layers of the seed (Singh *et al.,* 2011a, b). Flaxseed mucilage has gained momentum due to its superb health benefits and potential functional properties (Susheelamma, 1987; Mazza and Biliaderis, 1989). It contains 35–45 % of fibre and two-third is insoluble and one third is soluble fiber. Insoluble fiber consists of cellulose, hemicellulose and lignin (Morris, 2007). Most of the soluble fiber of flaxseed appears to be the mucilage of seed coat. It makes up 7–10 % of seed weight (Mazza and Biliaderis, 1989). Soluble fiber in the form of mucilaginous material consists mainly of water soluble polysaccharides; its recovery and purity vary with the extraction conditions. The water binding capacity of flaxseed mucilage is reported to be about 1600–3000 g of water/ 100 g of solids. High water binding capacity of flaxseed is attributed due to the presence of polysaccharides in the seed coat (Fedenuik and Biliaderis, 1994; Wanasundara and Shahidi, 1997). Mucilage of flaxseed consists of acidic and neutral polysaccharides. The neutral fraction constitutes L-arabinose, Dxylose and D-galactose and arabinoxylan and acidic fraction contains L-rhamnose, L-fucose, L-galactose and D-galactouronic acid

(Wanasundara and Shahidi, 1997). Functionally, these polysaccharides possess similar properties to guar gum (Wanasundara and Shahidi, 1994; Tarpila *et al.,* 2005).

2.1.3.3(a) Metabolism

Dietary fiber of flaxseed reaches the large intestine and is fermented by colonic microflora with production of short chain fatty acids (SCFA), hydrogen, carbon dioxide, methane and biomass and exhibit laxative effects (Kritchevsky, 1979). In the large intestine, both soluble and insoluble fibers have their bulking effect resulting in increasing both dry and wet weight of the colon contents and faeces. Soluble fiber increases water binding, initially by the binding capacity of its macromolecules, later by increasing the mass of microbial cells.

2.1.3.3(b)Health benefits

Water-binding capacity of flaxseed insoluble fiber increases the intestinal bulk which is useful in the treatment of constipation, irritable bowel syndrome and diverticular disease. Soluble fiber from flaxseed mucilage increases the viscosity of intestinal contents and delays gastric emptying and nutrient absorption. Inclusion of flaxseed mucilage in the diet of broiler chicks resulted in decreased faecal digestibility of fat and fatty acids while protein digestion was unaffected. Intestinal viscosity of the broiler chicks increased on addition of flaxseed mucilage in the diet (Rebole *et al.,* 2002). Traditionally, dietary fiber is used for the treatment of constipation, irritable bowel syndrome (Cann *et al.,* 1984; Tarpila *et al.,* 2005). Dietary fiber delays gastric emptying, regulate post prandial blood glucose levels and helpful in prevention of constipation (Spiller, 1994). Flaxseed fiber plays an important role in lowering the blood glucose levels. Studies demonstrated that insoluble fiber slows down the release of sugar in the blood and thus help in reducing blood glucose levels to great extent (Thakur *et al.,* 2009; Kapoor *et al.,*2011). Soluble gum of the flaxseed may be helpful in the prevention of cardiovascular diseases by exhibiting hypocholesterolemic effect (Jenkins *et al.,*1987; Cunnane *et al.,*1995). Kristensen *et al.,* (2012) studied the effect of differently processed flax fibers on the fat excretion and energy balance. It was observed that flax fiber enriched drink

lowered the cholesterol to a large extent as compared to fiber enriched bread. However, the consumption of fiber bread increased the fecal fat excretion and maintained proper energy balance.

2.1.4 Flaxseed in prevention of cardiovascular diseases

The cholesterol lowering effects of flaxseed have been investigated in a few animal and human studies. The incorporation of approximately 20% whole flaxseed to the diet of rats resulted in a lower serum cholesterol concentration (Ratnayake *et al.,* 1992). The cholesterol lowering effects of flaxseed have been investigated in both men and women (Harper *et al.,*2006; Fukumitsu *et al.,* 2010). These human studies have demonstrated that the consumption of approximately 40–50 g of flaxseed resulted in a 5–9% reduction in total cholesterol. However, the dose-dependent effect of flaxseed in reducing ovarian hormone deficiency-induced hypercholesterolemia has not been established. In addition to its cholesterol lowering effect, flaxseed may directly act on the vessel wall to prevent atherosclerosis. Anti-atherogenic effects of flaxseed have been investigated in hypercholesterolemic rabbits (Prasad *et al*., 1998). However little knowledge is available about the incorporation of flaxseed in dairy products which has to be investigated in detail.

2.2 Mango

Mango is the national fruit of India, known as the 'King of Fruits'. It is one of the most important and popular Asian fruits. Cultivation of Mangoes is deeply embedded in Indian history. Mangoes thrive in tropical regions, and are cultivated throughout India.

2.2.1 Botanical description

Mango (*Mangifera indica*), family Anacardiaceae, is a large, branched perennial erect tree, flowers appear in large terminal inflorescences producing fruit. The fruits have a small point at one end, known as the beak. Mangoes vary in shape (nearly round, oval, ovoid-oblong), size and color, depending upon the variety.

The fruits possess a single large, flattened, kidney-shaped seed that is enclosed in a woody husk.

2.2.2 Nutritional composition of Mango

The fruit contains nearly 81 per cent moisture, 0.4 per cent fat, 0.6 per cent proteins, 0.8 per cent of fibers. It also contains nearly 17 per cent of carbohydrate. The fruit is rich with important minerals contains important minerals like potassium, magnesium, sodium, phosphorus, and sulphur. Mangoes have sumptuous tropical flavor, which makes healthy eating a delightful sensory experience. Mangoes are an excellent source of vitamins A and C, both important antioxidant nutrients. Vitamin C promotes healthy immune function and collagen formation. Vitamin A is important for vision and bone growth.

Mangoes are a good source of dietary fiber, therefore, it is associated with a reduced risk of some types of cancer, protecting against heart disease and cholesterol build up. Mangoes contain over 20 different vitamins and minerals.

In the past few years, there has been increasing interest in the study of mango phenolics from mango fruits, due to their antioxidative and health promoting properties. Bioactive compounds found in the mangoes, among other plants and herbs have been shown to have possible health benefits with antioxidative, anticarcinogenic, antiatherosclerotic, antimutagenic, and angiogenesis inhibitory activities (Cao and Cao, 1999).

Polyphenols are secondary metabolites of plants and are widely distributed in beverages and plant-derived foods. Phenolic compounds have the capacities to quench lipid peroxidation, prevent DNA oxidative damage and scavenge free radicals (Cao and Cao, 1999) which are precursors to degenerative diseases. Free radicals cause depletion of the immune system antioxidants, change in gene expression, and induce abnormal proteins resulting in degenerative diseases and aging. Antioxidant nutrients and phytonutrients inhibit the oxidation of living cells by free radicals by protecting the lipids of the cell membranes through free radical scavenging, blocking

the initiators of free radical attack, neutralizing or converting free radicals into less active, stable products thus breaking the chain reaction and assisting in salvaging oxidized antioxidants enabling them to continue to be of benefit (Halliwell *et al.,* 1992). The main classes of polyphenols are defined according to the nature of their carbon skeleton and they are: phenolic acids, flavonoids, stilbenes, and lignans (Lee *et al.,* 2003). Other dietary polyphenols are not well-defined chemical entities and result from the oxidative polymerization of flavonoids and phenolic acids (Santos-Buelga and Scalbert, 2000).

2.2.2(a) Polyphenolic composition

Polyphenolic composition of mango pulp constitutes mangiferin, gallic acids (m-digallic and m-trigallic acids), gallotannins, quercetin, isoquercetin, ellagic acid, and β-glucogallin are among the polyphenolic compounds already identified in the mango pulp (Schieber *et al.*, 2000). Gallic acid has been identified as the major polyphenol present in mango fruits, followed by 6 hydrolysable tannins and 4 minor compounds,p-OH-benzoic acid, m-coumaric acid, p- coumaric acid, and ferulic acid (Kim *et al.*, 2007). Schieber *et al.*, (2000) found 6.9 mg/kg of gallic acid and 4.4 mg/kg of mangiferin in mango pulp. In a polyphenol screening of 20 mango varieties, Saleh and El-Ansari, (1975) reported the co-occurrence of mangiferin, isomangiferin, and homomangiferin in mango fruit pulp.

2.2.2(b) Mangiferin

Mangiferin is a xanthone and xanthones are some of the most potent antioxidants known; they are thought to be more potent than both vitamin C or vitamin E. Xanthones are heatstable molecules, mangiferin, generally called C-glucosyl xanthone, is widely distributed in higher plants (Sanchez *et al.*, 2000). It is a pharmacologically active phytochemical and a natural polyphenolic antioxidant present in the bark, fruits, roots, and leaves of *Mangifera indica* Linn. and a few other medicinal plants.

Mangiferin has been investigated in vitro for its antioxidant (Rouillard *et al.*, 1998), immuno-stimulating, and antiviral properties (Zheng and Lu, 1990), and it was found to protect hepatocytes, lymphocytes, neutrophils, and macrophages from oxidative stress; reduce atherogenicity in streptozotocin diabetic rats; and to reduce the streptozotocin-induced oxidative damage to cardiac and renal tissues in rats (Muruganandan *et al.*, 2002).The iron-complexing ability of mangiferin was reported as a primary mechanism for protection of liver mitochondria against Fe^{2+} citrate-induced lipid peroxidation (Halliwell and Gutteridge, 1986). They also showed that in vitro antioxidant activity of mangiferin is related to its iron-chelating properties and not merely due to the scavenging activity of free radicals.

Mango pulp may be added as it provides sweetness to yoghurt (Moreno *et al.*, 2000) and increases its nutritional properties as it is high in β-carotene, vitamin B complex and also helps in improving dietary fiber and folic acid when added in yoghurt (Sanchez-Segarra *et al.*, 2000). Pereira *et al.*, (2013) observed increase in antioxidant activity of yoghurt with inclusion of mango pieces in it, and found the addition of fruit pieces to yogurt was favorable for antioxidant content, increasing the protection of the consumer against diseases related to oxidative stress. Kumar and Mishra, (2003) optimized the formulation of mango soy fortified yogurt (MSFY) using response surface methodology (RSM). Kabir *et al.*, (2014) suggested that *dahi* could be prepared successfully by adding different proportion of mango juice with skim milk and 10% mango juice addition showed better result. However, inclusion of mango in Indian curd-*dahi* has not been studied much and little knowledge is available about its textural, sensory, physico-chemical, functional properties and storage.

2.3 Honey

Honey is primarily composed of the monosaccharides glucose and fructose, which can be found in amounts of between 55 and 75%. A complex mixture of minor carbohydrates (10-25%), mainly disaccharides and trisaccharides, is also present (Sanz *et al*., 2004). Moreover, the presence of four tetrasaccharides, one pentasaccharide, and one hexasaccharide has been detected in New Zealand

honeydew honeys (Astwood *et al*., 1998). Honey has been considered to be an important source of energy, being used in medical therapies and as a valuable food ingredient (Yaniv *et al.,* 1996). Certain components of honey can provide antioxidant activities, seen as beneficial for human health (Gheldof *et al.,*2002; Schramm *et al.,*2003; Perez *et al.,*2003), and various studies have revealed the inhibitory properties against certain pathogens (Molan, 1999). Shamala *et al.,* (2002) carried out in vitro and in vivo studies in the small and large intestines of rats and proposed that honey enhanced the growth of lactic acid bacteria. Moreover, honey has been shown to support lactic acid production in skim milk fermented with lactic acid bacteria in a manner similar to that of other sweeteners such as sucrose and fructose. More recently, Kajiwara *et al.,* (2002) showed that the growth, in pure culture, of commercial strains of bifidobacteria was enhanced by honey in a manner similar to that of other commercial prebiotic oligosaccharides (fructooligosaccharide (FOS), galactooligosaccharides (GOS), and inulin). Luz Sanz *et al.*, (2005) observed that honey oligosaccharides seem to present potential prebiotic activity (PI values between 3.38 and 4.24), increasing the populations of bifidobacteria and lactobacilli, although not to the levels of FOS (PI of 6.89).

Besides, prebiotic effect honey has many other benefits for human health as reported by many research findings. Antioxidant activity, is the ability and potential of honey to reduce oxidative reactions within the food systems and human health (Gheldof and Engeseth, 2002) It has been found that honey ameliorates cardiovascular risk factors in healthy individuals and in patients with elevated risk factors.

Yaghoobi *et al.,* (2008) investigated the effect of natural honey on total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triacylglycerole, C-reactive protein (CRP), fasting blood glucose (FBG) and body weight in overweight individuals for a period of 30 days. Results showed that honey caused a mild reduction in body weight (1.3%) and body fat (1.1%). Honey reduced total cholesterol (3%), LDL-C (5.8), triacylglycerole (11%), FBG (4.2%) and CRP (3.2%), and increased HDL-C (3.3%) in subjects with

normal values. Meanwhile, in patients with elevated variables, honey caused reduction in total cholesterol by 3.3%, LDL-. C by 4.3%, triacylglycerole by 19% and CRP by 3.3% ($p<0.05$). Hence, concluded that consumption of natural honey reduces cardiovascular risk factors, particularly in subjects with elevated risk factors, and it does not increase body weight in overweight or obese subjects. In diabetic patients, honey caused a significantly lower, the rise of plasma glucose than dextrose (Al-Waili, 2004).

Therefore, the substitution of honey in some foods like yoghurt for traditional sweeteners could result in an enhanced antioxidant defense system as well as other health benefits in healthy adults (Schramm *et al.*, 2003).

2.4 Probiotics

A probiotic is defined as a 'living organism which when administered in certain numbers exerts health benefits in the host' (FAO, 2001). Bacterial strains from the genera *Lactobacillus*, *Bifidobacterium*, and *Bacillus* have been widely studied and are used to prepare ready-to-eat foods. In order to produce therapeutic benefits, a suggested range for the minimum level for probiotic bacteria in probiotic milk is from 10^6 to 10^7 colony-forming units (cfu)/mL (IDF 1992). Several factors have been reported to affect the viability of probiotic cultures in fermented milks. Acidity, pH, dissolved oxygen content, redox potential and hydrogen peroxide have been identified as having an effect during the manufacture and storage of fermented milks (Lankaputhra and Shah, 1995). However, the physicochemical stability and bioavailability of these bacteria have represented a challenge for many years particularly in acidic refrigerated and nonrefrigerated foodstuffs. During cold storage of fermented bio-products, the number of viable probiotic cells often drops far below the minimum therapeutic level (Rybka, 1994). Microencapsulation (ME) helps to improve the survival of these bacteria because it protects them from harsh conditions, such as high temperature, pH, or salinity, during the preparation of a final food product and its gastrointestinal passage. The most common coating materials used in the ME of probiotics are ionic polysaccharides, microbial exopolysaccharides, and

milk proteins, which exhibit different physicochemical features as well as mucoadhesion.

Due to the adverse physicochemical conditions that probiotic bacteria are exposed to during the preparation and gastrointestinal (GI) passage of a food product, their survival is at risk (Prakash *et al.*, 2011). Therefore, in the characterization of a probiotic food, both the viability (within foods) and bioavailability (within the host) of the microorganism involved must be examined, among other parameters (Figueroa-Gonzalez *et al.*, 2011). Fortunately, there are several technologies that help to improve the survival of these bacteria during the manufacturing process. Microencapsulation (ME) by means of electrostatic, extrusion/coacervation, or emulsification (freeze-, fluidized-, or spray-drying) treatments are methods used to improve survival of probiotics in foods. However, the addition of microencapsulated live bacteria to prepared foods is a relatively new alternative, although it presents huge potential within the probiotic market (Heidebach *et al.*, 2012). The bacterial survival rate also depends on the bacterial strain involved and the chemical nature of the artificial or natural matrix in which it will be contained (Del Piano *et al.*, 2006; Gebara *et al.*, 2013). Lastly, whatever technology is used to increase the viability of probiotic strains in prepared foods, the resulted products must be subjected to several resistance tests (such as against temperature, pH, salinity, and bile salts) to demonstrate their potential bioavailability (Ding and Shah, 2007; Riaz and Masud, 2013).

Once the critical requirements are established and immobilized probiotic bacteria have been obtained, the shelf-life (viability within foods) and resistance to GI conditions (bioavailability within the host) must be analyzed. At temperatures ≤4 °C, the survival of *Lactobacillus acidophius* (LA) and some bifidobacteria improves for several weeks (Sakai *et al.*, 1997), but freeze-drying at lower temperatures results in a lower survival rate (Dianawati and Shah, 2011). Mosilhey, (2003) found that microencapsulated LA was stable up to 15 wk at 5 °C, which makes it ideal for use in refrigerated foods, such as dairy products (Shah, 2000).

Recoating of "primary" microcapsules with additional materials can help to prevent the exposure of probiotics to oxygen during storage and improve their stability at a low pH and high temperature. Some of the materials employed for recoating include chitosan, poly-L-lysine, alginate, and several starches, gums, and gelatins (Krasaekoopt *et al.*, 2004; Mortazavian *et al.*, 2007, 2008). Mokarram *et al.*, (2009) studied the influence of the number of layers of alginate on the survival of *LA*(PTCC1643) and *L. rhamnosus* (PTCC1637) in simulated GI conditions. In this study, ME with a double layer of alginate (about 100 μm) was found to provide the best protection against a loss of viability for both bacteria in gastric (pH 1.5, 2 h) and intestinal (pH 7.25, 2 h) juices.

Chandramouli *et al.*, (2004) and Kim *et al.*, (2008) also showed that ME with alginate increased the survival of *LA* CSCC 2400 and ATCC 43121, respectively, in GI conditions. Lyer and Kailasapathy, (2005) reported that *LA* microencapsulated in 1 or 2 layers of chitosan and resistant starch showed a loss of viability of 1×10^2 or 3×10^3 CFU/g, respectively, in simulated GI conditions, while in non-microencapsulated *LA* the observed loss of viability was 1×10^5 CFU/g.

Ding and Shah, (2009) evaluated the effect of acid (pH 2.0) on the viability (10^{10} CFU/g) of *LA, L. rhamnosus, B. longum, L. salivarius, L. plantarum, L. paracasei*, and *B. lactis* (type BI-O4 and Bi-07) microencapsulated in guar and xanthan gums, carob, alginate, and carrageenan. *LA* was one of the microorganisms that was most tolerant (10^5 to 10^8 CFU/g) to acidity (pH 2.0, 2 h) and to bile salts (taurocholic acid), especially when it was microencapsulated in alginate, xanthan gum, and carrageenan.

Murata *et al.*, (1999) coated chitosan with alginate and observed that chitosan has the ability to bind bile salts. This finding could indicate that the effectiveness of alginate in protecting a probiotic within its matrix may be decreased due to the incorrect selection of a second material, as in the case of chitosan.

2.5 Synbiotics

Synbiotics have been defined as "mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live

microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health - promoting bacteria, thus improving host welfare" (Gibson and Roberfroid, 1995). Many studies indicated that *synbiotics* have higher beneficial effects on the host health than probiotic or prebiotic products alone (Schaafsma *et al.*, 1998; Gmeiner *et al.*, 2000). Having both probiotic and prebiotic in a food product can certainly improve the survival rate of probiotic during storage and the passage through the intestinal tract. Akalin and Erisir, (2008) indicated that adding inulin and oligofructose at the 4% level significantly improved the survival rates of *Lactobacillus acidophilus* and *Bifidobacterium lactis*.

Also, prebiotics have been shown to sufficiently increase the implantation of probiotic bacteria in colonic microflora (Roberfroid *et al.*, 1998). Anandharaj *et al.*, (2014) have been suggested to reduce cholesterol via various mechanisms without any deleterious effect to the human health and may throw some light to show the ability of these *synbiotics* as a novel alternative or adjuvant to chemical drugs to help fight the hypercholesterolemic problem. Maassen *et al.*, (2000) demonstrated that a synbiotic combination of *L. paracasei* and maltodextrin decreased *E. coli* colonization, while a combination of *L. Paracasei* and FOS led to an increase in Lactobacillus and Bifidobacterium and decreased Clostridium and Enterobacterium in the jejunum of piglets. Furthermore, another determined that treatment with the prebiotics soybean oligosaccharide, FOS, and inulin were able to increase both survival time and retention period of the probiotics *B. lactis* LAFTI B94*, L. casei* L26 LAFTI, and *L. acidophilus* LAFTI L10.

Ooi *et al.*, (2010) investigated that the effect of a synbiotic product containing *Lactobacillus acidophilus* CHO-220 and inulin on the irregularity in shape of red blood cells (RBC) in hypercholesterolemic subjects. Morphological representation via scanning electron microscopy showed that the occurrence of spur RBC was improved and reduced the cholesterol: phospholipids ratio of the RBC membrane by 47.02% upon supplementation of the synbiotic over 12 wk. The study also showed that supplementation of the synbiotic reduced the concentration of saturated fatty acids

(SFA), increased unsaturated fatty acids (UFA), and increased the ratio of UFA:SFA over 12 wk.

2.6 *Dahi*

(a) Definition

Dahi or curd is an Indian fermented milk product which is equally known for its palatability, refreshing taste and therapeutic importance as claimed in the ayurvedic literature. Some of its characteristics are similar to other fermented milk products such as yoghurt and acidophilus milk but it differs with regard to heat treatment of milk, starter culture, chemical composition and taste. *Dahi* differs from yogurt in its use of mixed starters of mesophilic lactococci. A principle flavour-inducing metabolite is diacetyl, which is appreciated more by people of South Asian origin compared to the acetaldehyde flavour in yogurt. In addition, *dahi* also has antibacterial properties against pathogenic and non-pathogenic organisms.

(b) Types of *Dahi*

Some of the fermented milks and different types of *dahi* consumed throughout India have been categorized as follows:

North Zone : *Dahi*, Lassi

South Zone : *Dahi*, Buttermilk (Mattha)

East Zone : Payodhi or Lal *dahi* or Mishti *dahi*

West Zone : Shrikhand, Chakka, Chhash, *Dahi*

Based on the acidity level (% lactic acid), *dahi* has been classified into categories such as sweet *dahi* with a maximum acidity of 0.7 per cent and sour *dahi* with 1.0 per cent acidity.

(c) Properties and composition of *Dahi*

According to the Food Safety and Standard Authority of India (FSSAI) rules, *dahi* shall contain the same percentage of fat and solids-not-fat as the milk from which it is prepared. It should have a pleasing flavour and a clean acid taste, devoid of undesirable flavour, should have firm, solid body and texture and be uniform with negligible whey separation. Other characteristics should be as follows:

Table 2.2 Characteristics of sweet and sour *dahi*.

Characteristics	Sweet *dahi*	Sour *dahi*
Acidity (% lactic acid)	0.7	1.0
Yeast and molds (per gram) Max.	100	100
Coliforms(per gram) Max.	10	10
Phosphatase test	Negative	Negative

2.6.1 *Dahi* (Indian yoghurt) as a functional food

Dahi is an Indian counter part of yogurt and is very popular fermented product in India.The healthy image of *dahi*/yoghurt is endorsed by the addition of various fruit preparations in yoghurt to include the health benefits of fruits such as providing fiber and antioxidants (O'Rell and Chandan, 2006). A good quality *dahi* made from whole milk has a cream layer on the top, the rest being made up of a homogenous body of curd and the surface being smooth and glossy, while the cut surface should be firm and free from cracks of gas bubbles and it should have a pleasant acid taste with sweetish aroma. Composition and quality of *dahi* vary widely from one locality to another as it is being prepared under different domestic conditions as well as milk, with variable chemical and bacteriological quality used for the preparation. However, the chemical composition of *dahi* has been reported as fat ranging from 5 to 8 per cent, protein 3.3 to 3.4 per cent, ash 0.75 to 0.79 per cent and lactic acid 0.5 to 1.1 per cent.

The occurrence of various bioactive peptides in fermented milks, e.g., yoghurt, sour milk and ''*Dahi*'', has been reported in many studies. "*Dahi*" has been found to contain ACE inhibitory bioactive peptide Ser-Lys-Val-Tyr-Pro (Ashar and Chand, 2004) naturally. To increase the concentration of these in fermented product, studies have been employed strongly on proteolytic *Lactobacillus spp. (helveticus, rhamnosus, plantarum)* strains for the production of antihypertensive peptides in fermented milk products. Ashar and Chand, (2004) identifed antihypertensive penta-peptide (Ser-Lys-Val-Tyr-Pro) from *dahi* fermented by *Lb. delbruekii subsp. bulgaricus* along with *dahi* starters and it was found within β-casein f (57–61) primary structure with IC_{50} value of 1.4 mg mL^{-1}.

2.6.2 Manufacturing process of *Dahi*/Yoghurt

The main processing steps involved in *dahi*/yoghurt manufacture include the standardization of milk (fat and protein content), homogenization, heat treatment of milk, inoculation, incubation/ fermentation, cooling, and storage.

2.6.2.1 Milk standardization

Milk is often mixed with skim milk and cream to standardize (or adjust) the fat content to the desired level. The milk solids content (including the fat content) for *dahi*/yoghurt ranges from around 9% for skim milk yoghurt to more than 20% for certain types of concentrated yoghurt. The minimum milk solids non- fat content required in standards or regulations in many countries ranges from 8.2 to 8.6% (Tamime and Robinson, 1999). Codex regulations for yoghurt indicate that the minimum milk protein content is 2.7% (except for concentrated yoghurt where the minimum protein content is 5.6% after concentration) and the maximum fat content is 15% (Codex, 2007).

2.6.2.2 Homogenization

Homogenization of the milk base is an important processing step for *dahi*/yoghurts containing fat. Milk is typically homogenized using pressures of 10-20 and 5MPa first and second stage pressures, respectively, and at a temperature range

between 55 and 65°C. Homogenization results in milk fat globules being disrupted into smaller fat globules and the surface area of homogenized fat globules greatly increases. The use of homogenization prevents fat separation (creaming) during fermentation or storage, reduces whey separation, increases whiteness, and enhances consistency of *dahi*/yoghurts (Vedamuthu, 1991). When milk is homogenized, caseins and whey proteins form the new surface layer of fat globules, which increases the number of possible structure-building components in yoghurt made from homogenized milk (Walstra and Jenness, 1984). Homogenized milk fat globules act like protein particles due to the presence of protein on the fat surface.

2.6.2.3 Heat treatment of milk

Heating of milk is an important processing variable for the preparation of yoghurt since it greatly influences the physical properties and microstructure of yoghurt (Lucey *et al.,* 1998). In *dahi*/yoghurt manufacture, milk is heated prior to culture addition. The temperature/time combinations for the batch heat treatments that are commonly used in the yoghurt industry include 85°C for 30min or 90-95°C for 5min (Tamime and Robinson, 1999). However, very high temperature short time (100°C to 130°C for 4 to 16s) or ultra-heat temperature (UHT) (140°C for 4 to 16s) are also sometimes used (Sodini *et al.,* 2004). The heat treatment of milk is also used to destroy unwanted microorganisms, which provides less competition for the starter culture. *Dahi* starter cultures are sensitive to oxygen so heat treatment helps to remove dissolved oxygen assisting starter growth.

2.6.2.4 Inoculation of starter culture for *Dahi*/Yoghurt production

During the manufacture of *dahi*/yoghurt, the heat-treated milk is cooled to the incubation temperature of the starter culture. In general, it has been found that *dahi* culture is dominated by streptococci and lactobacilli. In sour *dahi*, however, lactobacilli predominate. For commercial manufacture by organized dairy, single starter culture (*Lactococcus lactissubsp. diacetylactis*) or mixed culture is used. Mesophilic Starter cultures such as *Streptococcus lactis, S. diacetylactis, S. cremoris* in single or in combination with or without *Leuconostoc* species along with

Lactobacillus acidophilus, L. bulgaricus, and *S. thermophilus* may be used for *dahi* preparation. In India it is produced using a mixed starter culture containing *S. thermophilus, Lactococcus lactis* and *Lactococcus lactis subsp. cremoris* (Tamime and Marshall, 1997). The traditional method for preparation of *dahi* invariably involves a small scale, either in consumers' household or in the sweet makers shop in urban areas. In the household, milk is boiled, cooled to about 37°C and inoculated with 0.5 – 1 per cent of starter (previous day's *dahi* or butter milk) and allowed to set overnight. It is then stored under refrigeration and consumed.

2.6.2.5 Fermentation process

In general, the milk is fermented at 35–37°C, that is, the optimum growth condition for the mixed culture and the short incubation method. However, the longer incubation method, (i.e. overnight) can be used and the incubation conditions are 30°C for around 16-18 hours, or until the desired acidity is reached (Hrabova and Hylmar, 1987). The actual fermentation stage can take place either in the retail container for the production of set yoghurt, or the milk is incubated in bulk for the manufacture of stirred yoghurt. However, no matter what type of yoghurt is being produced, the biochemical reactions responsible for the formation of the gel/coagulum are exactly the same. Bacterial fermentation converts lactose into lactic acid, which reduces the pH of milk. During acidification of milk, the pH decreases from 6.7 to ≤ 4.7. Gelation occurs at pH 5.2 to 5.4 for milk that was given a high heat treatment.

2.6.2.6 Cooling of *Dahi*/Yoghurt

When *dahi*/yoghurts reaches the desired pH (e.g., ~ 4.7), they are partially cooled (~20°C) before fruit or flavoring ingredients are added. Yoghurt products are often blast chilled to <10°C (e.g., 5°C) in the refrigerated cold store to reduce further acid development (Tamime and Robinson, 1999).

2.7 Studies on physicochemical properties of *Dahi*

Mohania *et al.*, (2013) examined the effects of probiotic *dahi* prepared by Lactobacillus plantarum Lp9 and *dahi* culture in buffalo milk on lowering cholesterol in rats fed a hypercholesterolemic basal diet. The experimental animal, male wistar rats were divided into 3 groups and fed with probiotic *dahi*, *dahi*, or buffalo milk for 120 days. Following the consumption of supplements (probiotic *dahi*, *dahi* or buffalo milk), the animals were fed a basal hypercholesterolemic diet. Plasma total cholesterol and triglycerides (TAGs) were decreased by 35% and 72% in rats fed with probiotic *dahi* group, while cholesterol levels increased by 70% and TAGs increased by 97% in buffalo milk and 59% in *dahi* fed groups. Supplementation of probiotic *dahi* further lowered plasma low-density lipoprotein (LDL) + very-low-density lipoprotein (VLDL)- cholesterol by 59%, while it elevated plasma high-density lipoprotein (HDL)-cholesterol by 116%. As a result, atherogenic index, the ratio of HDL to LDL + VLDL was markedly improved. Deposition of cholesterol and TAGs in liver and aorta were significantly reduced in rats fed with probiotic *dahi*. These observations suggest that probiotic *dahi* may have therapeutic potential to decrease plasma, hepatic and aortic lipid profile, and attenuate diet-induced hypercholesterolemia.

Yadav, 2007 investigated the effect of low-fat (2.5%) *dahi* containing probiotic Lactobacillus acidophilus and Lactobacillus casei on progression of high fructose-induced type 2 diabetes in rats. They observed that probiotic *dahi*-supplemented diet significantly delayed the onset of glucose intolerance, hyperglycemia, hyperinsulinemia, dyslipidemia, and oxidative stress in high fructose-induced diabetic rats, indicating a lower risk of diabetes and its complications.

Soomro and Masud, (2012) evaluated the probiotic potential of 35 strains of Lactobacillus spp. isolated from *dahi* a traditional fermented milk product. In order to select the candidate, probiotic strains Lactobacillus spp. isolated belonged to the *L. delbrueckiisubsp. bulgaricus, L. acidophilus, L. casei* and *L. helveticus* were examined for antimicrobial activity against selected pathogens, acid and bile tolerance and antibiotic susceptibility. It was observed that *L. acidophilus* LA 02

produced antimicrobial activities against the test strains and tolerated pH 2 and 3 and survived the bile salt concentration of 0.1, 0.2 and 0.3 %. It was resistant to vancomycin, erythromycin and chloramphenicol. They concluded that L. *acidophilus* LA 02 has potential probiotic value and have the best characteristics to fulfill the criteria of a probiotic strain.

Ramanathan and Sivakumar, (2013) evaluated organoleptic, chemical and microbiological properties of fibre enriched and vitamin-c fortified sweetened probiotic *dahi* and found that inclusion of oats at two percent level in milk produced *dahi* of high nutritive value as well as desirable sensory attributes.

Hossain, (2012) aimed to develop fruit *dahi* (yoghurt) with acceptable chemical, physical and microbiological characteristics and concluded that 10% of orange juice *dahi* was found to be best in all aspects of quality in comparison to strawberry and grape juice *dahi*. They also found that inclusion of fruit increased the acidity, total solids while resulted in decrease in viable count and shelf life of product.

Many other research findings on similar fermented product, yoghurt have been conducted which may help to understand the physical, chemical, functional and microbiological attributes of Indian yoghurt- *dahi* more efficiently.

Kumar and Mishra (2003) optimized the formulation of mango soy fortified yogurt (MSFY). The independent variables were proportions of mango pulp (1.2–13.8%), soymilk (2.39–27.61%), and fat content (0.48–5.52%) of buffalo milk. Statistical analysis revealed that mango pulp, soymilk, and fat content of buffalo milk significantly affected (95% confidence levels) all the responses. The optimum formulation conditions of 7.1 kg mango pulp (18% total solids), 14.7 kg soymilk (8.2% total solids), and 78.2 kg buffalo milk (2.95% fat content and 9% solid not fat) per 100 kg are recommended for the blend formulation yielding an acceptable and good quality MSFY.

Staffolo *et al.* (2004) observed the yoghurt fortified with 1.3% wheat, bamboo, inulin and apple fibres appeared to be promising avenue for increased fibre intake, with higher consumer acceptability.

Addition of 0.5% barley β - glucan or inulin and guar gum (>2%) were effective in improving serum retention and viscoelastic properties of low-fat yogurt (Brennan and Tudorica, 2008). Incorporation of fibre obtained from asparagus shoots increased yoghurt consistency and imparted a yellowish greenish colour to the yoghurt (Sanz *et al.*, 2008).

Hashim *et al.*, (2009) studied the effect of fortification with date fibre, a by-product of date syrup production, on fresh yoghurt. Control yoghurt (without fibre), yoghurt fortified with 1.5, 3.0 and 4.5% date fibre and yoghurt with 1.5% wheat bran were prepared. Yoghurt fortified with 3% date fibre resulted with similar sourness, sweetness, firmness, smoothness and overall acceptability as the control yoghurt. As both fibre and yoghurt are well known for their beneficial health effects, together will constitute a functional food with commercial applications..

2.8 Quality and shelf life of *Dahi*/Yoghurt

Knowledge of the behaviour of *dahi* during storage is important because its shelf life is based on whether the products display any of the physical, chemical or sensory characteristics that are unacceptable for consumption. Studies of the changes in these quality characteristics during storage would enable producers to predict the shelf life of the product more accurately.

Several investigations on the shelf life of different yogurts have been conducted. Salvador and Fiszman, (2004) studied the long duration of storage of yogurt (at 10°C for 91 days) prepared from whole and skim milk. They reported that whole milk yogurt was being accepted even after 91 days of storage by around 40% of consumers. Yadav *et al.,* (1994) reported that the sensory attributes and microbiological and biochemical changes of yogurt made with soymilk and buffalo milk demonstrated a positive relationship, during 15 days of storage at 7°C. The

addition of probiotic cultures to milk and milk products inhibits the growth of different pathogenic micro-organisms by producing some bacteriostatic molecules and many bioactive substances, e.g. bioactive peptides, free fatty acids, free amino acids and oligosaccharides (prebiotics) during fermentation and storage. The addition of probiotic cultures (*Bifidobacterium longum*, *Bifidobacterium bifidum*, *Bifidobacterium infantis*, *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) inhibits the growth of pathogenic bacteria (Sartor, 2005). Sartor, (2005) suggested that the probiotic cultures had a bacteriostatic effect against pathogenic bacteria.

During storage, the bacterial counts of fermented milk were increased. Sodini *et al.,* (2004) studied the effect of storage on probiotic cell counts in fermented milk processing. They reported that storage of fermented milk containing probiotic cultures (*L. acidophilus* and *L. rhamnosus*) significantly increased the probiotic cell count. It is necessary for optimization of the bacterial flora in milk products that the duration of storage and conditions should be standardized.

Yoghurt is classified as fresh with a shelf life of up 16 – 21 days under refrigeration or thermized yoghurt with shelf life of 8-12 weeks (Alakali *et al.,* 2008).

Hanif *et al.*, (2012**)** studied that the yoghurt was prepared from cow and buffalo milk and compared for rheological and sensorial characters during storage. Means for viscosity (6169-3701cp), flavour (37.78-14.56), texture (24.67-13.00), appearance (11.00-4.44) and taste (6.94-3.61) decreased while firmness (14.11-15.22) increased during storage. Buffalo milk yoghurt showed more viscosity (8944) and firmness (20.17) than cow milk yoghurt (2339, 10.08 viscosity and firmness respectively). Yoghurt from cow milk got higher score for texture (19.00) and taste (5.87) than buffalo yoghurt (17.50, 4.25 texture and taste respectively) while flavor (26.91) and appearance (7.75) of buffalo was better than that of cow milk yoghurt (25.91, 6.67 for texture and appearance respectively). On overall basis yoghurt made from buffalo milk showed better rheological and sensory characteristics and liked by the panel of judges.

Kumar and Mishra, (2004) stated that mango soy fortified *yoghurt* (MSFY) powder was obtained after recirculatory convective drying, conditioning and grinding and was packaged in pouches of high density polypropylene (HDPP) and pouches of aluminium laminated polyethylene (ALP) pouches. The shelf life of MSFY powder was predicted on the basis of free flowness of product under accelerated storage condition (38± 1°C, 90% relative humidity) and was calculated to be 45 and 54days in HDPP and ALP respectively. The storage stability of MSFY powder in terms of quality parameters free fatty acid (FFA), thiobarbituric acid (TBA), hydroxymethyl furfural (HMF), starter counts and colour change was studied in both packaging materials.

Garcia Perez *et al.,* (2005) studied the incorporation of orange fibre obtained from orange juice by- product in to yoghurt. Fiber (0%, 0.6%, 0.8%, and 1% doses and different fiber size: 0.417–0.701 and 0.701–0.991mm) effects on color during yoghurt fermentation and cold storage were studied. Overall composition, pH, acidity, syneresis, L*, a*, and b* values were determined. Sensory evaluation of yoghurts was carried out. Fibre addition did not cause changes in yoghurt acidification and color during fermentation process, though decreased L* value and increased b* value of the milk. Colour evaluation along fermentation is pH dependent (R 0.870). pH decreased and syneresis increased along cold storage. Because of the acidification process, L* value decreased and a* and b* values increased in all yogurts. Yoghurts with 1% fiber were significantly different from the others along cold storage, presenting lower L*, higher a* and b* values, and lower syneresis.

Saint-Eve *et al.,* (2008) studied the influence of packaging polymers (polypropylene or polystyrene) on the sensory and physicochemical characteristics of flavoured stirred yoghurts with either 0% or 4%-fat content was investigated during the 28 days of storage at 4°C. Regardless of the packaging type, complex viscosity and thickness perception increased during storage due to exopolysaccharide production, whereas the pH of yoghurts decreased. Packaging type had a greater impact on 0%-fat yogurts than on 4%-fat yogurts for both sensory and physicochemical characteristics. During storage, 0%-fat yogurt conditioned in glass

displayed the lowest aroma quantity decrease of the three types of pack- aging, in accordance with the olfactory properties.

Mataragas *et al.*, (2011) developed a predictive model to quantify the spoilage of yoghurt with fruits. Product samples were stored at various temperatures (5-20°C). Samples were subjected to microbiological (total viable counts, lactic acid bacteria-LAB, yeasts and moulds) and physico-chemical analysis (pH, titratable acidity and sugars). LAB was the dominant micro-flora. Yeasts population increased at all temperatures but a delay was observed during the first days of storage. Titratable acidity and pH remained almost constant at low temperatures (5 and 10°C). However, at higher temperatures (>10°C), an increase in titratable acidity and reduction in pH was observed. Sugar concentration (fructose, lactose and glucose) decreased during storage. A mathematical model was developed for shelf life determination of the product.

Apple, wheat and bamboo fibres were used in the production of strained yogurt at different ratios (1%, 2% and 3%). Colour, texture values and sensory evaluation scores of samples were analyzed at the 1st, 7th, 14th and 21st days of storage. Depending on storage, the most changed textural parameter is consistency in bamboo, wheat and apple fibrous strained yogurt. The type of dietary fibre caused statistically significant changes in colour, texture values and sensory evaluation scores. Apple fibrous strained yogurts weren't preferred by panelists because of their ragged structure, dominant apple taste and strong odour. Panelists found bamboo and wheat fiber strained yogurts acceptable. Analyses were done in two replications with their parallels (Seckin and Baladura, 2012).

Using probiotic cultures, no investigation has been reported on sensory, biochemical, and textural changes during storage of flaxseed fortified synbiotic flavoured *dahi*, together. The aim of this study is to follow these changes simultaneously during storage.

Chapter III

MATERIAL AND METHODS

An experiment entitled "**Development of Flaxseed fortified Synbiotic Flavoured *Dahi* and its Impact on Cholesterol-fed Mice Model**" was conducted in the Laboratory, Centre of Food Science and Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (U.P.), India.

The objectives of the present study was to develop a product with cholesterol lowering properties, increase the functional value, protein content and dietary fibre content by incorporation of flaxseed, honey, mango and synbiotic microcapsules of probiotic bacteria in *dahi*/yoghurt that makes it functional food.

To know the impact of the flaxseed fortified synbiotic flavoured *dahi*, experiments have also been conducted at this station on albino swiss mice.

3.1 Technical programme

The whole experiment was conducted in four phases:

Phase I

With the help of Response Surface Methodology (RSM), process of production of flaxseed fortified synbiotic flavoured *dahi* was optimised on the basis of following parameters:

***Sensory parameters*:** Colour, taste, texture and overall acceptability.

***Textural parameters*:** Firmness and cohesiveness

Antioxidant characteristics as % DPPH inhibition activity, probiotic viable count and whey separation were also studied for optimization of the developed product.

Phase II

Following compositional, physico-chemical and microbial properties of the developed *dahi* were studied.

***Compositional properties*:** proximate composition of developed optimized yoghurt i.e moisture, fat, protein ash, dietary fibres and total carbohydrate.

***Physico-chemical properties*:** moisture, pH, titratable acidity, %DPPH inhibition activity.

***Kinetic parameters*:** Influence of flaxseed powder on acifidication kinetics of FFSFD was evaluated on basis of following parameters - V_{max} (10^{-3}pHunits/min) t_{Vmax}(h) pH_{Vmax} $T_{pH5.0}$ (h) $T_{pH4.5}$ (h)

Phase III

Following physico-chemical properties and microbial properties were studied during storage at 4 ºC for the period of 28 days at the interval of 7 days. *Dahi*/Yoghurts were packed in (100ml) plastic cups for storage.

***Compositional properties*:** moisture, fat and protein

***Sensory characteristic*:** colour, flavor, texture and overall acceptability**.**

***Physico-chemical properties*:** pH, titratable acidity, syneresis, %DPPH inhibition activity, total solids and total phenolic content.

***Textural properties*:** firmness, consistency, cohesiveness and index of viscosity

Microbial properties: *Lactobacillus plantarum* count and *Lactobacillus acidophilus* count, total plate count, yeast and mould count and coliform count.

Phase IV

The impact of flaxseed fortified synbiotic flavoured *dahi* on high cholesterol-fed mice model was studied under the following heads:

***Performance*:** Feed intake, body weight gain feed efficiency, and impact on lipid profile of mice.

***Biochemical parameter*:** Blood sugar, serum cholesterol and triglyceride, HDL-C, LDL-C, VLDL-C, total lipids and total cholesterol content in various organs (heart, kidney, liver, spleen) and faeces of mice were evaluated.

3.2 Procurement of ingredients for manufacturing of flaxseed fortified synbiotic flavoured *Dahi*

3.2.1 Milk

Low-fat double toned milk of Amul brand was bought from the local market of Varanasi, India.

3.2.2 Starter culture

The mixed *dahi* starter (DS) culture NCDC-167, *Lactobacillus acidophilus* (LA) NCDC 195 and *L. plantarum* (LP) NCDC 221 separately procured from National Collection of Dairy Cultures (NCDC), Dairy Microbiology Division at NDRI Karnal, Haryana, India were used as starter culture.

3.2.3 Flaxseed

Preparation of flaxseed powder

Flaxseed (*Linum usitatissimum* Linott.) was obtained from the local market of Varanasi. It was roasted at 65°C for 30 min and then reduced to fine powder using grinder (HL 1632, Philips, New Delhi, India).The particle size was standardized to

less than 42 µm, measured through sieves (Granutest, São Paulo, Brazil) and stored in air-tight plastic containers at 4°C until used.

3.2.4 Honey

Natural honey was procured from the local market of Varanasi, India.

3.2.5 Mango

Fresh mango fruits of *Totapuri* variety were obtained from local market of Varanasi, India. Fruits were cleaned washed in running water, peeled, cut into small pieces and deseeded. These cut pieces were pulped in pulper (HL/1631/00, Philips, New Delhi, India). Pulp was with total soluble solids of 15° Brix, acidity 0.5 and pH 4.3. The pulp thus obtained was pasteurized at 80-83°C for 30 s then cooled rapidly to 35°C and kept in glass bottles with stopper at -23°C until use.

3.2.6 Basal feed

Materials: Corn starch, bengal gram, cane sugar, soybean oil was procured from local market of Varanasi, India.

Cholesterol and bile salts were purchased from Sigma Aldrich Chemical Co. (Germany).

Casein was purchased from Nimesh Corporation (Mumbai, India).

Vitamin and Mineral mixture was procured from Virbac Animal Health India Pvt. Ltd, Mumbai, India.

Nutritional value per kg of mix is given in section 3.6.1 and nutritional value of mineral and vitamin mixture (mg/ kg of diet)is given in appendix7.1 and 7.2 respectively.

3.3 Production of control and flaxseed fortified synbiotic flavoured *Dahi*

3.3.1 Preparation and maintenance of starter culture for *Dahi*

The mixed *dahi* starter culture NCDC-167 and *L. plantarum* NCDC 221 was procured from National Collection of Dairy Cultures, NDRI, Karnal, Haryana The culture was mixed thoroughly with reconstituted skim milk and incubated at 37°C for 12–14 h thereafter stored in refrigerator at 4°C for further use and treated as seed culture. Mother culture (50mL) was prepared using sterilized reconstituted skim milk inoculated with 1ml of seed culture and incubated under same conditions, Further, bulk culture (200 mL) was prepared using 1ml of mother culture as inoculums following the same method and stored (4°C) for further use.

3.3.2 Microencapsulation of probiotic bacteria

3.3.2.1 Preparation of probiotic bacterial culture

Culture of *Lactobacillus acidophilus* NCDC 195 was purchased in lyophilized form from National Collection of Dairy Cultures, NDRI, Karnal, and Haryana. *L. acidophilus* 195 culture was reconstituted in MRS broth and incubated for 24 h at 37°C. Cells were then cultured in the same conditions for three successive transfers in MRS broth at 37°C for 20-24 h under aerobic conditions to obtain a cell density of about 10^8 colony forming units per mL (cfu/mL).. Then, they were grown in fermenter (BioFlo® /CelliGen® 115, New Brunswick,Germany) using MRS broth for 48 h. The cells were harvested by centrifugation at 3000 × g for 10 min at 4°C and then washed twice with sterile 0.1% peptone solution and re-suspended with peptone water to obtain the final concentration of inoculums as 9.25 to 9.98 log cfu/ml to be used further in microencapsulation process.

3.3.2.2 Microencapsulation procedure of *Lactobacillus acidophilus*

Alginate beads or microcapsules were produced using a modified extrusion technique reported by Lisere *et al.*, (2007). Solutions of sodium alginate (1.8% w/v) containing approximately 10^9 cfu/mL of *Lactobacillus acidophilus*, with 1% of

prebiotic (FOS), were atomized in 0.1 M calcium chloride. The atomization was achieved by forcing the sodium alginate solution to the stainless steel tube with the aid of a peristaltic pump at a flow rate of 2.5 mL/min and compressed air at a flow rate of 2.5 m ^{3}/min. The solution of calcium chloride remained under constant magnetic stirring until the end of the encapsulation. Alginate beads remained at rest for 30 minutes, after that were separated from the calcium chloride solution with stainless steel sieves (mesh of 250, 355 and 500 mm), washed with distilled water and were coated with chitosan by the method reported by Krasaekoopt *et al.,* (2004).

3.3.3 Manufacturing of control and flaxseed fortified synbiotic flavoured *Dahi*

Control *dahi* (CD) was prepared from fresh, Amul milk containing 1.5% fat and 9% SNF . Milk was heated at 95ºC for 5 minutes. It was then cooled down at 37±1ºC and inoculated by the starter culture Dairy Starter (*Dahi*) NCDC-167 at the rate of 2.0% and incubated at 37ºC up to a desired acidity of 0.70% lactic acid, packed and stored at 4°C for storage study.

Preparation of flaxseed fortified synbiotic mango *Dahi* (FFSMD)

Fresh, Amul milk containing 1.5 % fat and 9% SNF was procured from the market of Varanasi, India. Different level of flaxseed powder (1-3%), mango pulp (3-7%) and honey (2-4%) were the variables used for the preparation of FFSFD, according to the experimental design. Process flowchart for the preparation of FFSFD is presented in Fig. 2. Twenty batches containing 3 sets of *Dahi* each were prepared.

Flaxseed fortified synbiotic flavoured *dahi* (FFSFD) was prepared using standardized milk, which was heat treated at 95°C for 5 min and then placed into sterilized wide-mouth glass jars (100 mL) with stopper and cooled to 37°C without exposing it to the atmosphere. Then flaxseed powder and honey was added and was two stage homogenized (at 2500 psi (1st stage) and 500 psi (2nd stage)). The blends were inoculated with 2% (v/v, Dairy Starter (*Dahi*) NCDC-167 and *Lactobacillus plantarum* (LP) NCDC 221(9.38 log cfu/ml) in 1:1 ratio of 24 h old culture. The *acidophilus* (LA) NCDC 195 was incorporated (10^9 CFU/ml) as free and encapsulated form in the different treatments of FFSFD as given in section 3.4.10. The blends were

then incubated at 37°C for 5-6 h in the plastic cups (100 mL) until previously determined final pH of 4.8 was obtained. The pH was constantly observed by pH meter (B21899, Orion Star series, Singapore) to measure the final pH. After final pH was obtained, mango pulp was added to the set *Dahi* and mixed thoroughly with high speed stirring. Thereafter, *dahi* samples were stored at 4°C for 1 h and then used for texture profile analysis, antioxidant activity and probiotic viable count. The FFSFD samples were packed and stored at 4°C for storage study.

With the help of response surface methodology (RSM), FFSFD was optimized on the basis of sensory, textural properties, antioxidant activity and probiotic microbial count.

PHASE - I: OPTIMISATION OF FLAXSEED FORTIFIED SYNBIOTIC FLAVOURED *DAHI*

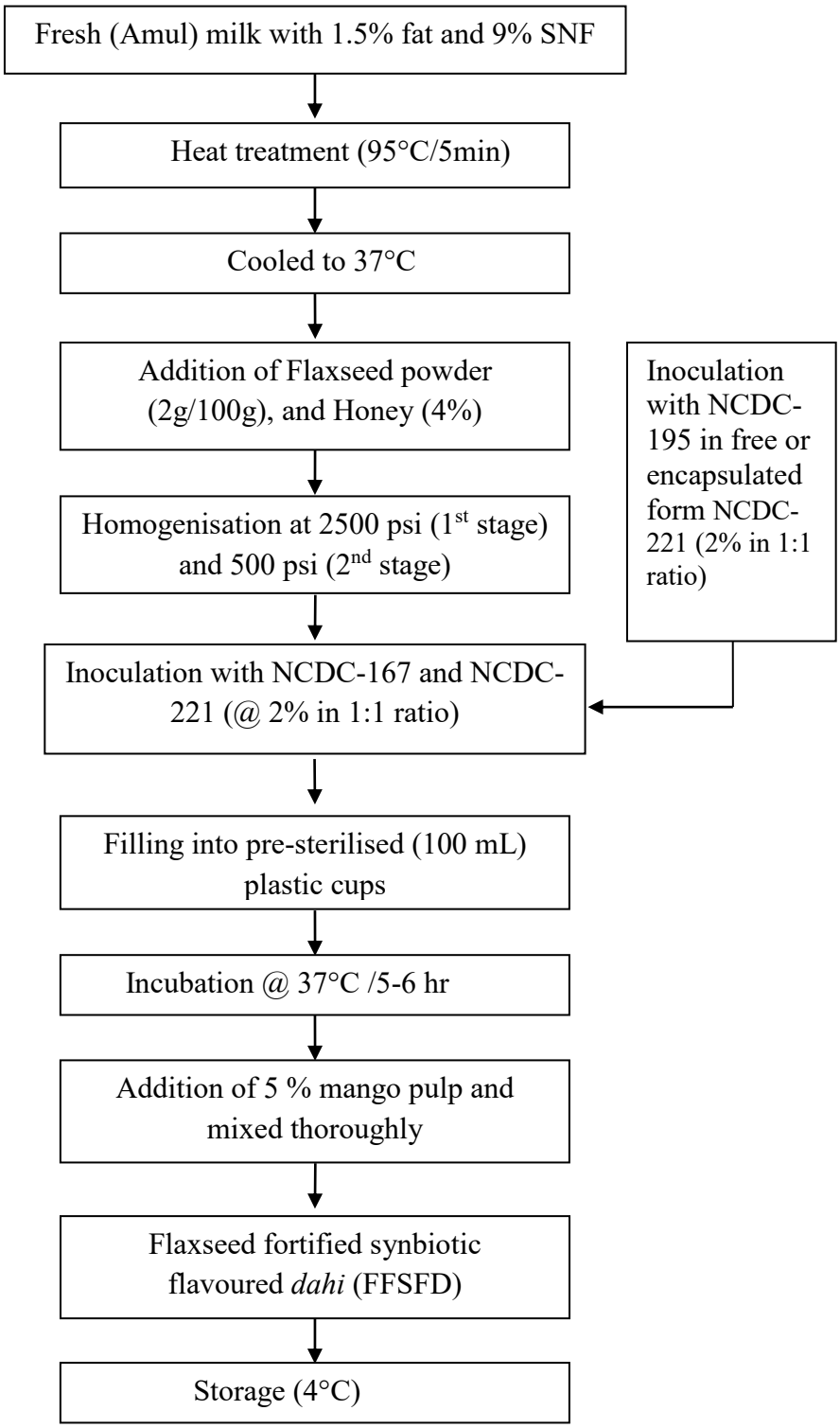
3.3.4 Sensory evaluation

The sensory evaluation was conducted in a quiet and well-lit room free from any odour. Panel booths were illuminated uniformly with special daylight bulbs for the evaluation of colour. Sensory evaluation was performed by a panel of 20 semi-trained panelists, from the Centre of Food Science & Technology and Department of Animal Husbandry & Dairying, Banaras Hindu University, Varanasi (India). Samples were placed in closed containers, coded with three-digit random numbers. Each panelist assessed three samples for each treatment. Sensory evaluation was done at 25^{o}C and 60 per cent relative humidity. Hedonic rating (9-point scale; 1 = dislike extremely, 9 = like extremely) (Amerine *et al.,* 1965) was used for colour, flavour, texture, sweetness and overall acceptability.

3.3.5 Texture profile analysis (TPA)

Textural attribute such as firmness and cohesiveness was determined by back extrusion method using a texture analyzer, (TA-XT plus, M/s Stable Micro Systems ltd, Godalming, United Kingdom) fitted with a 25 kg load cell and was calibrated with 5 kg standard dead weight prior to use.

Fig. 2. Flow diagram for manufacture of Flaxseed Fortified Synbiotic Flavoured *Dahi* (FFSFD)



For determining the firmness and cohesiveness, the pasteurized and cooled *dahi* mix was filled up to 5 cm in a pre-sterilized glass beaker and incubation was carried out. The beakers were tempered at 25°C for 2 h prior to analysis. The probe (A/BE 35) was penetrated up to 10 mm (20% compression) into the set FFSFD at a cross head speed of 1.0 mm/s. The probe displaced the material by compression followed by back-extrusion, so that the fluid flowed upwards through the concentric annular space (Exponent Lite XT PLUS, Ver. 4.0.13.0 lite). All measurements were done in triplicate per each sample (Raju and Pal, 2014).

3.3.6 Antioxidant activity (DPPH inhibition)

The antioxidant activity in sample was estimated as DPPH inhibition activity by following the protocol given by Nishino *et al.*, (2000). Two hundred mg of sample was taken in centrifuge tube (in replications). In blank, 0.2 ml distilled water was taken instead of sample. 1ml of DPPH (8 mg/100 mL ethanol) solution was added to the sample and tubes were left for 30 min (vortexed in between). Tubes were then centrifuged at 4000 rpm for 10 min. 0.5 ml supernatant was poured in fresh tubes and 1.0 ml ethanol to dilute the content. Absorbance was measured at 517 nm by using UV-1800 spectrophotometer (Shimadzu, Japan) against the ethanol.

Calculation

$$\% \text{ Antioxidant Activity} = \frac{A_b - A_s}{A_b} \times 100$$

Where, antioxidant activity is radical scavenging activity or % inhibition of DPPH,

A_b is absorbance of blank and

A_s is the absorbance of sample

3.3.7 Syneresis

Dahi/Yoghurt (100 g) was taken on filter paper and kept over a glass beaker in refrigerator for 12 h to separate the watery fluid from the yoghurt. The watery fluid

collected in glass beaker was measured using the measuring cylinder, and the percent syneresis was calculated on weight basis as follows:

$$\text{Syneresis (\%)} = \frac{\text{Volume of whey collected after drainage}}{\text{Volume of curd sample before drainage}} \times 100$$

3.3.8 Enumeration of probiotic viable bacteria

The 2g of *dahi* samples were suspended in phosphate buffer solution and mixed by mechanical shaking for 10 min at room temperature to ensure complete dissolution of the *dahi*. Serial dilutions were prepared from the initial suspension and pour plated on MRS agar (MRS Agar, Himedia, Mumbai, India) in triplicate was done. *L. acidophilus* was enumerated on a modified MRS agar with clindamycin (Oliveira *et al*., 2011). Plates were incubated at 37°C for 72 h under aerobic conditions and the numbers of colonies were counted (Champagne *et al.,* 2009). Enumeration of *L. plantarum* was done in modified MRS agar with ciproflaxin antibiotics (Bujalance et al., 2006).The plates were anerobically incubated for 42 h at 37 °C (Dave and Shah, 1996) , the bacteria enumerated as CFU/g and the numbers of colonies were counted (Champagne *et al.,* 2009). Results are expressed as log colony forming units per g (log cfu/g).

3.4 Analytical technique for determination of physical and chemical composition of FFSFD

3.4.1 Moisture content

Moisture content was calculated as per the method of AOAC, (1995). Approximately 5 g well-mixed sample was accurately weighed into a cooled and tare aluminum dish. The sample was heated in an oven maintained at 105 ± 2°C until constant weight. The dish was transferred to desiccators and upon cooling, weighed. Moisture content was calculated as under:

$$\text{Present moisture} = \frac{(W_1 - W_2 \times 100)}{W_1 - W}$$

Where, W = Weight of empty dish (g),

W_1 = Weight of dish with the sample (g), and

W_2 = Final weight of dish (g).

3.4.2 Fat content

The fat content of the samples was determined by using Soxhlet apparatus (Socs-plus). Soxhlet method is one of the standard methods for analysis of fat in food. The method is recognized by the AOAC, (1995). In this method fat content is determined by extracting the fat from the sample using solvent extraction. 5 g of sample was weighed in to the thimble and inserted in soxhlet extractor. A clean and dry 150 ml Soxhlet flask was accurately weighed and approximately 90 ml of petroleum ether was poured into the flask. The content of the flask was heated at 70 °C for one and half hour and then the temperature was increased to 140°C for evaporation for next one hour. The flask was removed from the soxhlet extractor and the remaining solvent was evaporated off by placing the flask in an oven at 102°C until a final weight is reached (1-2 h). Then the flask was cooled in a desiccator and the flask content was weighed. The fat content was calculated by using the following formula.

$$\text{Fat (\%)} = \frac{W_2 - W_1}{S} \times 100$$

Where, Weight of empty flask (g) = W_1

Weight of the flask and extracted fat (g) = W_2

Weight of sample = S

3.4.3 Protein content

The protein content of the samples was estimated by using Kjeldahl method (AOAC, 1995). Approximately 0.3 g of the sample was taken in a clean dry Kjeldahl flask. Thereafter 10 ml pure nitrogen free sulphuric acid (H_2SO_4), 4 g of pure

potassium sulphate (K_2SO_4) crystals and copper sulphate crystals ($CuSO_4$) were added into Kjeldahl flask. Then the Kjeldahl flask was transferred to digestion chamber for digestion of the content. Upon digestion, when the content of flask became carbon free, the Kjeldahl flask was allowed to cool down. To this flask approximately 400 ml of distilled water added and then the content transfer to distillation flask. 25 ml of 4% Boric acid with Methylene red indicator was taken in conical flask. Approximately 90 ml of 40% NaOH solution was then added to the distillation flask. The conical flask was placed below condenser to collect the condensate. The distillation head was fixed on distillation flask and condenser. The distillation process was continued until about 300 ml distillate was collected in the conical flask.

Following the usual precautions the beaker was removed from the assembly. The evolved N_2 was determined by titrating condensate with 0.1 N HCl. Percentage protein was calculated by following formula:

$$\text{Nitrogen (\%)} = \frac{(\text{Sample titre} - \text{Blank titre}) \times \text{Normality of Hcl} \times 14.01 \times 100}{\text{Wt. of sample} \times \text{Aliquot taken for distillation} \times 1000}$$

Protein % = Nitrogen % × 6.27

Protein % = Nitrogen% x 6.38 (for Milk)

3.4.4 pH

The digital pH meter was used to determine the pH of the samples. It has an electrode which was dipped in standard buffer solution during measurement of pH. pH meter was calibrated using buffer of pH 4, 7, and 10. After calibration pH meter was washed carefully with distilled water. Then the pH electrode was dipped into the sample. When the pH icon stopped flashing the reading was noted down.

3.4.5 Titratable acidity and total solids

Two gram of the sample was taken in conical flask. 10 ml distilled water and a few drops of phenolphthalein indicator were added to it, and then titrated against

0.1N NaOH till the color changed to light pink (end point) (Ranganna, 2001). Total solid content was analyzed as per the standard method of AOAC, (1995).

Calculation

$$\text{\% Acidity (in terms of lactic acid)} = \frac{\text{Titre reading} \times \text{Normality of NaOH} \times \text{Volume made upto} \times 90}{\text{Weight of sample taken} \times \text{volume of aliquot taken} \times 1000} \times 100$$

3.4.6 Ash content

Approximately 3 g sample was accurately weighed into a silica crucible and kept for charring on hot plate for 2 h. Now the sample was kept for ashing in a muffle furnace at 550 ± 2°C for 4 h (AOAC, 1995).

3.4.7 Dietary fibre

Total dietary fibre content was analyzed as detailed in AOAC (2008). 1 g sample was weighed accurately into a tall beaker, 50 ml phosphate buffer (pH 6.0) followed by 0.10 ml α amylase added and mixed well. Beaker was covered with aluminum foil, incubated in a water bath until 15 min after the internal temperature reached 95°C, with intermittent gentle mixing after each 5 min. After cooling to room temperature, pH was adjusted to 7.5 ± 0.2 and 0.10 ml of freshly prepared protease solution (50 mg/ml in phosphate buffer – 0.08 M, pH 6.0) added. Beaker was covered and incubated with intermittent mixing in a water bath at 60°C until 30 min after the internal temperature reached 60°C. After cooling to room temperature, pH was adjusted to 4.0 using 0.325 M HCL (~10 ml); 0.10 ml of amyloglucosidase added and the beaker covered and incubated at 60°C for 30 min after the internal temperature reached 60°C. Four volumes of 95% ethanol were added to the beaker before storing overnight at room temperature to allow complete precipitation.

Glass crucibles were thoroughly cleaned, taken in muffle at 525°C for 1 h, cooled, rinsed with water and dried in air. About 0.5 g diatomaceous flour (celite) is added and the crucible is dried at 130°C in an oven to constant weight (~1 h). The

crucible were cooled, weighted (W_1 = celite + crucible weight) and stored in the desiccator till use. The bed of celite was wetted and redistributed in each crucible using 78% ethanol. Using gentle suction the celite was drawn on to crucible as an even mat and maintaining that suction the precipitate and suspension were transferred from each beaker to its respective crucible. The residue was washed with three 20 ml portion of 78% ethanol, two 10 ml portion of 95% ethanol and two 10 ml portion of acetone before oven drying at 105°C overnight, then cooled and weighed (W_2 = residue + celite + crucible weight). The residue from two samples and two blanks were analyzed for protein by Kjeldahl method (AOAC, 1995). The residue in the crucible from the remaining two samples and two blanks were ignited for 5 h at 525° C in a muffle furnace. After cooling in a desiccator, weighed (W_3 = ash + celite + crucible weight).

$$\text{Total Dietary Fibre (\%)} = \frac{R - P - A - B}{Sw} \times 100$$

Where, R is residue weight (W_2 - W_1)

P is protein weight,

A is ash weight (W_3- W_1)

B is blank (R_{blank} - P_{blank} - A_{blank}) and

Sw is weight of sample

3.4.8 Carbohydrates

Carbohydrate was analyzed by difference method.

CHO = 100 - (Protein% + Moisture%+ Fat% + Ash %)

3.4.9 Total phenolic content

The total phenolic content of the samples were determined by the Folin-Ciocalteau method Cliffe *et al*. (1994) with some modifications. The sample (five

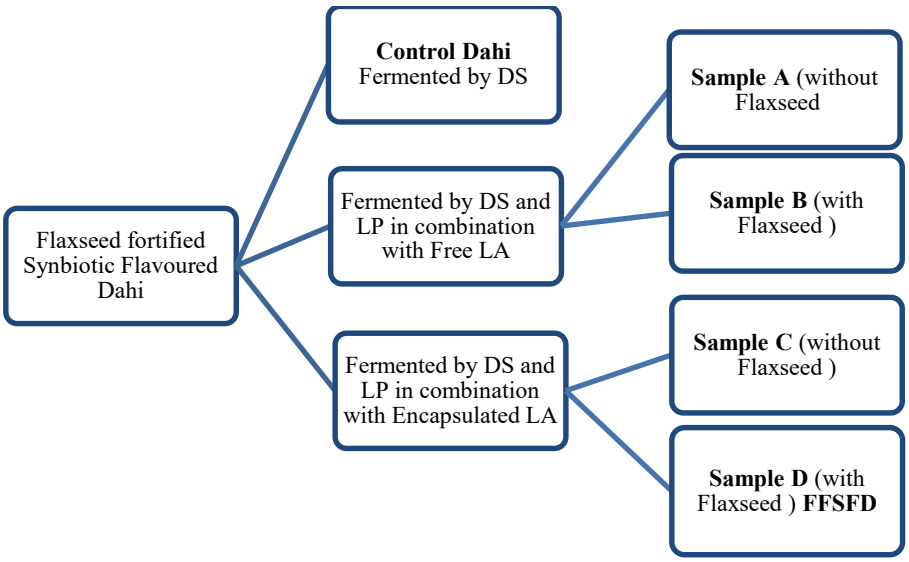
hundred microlitre) was added to 2.5 ml of 0.2 N Folin-Ciocalteau reagent and placed for 5 minutes. 1 ml of 75 g/l of Na_2CO_3 was then added. The above solution was then kept for incubation at room temperature for 2 hours. Absorbance was measured at 760 nm using 1 cm cuvette UV-1800 spectrophotometer (Shimadzu, Japan). Gallic acid (0 – 800 mg/L) was used to produce standard calibration curve. The total phenolic content was expressed in mg of Gallic acid equivalents (GAE)/g of extract.

3.4.10 Acidification kinetics parameters

To evaluate acidification kinetic parameters during fermentation, changes in pH were continuously monitored at 15 mins of time interval using a pH meter (B21899, Orion Star series, Singapore) (Varghese and Mishra, 2008). The results were expressed as the means of six replicates. The acidification rate (Vmax) was calculated as the time variation of pH (dpH/dt) and expressed as pH units/min. At the end of incubation, the following kinetic parameters were calculated: (1) t_{Vmax}, time to reach Vmax (h); (2) pH $_{Vmax}$, pH at V_{max} (3) $t_{pH\ 5.0}$, time to reach pH 5 (in hours) (4) $t_{pH\ 4.5}$, time to reach complete the fermentation (in hours).

Treatment combination for Study of Acidification Kinetics and Storage study of FFSFD

Four different *dahi* samples were prepared using following combination:



Control *Dahi* : The control sample was prepared by inoculation with 2% Dairy Starter (*Dahi*).

1. *Sample A* : Mango juice 5% and inoculated with 2% Dairy Starter (*Dahi*) and *L. plantarum* v/v, 1:1 ratio and 2% of free probiotic strains of *L. acidophilus* to obtain approximately 10^9cfug^{-1};

2. *Sample B*: Mango 5% and inoculated with 2% Dairy Starter (*Dahi*) and *L. plantarum* v/v, 1:1 ratio and 4% (previously optimized by experiment) of synbiotic microcapsules of probiotic strains of *L. acidophilus* to obtain approximately 10^9cfug^{1};

3. *Sample C*: Mango 5% and inoculated with 2% Dairy Starter (*Dahi*) and *L. plantarum* v/v, 1:1 ratio and 2% of free probiotic strains of *L. acidophilus* to obtain approximately 10^9cfug^{-1} with 2% of flaxseed powder;

4. *Sample D*: Mango 5% and inoculated with 2% Dairy Starter (*Dahi*) and *L. plantarum* v/v, 1:1 ratio and 4% of synbiotic microcapsules of probiotic strains of *L. acidophilus* to obtain approximately 10^9cfug^{-1} with 2% of flaxseed powder.

The blends were mixed thoroughly with high speed stirring.

After inoculation, glass flasks were transferred to incubator and two independent batch fermentations were performed at 37 °C up to pH 5.0 (selected as the condition to stop the fermentation). When the desired pH was reached (pH 5.0), the flasks with the *dahi* sample were cooled to 15°C in ice bath, and then the clot was broken. Afterwards, the fermented milk was packed and sealed in 100 mL polypropylene pots and cooled to 4 °C. All sampling and analyses were performed after 0 day (after 12 hr of fermentation), 07 days, 14 days, 21 days and 28 days of cold storage at this temperature.

3.5 Shelf-life study of flaxseed fortified synbiotic flavoured *Dahi*/Yoghurt (FFSFD)

3.5.1 Storage temperatures and sampling times

The samples of control *dahi* (CD) and flaxseed fortified synbiotic flavoured *dahi*/yoghurt (FFSFD) were stored in plastic cups at 4±1°C temperature. The samples were analyzed at every 7th days of interval up to 28 days during storage.

Colour, flavour, texture, and overall acceptability of flaxseed fortified synbiotic flavoured *dahi*/yoghurt (FFSFD) and control *dahi* was judged by a panel of 10 semi-trained judges by using 9-point hedonic rating (1 = dislike extremely, 9 = like extremely) scale.

Proximate analyses of flaxseed fortified synbiotic flavoured *dahi* (FFSFD) and control *dahi* (fat, protein, moisture, pH, total solids and titratable acidity, syneresis) were analyzed using standard procedures (AOAC, 1995). Textural properties i.e. firmness, cohesiveness, consistency and index of viscosity of optimized *dahi* and different treatments were studied by same method as given in section 3.3.5.

3.5.2 Thiobarbituric acid (TBA)

The extent of oxidation of fat in *dahi*/yoghurt was measured in terms of increase in TBA value. The extraction method of Strange *et al.* (1977) was followed with slight modification. For TBA value determination about 2 g sample was taken and blended with 50 ml of 20 % TCA (Trichloroacetic acid) and 50 ml of distilled water and left undisturbed for 10 min. Then the contents were filtered through Whatman No.1 filter paper. The filtrate (5 ml) was pipette out in test tube and added with 5 ml of 0.01M 2-Thiobarbituric acid. Colour was developed by incubating the tubes in boiling water bath for 30 min at 100 °C. The contents were cooled to room temperature and absorbance was determined at 532 nm (UV-1800, UV spectrophotometer, Shimadzu corporation, Japan). Blank determinations were made using distilled water in place of sample. TBA value was expressed as absorbance at 532 nm.

3.5.3 Free fatty acid (FFA)

The method prescribed by Deeth *et al.,* (1975) was used to estimate the FFA content of the *dahi*/yoghurt. The method consisted of accurate weighing of 0.5 g of the sample into a 60 ml stoppered test tube. 10 ml of extraction mixture (Iso propanol: Petroleum ether: 4N H_2SO_4 in the ratio of 40:10:1) was added and mixed thoroughly. This was followed by the addition of 6 ml petroleum ether and 4 ml distilled water. The test tube was stoppered and tempered at 40^oC for 10 min. The contents were vigorously shaken for 20 sec. The two layer were allowed to separate for 10-15 min and an aliquot of the upper layer (5-8 ml) was withdrawn and titrated against 0.02N methanolic KOH solution using 1% methanolic phenolphthalein indicator. A blank, in which the sample was replaced with distilled water, was used to obtain the background titration.

3.5.4 Enumeration of probiotic viable bacteria

Loss in viability was determined by the method discussed in section 3.3.8

3.5.3 Microbial analysis

The samples stored at 4°C was analyzed for total plate count, yeast and mould count and coliform count using plate count agar (PCA), potato dextrose agar (PDA) after incubation at 25°C for 24-48 hrs and violet red bile agar (VRBA) after incubation at 37°C for 24-48 hrs respectively. The medium used for the all the microbial tests were procured from Himedia Laboratories Private Ltd., Mumbai, India (AOAC, 2000).

3.6 Impact of flaxseed fortified synbiotic flavoured *dahi* on lipid profile of mice

3.6.1 Preparation of basal feed

A standard semi synthetic diet was prepared according to the AIN-76A formulation (g/kg dry basis), containing 31% corn starch, 10% Bengal gram, 10% casein, 5% cane sugar powder, 10% soybean oil, 1% vitamin mix, 4% mineral

mixture. Hypercholesterolaemic diet was made by supplementing 1% per cent cholesterol and 0.20 per cent bile salts (1:1 mixture of sodium cholate and sodium deoxycholate) to the above basal diet substituting the same quantity of starch. FFSFD was given as 30% of total diet while control group (control diet) mice received equal amount of milk.

Table 3 Composition of the control diet (CD), hypercholesterolaemic diet (HCD), control FFSFD diet and HCD with FFSFD (g/kg)

Ingredients	**Constituents**			
	Control diet	**Hyper-cholesterolaemic diet**	**Test diet**	**Hyper-cholestrolaemic diet with test diet**
Corn starch	310.692	298.692	295.692	283.692
Bengal gram	105	105	90	90
Casein	140	140	140	140
Cane sugar	100	100	100	100
Soyabean oil	40	40	40	40
Vitamin mix	10	10	10	10
Mineral mix	35	35	35	35
L-Cys	1.8	1.8	1.8	1.8
Cellulose	100	100	100	100
Dextrose	155	155	155	155
Choline bitartrate	2.5	2.5	2.5	2.5
t-BHQ*	0.008	0.008	0.008	0.008
Cholesterol		10		10
Bile Salts		2		2
FFSFD			300	300

[a] According to the AIN-76A formulation (g/kg dry basis). *T-BHQ (tetra-butyl hydroquinone). Hypercholesterolaemic diets were supplemented with 10g of cholesterol and 2g of bile salts (sodium cholate and sodium deoxycholate) at the expense of starch.

After ascertaining the best quality of flaxseed fortified synbiotic flavoured *dahi* (chemically and organoleptically) from the different treatment combinations tried, it was thought essential to find out the influence of this product on the growth performance and lipid profile of the animals. For this purpose, 24 albino swiss mice were chosen. The treatment combinations were as follows: 1. Basal diet with 30% milk (G_1-Control), 2. Basal diet supplemented with 1% cholesterol and 0.20% bile salts (G_2-High Cholesterol diet) with 30% control *dahi* 3. Basal diet supplemented with 30% FFSFD (G_3) 4. Basal diet supplemented with 1% cholesterol and 0.20% bile salts and 30% of FFSFD (G_4-High Cholesterol diet with FFSFD)

3.6.2 Selection of experimental animals

Out of 100 albino mice, 24 mice of similar sex, body weight and body conformation were selected at 8 weeks of age for the study. The mice having an average body weight of 32.56±0.30 gm were randomly divided into 4 groups of 6 each.

3.6.3 Equipments and light arrangements

During the present investigation the following equipments were selected:

(I) **Maintenance & cleaning of house:** The room was cleaned and disinfected before housing the mice.

(II) **Light arrangement:** For providing sufficient light to each group, two 40 watts of tube lights were used in the room during entire periods of the experiment. A lightness was maintained between 8:00 am to 8:00 pm daily.

(III) **Cage:** Four cages each of size 1.5'×1.5'×1.0' were kept at equal distance in two rows in a room.

(IV) **Feeders:** In each cage one feeder was provided.

(V) **Water:** One plastic bottle with a small siphon fitted on the mouth was provided in each cage. Each set of bottle was washed and clean daily. The bottle was filled up with fresh and purified water twice a day for drinking.

3.6.4 Preparation of treatment groups feed mixture

On daily basis, bengal gram was soaked in water a night before. Soaked bengal gram was coarsely ground in Willy type grinder and mixed with the rest of the components of basal feed mixture as mentioned in table 3.1. The experimental rations were prepared before one hour feeding of rats (Table 3.1).

Table 3.1 Feed combination in different groups

Group	Constitution
G_1 (Control)	Basal feed + 30% milk
G_2 (High cholesterol diet-HCD)	Basal feed + 1.0% Cholesterol + 0.2% sodium cholate+30% Control *dahi*
G_3(Control of FFSFD)	Basal feed + 30% FFSFD
G_4(HCD+ FFSFD)	Basal feed +1.0% Cholesterol + 0.2% sodium cholate+30% FFSFD

3.6.4.1 Weighing of mice, feed and feed residue

A digital electronic balance (of capacity 500 gm) was used for weighing of mice, feed, feed residue and faeces. The feed offered and the leftover feed residue were recorded daily whereas, mice were weighed weekly in the morning before offering the feed to the mice. Faeces were collected during the last 3 d of the experimental period, oven dried at 54ºC and stored at -18ºC.

3.6.5 Duration of experiment

The experiment was conducted on mice over a period of four weeks (10^{th}October to 12^{th} November, 2015).

3.6.6 Health

During the experimentation, each mice was critically examined daily for their normal health.

3.6.7 Sampling and analysis of feed

All the feed ingredients were ground and filtered in 100 mm sieves and collected for proximate analysis of feeds (AOAC, 2005).

3.6.8 Preparation of mice for carnage

At the end of experiment, mice were deprived of feed for 12 h, weighted and anesthetized with chloroform. Blood was withdrawn by cardiac puncture and mice were sacrificed by decapitation in order to minimize the handling stress, and blood samples were collected to determine the plasma lipid profile. Blood samples were centrifuged and serums were collected. The liver, heart, kidneys and spleen were excised, rinsed with physiological saline solution, blotted and weighed. All samples were stored at -18˚C until analyzed.

3.6.9 Blood collection and processing

Blood from all experimental animals was collected separately at the end of experimental period through cardiac puncture early in the morning before feeding was collected with addition of EDTA for hematological parameters and the remaining was taken in well cleaned, dry, sterilized test tubes and allowed for clotting. After clotting, the tubes were centrifuged to collect sera. The collected sera samples were stored in deep freeze for further analysis. The collection of serum from the heart, liver kidneys, spleen and faeces was also done in same manner for further analysis.

3.6.10 Biochemical parameter

Blood sugar, cholesterol and triglycerides.

3.6.10.1 Blood sugar (Glucose)

Glucose in plasma was determined after enzymatic oxidation in the presence of glucose oxidase (GOD). The hydrogen peroxide so formed reacts under catalysis of peroxidase (POD) with phenol and 4-aminoantipyrine (4-AAP) to form a red

coloured quinoneimine compound. The absorbance of the colour measured at 505 nm, is directly proportional to glucose concentration.

$$\text{Glucose} + O_2 + H_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{4-AAP} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4H_2O$$

3.6.10.2 Lipid profile (Triglyceride, Cholesterol, HDL-C and LDL-C)

Triglycerides TG, Total cholesterol TC, total lipids and HDL-C, VLDL-C and LDL-C were measured on an automated RX Imola Clinical System Analyzer.

3.7 Statistical analysis

3.7.1 Optimization

Response surface methodology which involves design of experiments, selection of levels of variables in experimental runs, fitting mathematical models and finally selecting variable levels by optimizing the response (Khuri and Cornell, 1987) was employed in the study. To optimize the various parameters for the product response surface methodology (RSM) was used. The experimental data obtained from the design were analyzed by the package Design-Expert® version 9.0.2 software, Stat-Ease, Inc., Minneapolis, MN, USA. The full quadratic equation of the response variables was derived by using RSM as following Eq.

$$Y = \beta_0 + \beta_1A_1 + \beta_2B_2 + \beta_3C_3 + \beta11A_1^2 + \beta22B_2^2 + \beta33C_3^2 + \beta12A_1B_2 + \beta13A_1C_3 + \beta23B_2C_3 \quad \ldots (1)$$

Where, Y= responses; β_0 = constant; β_1, β_2, β_3 = linear regression;

β_{11}, β_{22}, β_{33} = interaction regression; A_1, B_2, C_3 = variables.

3.7.2 Statistical study of acidification kinetics, shelf life and impact of FFSFD on lipid profile of Mice

The studies of acidification kinetics, shelf life and impact of FFSFD on lipid profile of mice were subjected to statistical analysis. One-way analysis of variance

(ANOVA) was applied and Duncan's multiple range tests was used to analyze the test of significance by using SPSS 17.0 software (SPSS Italia, Bologna, Italy). Correlation and regression analysis of physico-chemical and sensory data were subjected to standard statistical methods (O'Mahony, 1986).

Chapter IV

RESULTS AND DISCUSSION

The present study was done to utilize and investigate the properties of flaxseed powder and therefore, it was planned to develop flaxseed powder fortified synbiotic flavoured *dahi* (FFSFD) to enhance the therapeutic and nutritional value, so as to obtain a value-added dairy product that could be offered to the consumers as a convenience food. To obtain the results, the whole experiment was divided into four phases as given below:

PHASE – I: OPTIMISATION OF FLAXSEED FORTIFIED SYNBIOTIC FLAVOURED *DAHI*

4.1 Optimisation of parameters

Using a Central Composite Design (CCD), levels of variables *viz*., flaxseed powder, mango pulp and honey were selected through 20 experiments. The sensory, textural, antioxidant activity and probiotic microbial count scores as influenced by different levels of flaxseed powder, mango pulp and honey are presented in table 4.1. The results generated from table (Table 4.1) are presented in table 4.2, 4.3, 4.4 and 4.5. The ANOVA results for all responses are summarized in table 4.3. In order to quantify the curvature effects, the data from the experimental results were fitted to higher degree polynomial equations i.e. quadratic. In these polynomial equations A, B & C are coded terms for the three variables *i.e*. flaxseed powder, mango pulp and honey respectively.

The model terms in the equations are those remained after the elimination of insignificant variables and their interactions. Based on the statistical analysis, the models were highly significant with very low probability values ($p<0.0001$). It is shown that the model terms were significant at the 99% confidence level. The square of correlation coefficient for each response was computed as the coefficient of determination (R^2). It showed high significant regression at 95% confidence level.

Table 4.1 Experimental runs and actual values of factors used in CCD

Runs	FACTORS			RESPONSES							
	A:Flaxseed powder (g/100g)	B: Mango pulp (%)	C: Honey (%)	Taste	Colour	Overall Accept Ability	Anti-oxidant Activity	Firmness (g)	Cohesive-ness (gs)	Probiotic viable count (CFU/g)	Whey Separation (%)
1	3.68	5.00	4.00	7.01	6.50	6.75	80.59	253.26	38.56	9.45	4.15
2	2.00	5.00	7.36	7.80	6.52	7.65	84.26	231.57	30.59	9.85	6.45
3	2.00	8.36	4.00	7.00	6.10	7.06	78.25	247.36	36.87	7.05	5.76
4	1.00	7.00	2.00	6.05	6.56	8.86	72.23	245.20	31.09	6.25	5.65
5	2.00	5.00	0.63	5.00	6.23	5.04	63.85	252.45	34.32	6.51	4.56
6	2.00	5.00	4.00	8.95	8.49	8.52	83.29	243.60	35.60	9.62	5.25
7	2.00	5.00	4.00	8.75	8.53	8.47	84.25	244.38	35.48	9.05	5.09
8	2.00	5.00	4.00	8.25	8.15	8.75	84.21	242.53	36.25	9.64	5.03
9	0.31	5.00	4.00	9.01	9.05	9.00	79.36	223.64	25.98	7.19	6.54
10	3.00	7.00	2.00	5.59	5.26	5.66	78.32	259.45	35.12	7.32	4.92
11	2.00	5.00	4.00	9.03	8.06	8.05	83.49	245.19	34.45	9.25	5.29
12	2.00	1.64	4.00	6.78	5.28	7.36	67.98	232.92	32.57	9.52	4.47
13	1.00	3.00	6.00	6.65	7.15	6.85	75.25	238.29	28.59	8.06	6.72
14	3.00	3.00	6.00	6.71	6.45	6.15	78.12	235.78	30.97	8.76	5.36
15	3.00	3.00	2.00	5.81	6.08	5.35	70.02	240.87	32.24	7.23	4.02
16	2.00	5.00	4.00	7.10	8.15	8.24	83.26	244.61	36.56	9.56	4.85
17	3.00	7.00	6.00	6.95	6.25	7.01	85.75	251.64	40.24	7.26	7.96
18	1.00	7.00	6.00	7.48	7.59	7.45	82.51	241.56	34.29	6.13	7.12
19	1.00	3.00	2.00	8.23	7.43	6.21	72.94	243.35	26.35	5.89	7.46
20	2.00	5.00	4.00	8.42	8.25	8.04	83.54	247.89	35.36	8.41	5.01

Table 4.2 Coded and actual levels of factors used

Variables	**Actual factor level at coded factor level**					
	Symbol	**-1.68**	**-1**	**0**	**1**	**1.68**
Flaxseed powder (g/100g)	A	0.31	1	2	3	3.68
Mango pulp (%)	B	1.64	3	5	7	8.36
Honey (%)	C	0.63	2	4	6	7.36

Table 4.3 ANOVA for different predicted model for responses

Source	Degree of freedom	Taste		Colour		Overall Acceptability		Antioxidant Activity		Firmness		Cohesiveness		Probiotic Viable Count		Whey Separation	
		F value	P Value Prob>F	F value	P Value Prob>F	F value	P Value Prob>F	F value	P Value Prob>F	F value	P Value Prob>F	F value	P Value Prob>F	F value	P Value Prob>F	F value	P Value Prob>F
Model	9	22.57	0.000	35.76	0.000	71.65	0.000	31.94	0.000	47.61	0.000	125.43	0.000	27.22	0.000	34.87	0.000
A-Flaxseed	1	0.33	0.579	2.96	0.116	0.79	0.395	51.55	0.000	411.40	0.000	1077.9	0.000	10.13	0.010	100.74	0.000
B-Mango pulp	1	61.24	0.000	76.22	0.000	178.88	0.000	107.45	0.000	0.14	0.717	33.26	0.000	36.82	0.000	251.37	0.000
C-Honey	1	0.00	0.961	1.04	0.332	5.85	0.036	3.34	0.098	1.01	0.338	7.39	0.022	142.12	0.000	49.26	0.000
AB	1	2.69	0.132	13.97	0.004	0.51	0.492	15.02	0.003	0.41	0.537	0.83	0.384	4.79	0.053	1.30	0.281
AC	1	0.16	0.699	0.00	0.980	4.23	0.067	0.47	0.507	0.26	0.620	1.37	0.269	0.44	0.520	0.47	0.509
BC	1	0.77	0.402	0.08	0.787	7.52	0.021	0.38	0.554	0.05	0.835	0.01	0.918	9.51	0.012	1.90	0.198
A^2	1	95.47	0.000	113.71	0.000	333.98	0.000	97.25	0.000	13.72	0.004	2.41	0.152	0.96	0.349	3.12	0.108
B^2	1	44.98	0.000	136.20	0.000	147.22	0.000	17.35	0.002	2.15	0.173	6.15	0.033	1.58	0.237	1.16	0.307
C^2	1	21.05	0.001	5.39	0.043	25.81	0.000	6.56	0.028	0.97	0.348	0.56	0.471	40.72	0.000	3.43	0.094
Lack of Fit	5	3.17	0.115	3.71	0.088	3.77	0.086	1.19	0.425	3.41	0.102	4.06	0.075	3.93	0.080	2.72	0.148
Std. Dev.		0.44		0.27		0.25		0.83		0.26		1.03		0.22		0.38	
R-Squared		0.9531		0.9699		0.9847		0.966		0.9772		0.9912		0.96		0.96	
Adj R-Squared		0.91		0.94		0.97		0.93		0.95		0.98		0.92		0.94	
Pred R-Squared		0.71		0.80		0.89		0.83		0.85		0.94		0.80		0.88	
Model		Sig.		Sig.		Sig.		Sig.		Sig.		Sig.		Sig.		Sig.	

Table 4.4 Predicted score of the suggested formulation of flaxseed fortified synbiotic flavoured *dahi* by Minitab version 17

Number	A: Flaxseed powder (g/100g)	B: Mango pulp (%)	C: Honey (%)	Taste	Colour	Overall Acceptability	Antioxidant Activity	Firmness (g)	Cohesiveness (gs)	Probiotic viable count (CFU/g)	Whey separation (%)	Desirability	
1	**2.25**	**5.25**	**4.30**	**8.43**	**8.26**	**8.35**	**85.04**	**245.78**	**36.69**	**9.38**	**5.12**	**0.81785**	**Selected**
2	2.32	4.41	5.32	8.35	8.04	8.21	83.04	244.55	35.64	9.19	5.29	0.80225	
3	1.78	4.32	5.44	7.59	7.15	7.57	77.58	238.39	33.20	9.54	5.54	0.75824	
4	2.60	4.15	5.65	8.67	8.64	7.34	79.43	239.93	34.38	8.75	5.29	0.73814	
5	1.35	4.22	5.86	7.46	7.07	7.57	79.97	235.51	31.75	8.85	6.03	0.68557	

Table 4.5 Constraints used for the formulation of flaxseed fortified synbiotic flavoured *dahi* by Minitab version 17.

Name	Goal	Lower Limit	Upper Limit	Weight	Importance
A:Flaxseed powder	is in range	1	3	1	1
B:Mango pulp	is in range	3	7	1	1
C:Honey	is in range	2	6	1	1
Taste	maximize	5.01	9.03	1	1
Colour	maximize	5.26	9.05	1	1
Overall Acceptability	maximize	5.04	9.00	1	1
Antioxidant activity (%)	maximize	63.85	85.75	1	1
Firmness (g)	maximize	223.64	259.45	1	1
Cohesiveness (g)	maximize	25.98	40.24	1	1
Probiotic Viable Count (CFU/g)	maximize	5.89	9.85	1	1
Whey Separation (%)	minimum	4.02	7.96	1	1

Table 4.6 Coefficient estimate for different predicted model for responses

Factor	***Coefficient Estimate***							
	Taste	**Colour**	**Overall Acceptability**	**Antioxidant Activity**	**Firmness (g)**	**Cohesiveness (gs)**	**Probiotic viable count (CFU/g)**	**Whey separation (%)**
Intercept	8.432	8.269	8.357	83.620	244.55	35.637	9.292	5.061
A:Flaxseed powder	-0.069	-0.1303	-0.0620	1.625	36.66	9.203	-0.866	0.2923
B:Mango pulp	0.940	0.6614	0.9333	2.346	0.67	1.616	1.523	0.5571
C:Honey	0.006	-0.0772	0.1687	0.413	1.82	0.762	-0.198	1.0945
AB	-0.257	-0.3700	-0.0650	-2.173	-6.52	-0.424	0.711	-0.0877
AC	-0.063	-0.0025	-0.1875	-0.918	-2.58	-0.676	0.303	-0.1125
BC	0.137	0.0275	0.2500	-0.564	-1.73	-0.205	0.290	-0.5703
A^2	-1.143	-0.7865	-1.2414	1.146	1.51	0.334	0.716	-0.262
B^2	-0.779	-0.8607	-0.8242	-0.204	-1.21	0.429	-0.071	0.080
C^2	-0.537	-0.1713	-0.3451	0.181	0.50	0.039	0.266	-0.370

The value of the adjusted determination coefficient (adjusted R^2) was also high to prove the high significance of the model (Khuri and Cornell, 1987). The predicted versus actual plot for the eight responses is also plotted. It shows that the actual values are distributed close to the straight line (y = x) with relatively high values of R^2 (Table 4.3).

The models adequacy was tested through lack of fit F-test (Montgomery, 1997). Adequate precision is a measure of the range in predicted response relative to its associated error or, in other words, a signal to noise ratio. Its desired value is 4 or more (Mason *et al.,* 2003). The value was found to be desirable for the all models. Simultaneously, low values of the coefficient of variation (CV) indicated good precision and reliability of the experiments as suggested by Ahmad *et al.,* (2005). Detail analysis on the models is presented in the following sections:

4.2 Effect of variables on sensory, textural properties and microbial characteristics of flaxseed fortified synbiotic flavoured *Dahi* (FFSFD)

Sensory analysis helps defining the product characteristics which are important with respect to customer acceptance of the product (Yaakob *et al.,* 2012). The sensory profiles of FFSFD are given in table 4.1, 4.3, 4.4, 4.5 and 4.6.

4.2.1 Effect on Taste

The regression equation obtained in terms of coded factors for the effect of variables, flaxseed powder (A), mango pulp (B) and honey (C) on taste score of flaxseed powder fortified synbiotic flavoured *dahi* could be described by the following equation:

$$\text{Taste} = 3.77 + 1.715\,A + 1.074\,B + 1.001\,C - 0.1270\,A^2 - 0.04868\,B^2 - 0.1342\,C^2 - 0.0215\,AB - 0.0104\,AC + 0.0172\,BC \quad \ldots (1)$$

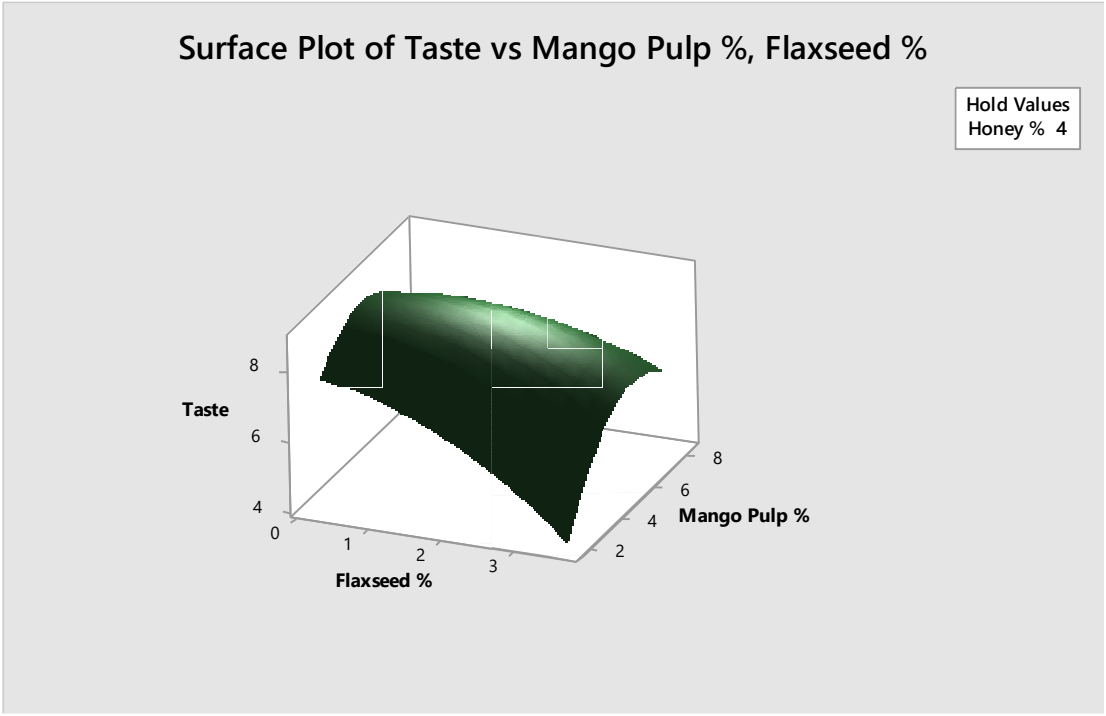
In table 4.1, the F-value was determined to examine the goodness of fit for the developed model. The F-value (22.57) for model of taste was significant ($p<0.0005$). The coefficient of determination (R^2) was 0.95 indicating that 95% of the variability in the response could be explained by the model. The "Pred. R-Squared" of 0.71 was in reasonable agreement with the "Adj R-Squared" of 0.91. A lack of fit value of 3.17 is found to be not significant. Hence, this model could be used to navigate the design space. The hedonic score for taste varied from 5.0 to 9.03 (Table 4.5).

The response surface plots for taste score presented in fig. 4.1 (a, b & c). The fig. 4.1 (a & b) clearly indicates that as the level of flaxseed powder increased from 0.31 to 2.0g, the taste score of *dahi* increased ($p<0.05$), but thereafter the taste score decreased. The fig. 4.1 (a) also showed that as the level of mango pulp increases (2 to 5%) in presence of flaxseed powder, the taste score increased. In the presence of mango pulp (fig 4.1 c), the taste score increased ($p<0.05$) when the level of honey increased from 2 to 4%.

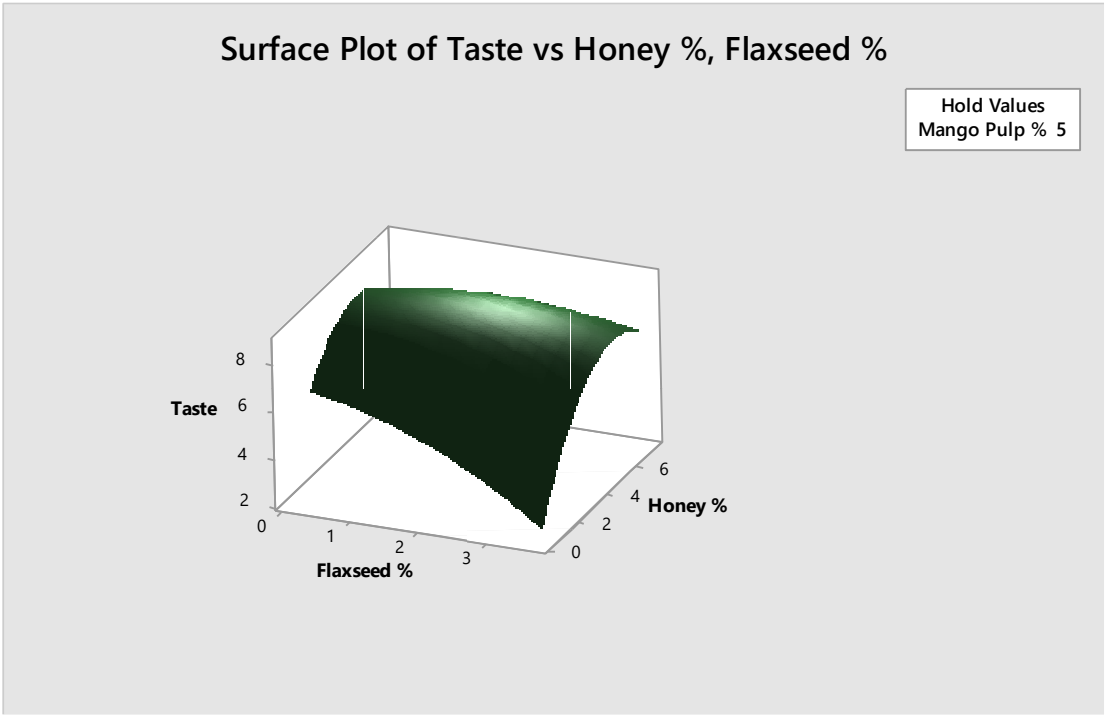
The coefficient estimate for the variables has been presented in Table 4.6. Variables i.e. flaxseed powder had negative effect, while mango pulp and honey had positive effect on taste of the *dahi* sample at linear level and all the variables had significant ($p < 0.01$) negative effect at quadratic level. Interactive effect of flaxseed powder with mango pulp and honey was slight negative.

Similar to the present findings, Raju and Pal, (2014) found significant effect of soy and oat fiber on flavour of yoghurt. Staffolo *et al.,* (2004) reported lower score in yogurt with apple fiber for flavour characteristics. The fruits added to flavored yogurt, significantly determines the taste (Tamime and Deeth, 1980). Hossain *et al.*, (2012) reported enhancement in taste and flavour characteristics of fruit fortified *dahi*. Ghadge *et al*., (2008) reported higher sensory scores for addition of various proportions of apple fruit pulp and honey to buffalo milk yoghurt.

a)

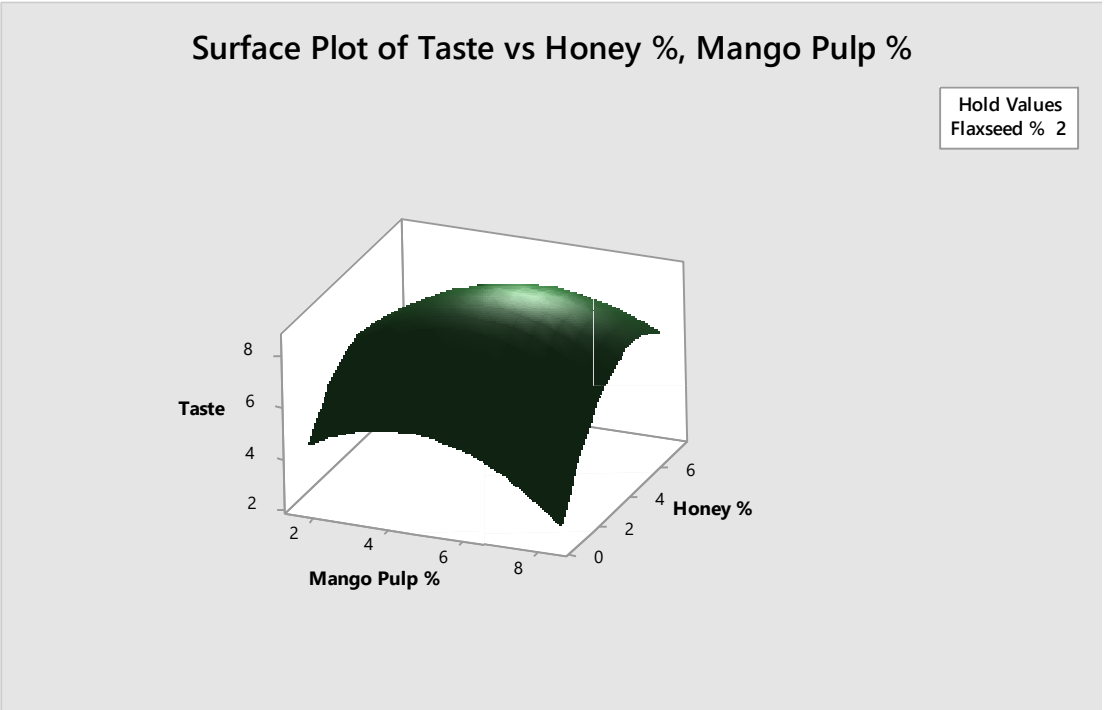

Surface Plot of Taste vs Mango Pulp %, Flaxseed %
Hold Values
Honey % 4
Taste
8
6
4
0
1
2
3
Flaxseed %
2
4
6
8
Mango Pulp %

b)


Surface Plot of Taste vs Honey %, Flaxseed %
Hold Values
Mango Pulp % 5
Taste
8
6
4
2
0
1
2
3
Flaxseed %
0
2
4
6
Honey %

Contd..

c)



d)

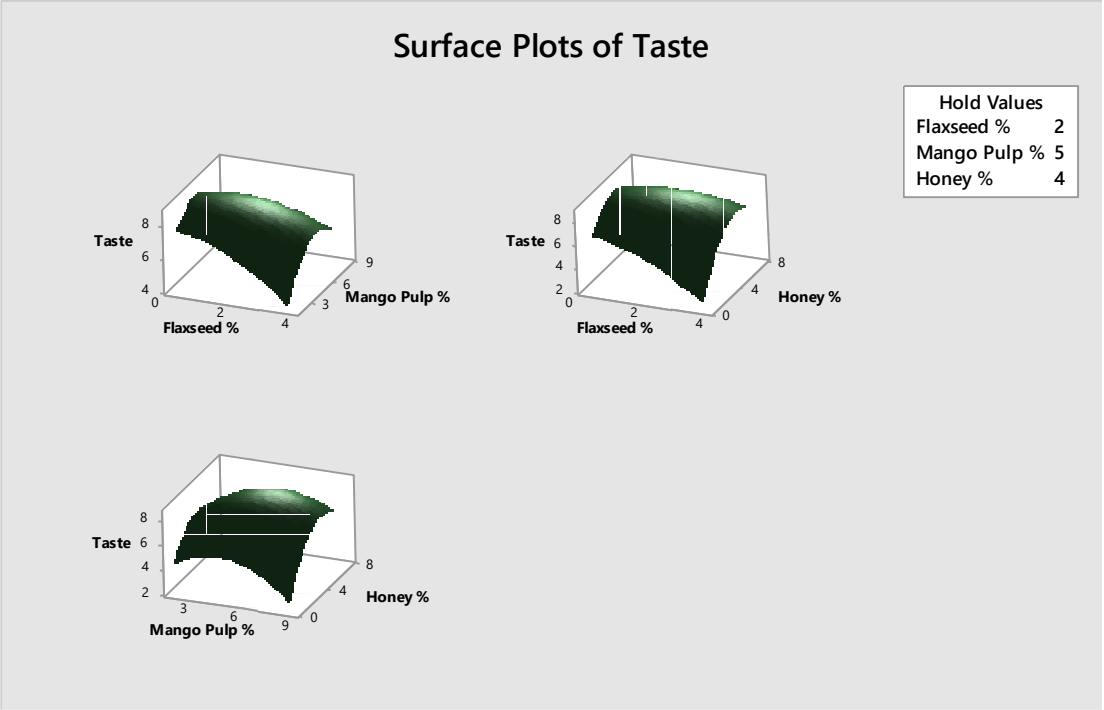
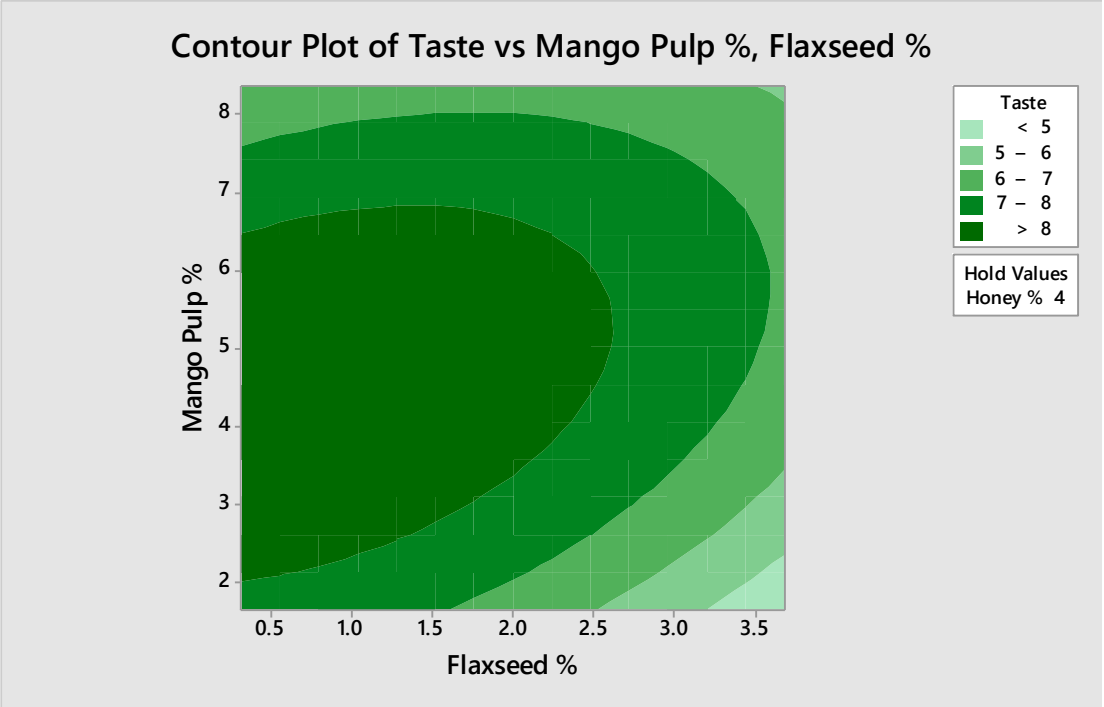
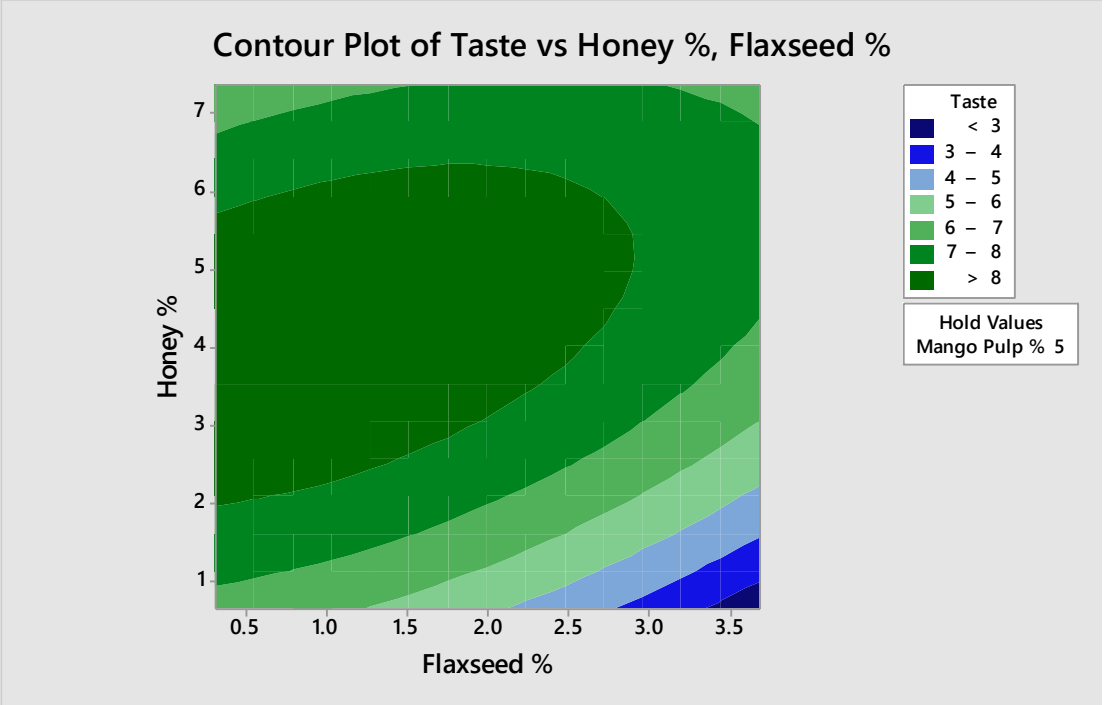


Figure 4.1 3-D plots representing the effect of flaxseed powder, mango pulp and honey on taste of FFSFD a) flaxseed powder and mango pulp b) flaxseed powder and honey c) mango pulp and honey d) surface plots of taste on the same panel.

a)

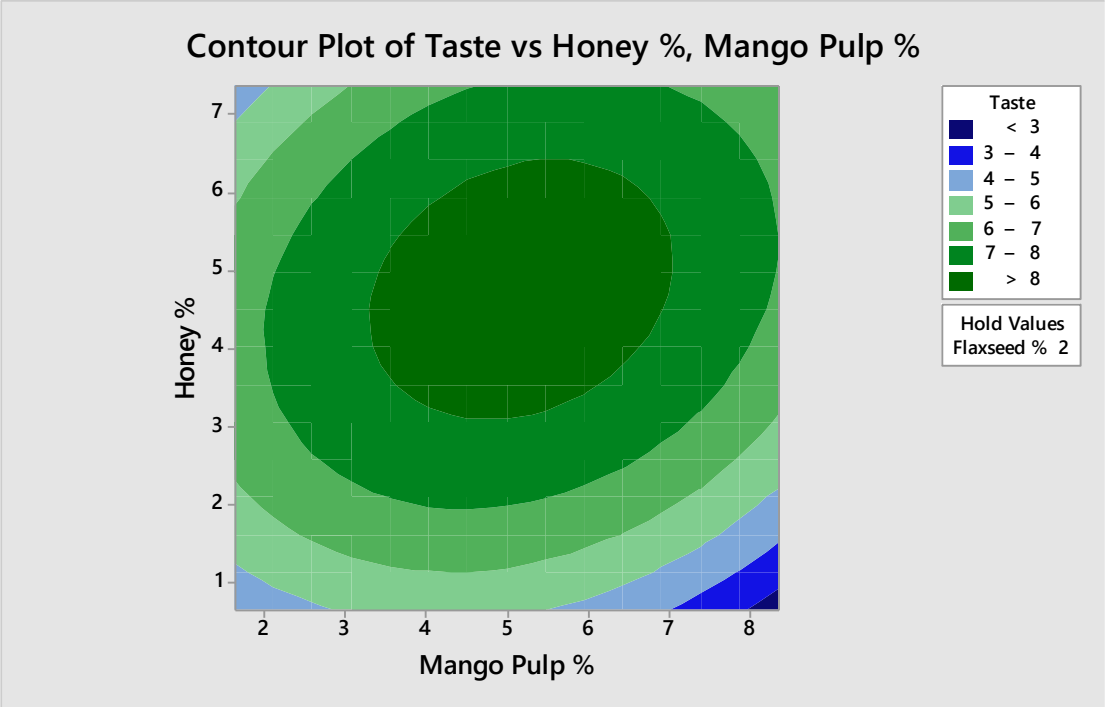

Contour Plot of Taste vs Mango Pulp %, Flaxseed %
Mango Pulp %
Flaxseed %
8
7
6
5
4
3
2
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Taste
< 5
5 – 6
6 – 7
7 – 8
> 8
Hold Values
Honey % 4

b)


Contour Plot of Taste vs Honey %, Flaxseed %
Honey %
Flaxseed %
7
6
5
4
3
2
1
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Taste
< 3
3 – 4
4 – 5
5 – 6
6 – 7
7 – 8
> 8
Hold Values
Mango Pulp % 5

Contd..

c)



d)

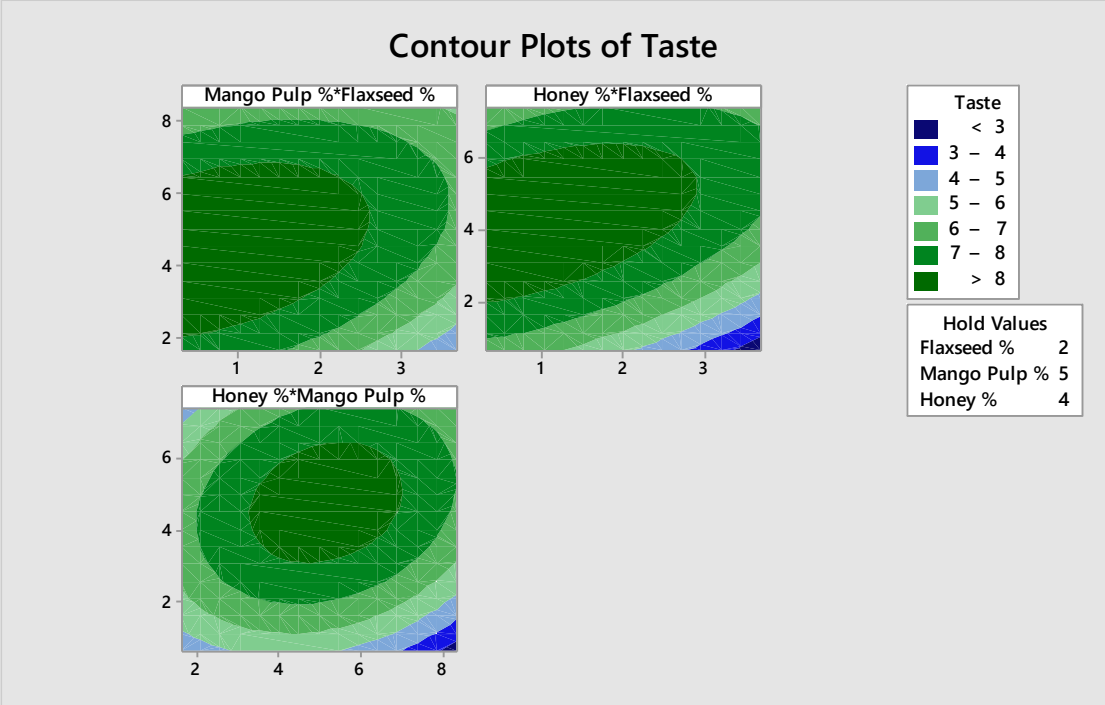


Figure 4.2 Contour plots representing the effect of flaxseed powder, mango pulp and honey on taste of FFSFD a) flaxseed powder, and mango pulp b) flaxseed powder and honey c) mango pulp and honey d) contour plots of taste on the same panel.

4.2.2 Effect on Colour

For colour score, a reduced quadratic model was selected to describe the variation of the response. The coefficient estimates for flaxseed fortified *dahi* showed that the quadratic model term for flaxseed (A^2) mango pulp (B^2) and honey (C^2) had significant effect ($P<0.0001$) on colour score (Table 4.6). The regression equation obtained in terms of coded factors for the effect of variables (flaxseed powder, mango pulp and honey) on colour score of flaxseed powder fortified synbiotic flavoured *dahi* could be described by the following equation given as:

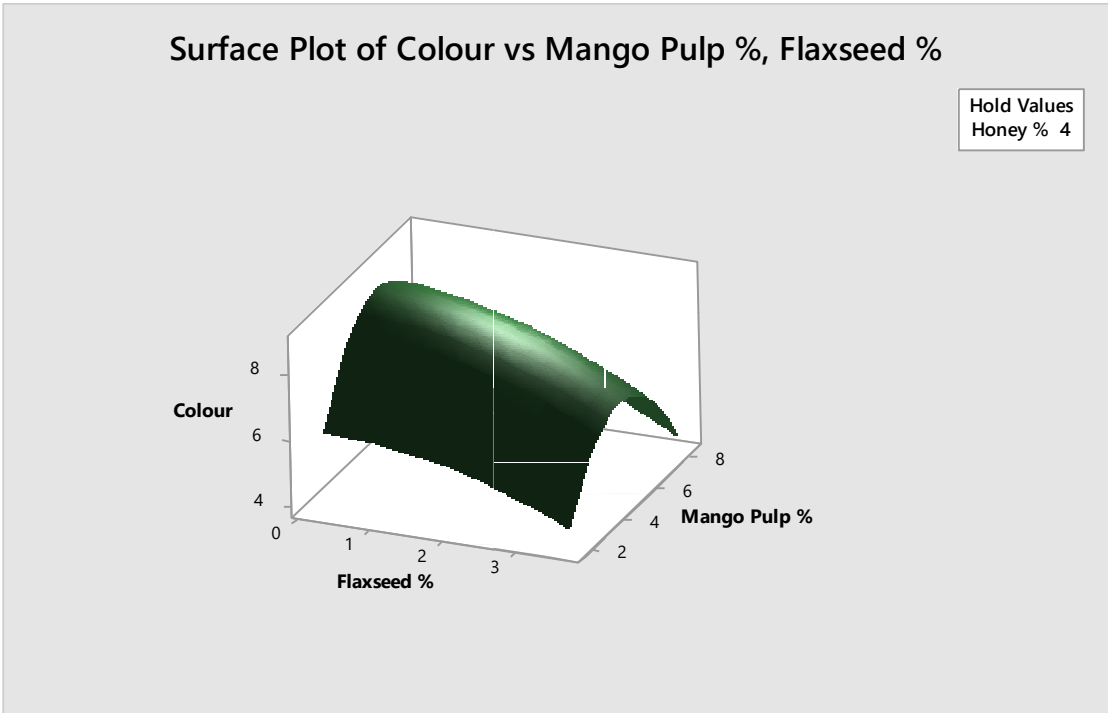
$$\text{Colour} = -1.821 + 1.340\,A + 1.225\,B + 0.334\,C - 0.08739\,A^2 - 0.05379\,B^2 - 0.0428\,C^2 - 0.03083\,AB - 0.0004\,AC - 0.0034\,BC \quad \text{…....(2)}$$

Here, A, B & C are coded terms for three variables i.e., flaxseed powder, mango pulp and honey, respectively.

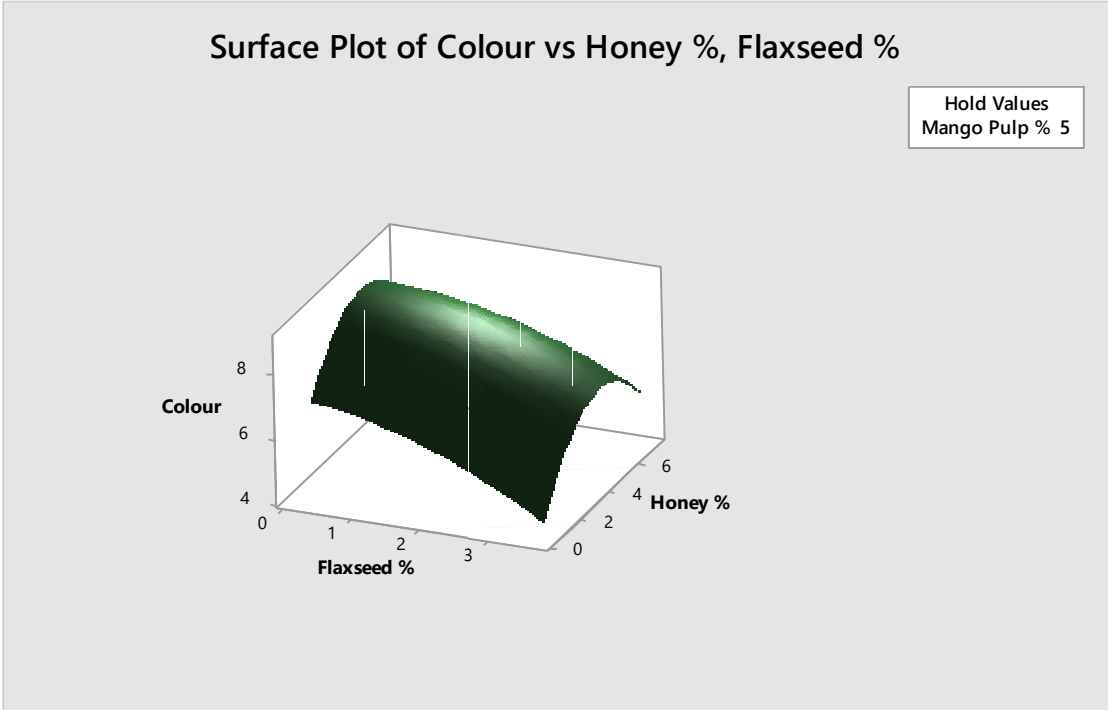
In table 4.3, the F-value was determined to examine the goodness of fit for the developed model. The F-value (35.76) for model of colour and appearance score was significant ($P<0.0001$). The coefficient of determination (R^2) was 0.969 indicating that 96.9% of the variability in the response could be explained by the model. The "Pred. R-Squared" of 0.76 was in reasonable agreement with the "Adj R-Squared" of 0.80. Hence, this model could be used to navigate the design space. The hedonic score for colour score was varied from 5.26 to 9.05 (Table 4.5).

The response surface plots for colour score was presented in fig. 4.3 (a, b & c). The values presented in table 4.1 depicts that as the level of flaxseed powder increased the colour score drastically decreased ($p<0.0001$). The fig. 4.2 (a) showed that as the level of mango pulp increases (3 to 5%) in the presence of flaxseed powder, the colour score increased gradually and thereafter decreased. The fig. 4.2 (b) showed that the interaction effect of flaxseed powder (0.31-2g) with different level of honey (2-4%) on colour score was non significant. The fig. 4.2 (c) clearly depicts that as the levels of flaxseed powder increased along with increasing level of honey (2 to 6%), the colour score was significantly decreased ($p<0.0001$).

a)

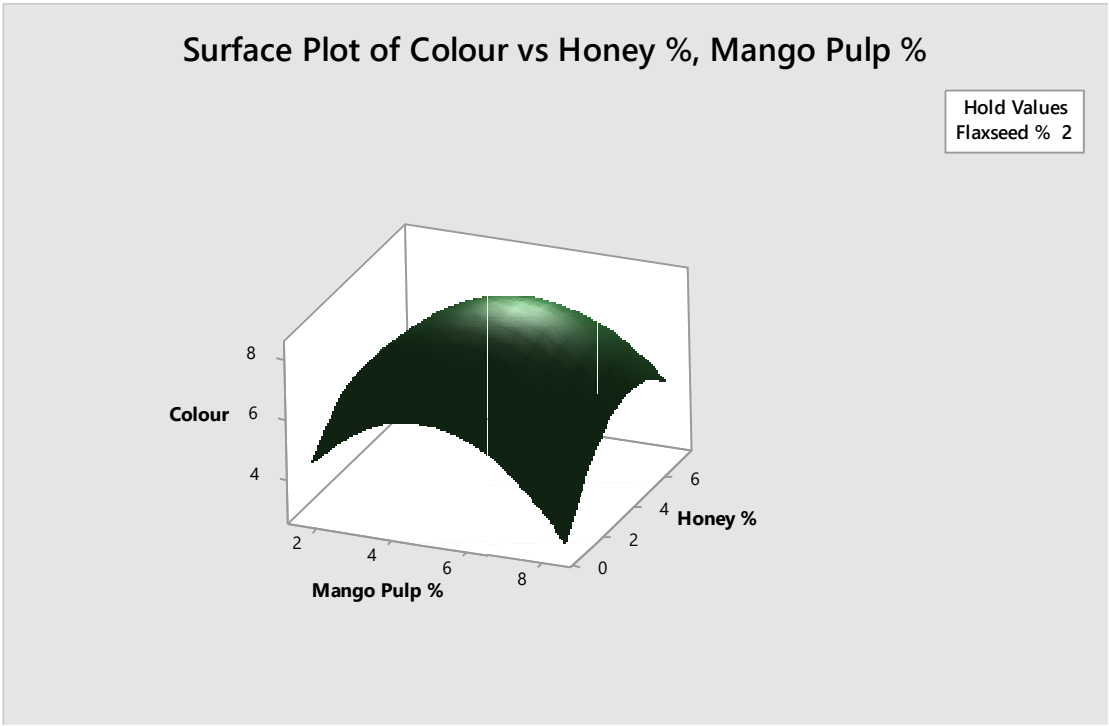

Surface Plot of Colour vs Mango Pulp %, Flaxseed %
Hold Values
Honey % 4
Colour
8
6
4
0
1
2
3
Flaxseed %
2
4
6
8
Mango Pulp %

b)


Surface Plot of Colour vs Honey %, Flaxseed %
Hold Values
Mango Pulp % 5
Colour
8
6
4
0
1
2
3
Flaxseed %
0
2
4
6
Honey %

Contd..

c)



d)

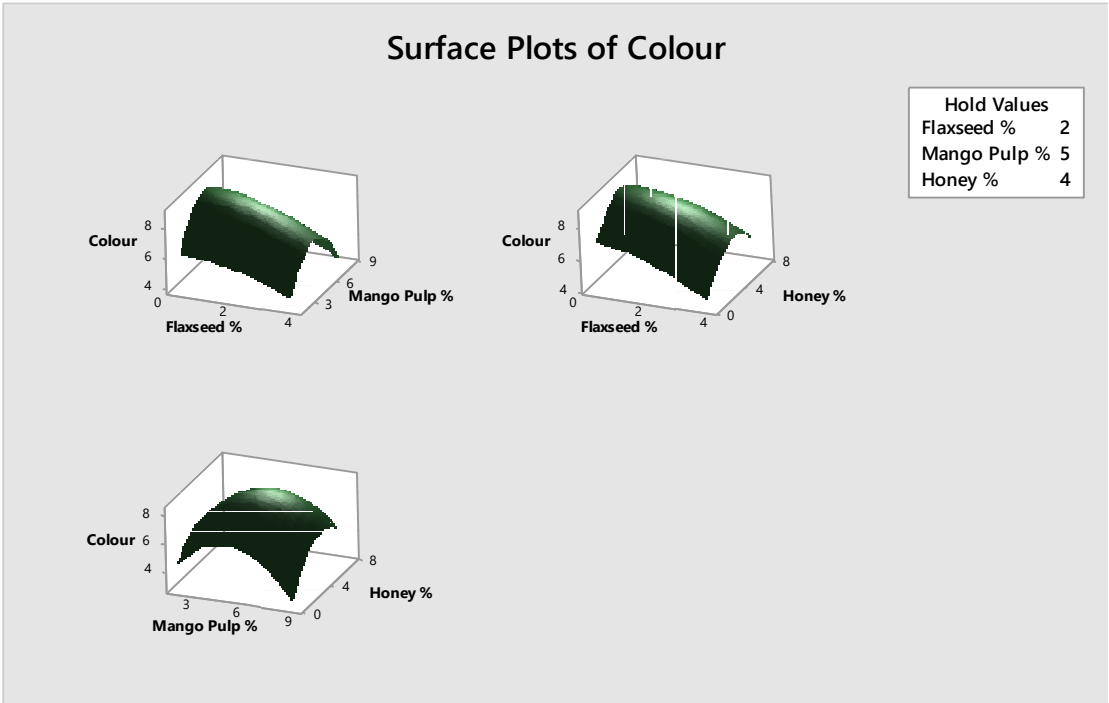
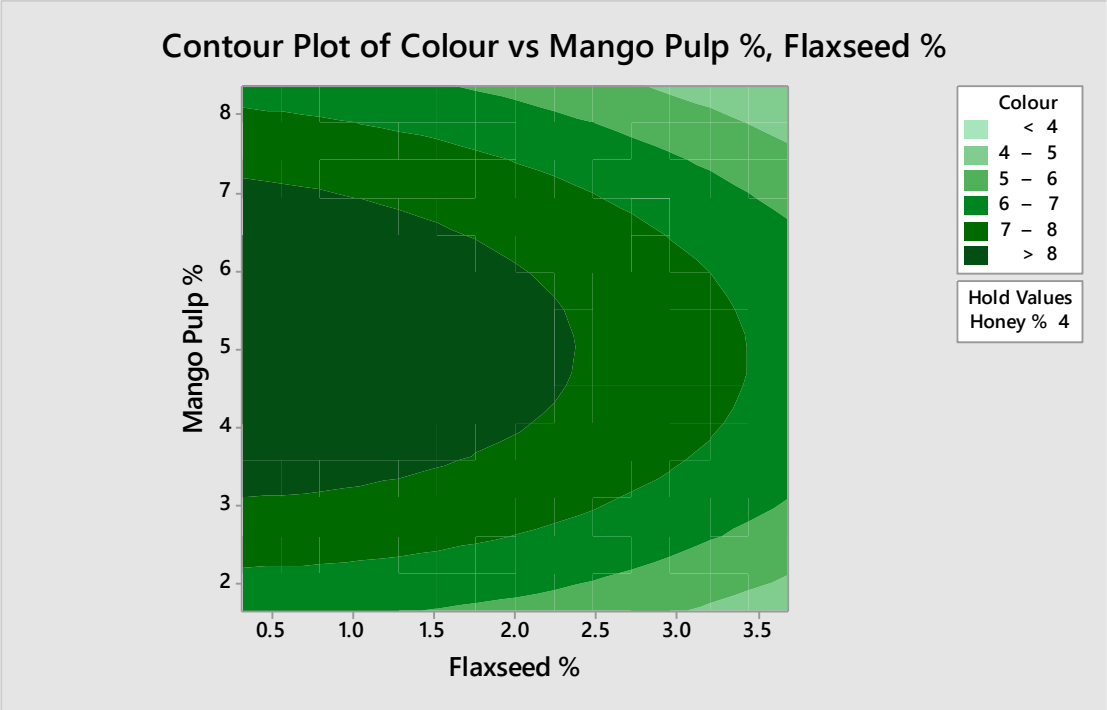
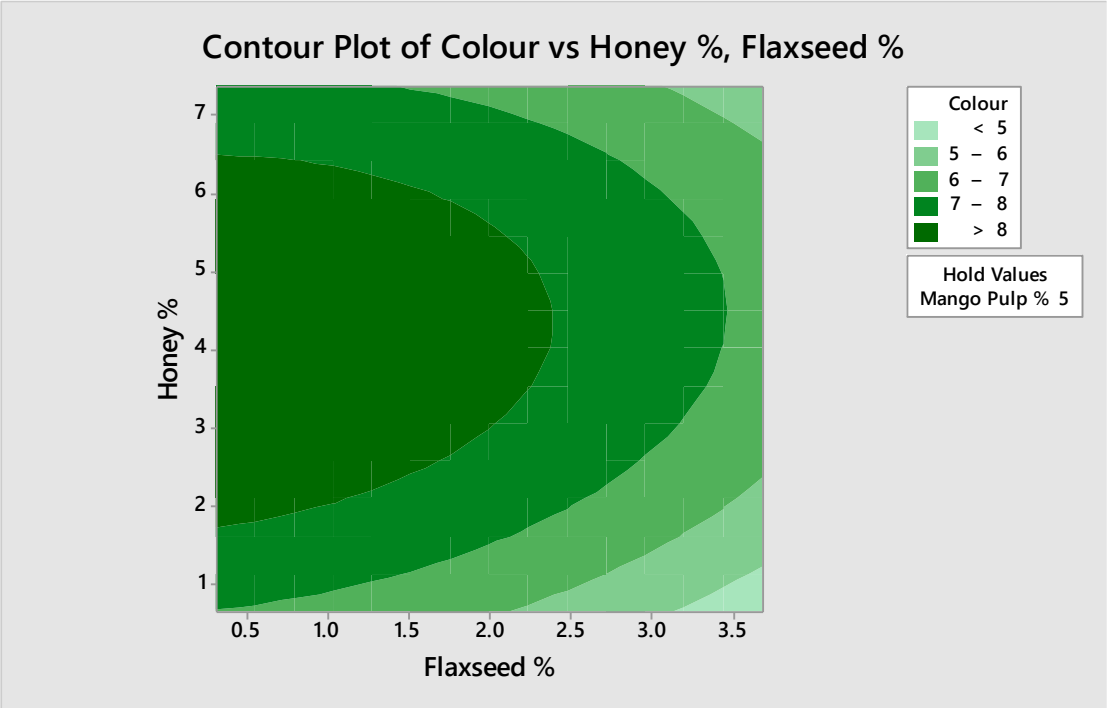


Figure 4.3 3-D plots representing the effect of flaxseed powder, mango pulp and honey on colour of FFSFD a) flaxseed powder and mango pulp b) flaxseed powder and honey c) mango pulp and honey d) surface plot of colour on the same panel.

a)

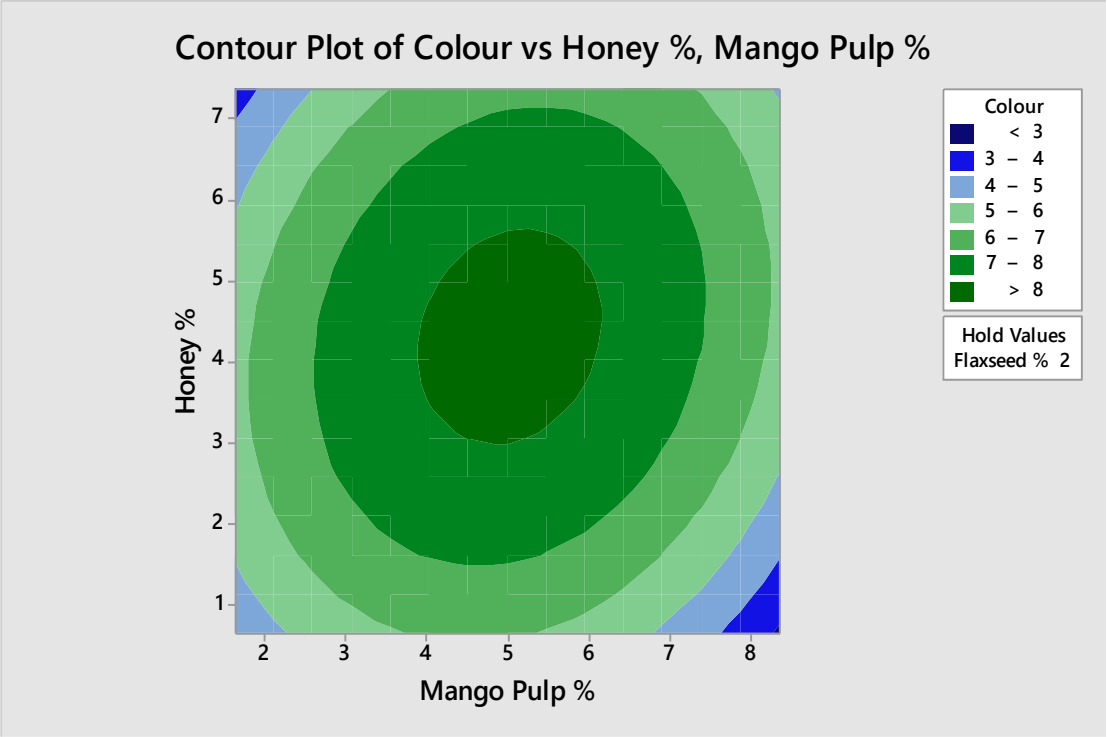

Contour Plot of Colour vs Mango Pulp %, Flaxseed %
Mango Pulp %
Flaxseed %
8
7
6
5
4
3
2
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Colour
< 4
4 – 5
5 – 6
6 – 7
7 – 8
> 8
Hold Values
Honey % 4

b)


Contour Plot of Colour vs Honey %, Flaxseed %
Honey %
Flaxseed %
7
6
5
4
3
2
1
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Colour
< 5
5 – 6
6 – 7
7 – 8
> 8
Hold Values
Mango Pulp % 5

Contd..

c)



d)

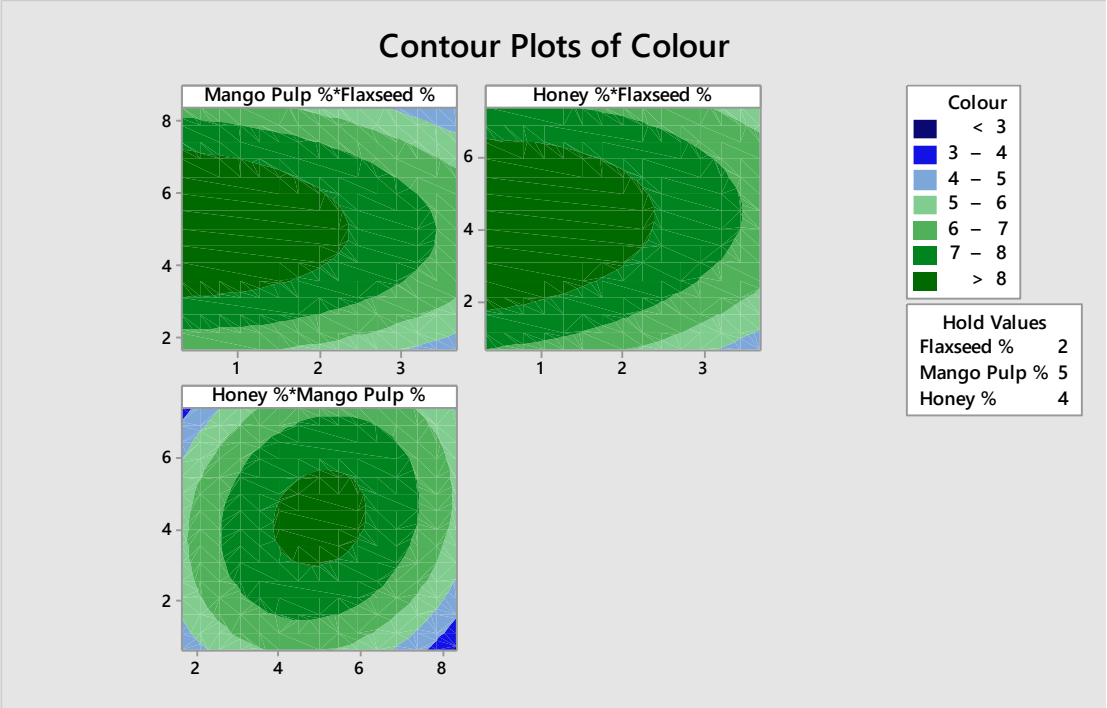


Figure 4.4 Contour plots representing the effect of flaxseed powder, mango pulp and honey on colour of FFSFD a) flaxseed powder, and mango pulp b) flaxseed powder and honey c) mango pulp and honey. d) contour plot of colour on the same panel.

The coefficient estimate for the variables has been presented in Table 4.9. Mango pulp had significant ($p< 0.01$) positive effect on colour of the *dahi* sample at linear level and flaxseed powder and honey had significant ($p< 0.01$) negative effect at quadratic level. Interactive effect of flaxseed powder with honey and flaxseed powder with honey was negative, while between mango pulp with honey, it was observed positive.

The colour attribute was enhanced by mango and honey while flaxseed powder had negative effect on colour of FFSFD.Results were in line with findings of Fernandez-Garcia and McGregor, (1997) who reported that addition of sugar beet fiber and rice fiber significantly lowered the appearance scores of sweetened plain yoghurt. Simlar findings were reported by Staffolo *et al.,* (2004); Ghadge *et al*., (2008) and Hossain *et al*., (2012) for colour characteristics of yoghurt. *Dahi* fortified with fiber and fruit pulp.

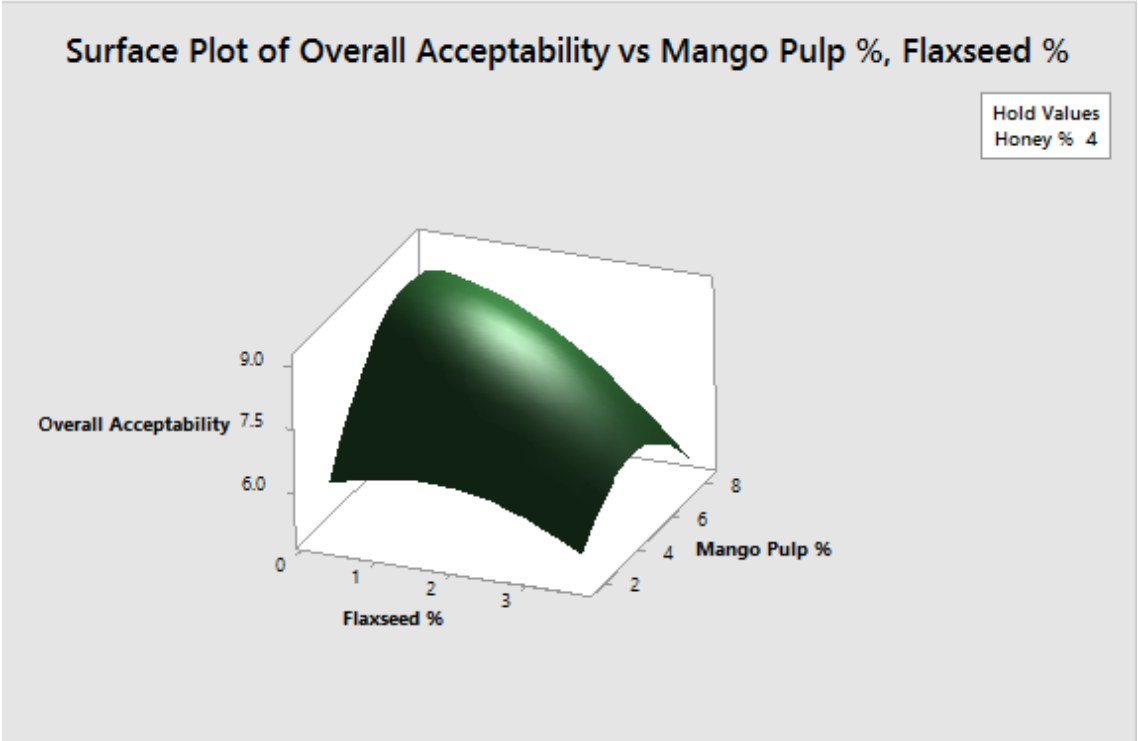
4.2.3 Effect on Overall acceptability

The average overall acceptability score of flaxseed powder fortified synbiotic flavoured *dahi* varied from 5.04 to 9.0 (Table 4.5). The coefficient estimates (Table 4.6) of overall acceptability showed that the linear model terms (A, B & C), quadratic interactive model terms (AB, AC and BC) and quadratic model terms (A^2, B^2 & C^2) had significant effect ($P<0.0001$) on overall acceptability. The quadratic equation obtained by the RSM of the data showing the effect of flaxseed powder, mango pulp and honey on the overall acceptability score of FFSFD could be described by the following equation given as:

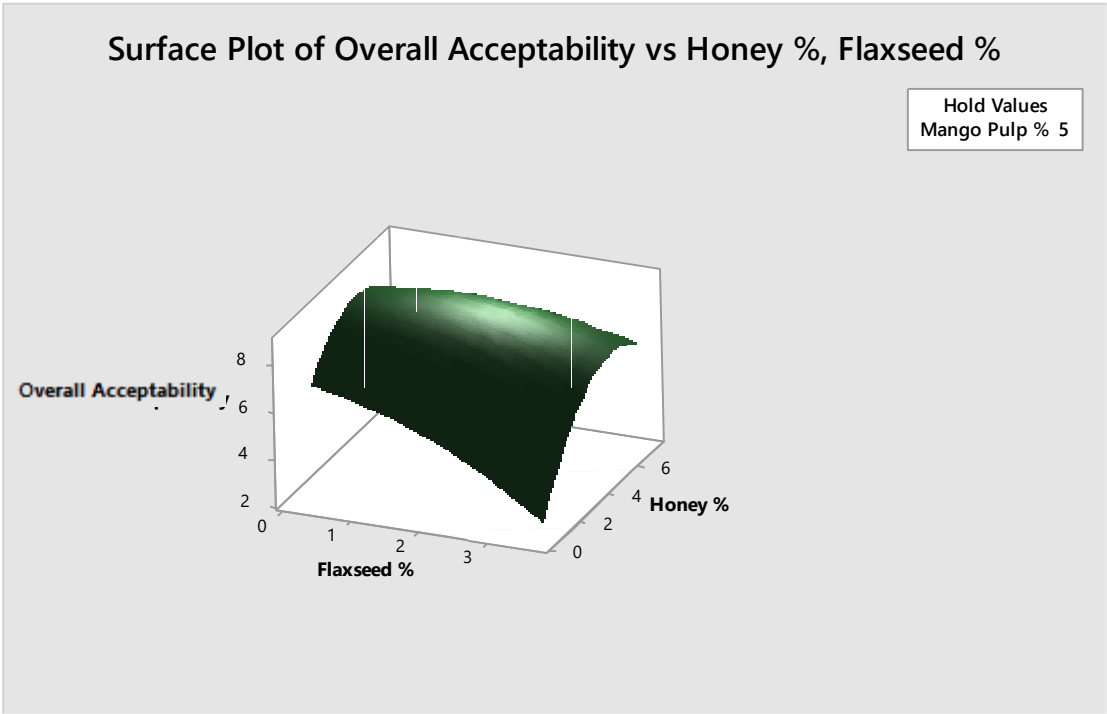
$$\text{Overall Acceptability} = -3.16 + 1.80\,A + 0.96\,B + 0.71\,C - 0.13\,A^2 - 0.05\,B^2 - 0.0863\,C^2 - 0.0054\,AB - 0.0313\,AC + 0.0312\,BC \quad .....(3)$$

Here, A, B & C are coded terms for the three variables, i.e. flaxseed powder, mango pulp and honey, respectively.

a)

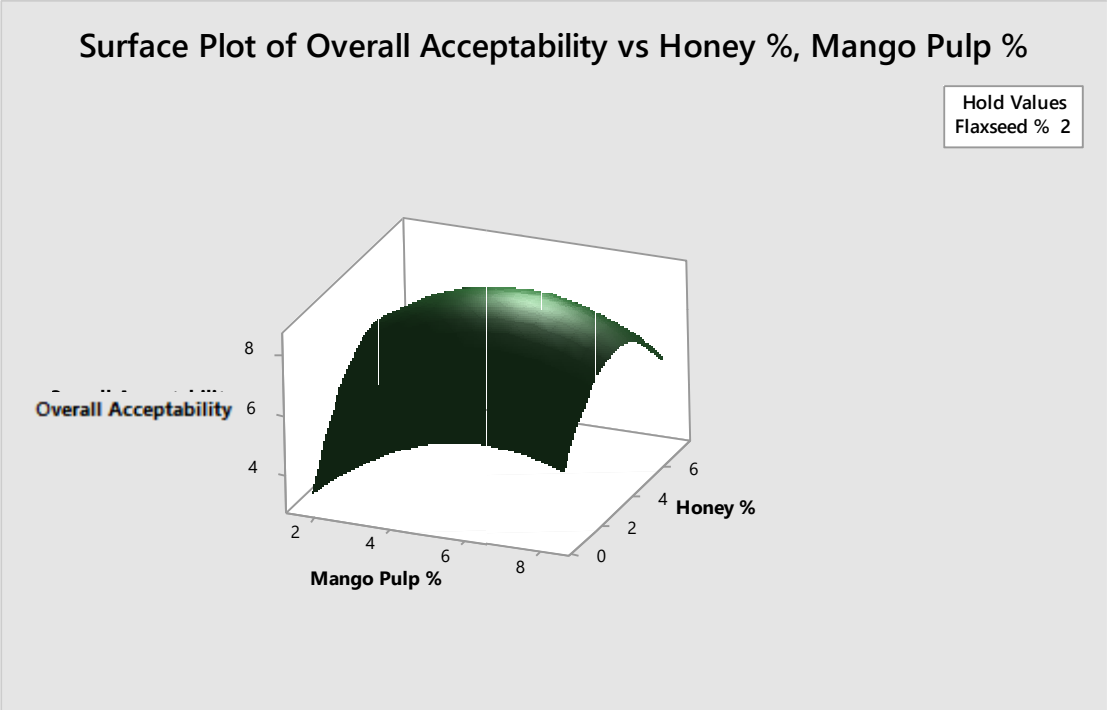

Surface Plot of Overall Acceptability vs Mango Pulp %, Flaxseed %
Hold Values
Honey % 4
Overall Acceptability
9.0
7.5
6.0
0
1
2
3
Flaxseed %
2
4
6
8
Mango Pulp %

b)


Surface Plot of Overall Acceptability vs Honey %, Flaxseed %
Hold Values
Mango Pulp % 5
Overall Acceptability
8
6
4
2
0
1
2
3
Flaxseed %
0
2
4
6
Honey %

Contd..

c)



d)

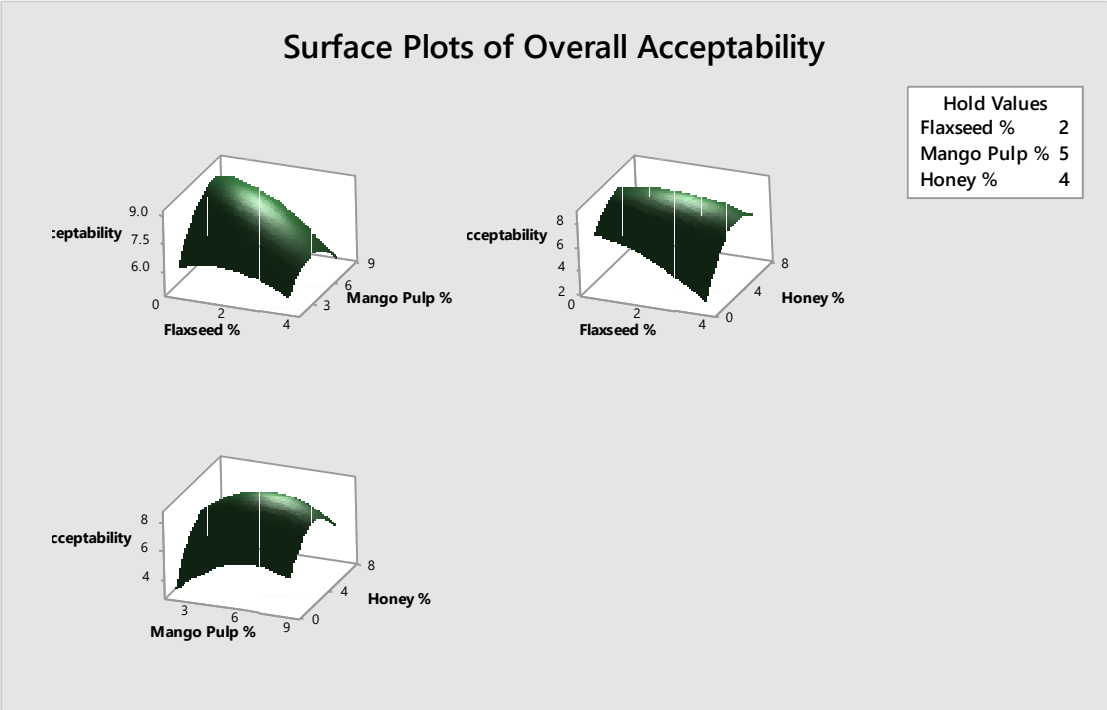
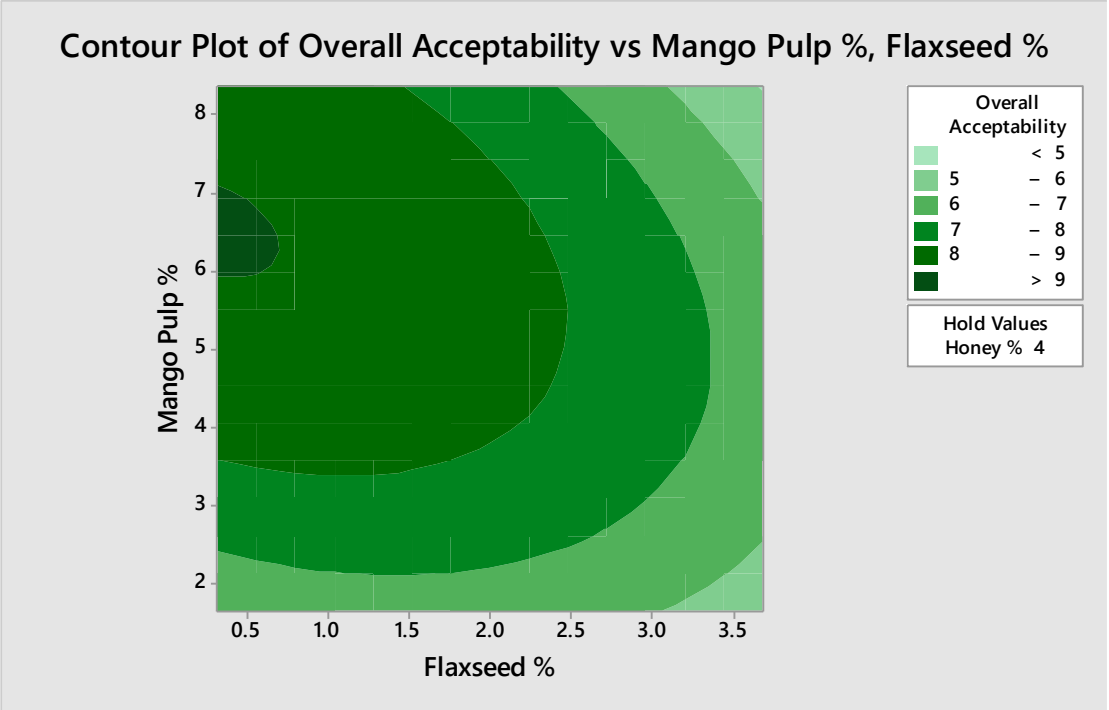
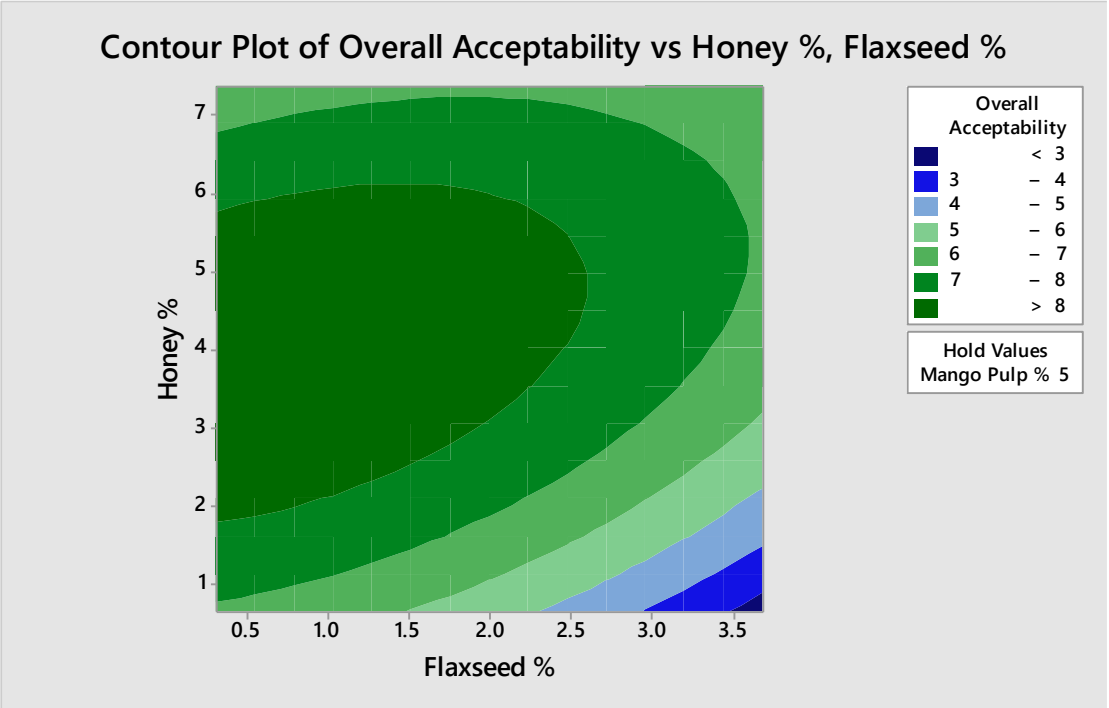


Figure 4.5 3-D plots representing the effect of flaxseed powder, mango pulp and honey on overall acceptability of FFSFD a) flaxseed powder and mango pulp b) flaxseed powder and honey c) mango pulp and honey d) surface plot of overall acceptability on the same panel.

a)

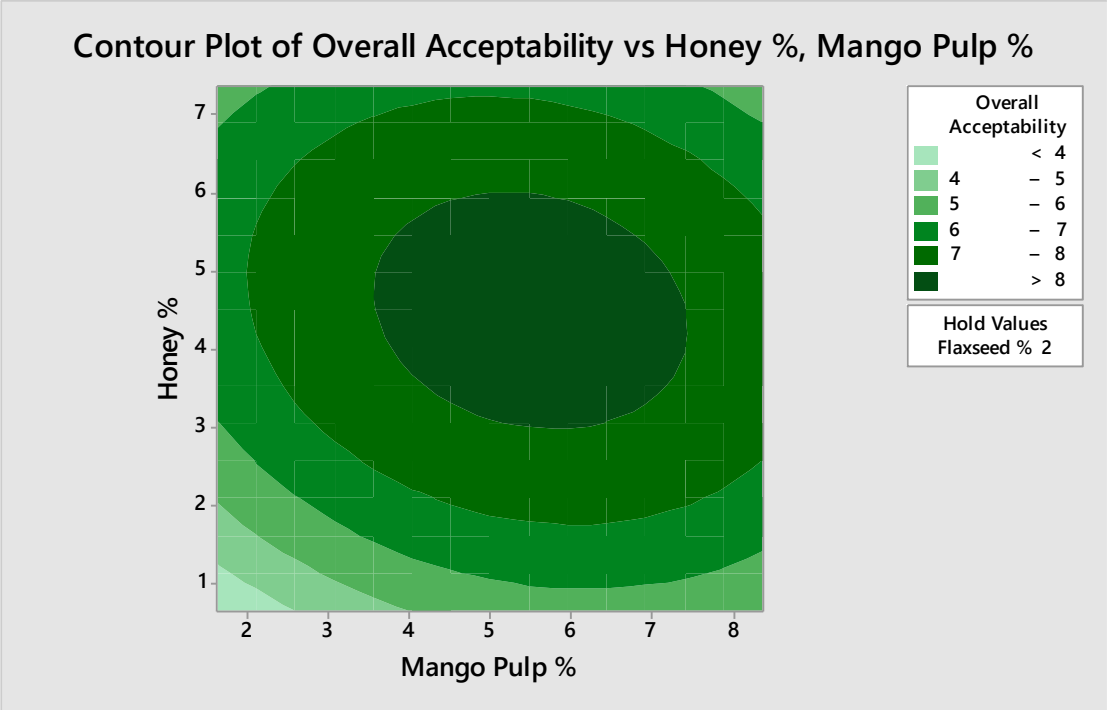

Contour Plot of Overall Acceptability vs Mango Pulp %, Flaxseed %
Mango Pulp %
Flaxseed %
8
7
6
5
4
3
2
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Overall Acceptability
< 5
5 – 6
6 – 7
7 – 8
8 – 9
> 9
Hold Values
Honey % 4

b)


Contour Plot of Overall Acceptability vs Honey %, Flaxseed %
Honey %
Flaxseed %
7
6
5
4
3
2
1
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Overall Acceptability
< 3
3 – 4
4 – 5
5 – 6
6 – 7
7 – 8
> 8
Hold Values
Mango Pulp % 5

Contd..

c)



d)

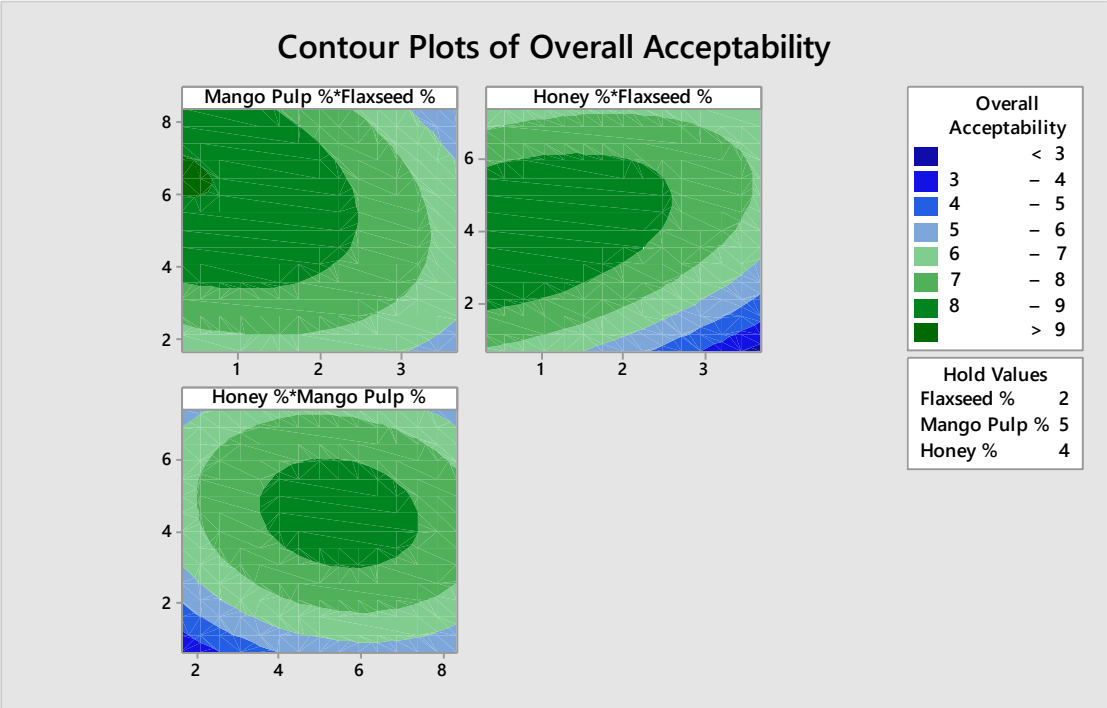


Figure 4.6 Contour plots representing the effect of flaxseed powder, mango pulp and honey on overall acceptability of FFSFD a) flaxseed powder, and mango pulp b) flaxseed powder and honey c) mango pulp and honey. d) contour plot of overall acceptability on the same panel.

In the table 4.2, the F-value was determined to examine the goodness of fit for the developed model. The F-value (71.65) for the model of overall acceptability was significant (P<0.0001). The coefficient of determination (R^2) was 0.9847 indicating that 98.47% of the variability in the response could be explained by the model. The "Pred. R-Squared" of 0.89 was in reasonable agreement with the "Adj R-Squared" of 0.94.

The response surface plots for overall acceptability score are presented in fig. 4.5 (a, b, c & d). In order to gain a better understanding of the interaction effects of variables on overall acceptability score of flaxseed fortified *dahi*, two dimensional contour plots for the measured response were formed based on the model (Eq. 3). The fig. 4.6 (a, b, c & d) shows the plots of the model for variation in overall acceptability score of flaxseed fortified *dahi* as a function of flaxseed powder, mango pulp and honey. It is clear from the fig. 4.6 (a, b, c & d) and Eq. (5) that the most significant factor on the response is the first-order interaction effects between flaxseed & mango pulp and flaxseed & honey. Similar to the present findings, Staffolo *et al.,* (2004); Ghadge *et al*., (2008) Imele and Atemnkeng, (2001); Kumar and Mishra, (2004); Mishra and Mishra, (2014); found significant effect of various dairy products on overall acceptability score.

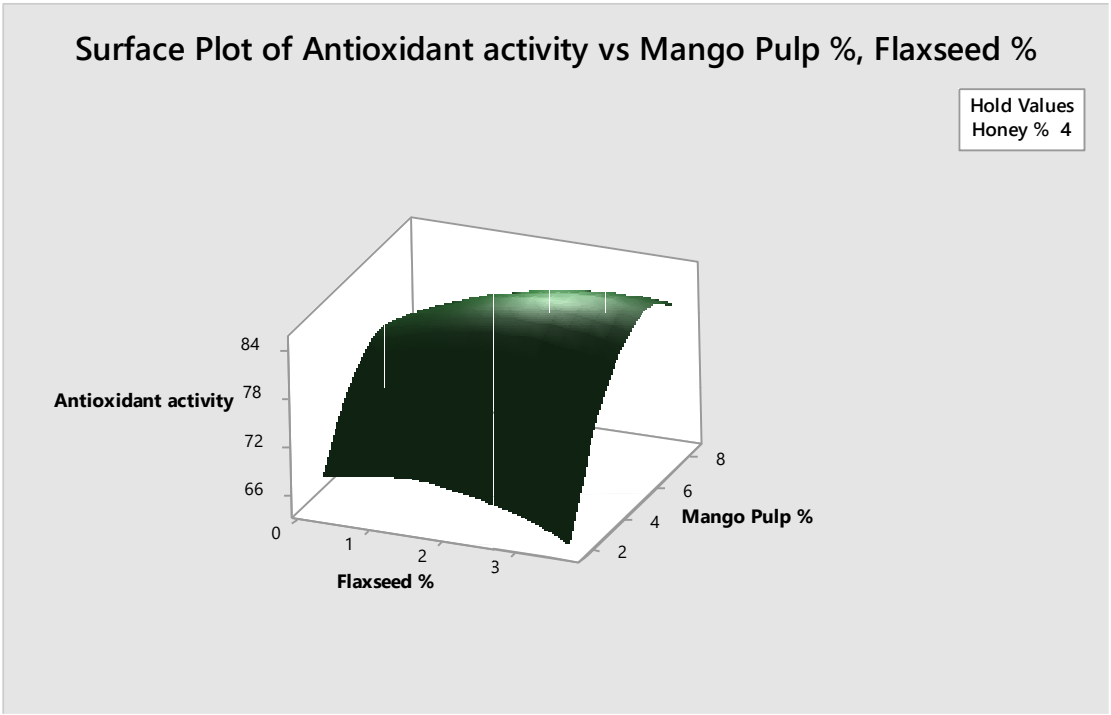
4.2.4 Effect on Antioxidant activity

Effect of flaxseed powder (A), mango pulp (B) and honey (C) on the antioxidant activity (DPPH) of FFSFD could be described by the following equation:

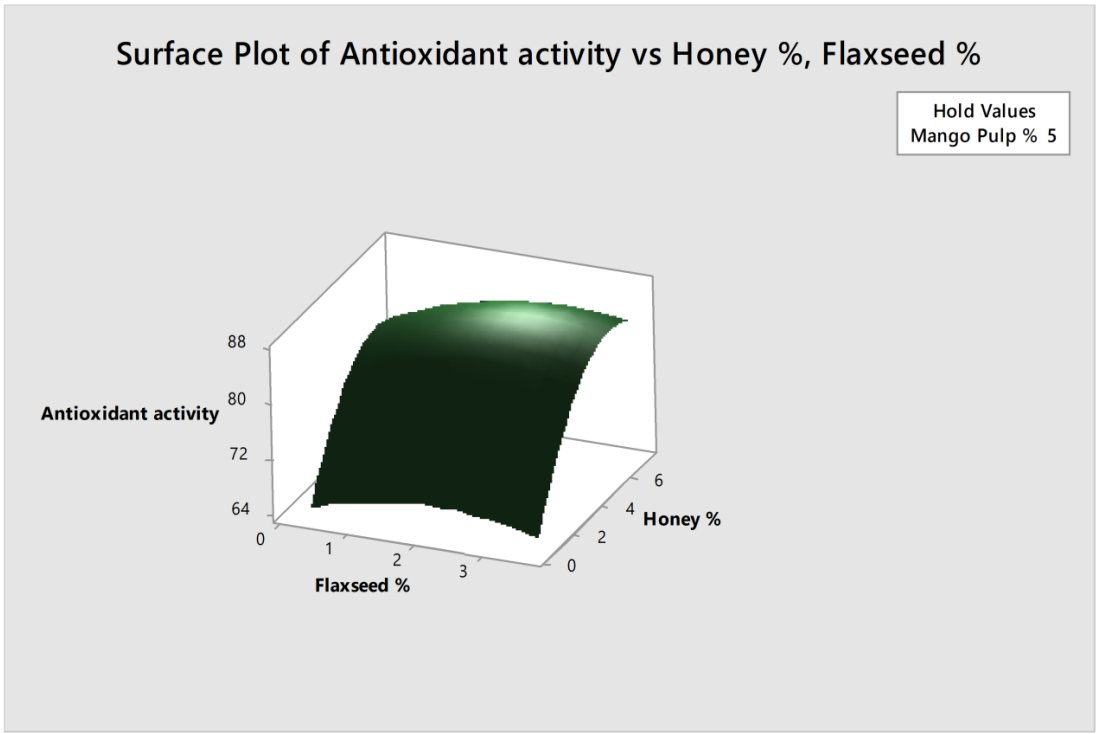
$$\text{Antioxidant activity} = 64.77 + 2.811A + 0.841B + 1.358C - 0.2414A^2 - 0.0574B^2 - 0.1411C^2 + 0.0955AB - 0.0340AC + 0.0227BC \quad \ldots\ldots\ldots\ldots (4)$$

In table 4.3, the F-value (31.94) for the model of antioxidant activity was significant (p<0.0001). The coefficient of determination (R^2) was 0.966 indicating that 96.6% of the variability in the response could be explained by the model. The "Pred. R-Squared" of 0.83 was in reasonable agreement with the "Adj. R-Squared" of 0.93. A lack of fit value of 1.19 is found to be not significant. Hence, this model could be used to navigate the design space.

a)

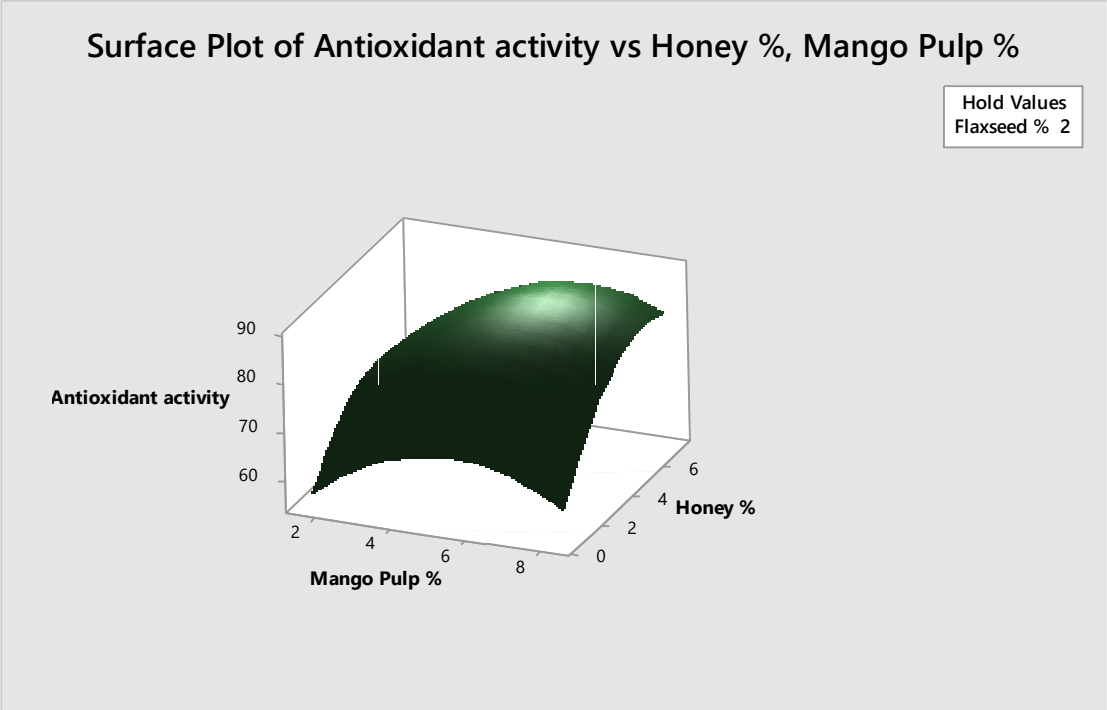

Surface Plot of Antioxidant activity vs Mango Pulp %, Flaxseed %
Hold Values
Honey % 4
Antioxidant activity
84
78
72
66
0
1
2
3
Flaxseed %
2
4
6
8
Mango Pulp %

b)


Surface Plot of Antioxidant activity vs Honey %, Flaxseed %
Hold Values
Mango Pulp % 5
Antioxidant activity
88
80
72
64
0
1
2
3
Flaxseed %
0
2
4
6
Honey %

Contd..

c)



d)

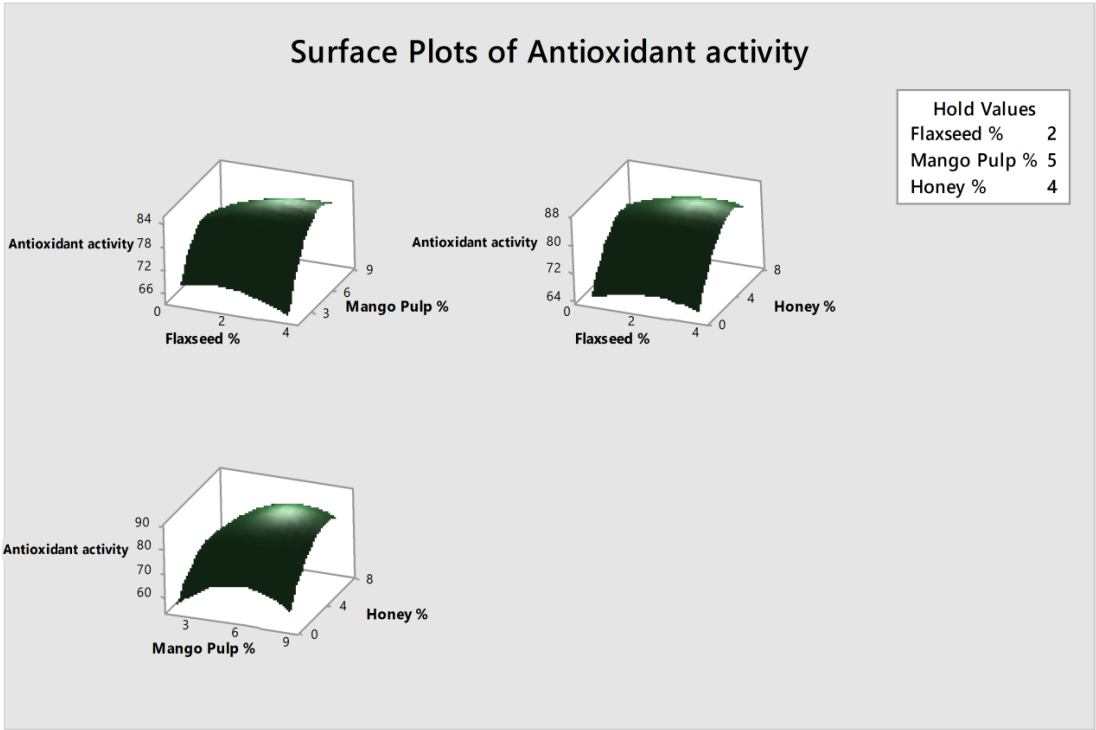
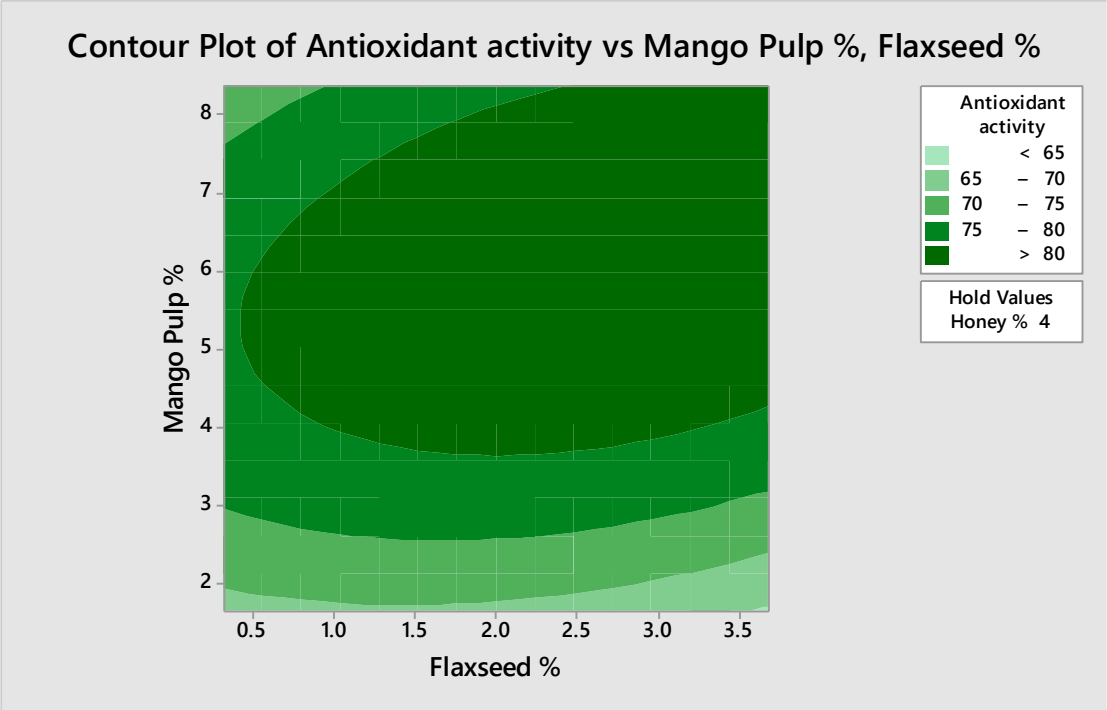
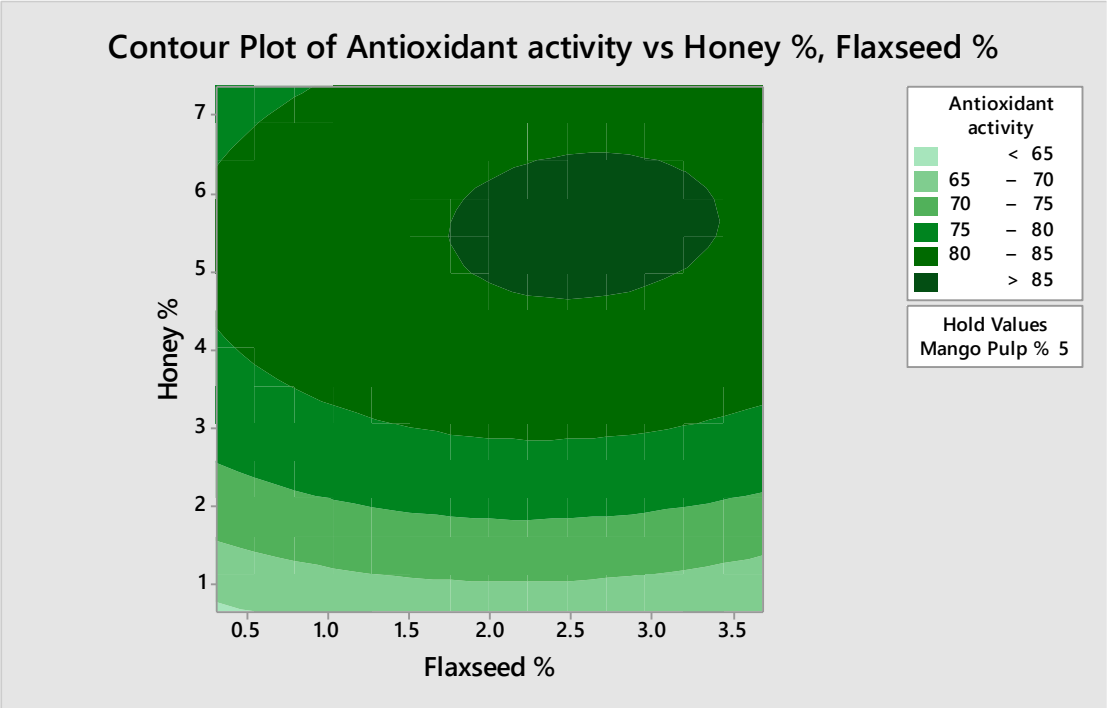


Figure 4.7 3-D plots representing the effect of flaxseed powder, mango pulp and honey on antioxidant activity of FFSFD a) flaxseed powder and mango pulp b) flaxseed powder and honey c) mango pulp and honey d) surface plot of antioxidant activity on the same panel.

a)

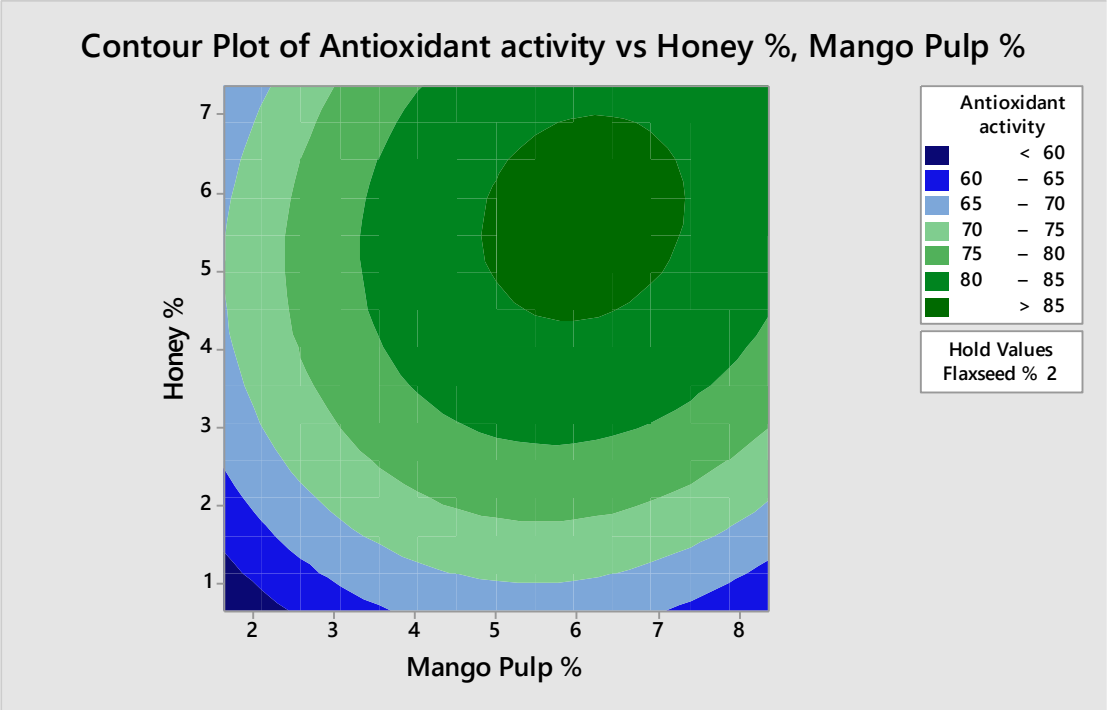

Contour Plot of Antioxidant activity vs Mango Pulp %, Flaxseed %
Mango Pulp %
Flaxseed %
8
7
6
5
4
3
2
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Antioxidant activity
< 65
65 – 70
70 – 75
75 – 80
> 80
Hold Values
Honey % 4

b)


Contour Plot of Antioxidant activity vs Honey %, Flaxseed %
Honey %
Flaxseed %
7
6
5
4
3
2
1
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Antioxidant activity
< 65
65 – 70
70 – 75
75 – 80
80 – 85
> 85
Hold Values
Mango Pulp % 5

Contd..

c)



d)

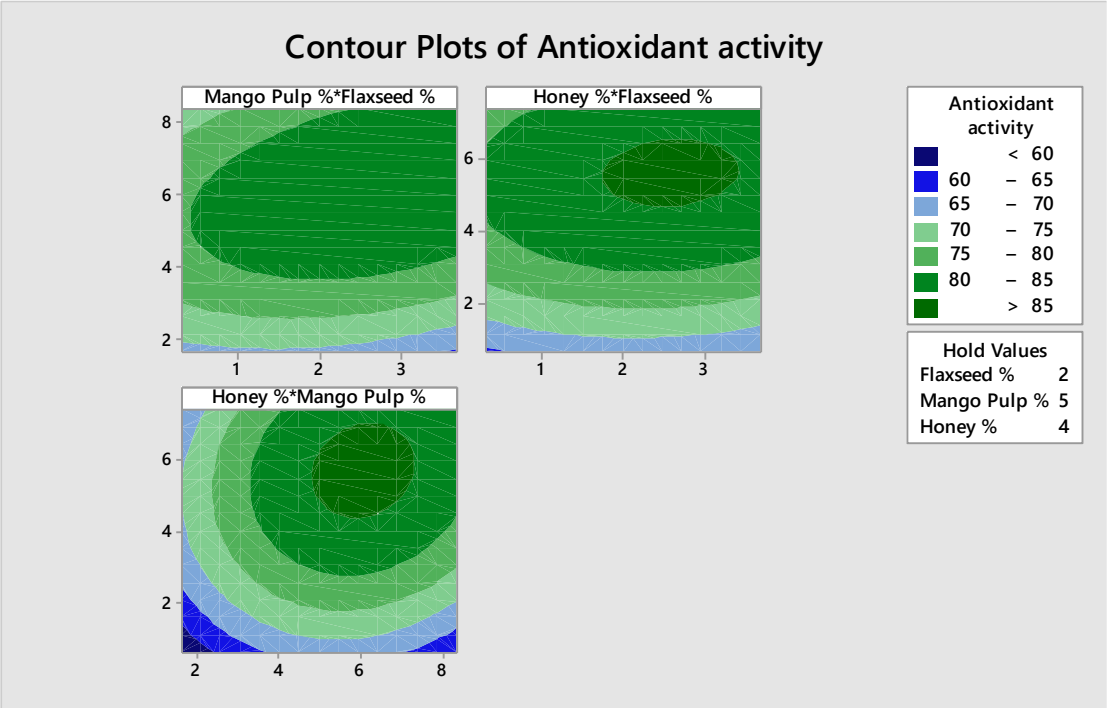


Figure 4.8 Contour plots representing the effect of flaxseed powder, mango pulp and honey on antioxidant activity of FFSFD a) flaxseed powder, and mango pulp b) flaxseed powder and honey c) mango pulp and honey. d) contour plot of antioxidant activity on the same panel.

The average of antioxidant activity of flaxseed powder fortified synbiotic flavoured *dahi* varied from 63.85% to 85.75% (Table 4.5). The highest antioxidant activity was recorded 85.75 at 3.0g, 7% and 6% of flaxseed powder, mango pulp and honey, respectively (table 4.1). The response surface plots for antioxidant activity are presented in fig. 4.7 (a, b & c). The antioxidant activity increased ($p<0.0001$) with increasing the level of flaxseed powder (1 to 3g), mango pulp (3 to 7%) and honey level from 2 to 6%.

All the three variables i.e. flaxseed powder , mango pulp and honey had significant ($P < 0.0001$) positive effect on antioxidant activity of *dahi* sample at linear level and flaxseed powder and mango pulp had positive effect while honey had significant ($P < 0.0001$) negative effect at quadratic level. Interactive effect ($P < 0.0005$) of flaxseed powder with mango pulp and flaxseed powder with honey was positive, while between mango pulp and honey, it was observed negative.

Eliana *et al.,* (2013) reported that yoghurt fortified with mango pulp had high antioxidant activity (42.47%). Similar finding was reported by Madhu *et al.,* (2012) who showed that the antioxidant activity (85%) in synbiotic yoghurt containing *L. plantarum* and fructooligosaccharide was significantly higher in comparison with that of control yoghurt (72 %).

4.2.5 Effect on Firmness

Effect of flaxseed powder (A), mango pulp (B) and honey (C) on the firmness of FFSFD could be described by the following equation:

$$\text{Firmness(g)} = 128.0 + 20.71A + 1.74B - 5.08C - 0.724A^2 - 0.161B^2 + 0.43C^2 + 0.126\,AB - 0.201AC - 0.063BC \quad \text{…….(5)}$$

In table 4.3, the F-value (47.61) for the model of firmness was significant ($p<0.0001$). The coefficient of determination (R^2) was 0.977 indicating that 97.7% of the variability in the response could be explained by the model. The "Pred. R-Squared" of 0.85 was in reasonable agreement with the "Adj. R-Squared" of 0.95. A lack of fit value of 3.41 is found to be not significant. Hence, this model could be used

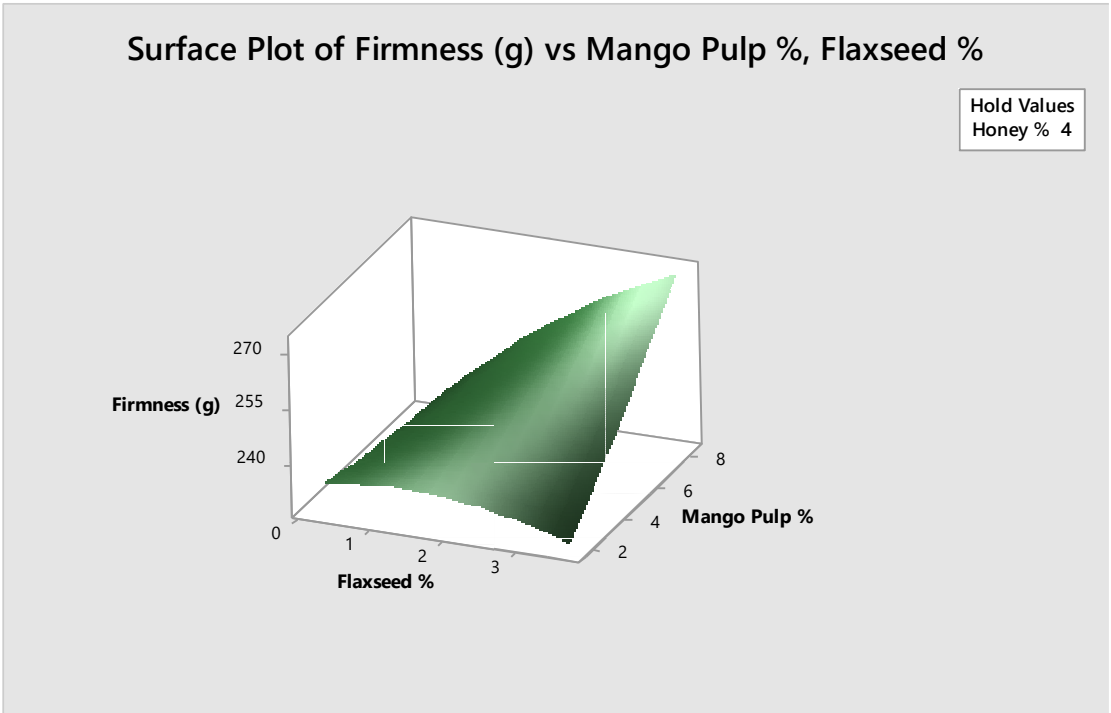
to navigate the design space. The firmness of *dahi* varied from 223.64 to 259.45g (Table 4.5).

The highest firmness was recorded 259.45g at 3.0g, 7% and 2% of flaxseed powder, mango pulp and honey, respectively (table 4.1). The response surface plots for overall acceptability are presented in fig. 4.9 (a, b, c & d). The firmness increased ($p<0.0001$) with increasing the level of flaxseed powder (0.31 to 3.68g) and mango pulp yoghurt culture (3 to 7%), while slight decrease in firmness value was observed with increased level of honey from 2 to 6%.

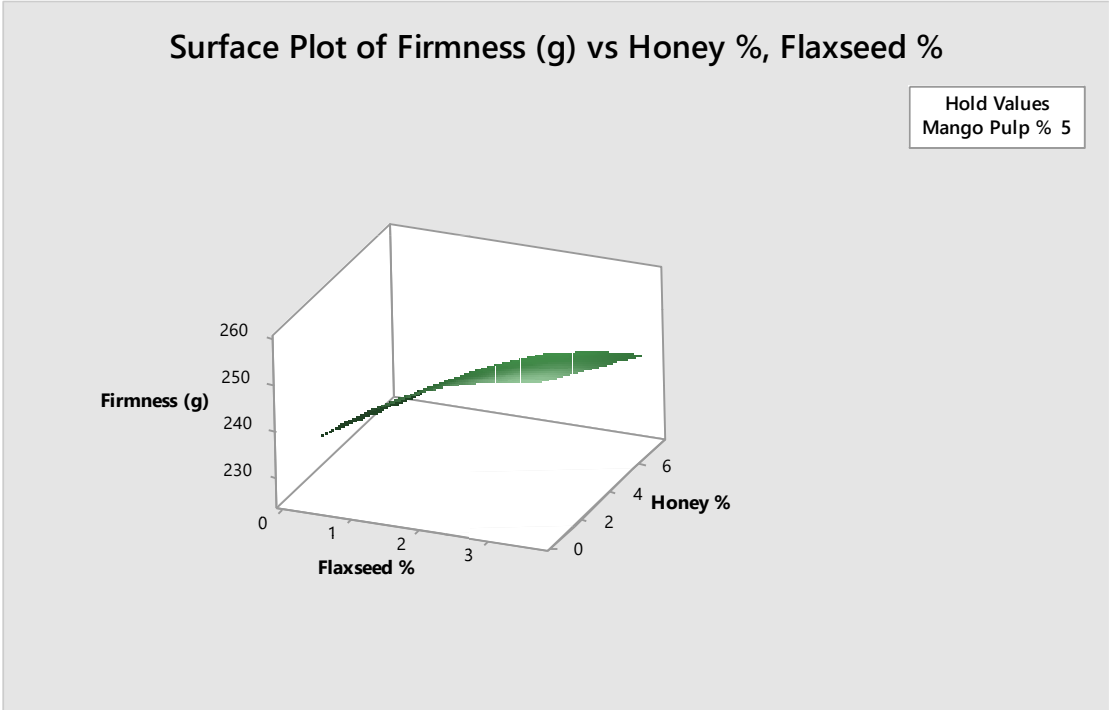
The coefficient estimate for the variables has been presented in Table 4.5. Flaxseed powder and mango pulp had significant ($p< 0.0001$) positive effect and honey had significant ($p< 0.0001$) negative effect on firmness of the *dahi* sample at linear level. Flaxseed powder and mango pulp had significant ($p< 0.0001$) negative effect while honey had positive effect at quadratic level. Interactive effect of flaxseed powder with mango pulp and flaxseed powder with honey was positive, while between mango pulp with honey, it was observed negative.

The firmness of *dahi* and yoghurt is directly dependent on its total solids and specifically protein content and the type of proteins. Higher protein content would cause a higher degree of cross linkage of the gel network, resulting in a much denser and more rigid gel structure (Tamime, 2006). Firmness increased with the increase in concentration of fibre content, this result is in agreement with the results of Aportela-Palacios *et al.,* (2005), as stated that the particular structure of the fiber may be involved in the entrapment of water molecules as part of the three dimensional network there by increasing the firmness of the yogurt gel. Firmness of FFSFD also might have increased due to presence of alginate-chitosan microcapsules (Sultana *et al*., 2000). Sodium alginate used in the encapsulation of the probiotic cultures has been reported to form a gel with a number of cations such as calcium which is present in the yoghurt gel (Kailasapathy, 1996) resulting in increased firmness.

a)

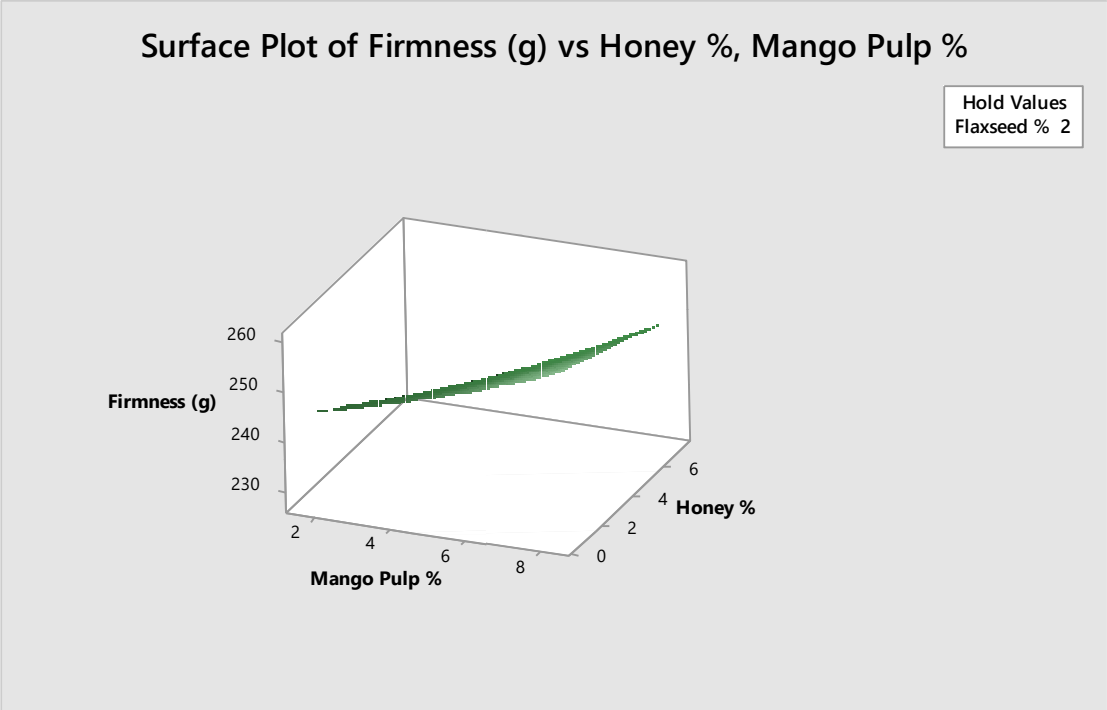

Surface Plot of Firmness (g) vs Mango Pulp %, Flaxseed %
Hold Values
Honey % 4
Firmness (g)
270
255
240
0
1
2
3
Flaxseed %
2
4
6
8
Mango Pulp %

b)


Surface Plot of Firmness (g) vs Honey %, Flaxseed %
Hold Values
Mango Pulp % 5
Firmness (g)
260
250
240
230
0
1
2
3
Flaxseed %
0
2
4
6
Honey %

Contd..

c)



d)

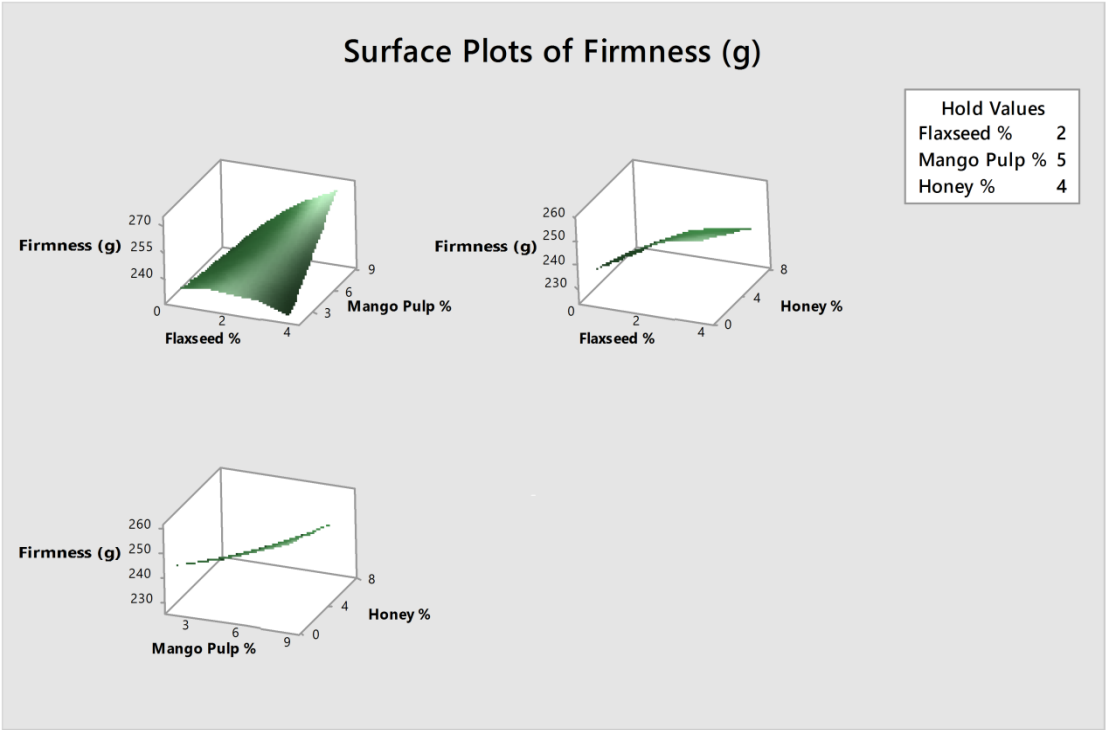
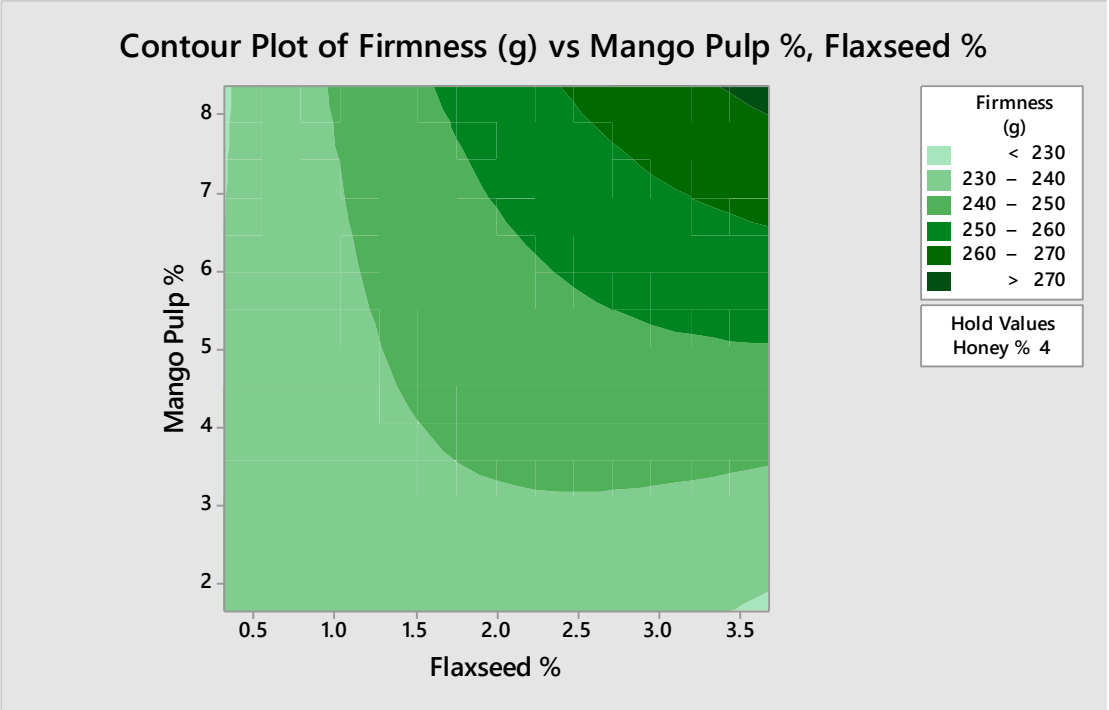
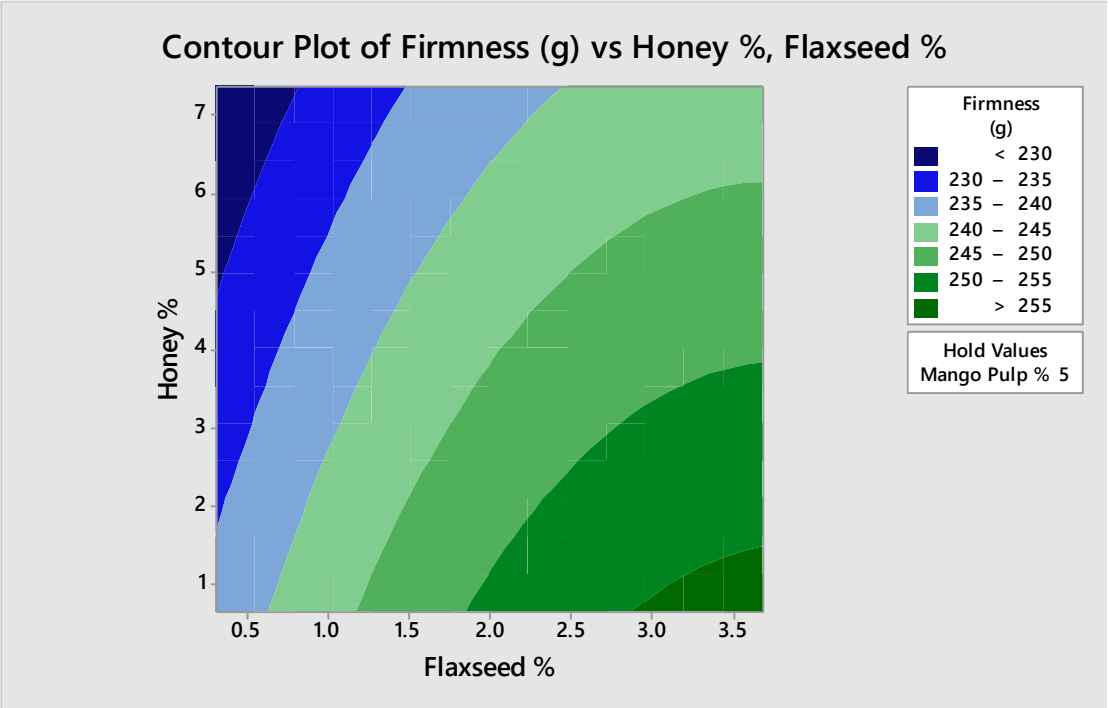


Figure 4.9 3-D plots representing the effect of flaxseed powder, mango pulp and honey on firmness of FFSFD a) flaxseed powder and mango pulp b) flaxseed powder and honey c) mango pulp and honey d) surface plot of firmness on the same panel.

a)

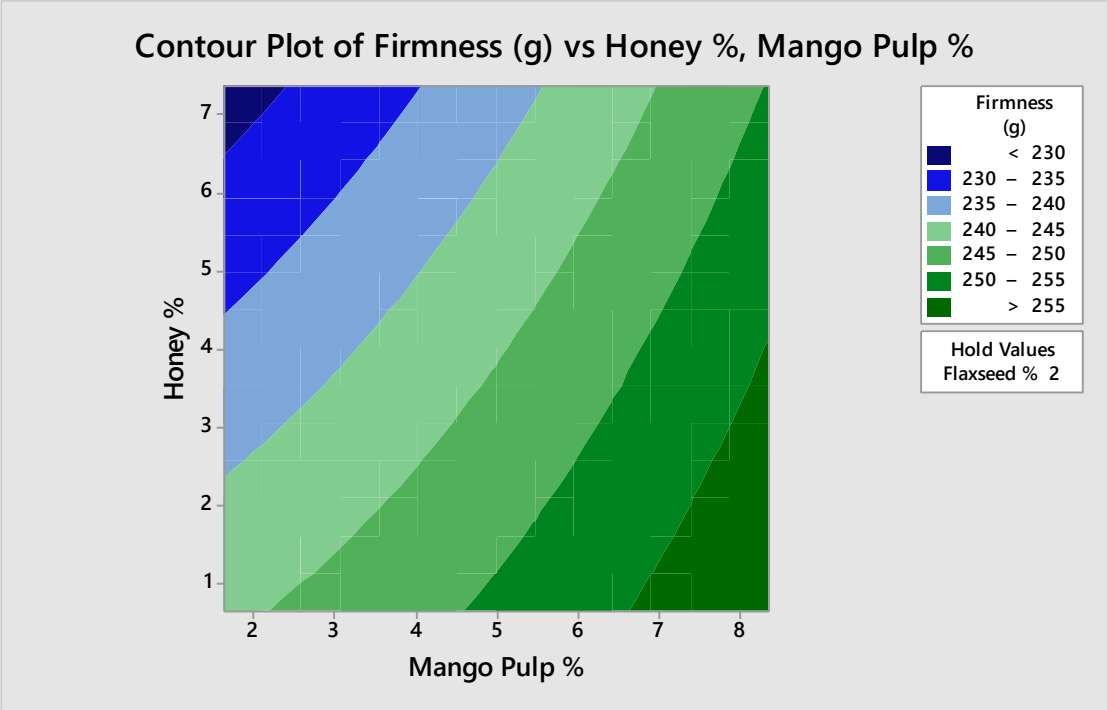

Contour Plot of Firmness (g) vs Mango Pulp %, Flaxseed %
Mango Pulp %
Flaxseed %
8
7
6
5
4
3
2
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Firmness (g)
< 230
230 – 240
240 – 250
250 – 260
260 – 270
> 270
Hold Values
Honey % 4

b)


Contour Plot of Firmness (g) vs Honey %, Flaxseed %
Honey %
Flaxseed %
7
6
5
4
3
2
1
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Firmness (g)
< 230
230 – 235
235 – 240
240 – 245
245 – 250
250 – 255
> 255
Hold Values
Mango Pulp % 5

Contd..

c)



d)

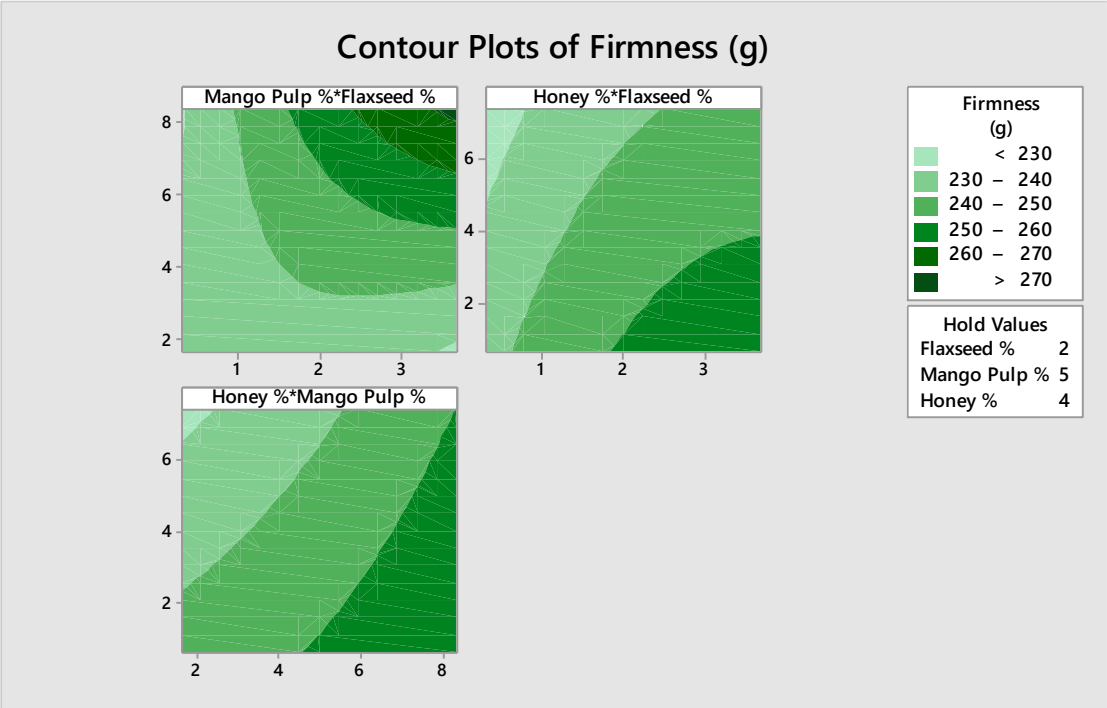


Figure 4.10 Contour plots representing the effect of flaxseed powder, mango pulp and honey on firmness of FFSFD a) flaxseed powder, and mango pulp b) flaxseed powder and honey c) mango pulp and honey. d) contour plot of firmness on the same panel.

4.2.6 Effect on Cohesiveness

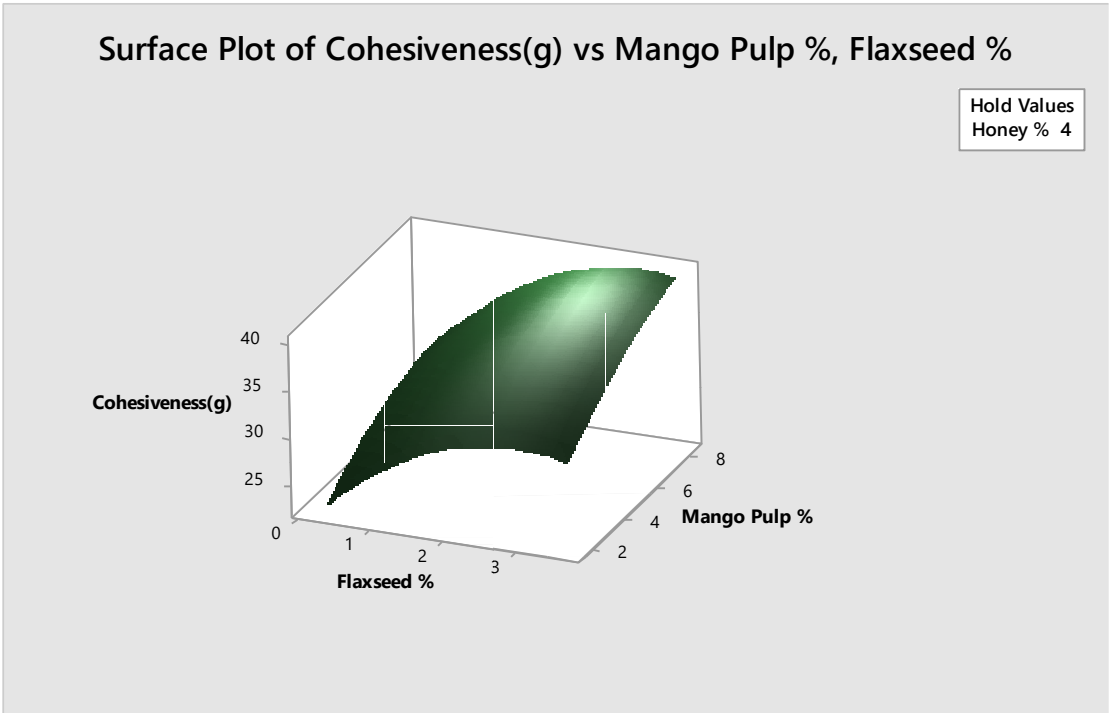
The quadratic equation obtained by the Response surface analysis of the data showing the effect of flaxseed powder (A), mango pulp (B) and honey (C) on the cohesiveness of flaxseed fortified synbiotic flavoured *dahi* that could be described by the following equation:

$$\text{Cohesiveness (g)} = 10.64 + 3.124A + 0.894B + 0.323C - 0.0471A^2 - 0.0423B^2 - 0.0511C^2 + 0.0278AB - 0.0715AC + 0.0048BC \quad \ldots (6)$$

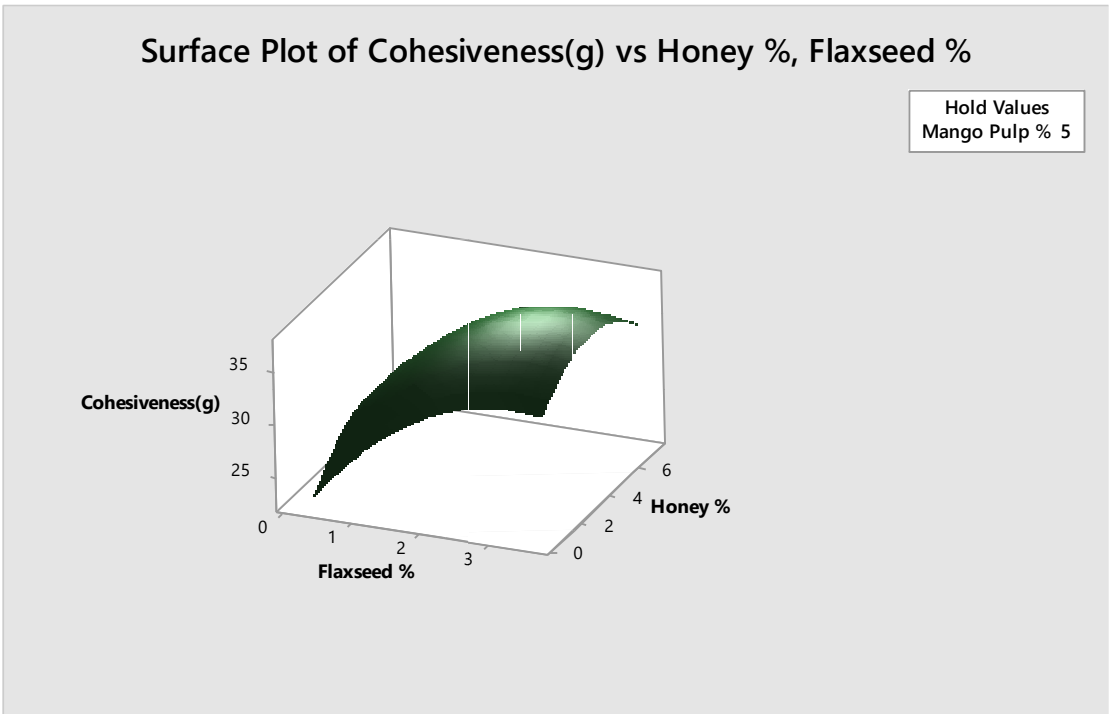
In table 4.3, the F-value (125.43) for the model of cohesiveness was significant ($p<0.0001$). The coefficient of determination (R^2) was 0.99 indicating that 99.12% of the variability in the response could be explained by the model. The "Pred. R-Squared" of 0.98 was in reasonable agreement with the "Adj R-Squared" of 0.94. A lack of fit value of 4.06 is found to be not significant. Hence, this model could be used to navigate the design space. The cohesiveness of FFSFD varied from 25.98 to 40.24gs (Table 4.5).

The cohesiveness increased ($p<0.0001$) with increasing the level of flaxseed powder (0.31 to 3.68g) and mango pulp (3 to 7%), while slight decrease in cohesiveness value was observed with increased level of honey from 2 to 6%. The highest cohesiveness was recorded to be 40.24gs at 3.0g, 7% and 6% of flaxseed powder, mango pulp and honey, respectively. The response surface plots for cohesiveness are presented in fig. 4.11 (a, b, c & d). In order to gain a better understanding of the interaction effects of variables on cohesiveness of FFSFD, two dimensional contour plots for the measured response were formed based on the model Eq. 6. The fig. 4.12 (a, b, c & d) shows the plots of the model for variation in cohesiveness values of FFSFD as a function of flaxseed powder, mango pulp and honey.

a)

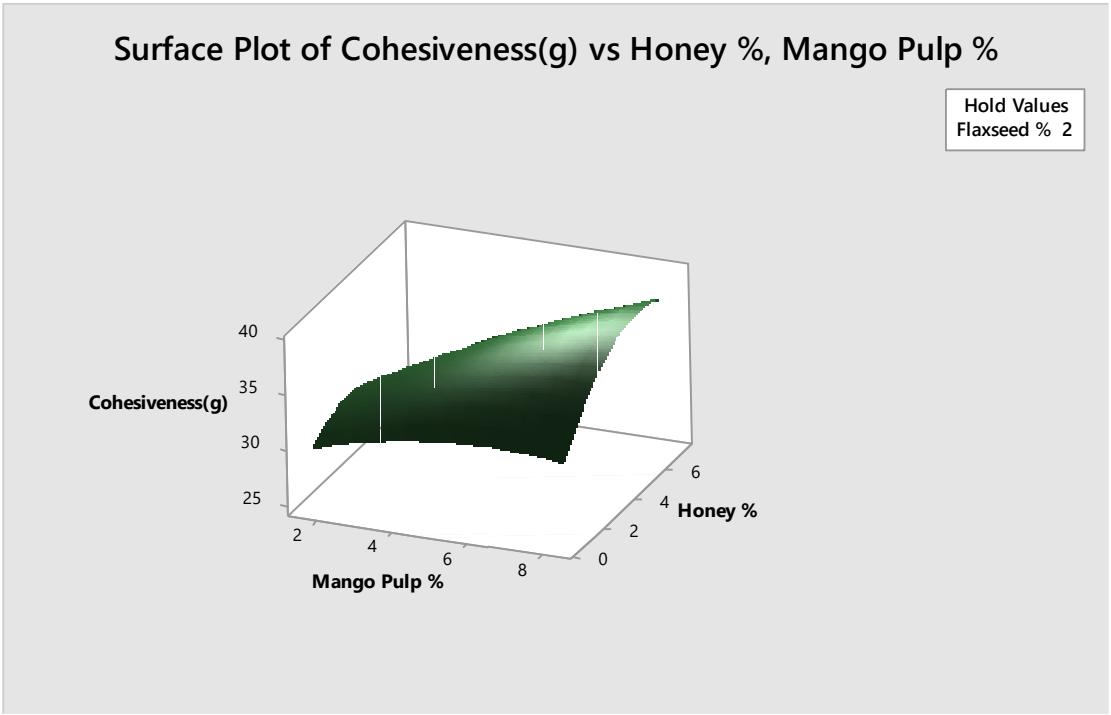

Surface Plot of Cohesiveness(g) vs Mango Pulp %, Flaxseed %
Hold Values
Honey % 4
Cohesiveness(g)
40
35
30
25
0
1
2
3
Flaxseed %
2
4
6
8
Mango Pulp %

b)


Surface Plot of Cohesiveness(g) vs Honey %, Flaxseed %
Hold Values
Mango Pulp % 5
Cohesiveness(g)
35
30
25
0
1
2
3
Flaxseed %
0
2
4
6
Honey %

Contd..

c)



d)

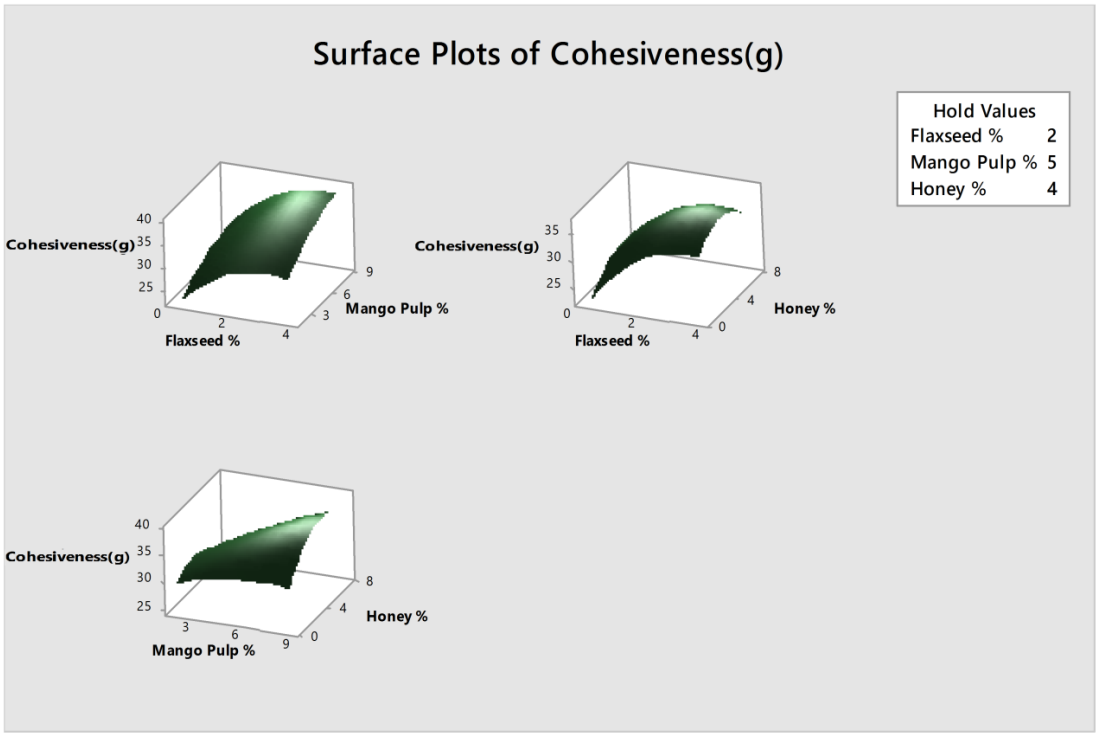
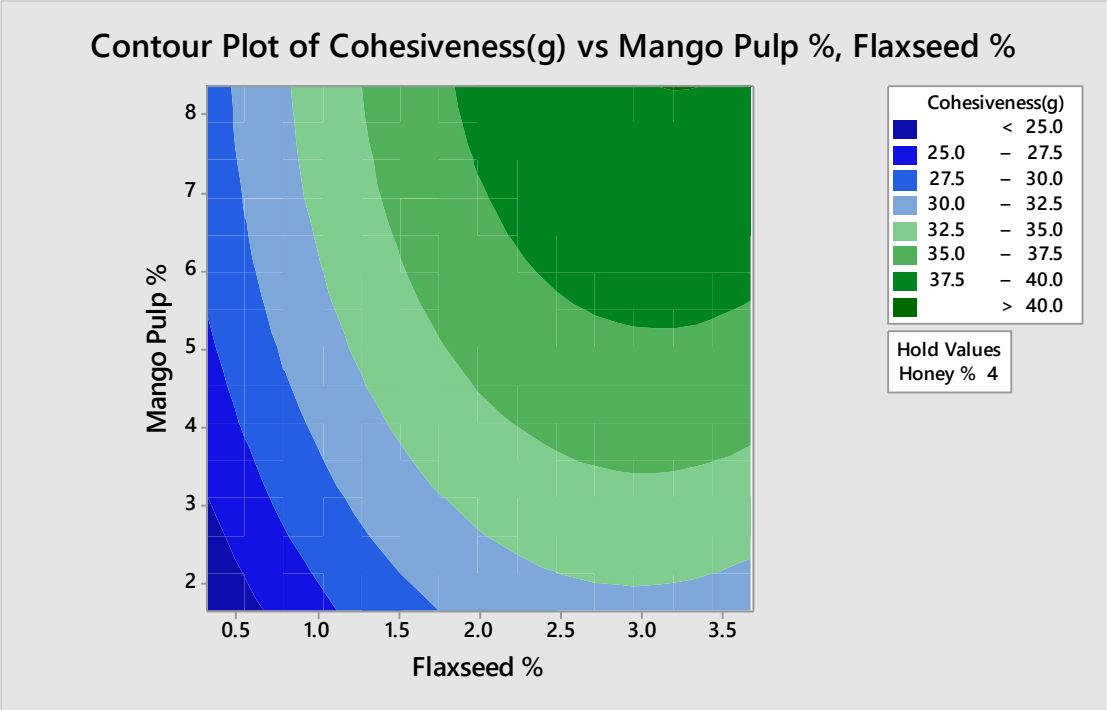
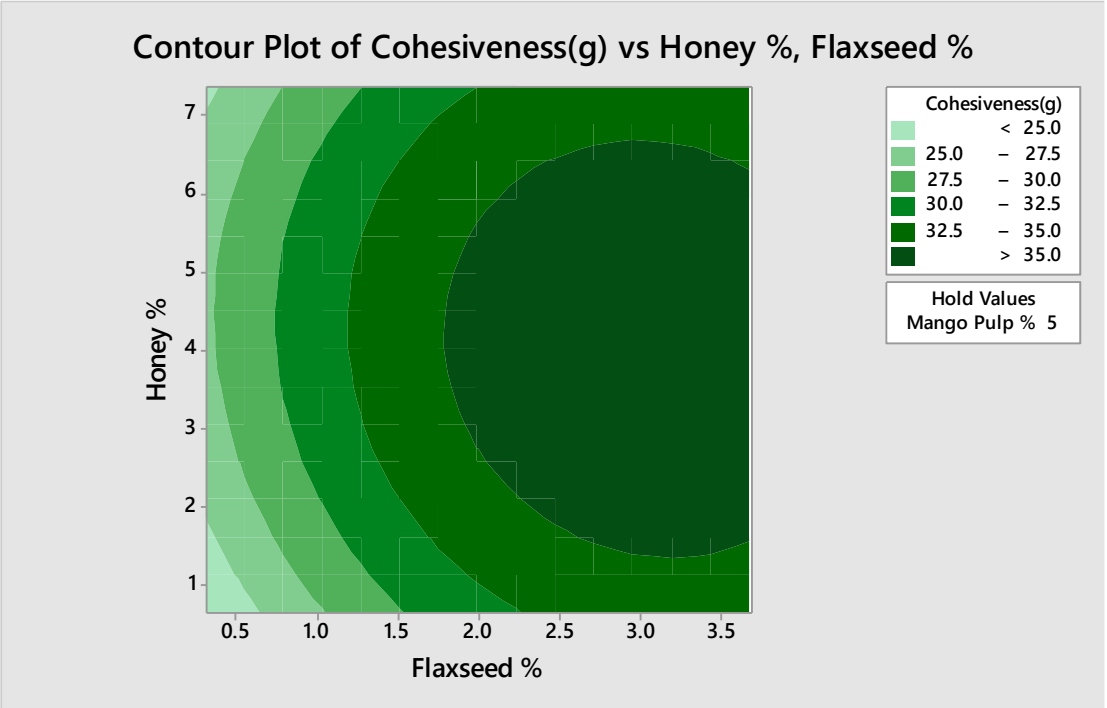


Figure 4.11 3-D plots representing the effect of flaxseed powder, mango pulp and honey on cohesiveness of FFSFD a) flaxseed powder and mango pulp b) flaxseed powder and honey c) mango pulp and honey d) surface plot of cohesiveness on the same panel.

a)

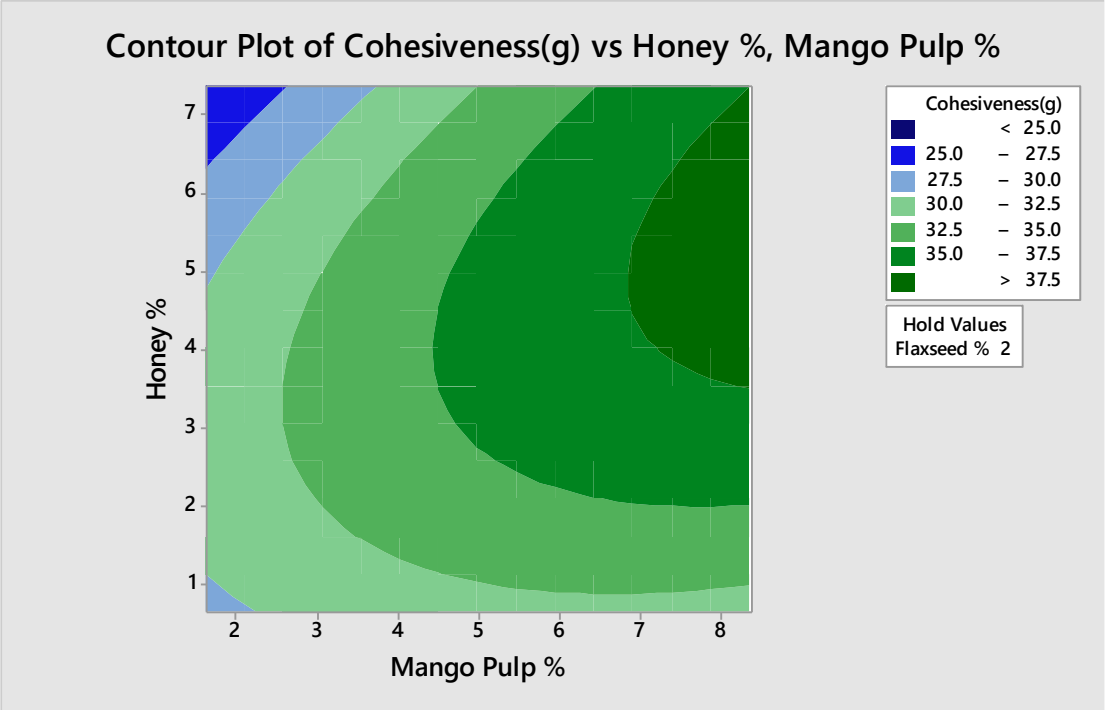

Contour Plot of Cohesiveness(g) vs Mango Pulp %, Flaxseed %
Mango Pulp %
Flaxseed %
8
7
6
5
4
3
2
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Cohesiveness(g)
< 25.0
25.0 – 27.5
27.5 – 30.0
30.0 – 32.5
32.5 – 35.0
35.0 – 37.5
37.5 – 40.0
> 40.0
Hold Values
Honey % 4

b)


Contour Plot of Cohesiveness(g) vs Honey %, Flaxseed %
Honey %
Flaxseed %
7
6
5
4
3
2
1
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Cohesiveness(g)
< 25.0
25.0 – 27.5
27.5 – 30.0
30.0 – 32.5
32.5 – 35.0
> 35.0
Hold Values
Mango Pulp % 5

Contd..

c)



d)

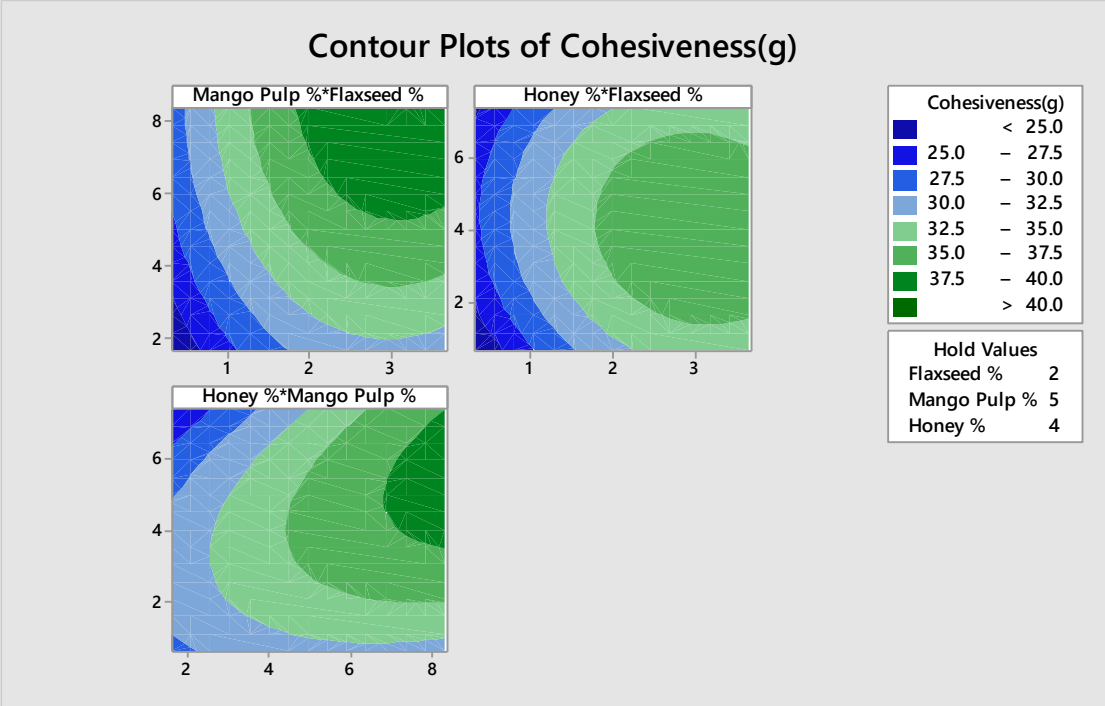


Figure 4.12 Contour plots representing the effect of flaxseed powder, mango pulp and honey on cohesiveness of FFSFD a) flaxseed powder, and mango pulp b) flaxseed powder and honey c) mango pulp and honey. d) contour plot of cohesiveness on the same panel.

The coefficient estimate for the variables has been presented in Table 4.5. All the three variables i.e. flaxseed powder, mango pulp and honey had significant ($P < 0.0001$) positive effect on cohesiveness of the *dahi* sample at linear level. All the three variables i.e. flaxseed powder, mango pulp and honey had significant ($P < 0.0001$) negative effect at quadratic level. Interactive effect of flaxseed powder with mango pulp and mango pulp with honey, was positive, while between flaxseed powder with honey it was observed negative.

Incorporation of fibre obtained from asparagus shoots increased yogurt cohesiveness and imparted a yellowish greenish colour to the yogurt (Sanz *et al.,* 2008). Guven *et al.,* (2005) investigated that the cohesiveness of yoghurt increased with inulin concentration. The result of study were in line with findings of Kumar and Mishra, (2003) who reported that cohesiveness increased with increase in proportion of soymilk and mango pulp in soymilk fortified mango yoghurt.

4.2.7 Effect on Probiotic viable count

The quadratic equation obtained by the response surface analysis of the data showing the effect of flaxseed powder (A), mango pulp (B) and honey (C) on the viable count of *Lactobacillus acidophilus* could be described by the following equation:

$$\text{Probiotic Viable Count} = -0.87 + 0.336A + 0.568B + 1.978C - 0.00975A^2 - 0.00703B^2 - 0.1426C^2 - 0.0219AB + 0.0133AC - 0.0463BC \quad \ldots\ldots (7)$$

In table 4.3, the F-value (27.22) for the model of LA was significant ($P<0.0001$). The coefficient of determination (R^2) was 0.96 indicating that 96.0% of the variability in the response could be explained by the model. The "Pred. R-Squared" of 0.80 was in reasonable agreement with the "Adj. R-Squared" of 0.92. The probiotic viable count range of *dahi* sample varied from 5.89 to 9.85cfu/g.

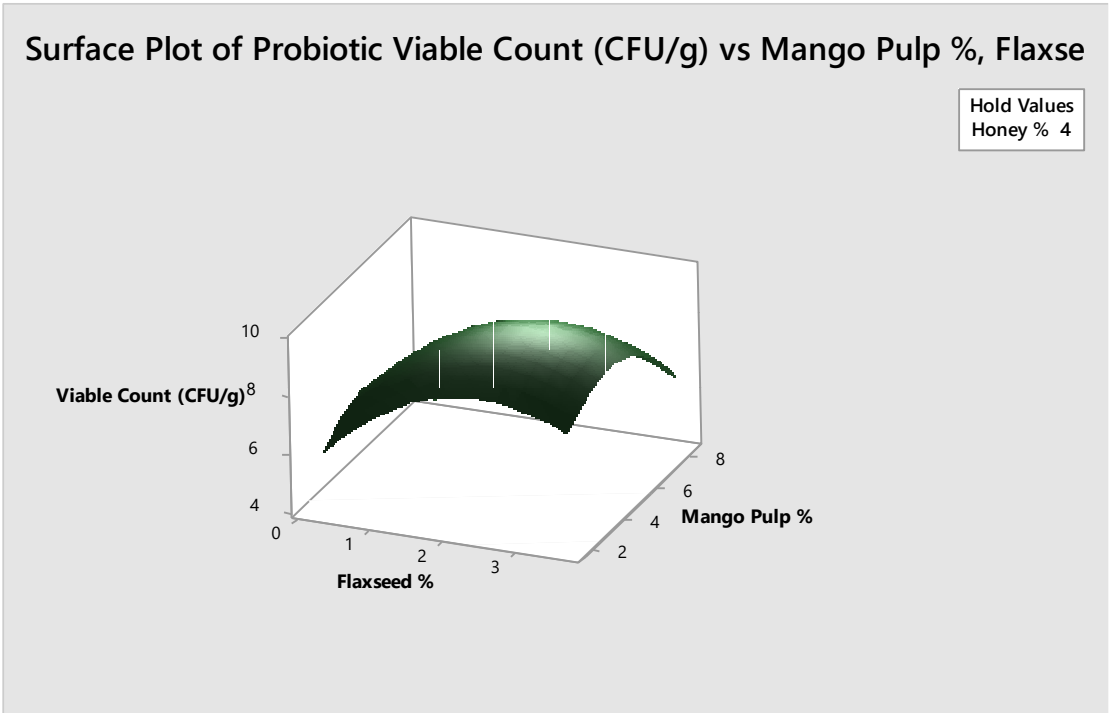
The coefficient estimate for the variables has been presented in Table 4.5. All the three variables i.e. flaxseed powder, mango pulp and honey had significant ($P < 0.0001$) positive effect on probiotic viable count of *dahi* sample at linear level and all

the variables had significant ($P < 0.0001$) negative effect at quadratic level. Interactive effect ($P < 0.0005$) of flaxseed powder with mango pulp and mango pulp with honeys had negative effect while flaxseed powder with honeys was found positive.

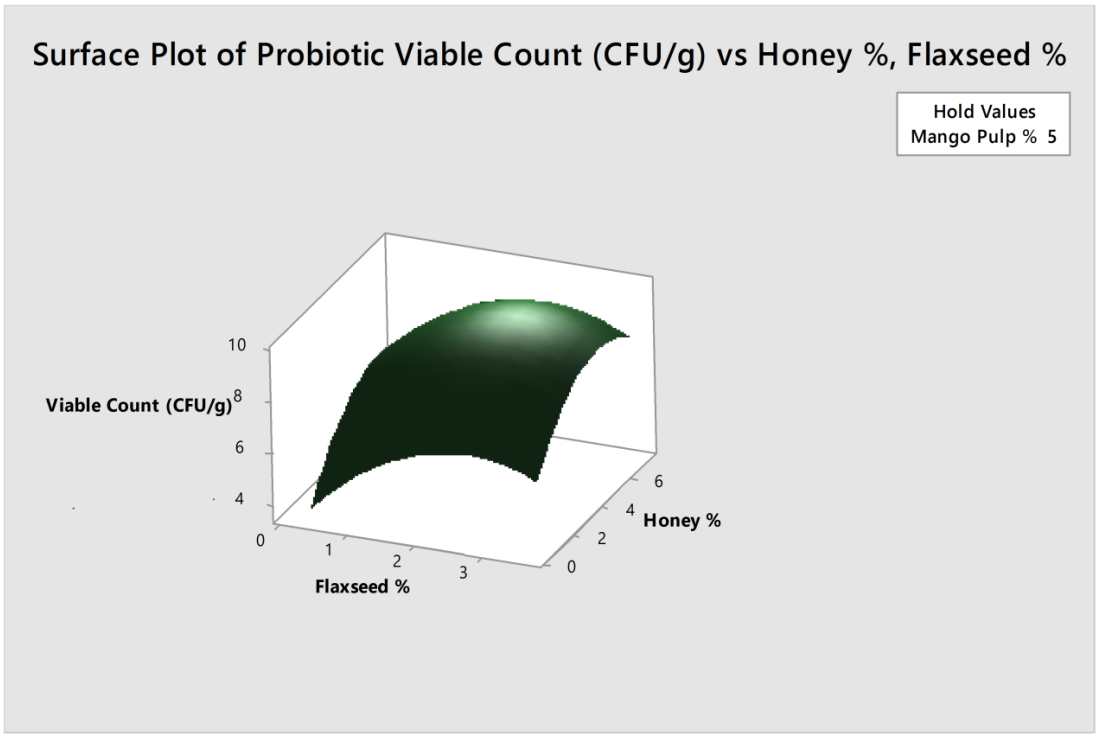
Fig. 4.13 (a, b, c & d) shows response surface plots of all three variables on probiotic viable count. The probiotic viable count increased highly when the level of honey increased in *dahi* sample. This may be due to fructooligosaccharides and oligosaccharides naturally present in honey. As the level of flaxseed powder increased from 1 to 3g, probiotic viable count was increased. This may be due to flaxseed powder acts as a prebiotic for the probiotic bacteria (Nezhad *et al.,* 2013). The maximum probiotic viable count predicted by response surface analysis was 9.85cfu/g with flaxseed powder of 2g, mango pulp of 5% and honeys of 7.36%.

The increase in probiotic viable count in FFSFD is in accordance with the findings of Nezhad *et al.,* (2013) who showed that after 21days of cold storage kefir samples with crude flaxseed mucilage, with or without probiotics, showed lower pH values compared to their respective treatments supplemented with pure mucilage. This indicates that LA utilizes flaxseed mucilage as prebiotic for their growth. Prebiotics, (oligosachharides from honey) may stimulate the metabolism of probiotics, by release of increased level of fructose as result of its partial hydrolysis, which gets metabolized as an additional carbon and energy source (Tamime, 2005). Luz Sanz *et al.*, (2005) observed that honey oligosaccharides seem to present potential prebiotic activity (PI values between 3.38 and 4.24), increasing the populations of bifidobacteria and lactobacilli. Contrary to the present findings, Kumar and Mishra, (2003), reported that an increase in the mango pulp content in the yoghurt increased the counts of *S. thermophilus* and *L. bulgaricus*.

a)

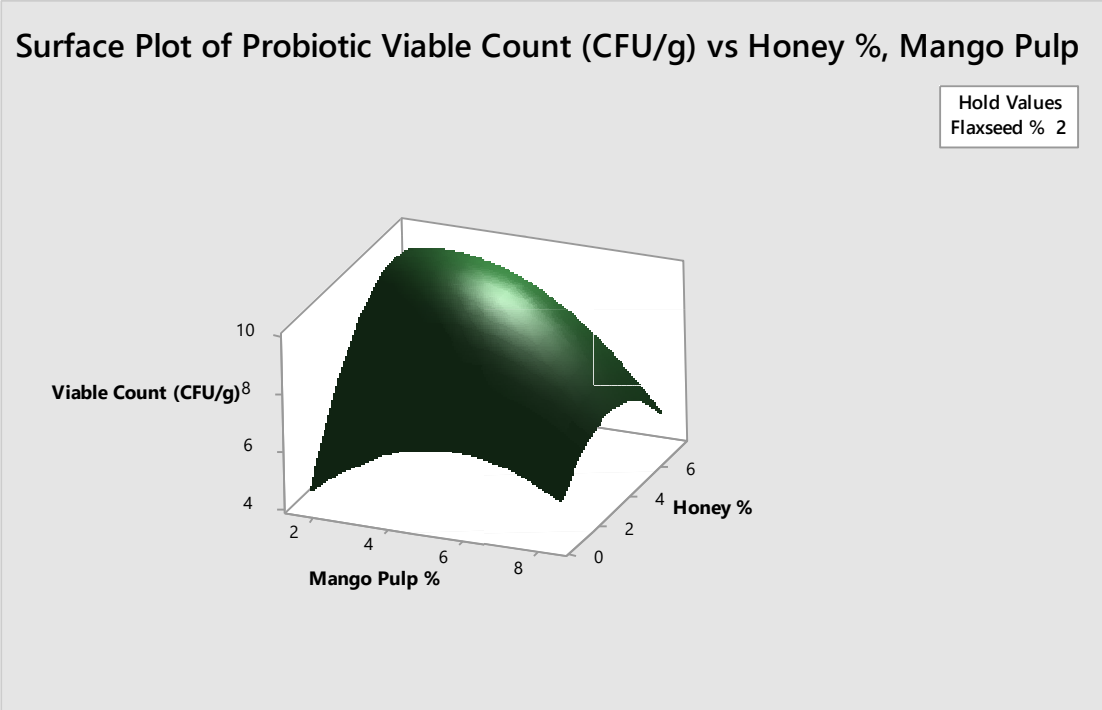

Surface Plot of Probiotic Viable Count (CFU/g) vs Mango Pulp %, Flaxse
Hold Values
Honey % 4
Viable Count (CFU/g)
10
8
6
4
0
1
2
3
Flaxseed %
2
4
6
8
Mango Pulp %

b)


Surface Plot of Probiotic Viable Count (CFU/g) vs Honey %, Flaxseed %
Hold Values
Mango Pulp % 5
Viable Count (CFU/g)
10
8
6
4
0
1
2
3
Flaxseed %
0
2
4
6
Honey %

Contd..

c)



d)

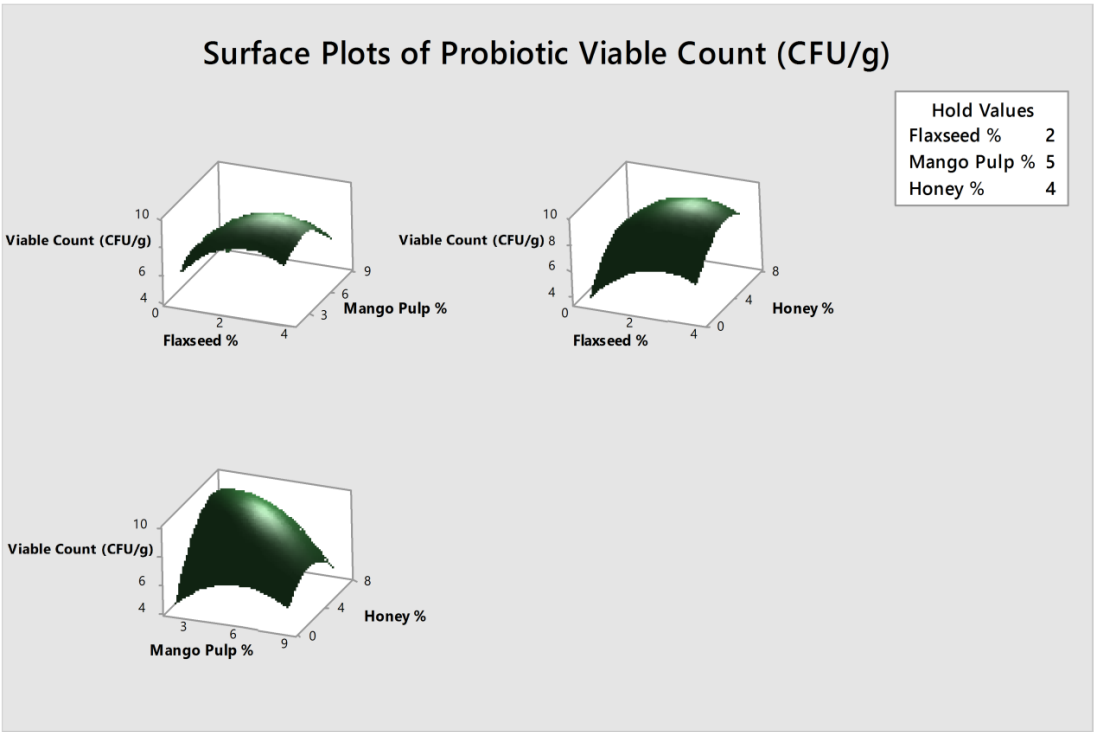
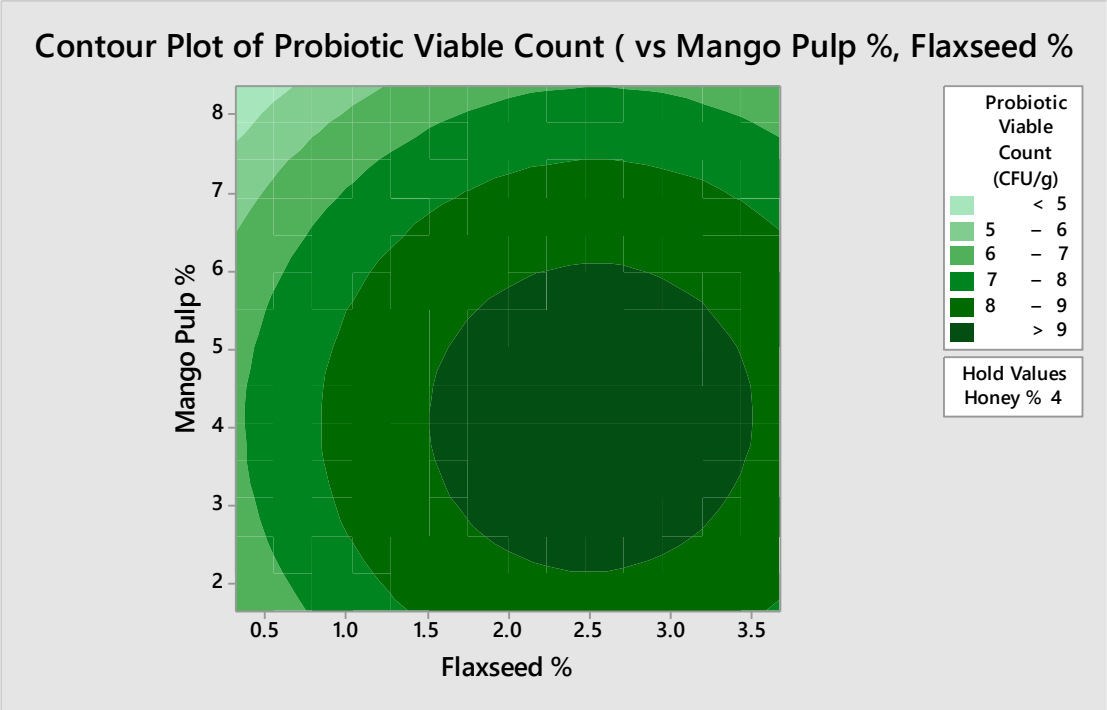
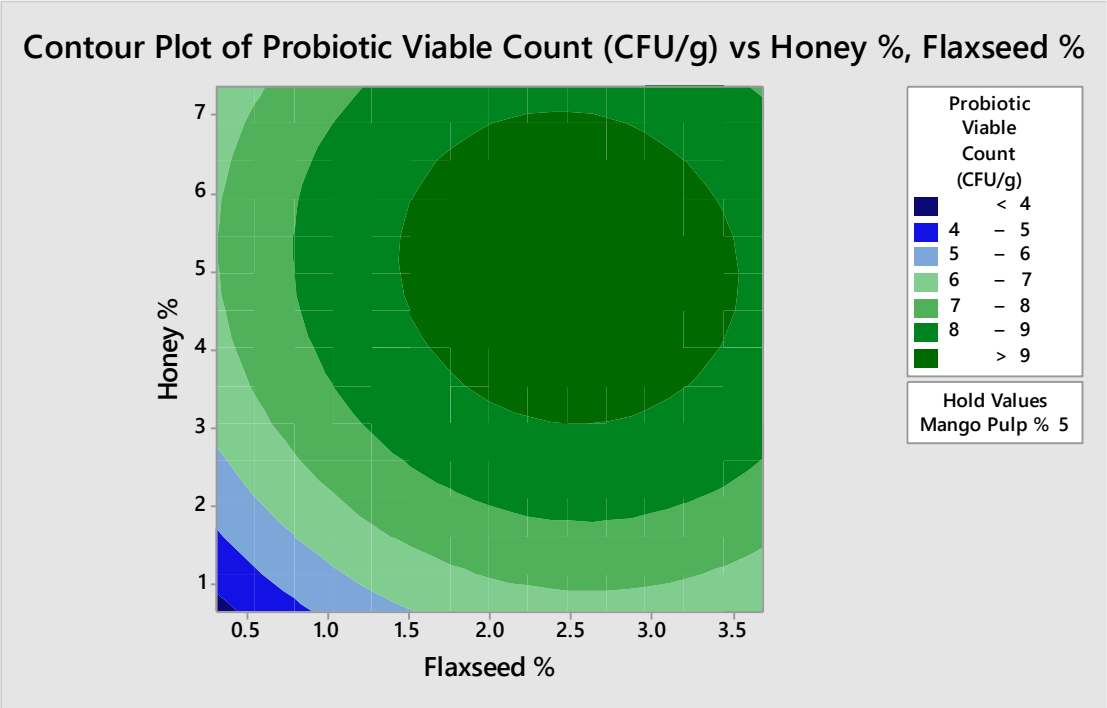


Figure 4.13 3-D plots representing the effect of flaxseed powder, mango pulp and honey on probiotic viable count of FFSFD a) flaxseed powder and mango pulp b) flaxseed powder and honey c) mango pulp and honey d) surface plot of probiotic viable count on the same panel.

a)

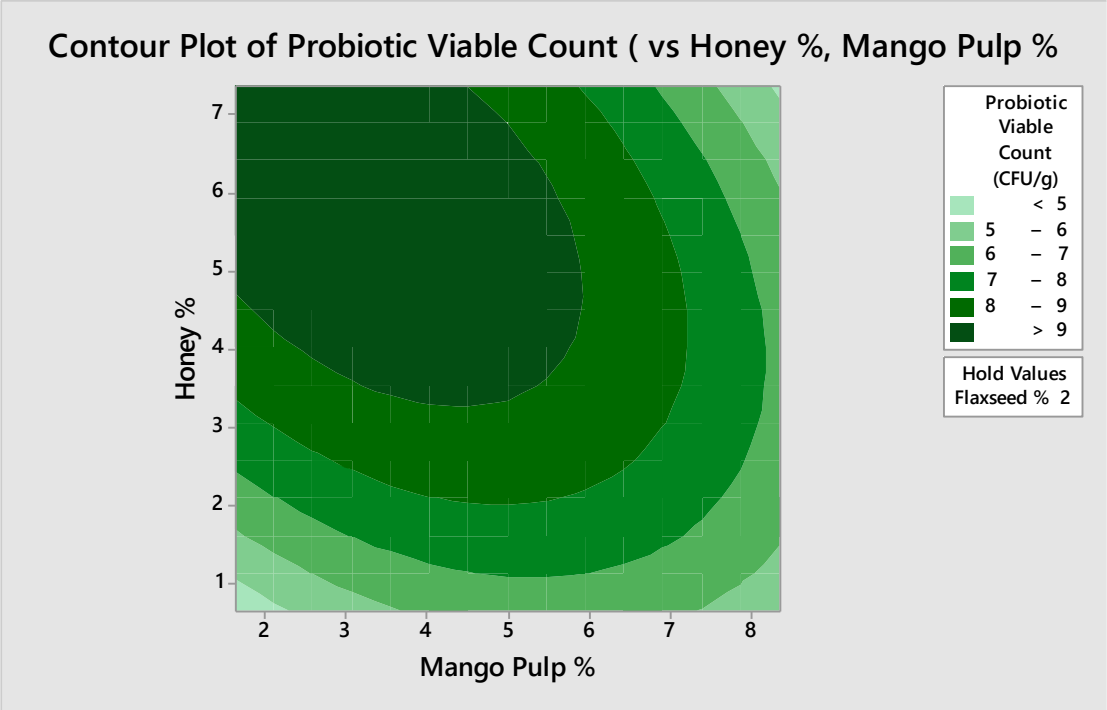

Contour Plot of Probiotic Viable Count (vs Mango Pulp %, Flaxseed %
Mango Pulp %
Flaxseed %
8
7
6
5
4
3
2
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Probiotic Viable Count (CFU/g)
< 5
5 – 6
6 – 7
7 – 8
8 – 9
> 9
Hold Values
Honey % 4

b)


Contour Plot of Probiotic Viable Count (CFU/g) vs Honey %, Flaxseed %
Honey %
Flaxseed %
7
6
5
4
3
2
1
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Probiotic Viable Count (CFU/g)
< 4
4 – 5
5 – 6
6 – 7
7 – 8
8 – 9
> 9
Hold Values
Mango Pulp % 5

Contd..

c)



d)

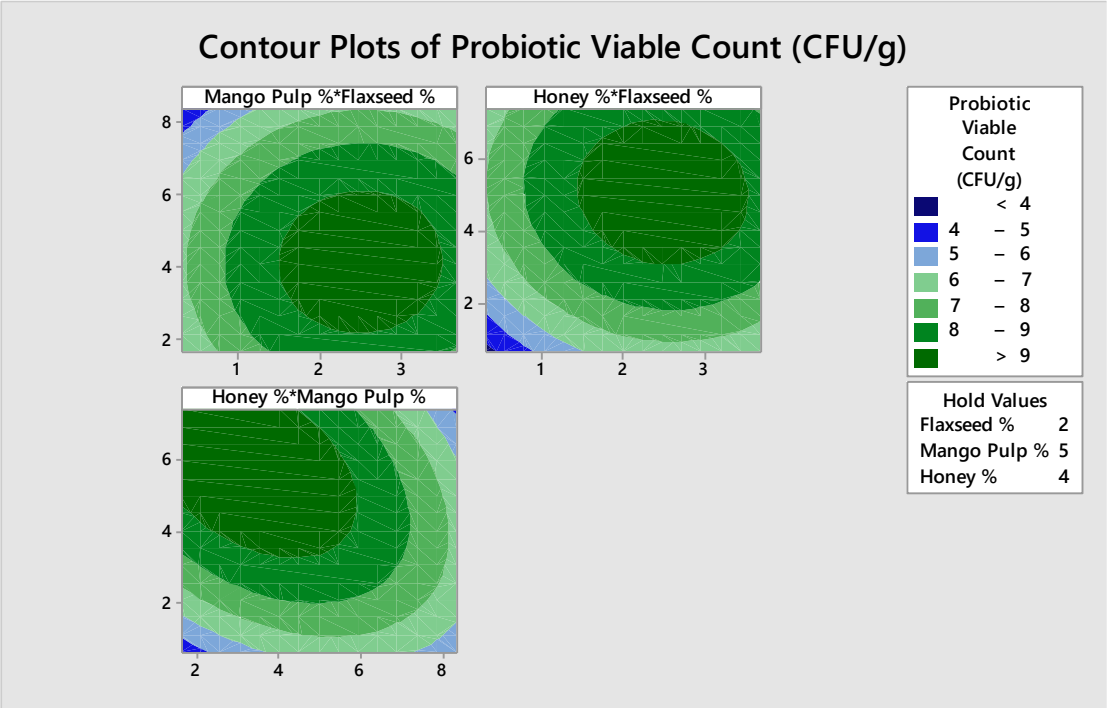


Figure 4.14 Contour plots representing the effect of flaxseed powder, mango pulp and honey on probiotic viable count of FFSFD a) flaxseed powder, and mango pulp b) flaxseed powder and honey c) mango pulp and honey. d) contour plot of on probiotic viable count the same panel.

4.2.8 Effect on Whey separation (Syneresis)

Whey separation (wheying-off) is defined as the expulsion of whey from the network which then becomes visible as surface. Wheying-off negatively affects consumer perception of yoghurt. Spontaneous whey separation is related to an unstable network, which can be due to an increase in the rearrangements of the gel matrix or it can be induced by damage to the weak gel network e.g., by vibration or cutting (Lucey *et al.,* 1998, 2001). The quadratic equation obtained by the response surface analysis of the data showing the effect of flaxseed powder (A), mango pulp (B) and honeys (C) on the fermentation time resulted in the following equation:

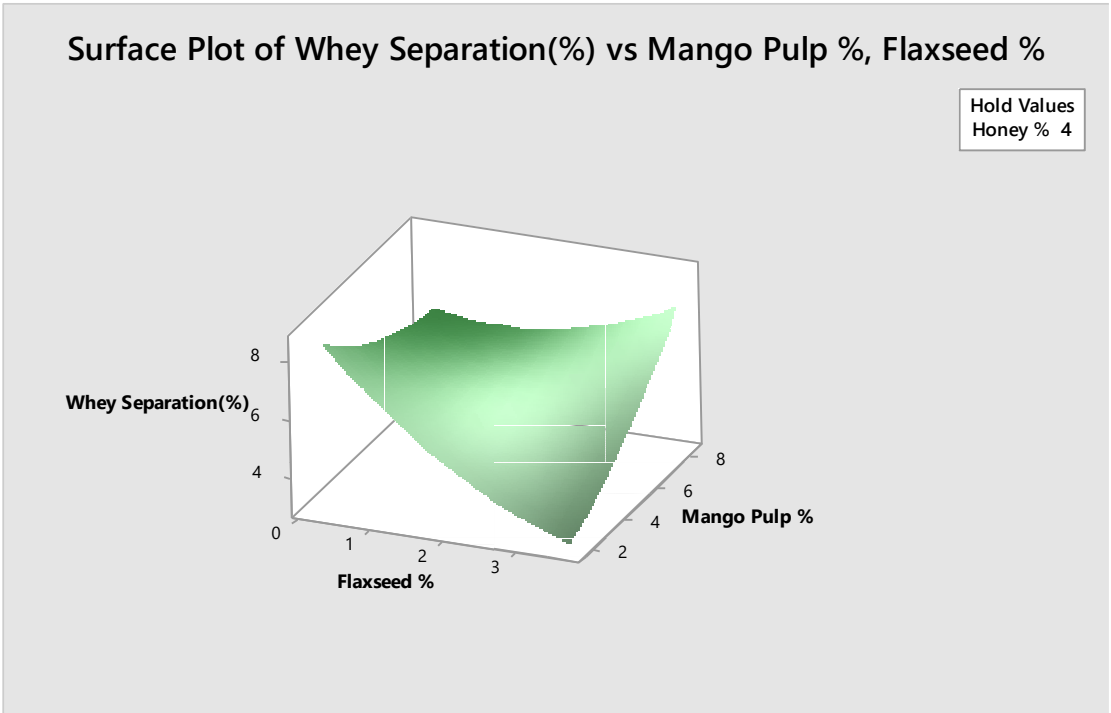
$$\text{Whey Separation (\%)} = 20.16 - 1.596A - 0.613B - 3.49C + 0.079A^2 + 0.018B^2 + 0.290C^2 + 0.059AB - 0.023AC + 0.0666BC \quad \text{..... (8)}$$

In table 4.3, the F-value was determined to examine the goodness of fit for the developed model. The F-value (34.87) for the model of whey separation was significant (p<0.0001). The coefficient of determination (R^2) was 0.96 indicating that 96% of the variability in the response could be explained by the model. The "Pred. R-Squared" of 0.88 was in reasonable agreement with the "Adj R-Squared" of 0.94. A lack of fit value of 2.72 is found to be not significant. Hence, this model could be used to navigate the design space. The values for whey separation varied from 4.02 to 7.96 (Table 4.5). The whey separation score was the highest (7.96) at 3.0g, 7% and 6% of flaxseed powder,mango pulp and honey, respectively (Table 4.1).

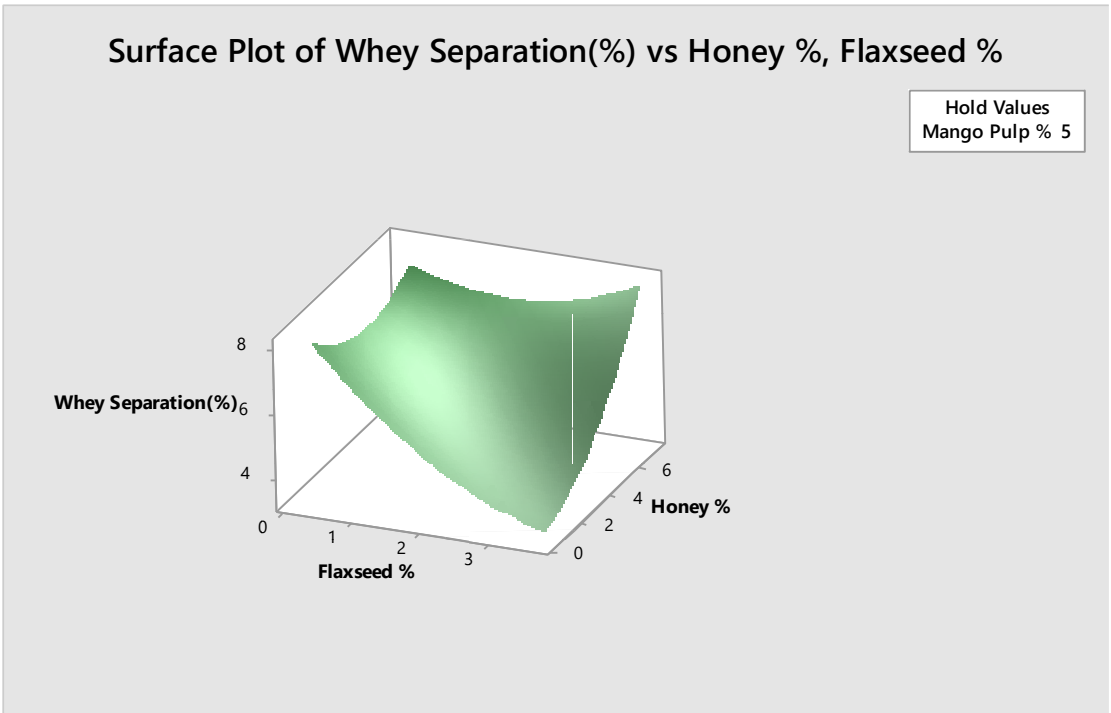
The response surface plots for whey separation score presented in fig. 4.15 (a, b, c & d).The interaction effect with mango pulp level (Fig. 4.11 a), as the level of flaxseed powder increased (0.31 to 2g) the whey separation score decreased (P<0.0001) while as the level of honey (Fig. 4.15 b) increases, the whey separation score increased. As the level of mango pulp increased from 3 to 7%, whey separation score increased (p<0.0001) with the interaction effect of honey and mango pulp.

Garcia-Perez *et al.,* (2005) reported that addition of orange fiber at 0.6 % and 0.8 % had a breakening effect in the gel structure of yoghurt which led to increase in syneresis. Further, when added at 1 % level, the syneresis decreased due to increased water holding of fiber that absorbed the whey released by the gel structure. Raju and Pal, (2014) reported that soy fiber significantly reduced the syneresis of misti *dahi* compared to control sample. Decreased syneresis of soy fibre fortified misti *dahi* samples could be attributed to the water holding capacity of soy fiber.

a)

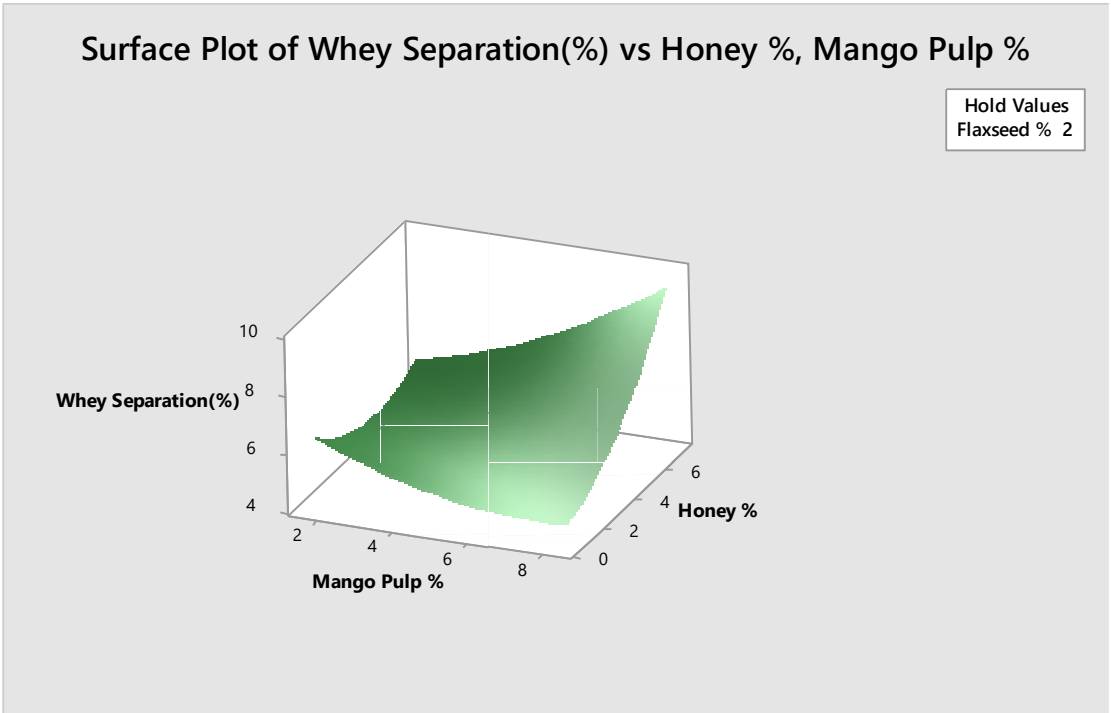

Surface Plot of Whey Separation(%) vs Mango Pulp %, Flaxseed %
Hold Values
Honey % 4
Whey Separation(%)
8
6
4
0
1
2
3
Flaxseed %
2
4
6
8
Mango Pulp %

b)


Surface Plot of Whey Separation(%) vs Honey %, Flaxseed %
Hold Values
Mango Pulp % 5
Whey Separation(%)
8
6
4
0
1
2
3
Flaxseed %
0
2
4
6
Honey %

Contd..

c)



d)

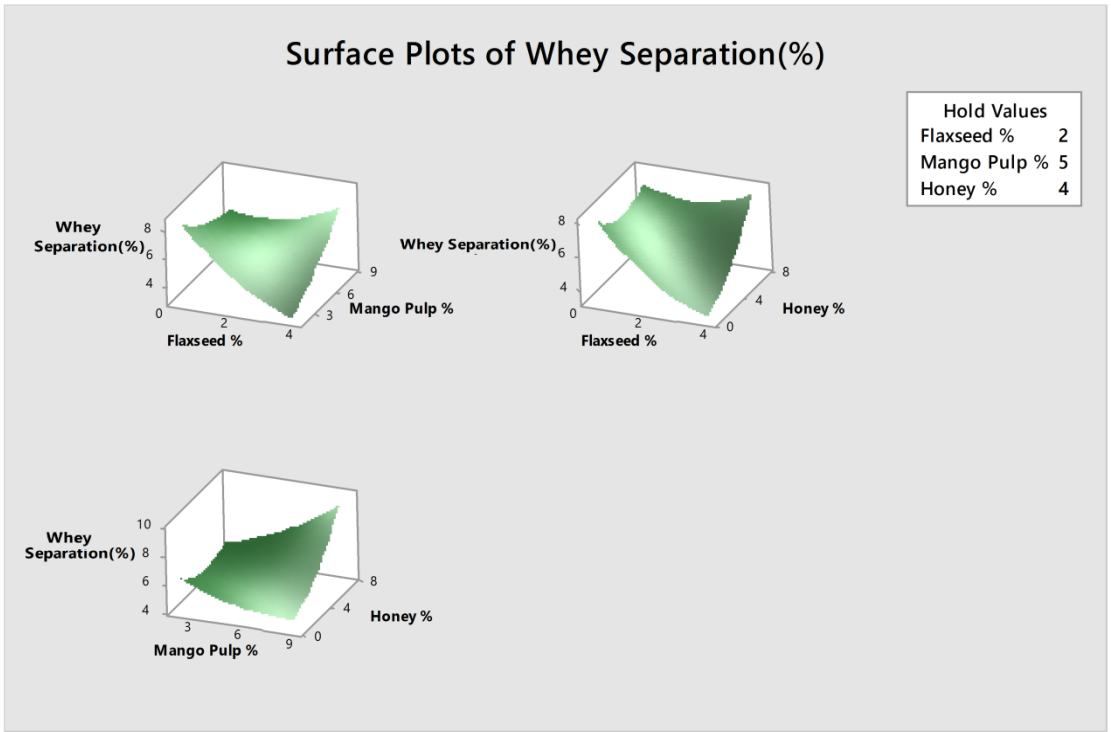
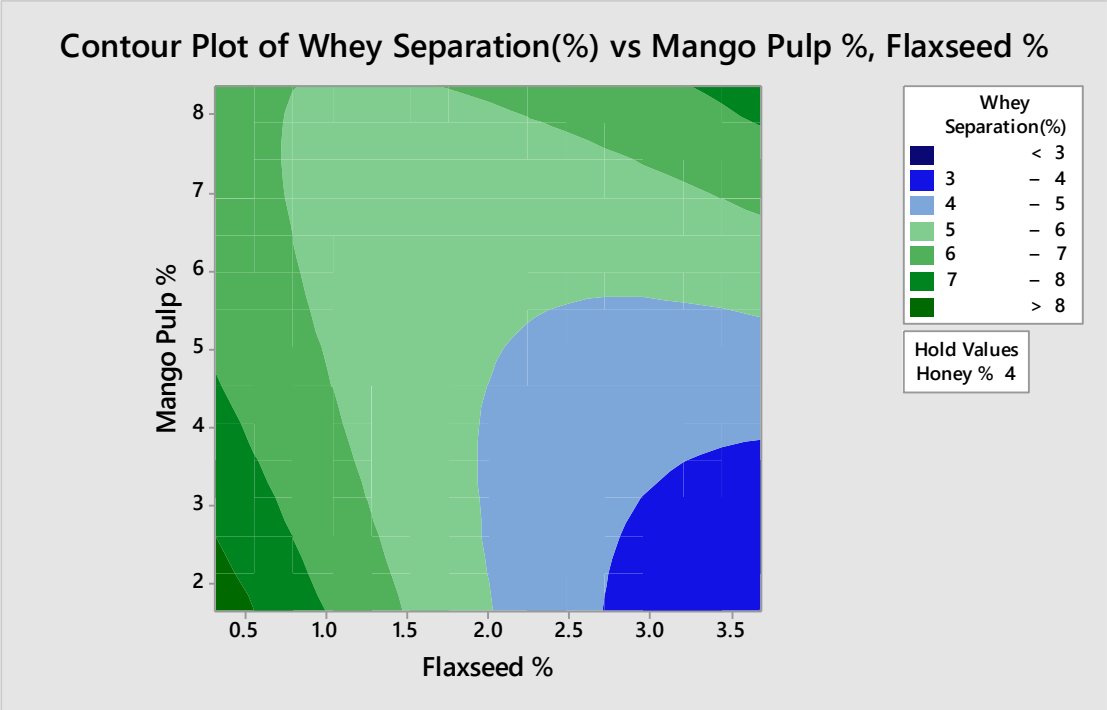
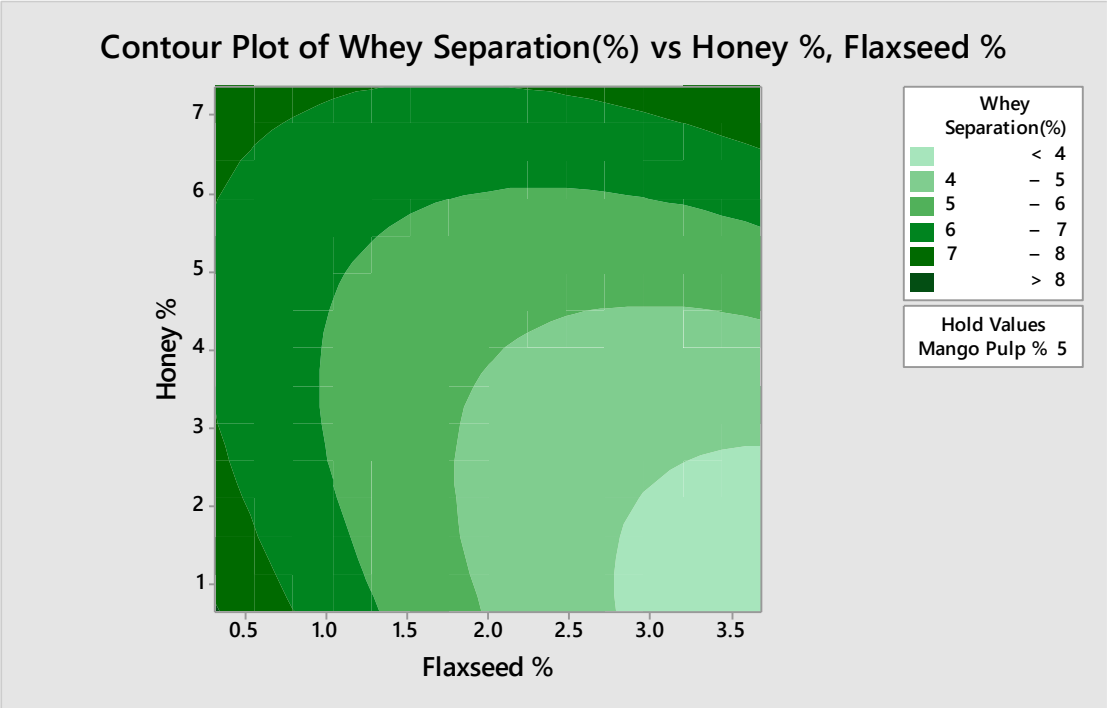


Figure 4.15 3-D plots representing the effect of flaxseed powder, mango pulp and honey on whey separation of FFSFD a) flaxseed powder and mango pulp b) flaxseed powder and honey c) mango pulp and honey d) surface plot of whey separation on the same panel.

a)

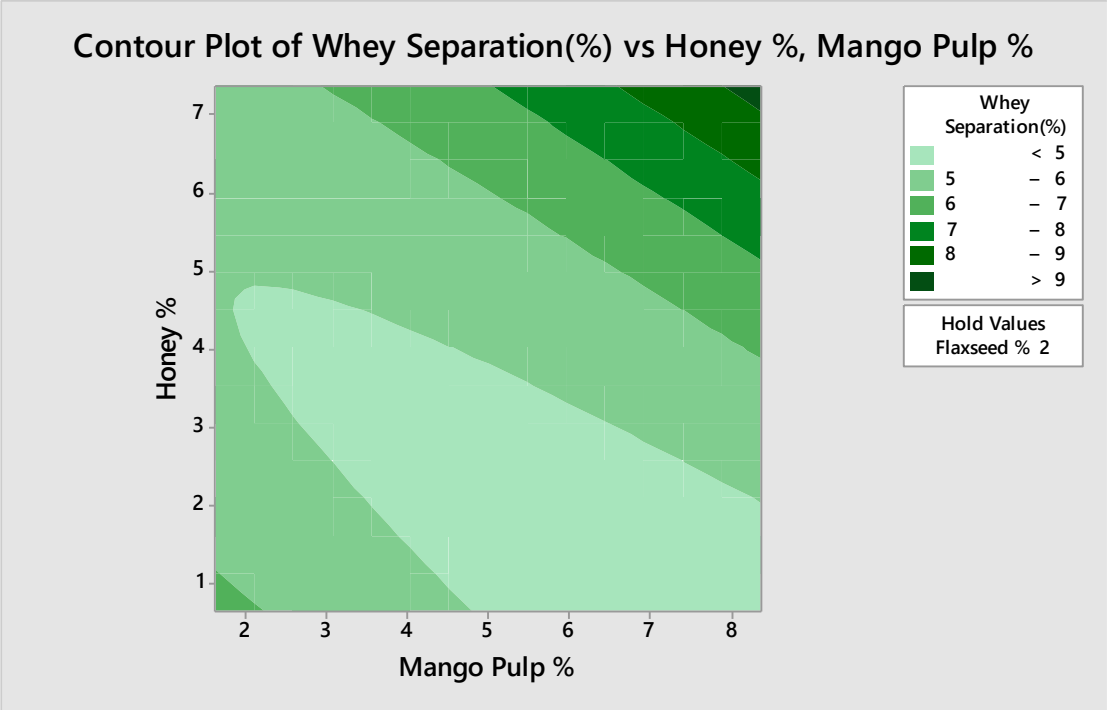

Contour Plot of Whey Separation(%) vs Mango Pulp %, Flaxseed %
Mango Pulp %
Flaxseed %
8
7
6
5
4
3
2
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Whey Separation(%)
< 3
3 – 4
4 – 5
5 – 6
6 – 7
7 – 8
> 8
Hold Values
Honey % 4

b)


Contour Plot of Whey Separation(%) vs Honey %, Flaxseed %
Honey %
Flaxseed %
7
6
5
4
3
2
1
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Whey Separation(%)
< 4
4 – 5
5 – 6
6 – 7
7 – 8
> 8
Hold Values
Mango Pulp % 5

Contd..

c)



d)

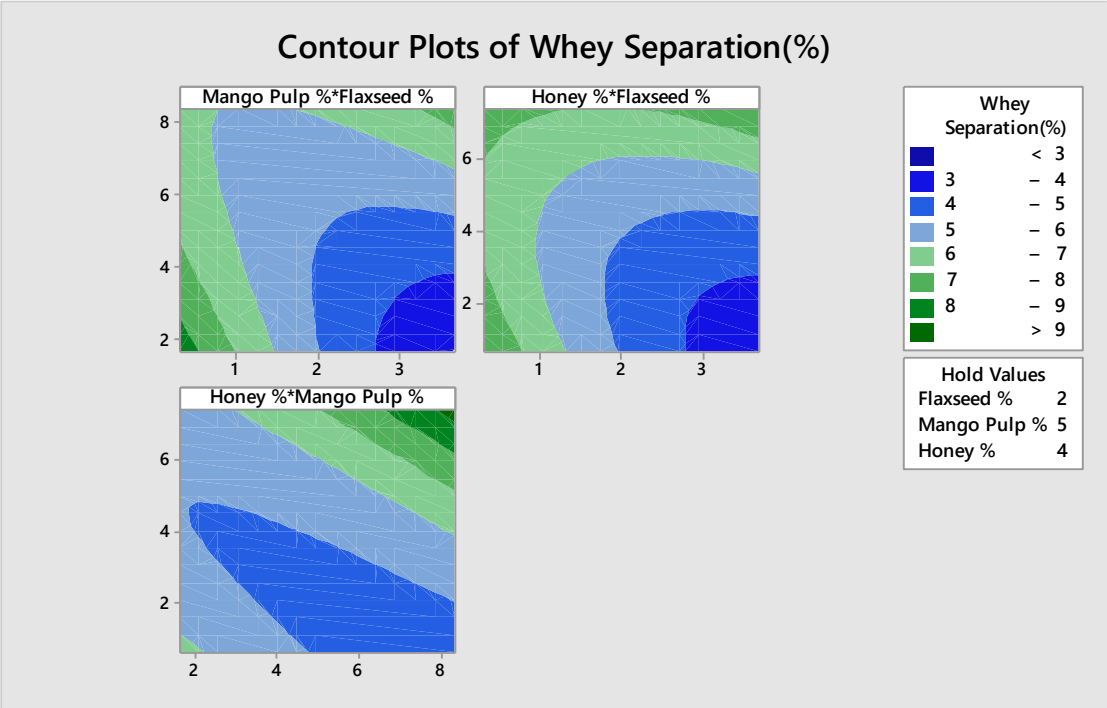


Figure 4.16 Contour plots representing the effect of flaxseed powder, mango pulp and honey on whey separation of FFSFD a) flaxseed powder, and mango pulp b) flaxseed powder and honey c) mango pulp and honey. d) contour plot of whey separation on the same panel.

PHASE –II: PHYSICO-CHEMICAL AND FUNCTIONAL PROPERTIES OF FLAXSEED FORTIFIED SYNBIOTIC FLAVOURED *DAHI*

4.3 Chemical composition of flaxseed powder

Full fat flaxseeds contained 4.5 % moisture, 19.26% protein, 37.26% fat, 3.42% ash, 28.76% carbohydrate and 6.29% dietary fibre (Table 4.7). Hussain *et al.,* (2006) have reported similar values (4.23% moisture, 21.27% protein, 38.53% fat, 30.25% carbohydrates, 3.48% ash and 8-12% dietary fibre) in full fat roasted flaxseed. Morris (2007) have also reported similar values (20.3% protein, 37.1% fat, 3.58% ash, 28.9% carbohydrate and 4.8% dietary fibre) in flaxseeds. Ho *et al.,* (2007) reported similar value for total phenolic content 20.82 %.

Table 4.7 Chemical properties of whole roasted flaxseed powder (FP)

	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	CHO (%)	DF (%)	TPC (%)	AA (%)
Full roasted flaxseed powder (FP)	4.5 ±0.51	19.26 ±0.88	37.26 ±0.31	3.42 ±0.2	28.76 ±0.53	6.29 ±0.83	20.03 ±0.45	46.27 ±0.54

Values are mean ± standard deviation of 3 replicates
CHO = carbohydrates, DF = dietary fibre, TPC = total phenolic content, AA = antioxidant activity

4.4 Composition and acidification kinetics of flaxseed fortified synbiotic flavoured *Dahi*

4.4.1 Physico-chemical composition of flaxseed fortified synbiotic flavoured *Dahi* (FFSFD)

The physico-chemical parameters analysed for the *dahi* samples are summarised in Table 4.8. The composition of foods is known exert considerable influence on their physical, nutritional, sensory and shelf characteristics (Prodaniuc, 2009; El Bakri and Zubeir, 2009). The moisture content of the *dahi* samples ranged between 80.03 to 87.42%. This was due to incorporation of flaxseed powder, honey and mango pulp which lead to the reduction of moisture content. The protein content was higher in FFSFD 5.26% than control *dahi* 3.65%. The protein content increased as the proportion of the flaxseed powder increased in the *dahi*. The fat content ranged between 4.28 to 1.5% in both the *dahi* samples. Fat content of FFSFD increased due

to incorporation of flaxseed powder which has high omega-3 fatty acid content. Fat content has been reported by other researchers to have positive influence on the physical and sensory characteristics (Bille and Keya, 2002; Marinescu and Pop, 2009) and negative impact on the shelf stability of yoghurts (Saint-Eve, 2008; Farinde *et al.,* 2009).

The ash content also increased as the proportion of flaxseed powder increased in the FFSFD *dahi* (3.42%). This could be due to the fact that flaxseed powder and mango pulp is high in mineral content (Morris*,* 2007). The carbohyadrate content of the FFSFD *dahi* samples increased with flaxseed powder, mango pulp, honey and alginate-chitosan microcapsules fortification from 16.11% in FFSFD while 6.07% in control *dahi*. The pH values of the *dahi* samples ranged from 4.65 to 4.73. FFSFD sample had the lowest value, when compared with the control sample. Lactic acid bacteria produce lactic acid during fermentation of milk-lactose, thus lowering the pH (Eke *et al.,* 2013). The titratable acidity also ranged from 0.75 to 0.68% in the *dahi* samples. The control *dahi* samples had lower acidity values (0.68%) than FFSFD (0.75%). This could be due to more availability of growth promoting substances to the fermenting microbes in FFSFD.

There was increased dietary fibre content in the *flaxseed powder fortified synbiotic flavoured dahi* by 0.81% compared to control *dahi* (0.00%). It may be due to high amount of dietary fibre present in flaxseed powder (Gopalan *et al.,* 2004). There was increase in antioxidant activity and phenolic content in the *flaxseed powder fortified synbiotic flavoured dahi* compared to control *dahi*. The increase in antioxidant activity and total phenolic content was contributed by flaxseed powder, mango pulp and honey which are known to be rich in antioxidant activity and total phenolic content (Chen *et al.,*1996). There was slight increase in syneresis in the control *dahi* by 10.36% compared to *flaxseed powder fortified synbiotic flavoured dahi* (5.17%). The reason might be the increase in water holding capacity due to high dietary fibre and increased total solid content in FFSFD.

The proximate composition of *dahi* as control (CD) and flaxseed fortified synbiotic flavoured *dahi* (FFSFD) was analyzed and presented in table (4.8).

Table 4.8 Compositional properties of optimised flaxseed fortified synbiotic flavoured *dahi* (FFSFD) and control *dahi* (CD)

Parameters	FFSFD	Control *Dahi*
Moisture (%)	80.03 ± 0.12	87.42 ± 0.31
Protein (g)	5.26 ± 0.02	3.65 ± 0.22
Fat (g)	4.28 ± 0.08	1.5 ± 0.1
Carbohydrate (g)	16.11 ± 0.12	6.07 ±0.48
Ash (%)	3.42 ± 0.04	0.64 ± 0.15
Dietary fibre (%)	1.96 ± 0.02	--
pH	4.65 ± 0.05	4.73 ± 0.05
Acidity (%)	0.71 ± 0.04	0.59 ± 0.02
Antioxidant activity (%)	80.18 ± 0.67	36.67±0.24
Total solids (%)	25.03 ± 0.40	10.16±0.10
Syneresis (%)	5.17 ± 0.01	10.36 ± 0.15

Values are Mean ± Standard deviation (SD) of three replicates
CD=Control *dahi* samples,
FFSFD= Flaxseed fortified synbiotic flavoured *dahi* samples (optimised level)

4.4.2 Influence of flaxseed powder on acidification kinetic parameters of FFSFD

Effect on acidification kinetics, pH and total solid

Acidification kinetics

The acidification kinetics of FFSFD *dahi* was characterized by V_{max}, t_{Vmax}, $pH_{Vmax,}$ $t_{pH\ 5.0}$ and $t_{pH\ 4.5}$ (Spinnler and Corrieu, 1989). Acidification profile of low fat milk supplemented with flaxseed powder, mango pulp, honey and synbiotic microcapsules of LA in different combinations of treatment are given in Table4.9. The highest value of V_{max} (25.73×10^{-3}pHunits/min) was obtained in sample C with mixed culture of *Dahi* starter, *L. plantarum* and *L. acidophilus* (in free form). The lowest V_{max} value was observed with the sample B with flaxseed powder and free LA (17.16×10−3pH units/min). As Table 4.9. shows, the maximum rate of acidification

(V_{max}) was significantly reduced ($P < 0.05$) by the addition of flaxseed powder in both samples B and D with free and microencapsulated bacteria, respectively which can probably be ascribed to the presence of substances with buffering capacity in FP such as organic acids and phenolic compounds (Zibadi & Watson, 2004) which might have accelerated the rate of fermentation the passion fruit peel, (Espirito Santo *et al.*, 2012).

Furthermore, it was observed that *dahi* samples A and B co-fermented by free cells of LA exhibited significantly ($P < 0.05$) lower V_{max} than the samples (C and D) co-fermented by microencapsulated LA and control. The ability of *L. acidophilus* to ferment FOS more effectively than other microorganisms (Barrangou *et al.,* 2003) might be the primary reason for the higher acidification rate of sample B with free cells of LA, which was significantly (P<0.05) higher from control and other samples. Nezhad *et al.,*, (2013) demonstrated that flaxseed mucilage acts as a good source of prebiotic, enhancing lactic acid bacteria growth in kefir model. The higher rate of acidification V_{max} of sample C and D is due to slow metabolic activity of bacteria within microcapsules. The observation was in line with findings of Sulatana *et al*., (2000) who reported the rate of acidification for the encapsulated cultures was slower than that observed for free cell incubated under similar conditions. The time taken for the encapsulated cells to arrive at the same end point of pH change is longer than that reached by the free cells. A similar pattern was also observed by Larisch *et al*., (1994).

The t_{Vmax} value for *dahi* samples ranged from 2.33 to 3.32 h. Nevertheless, the time to reach the maximum acidification rate (T_{Vmax}) was significantly reduced by the presence of the FP in samples B (2.59 h) and D (3.05 h) than the samples without FP i.e. sample A (3.13 h) and C (2.33 h). Same trend is shown in sample B and D for time to reach pH 5.0 ($T_{pH5.0}$) and end of fermentation pH 4.5 ($T_{pH4.5}$). The t_{Vmax} value of *dahi* samples was significantly affected by the fermenting culture strain and state of bacteria being as free cells or in encapsulated form. The t_{Vmax} value of control *dahi* (3.13 h) was significantly (P<0.05) higher than other samples co-cultured by LP and LA.

Champagne *et al.,* (2000) and Fonseca *et al.,* (2000) studied the acidification kinetics of different strains of S. thermophilus and Lactobacilli and reported V_{max}, t_{Vmax}, pH_{Vmax} and $t_{pH\ 4.5}$ strain dependent. Furthermore, the t_{Vmax} of sample A (2.59 h) and B (2.33 h) with free cells of probiotic bacteria LA was significantly lower than sample C (3.32 h) and D (3.05 h). This might be due to relatively lower metabolism of encapsulated bacteria within the microcapsule.

Thus, the present study of correlation analysis related to kinetic parameters indicates that multiple factors such as culture composition, culture state and presence of FP can affect acidification parameters of synbiotic *dahi*/yoghurt.

Total solids and pH

The content of total solids of *dahi* samples was found to be in range of 10.01±0.01 and 25.06±0.02. As expected, total solid content of *dahi* increased significantly ($P < 0.05$) in the presence of FP in samples B and D than in samples A and C. During the storage of FFSFD there was significant ($P < 0.05$) decrease in TS content of all samples but relatively less in sample D (FFSFD) with FP and microencapsulated LA. (Espirito Santo *et al.,* 2012; Sulatana *et al*., 2000).

The pH of all fresh *dahi* samples ranged between 4.8 and 4.56 after 12hr of fermentation. The FP addition reduced significantly the pH of sample B (4.56) and D (4.74) in comparison to samples A, C and control. Raju and Pal, (2014) reported that fiber (inulin) incorporation led to increased acidity of misti *dahi* ($P<0.05$) than the control. Franck, (2006) suggested that type of fibre added may constitute soluble fibre which might be consumed by bacteria resulting in formation of organic acids (Fernandez-Garcia and McGregor, 1997; Fernandez Gracia *et al.,*1998) including lactic acid leading to increased titratable acidity.

Table 4.9 Acidification kinetic parameters of low-fat milk fortified with mango pulp, honey and fermented by starter culture of *dahi* DS, co-cultures of DS and *Lactobacillus plantarum* (LP) along with free or microencapsulated culture of *Lactobacillus acidophilus* (LA) with (+FP) and without flaxseed powder (-FP).

Storage Days	**Fermented by DS**	**Fermented by DS and LP in combination with**			
		Free LA + Mango Pulp		**Encapsulated LA + Mango Pulp**	
	Control	**–FP (Sample A)**	**+FP (Sample B)**	**–FP (Sample C)**	**+FP (Sample D)**
V_{max} (10^{-3}pHunits/min)	18.90±0.02^{b}	21.82±0.04^{c}	17.16±0.30^{a}	25.73±0.25^{e}	23.25±0.67^{d}
t_{Vmax}(h)	3.13±0.06^{c}	2.59±0.03^{b}	2.33±0.06^{a}	3.32±0.02^{b}	3.05±0.01^{d}
pH_{Vmax}	5.56±0.03^{d}	5.27±0.06^{b}	5.17±0.04^{a}	5.59±0.05^{d}	5.47±0.05^{c}
$T_{pH5.0}$ (h)	4.06±0.02^{c}	3.44±0.03^{b}	3.27±0.01^{a}	4.25±0.04^{d}	3.49±0.02^{b}
$T_{pH4.5}$ (h)	5.23±0.12^{c}	4.36±0.06^{b}	4.20±0.01^{a}	5.43±0.06^{d}	5.26±0.05^{c}

All values are means±standard deviations of data from three independent experiments. Different lowercase letters (a–e) in the same row indicate significant difference ($P<0.05$).

V_{max}, maximum rate of acidification; T_{max}, time to reach V_{max}; pH $_{Vmax}$, pH at V_{max} ;$T_{pH5.0}$, time to reach pH 5.0;$T_{pH4.5}$, end time of fermentation.

Table 4.10 Post-acidification pH, titratable acidity, total solids and syneresis during shelf-life storage of control and different treatments of *dahi* samples

pH					
Storage Days	**Fermented by DS**	**Fermented by DS and LP in combination with**			
		Free LA + Mango Pulp		**Encapsulated LA + Mango Pulp**	
	Control	**–FP (Sample A)**	**+FP (Sample B)**	**–FP (Sample C)**	**+FP (Sample D)**
0 Day	4.80±0.01^{eC}	4.73±0.02^{eB}	4.56±0.02^{eA}	4.83±0.03^{dC}	4.74±0.02^{bB}
7th Days	4.63±0.02^{dC}	4.56±0.03^{dB}	4.34±0.03^{dA}	4.75±0.03^{cD}	4.68±0.02^{cC}
14th Days	4.46±0.02^{cB}	4.48±0.01^{cB}	4.19±0.01^{cA}	4.71±0.05^{cC}	4.63±0.03^{cD}
21st Days	4.32±0.03^{bB}	4.31±0.06^{bB}	3.74±0.04^{bA}	4.67±0.02^{bD}	4.56±0.01^{bC}
28th Days	4.06±0.05^{aB}	4.19±0.04^{aC}	3.05±0.02^{aA}	4.59±0.05^{aD}	4.50±0.04^{aE}
Titratable acidity (% lactic acid)					
Storage Days	**Control**	**(Sample A)**	**(Sample B)**	**(Sample C)**	**(Sample D)**
0 Day	0.59±0.01^{aA}	0.76±0.01^{aC}	0.82±0.01^{aD}	0.61±0.01^{aB}	0.71±0.01^{aC}
7th Days	0.65±0.02^{bA}	0.81±0.04^{bC}	0.95±0.02^{bD}	0.63±0.03^{aA}	0.76±0.03^{aB}
14th Days	0.73±0.02^{cB}	0.92±0.02^{cD}	1.04±0.01^{cE}	0.68±0.01^{abA}	0.83±0.01^{bC}
21st Days	0.84±0.04^{dB}	1.05±0.03^{dD}	1.16±0.02^{dE}	0.74±0.04^{cA}	0.90±0.05^{cC}
28th Days	0.98±0.01^{eB}	1.18±0.05^{eC}	1.32±0.02^{eD}	0.81±0.02^{dA}	1.02±0.04^{dB}

Results presented as a mean (n=3). Different small letter superscripts depict the statistical difference (P<0.05) between means for the same *dahi*/yogurt batches at different time intervals within a row. Different capital letter superscripts depict the statistical difference P<0.05 between means for different *dahi*/yogurt batches within a column.
* Abbreviations are as per Table4.9

Contd…

Total Solids (%)					
Storage Days	**Fermented by DS**	**Fermented by DS and LP in combination with**			
		Free LA + Mango Pulp + Honey		**Encapsulated LA + Mango Pulp+ Honey**	
	Control	**−FP (Sample A)**	**+FP (Sample B)**	**−FP (Sample C)**	**+FP (Sample D)**
0 Day	10.01 ± 0.01^{cA}	21.11 ± 0.02^{dB}	23.18 ± 0.02^{cD}	22.61 ± 0.03^{aC}	25.06 ± 0.02^{aE}
7th Days	11.23 ± 0.02^{eA}	22.62 ± 0.03^{eB}	25.24 ± 0.03^{dD}	24.37 ± 0.03^{bC}	27.54 ± 0.02^{bE}
14th Days	10.16 ± 0.02^{dA}	20.59 ± 0.01^{cB}	26.06 ± 0.01^{eD}	25.95 ± 0.05^{cC}	28.49 ± 0.03^{cE}
21st Days	9.45 ± 0.03^{bA}	18.36 ± 0.06^{bB}	20.37 ± 0.04^{bC}	26.08 ± 0.02^{eD}	29.12 ± 0.01^{eE}
28th Days	8.23 ± 0.05^{aA}	15.89 ± 0.04^{aC}	15.29 ± 0.02^{aB}	25.21 ± 0.05^{dD}	28.63 ± 0.04^{cdE}
Syneresis (%)					
Storage Days	**Control**	**(Sample A)**	**(Sample B)**	**(Sample C)**	**(Sample D)**
0 Day	10.36 ± 0.51^{eE}	8.12 ± 0.41^{eD}	7.25 ± 0.11^{eC}	6.75 ± 0.22^{eB}	5.81 ± 0.57^{eA}
7th Days	9.04 ± 0.65^{dE}	7.86 ± 0.34^{dD}	6.73 ± 0.12^{dC}	5.84 ± 0.53^{dB}	5.24 ± 0.35^{dA}
14th Days	7.13 ± 0.70^{cE}	6.43 ± 0.42^{cD}	5.26 ± 0.41^{cC}	4.53 ± 0.16^{cB}	4.38 ± 0.51^{cA}
21st Days	6.24 ± 0.24^{bE}	5.56 ± 0.63^{bD}	4.49 ± 0.52^{bC}	3.86 ± 0.54^{bB}	3.31 ± 0.45^{bA}
28th Days	5.15 ± 0.38^{aE}	4.62 ± 0.25^{aD}	3.81 ± 0.12^{aC}	3.02 ± 0.32^{aB}	2.26 ± 0.24^{aA}

Results presented as a mean (n=3). Different small letter superscripts depict the statistical difference ($P<0.05$) between means for the same *dahi*/yogurt batches at different time intervals within a row. Different capital letter superscripts depict the statistical difference $P<0.05$ between means for different *dahi*/yogurt batches within a column.
* Abbreviations : Dairy Starter (DS) , *Lactobacillus plantarum* (LP), *Lactobacillus acidophilus* (LA) with (+FP) and without flaxseed powder (-FP).

Table 4.11 Texture parameters of FFSMD *dahi* samples with different treatments during cold storage.

S. No.	Treatment	Firmness(g)					Consistency(gs)				
		0 d	**07 d**	**14d**	**21d**	**28d**	**0 d**	**07 d**	**14d**	**21d**	**28d**
1.	Control	171.05^{bA}	175.11^{dA}	177.12^{eA}	173.47^{cA}	164.29^{aA}	2222.28^{eA}	2087.08^{dA}	1822.70^{cA}	1654.23^{bA}	1326.51^{aA}
2.	Sample A	177.00^{bB}	183.78^{dB}	186.36^{eB}	179.26^{cB}	169.36^{aB}	2475.53^{eC}	2140.20^{dB}	2000.00^{cB}	1886.28^{bB}	1727.32^{aB}
3.	Sample B	211.92^{bC}	224.18^{cC}	232.04^{eC}	221.28^{dC}	205.94^{aC}	2846.36^{aD}	3046.60^{cD}	3186.83^{eD}	3089.35^{dD}	2862.57^{bD}
4.	Sample C	204.96^{aD}	211.71^{bD}	224.97^{eD}	220.35^{dD}	217.52^{cD}	2380.50^{aB}	2872.97^{dC}	2870.47^{dC}	2642.61^{cC}	2439.26^{bC}
5.	Sample D	235.97^{aE}	258.80^{bE}	282.05^{eE}	280.16^{dE}	277.68^{cE}	3072.46^{aE}	3267.10^{bE}	3420.03^{cE}	3391.35^{cE}	3263.51^{bE}

S. No.	Treatment	Cohesiveness (g)					Index of Viscosity (gs)				
		0 d	07 d	14d	21d	28d	0 d	07 d	14d	21d	28d
1.	Control	18.44^{bA}	19.23^{cA}	20.67^{dA}	19.80^{cA}	17.46^{aA}	20.60^{bA}	23.10^{dA}	24.79^{eA}	21.39^{cA}	18.35^{aA}
2.	Sample A	22.19^{bB}	24.86^{dB}	25.68^{eB}	23.65^{cB}	20.17^{aB}	24.57^{bB}	28.35^{dB}	29.71^{eB}	26.43^{cB}	23.51^{aB}
3.	Sample B	30.55^{bD}	31.29^{cD}	34.53^{eC}	32.16^{dC}	29.18^{aC}	35.53^{aD}	38.63^{bD}	41.92^{dD}	39.25^{cD}	35.31^{aD}
4.	Sample C	25.16^{aC}	29.99^{bC}	34.77^{eC}	33.47^{dD}	31.43^{cD}	28.70^{aC}	31.94^{bC}	34.16^{eC}	33.53^{dC}	32.16^{cC}
5.	Sample D	36.19^{aE}	38.71^{bE}	42.94^{dD}	41.76^{cE}	41.04^{cE}	42.15^{aE}	47.00^{bE}	51.43^{dE}	51.06^{dE}	49.83^{cE}

Standard deviations were under 0.05. Results presented as a mean (n=3). Different small letter superscripts depict the statistical difference ($P<0.05$) between means for the same *dahi*/yogurt batches at different time intervals between the column. Different capital letter superscripts depict the statistical difference $P<0.05$ between means for different *dahi*/yogurt batches within a row.

PHASE III: SHELF LIFE STUDY OF FLAXSEED FORTIFIED SYNBIOTIC FLAVOURED *DAHI*

4.5 Post acidification changes during storage

4.5.1 Effect of storage period on pH

The study of post-acidification and titratable acidity of flaxseed powder fortified synbiotic flavoured *dahi* (FFSFD) was conducted for 28 days of storage at 4 °C. Readings were taken at 0 day (after 12 hr of fermentation), 7^{th}, 14^{th}, 21^{st} and 28^{th} day and results are shown in Table 4.10. The values of pH at start of storage i.e. 0 days in control and samples A, B, C, D ranged in between 4.90 and 4.56, and the largest difference observed was between control (4.90) and sample B (4.56) which might be due to the rapid utilization of growth promoting substances of FP such as soluble fibre from FP by free cells of LA. The pH of sample D (4.64) with FP and encapsulated LA was higher than Sample B with FP and free cells of LA. This might be due to the lower metabolic activity of encapsulated LA which results in slow and steady consumption of growth promoting substances from the product into which microcapsules has been incorporated. During cold (4˚C) storage of 28 days, pH of Sample B and sample D significantly ($P < 0.05$) reduced from 4.56 to 4.19 and 4.64 to 4.48 respectively. The sample B shows that in presence of FP there was increased reduction in pH which indicated higher lactic acid production by free LA. This indicates, profuse consumption of growth promoting substances from FP by free cells of LA which results in drastic reduction in pH within short period of storage days. In similar study Oakenfull, (2001) reported that presence and type of fiber contributes to increase the pH value of the yogurt samples that is attributed to a reaction of the linked monosaccharide units that constitute the soluble fiber or polysaccharide. After 14 days of shelf-life the pH of *dahi* samples A and B decreased significantly ($P < 0.05$). This may alter the taste and flavour leading to unacceptability of product by consumers. However, in sample D there was steady reduction in pH from (4.64 to 4.56 in 7days and 4.44 in 14 days) resulted from controlled utilization of food resources by microencapsulated cells of LA. This stability of pH observed in sample

D with FP and microencapsulated LA during cold storage may prolong the shelf life of FFSFD.

Nazzaro *et al.,* (2009) demonstrated that the amount of acetic acid was lower in juice fermented with encapsulated cells (34% less than free cells), the level remained stable after 8 weeks of cold storage. The decrease of pH during the storage can be attributed to the high bacterial metabolic activity with the consumption of lactose and lactic acid production (Bakirci and Kavaz, 2008). The final pH (at end of 6 week storage) of *dahi* samples with encapsulated probiotic bacteria was higher than the samples inoculated with free probiotic bacteria. In conventional yoghurt formulations, acidity is a consequence of lactic acidification obtained at the end of incubation and post-acidification during storage. Lactic acidification is the result of lactose fermentation by the associative growth of the mesophilic bacteria of *dahi* starter and L. *plantarum.*

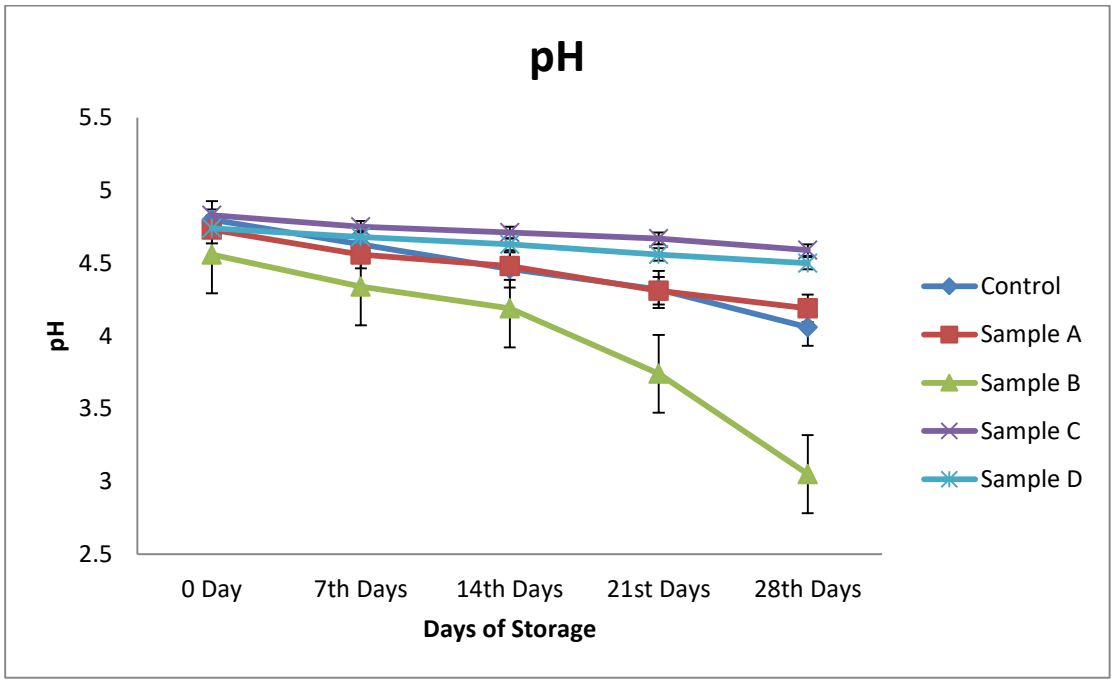


Fig. 4.17 Changes in pH of *dahi* samples during storage

Post-acidification during storage time can be linked to the progressive transformation of lactose into lactic acid. However, in yoghurts added with soluble fiber components, other mechanisms may take place that promote a further increase in

the acidity values. Soukoulis *et al.,* (2007) showed that yoghurts with pectin or guar gum used as stabilizers had higher acidities than those containing xanthan or j-carrageenan. Sendra *et al.,* (2008) found that the addition of citric fiber in fermented milks enhanced the growth and survival of probiotic bacteria, which on turn probably induced a more rapid transformation of lactose into lactic acid.

4.5.2 Effect of storage period on titratable acidity (% Lactic Acid)

After 12hr of fermentation i.e. on 0 day of cold storage all *dahi* samples exhibited a significant increase ($P < 0.05$) in their titratable acidity. After 28 days of storage the highest value of average titratable acidity 1.32 % was observed in sample B with FP and free cells of LA. This rise in lactic acid content is in accordance with findings of Nazzaro *et al.,*(2009) who reported higher lactic acid content in carrot juice samples fermented by free cells of bacteria than encapsulated bacteria. At 28 days of shelf life, titratable acidity of sample B with FP and free cells of LA and BB was significantly higher than sample A without FP. The possible explanation of rise in lactic acid content might be due to availability of soluble fiber of flaxseed for growth of bacteria and thereby increased production of lactic acid (Nezhad *et al.,* 2013). Fernandez-Gracia *et al.,* (1998) reported that oat fiber addition in sweetened yoghurt slightly increased the lactic acid content after 6 h of fermentation.

The problem of sensitivity to acidity of the probiotic culture is compounded by the fact that acidity may increase during storage, a phenomenon known as 'over acidification'. This post-acidification, during storage, is due to β-galactosidase which is still active at 0–5 °C. In this case, pH may decrease to less than 4.2, resulting in whey separation and affecting also the LAB viability, due more to hydrogen ions than ions of lactate (Rasic & Kurman, 1978). Titratable acidity of sample D with FP and encapsulated LA varied from 0.72 to 0.92% which is lower than sample B. The decreased pH 4.19 and increase in lactic content 1.32 mg lactic acid g^{-1} of sample B results in shorter shelf life affecting the sensory quality of product rendering it unacceptable to the consumers.

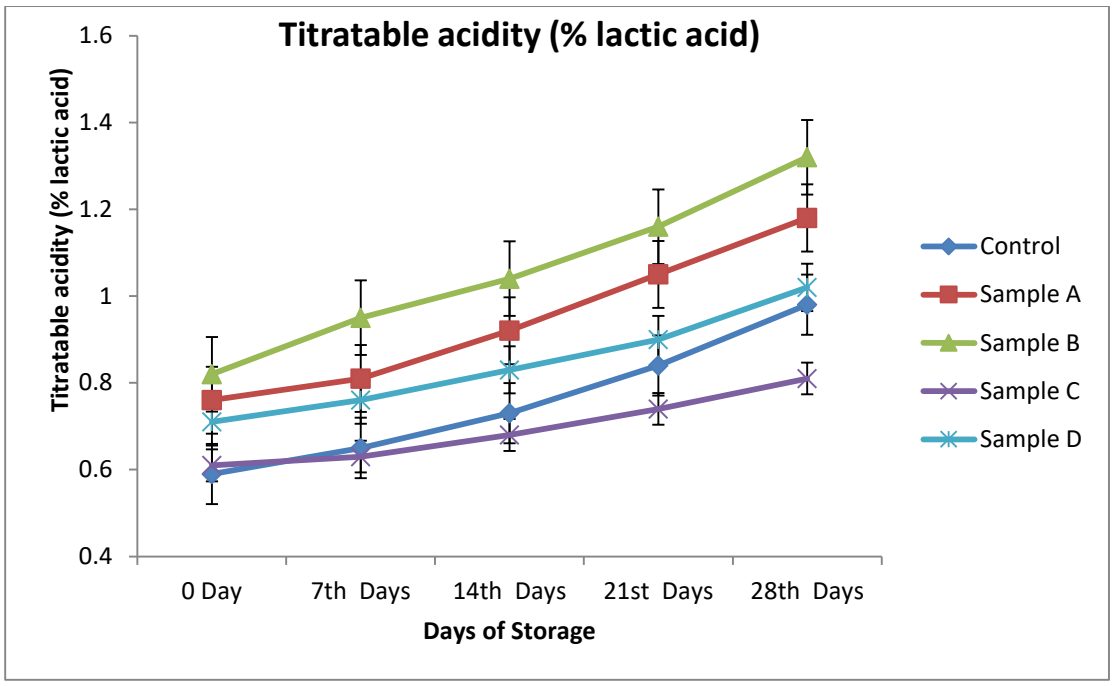


Fig. 4.18 Changes in acidity content of *dahi* samples during storage

4.5.3 Effect of storage period on syneresis

Syneresis provides an indication of the non-homogeneities in the fermented milk system. A higher water separation (syneresis) is related to higher gel instability (Lucey *et al.,* 1998). The syneresis of all *dahi* samples immediately after production and after 7, 14, 21 and 28 days of cold storage is presented in Fig.4.19. On 0 day, the highest syneresis was observed in sample A followed by control samples, then sample B and least syneresis was observed in sample D ($P<0.05$). Thus, the supplementation significantly affected the syneresis ($P<0.05$). The results, therefore, clearly show that supplementation of FP in sample B significantly improved gel stability of the fermented milk systems much more than the sample A and control samples. This can be possibly explained by fact that fiber may be involved in the entrapment of water as part of the three dimensional network (Oakenfull, 2001) of the yogurt gel.

In sample C and D syneresis percent decreased in presence of microcapsules of LA. This could be due to carbohydrate, of the alginate-chitosan microcapsule which binds to the hydrogen ions present in gel system decreasing serum expulsion. In sample B, there was greater change in syneresis after 14 days was potentially due

to the higher decrease in pH, as acid production was highest in the sample fermented by free LA during storage. Tamime and Robinson, (1999) have reported that greater acidification causes more initial water separation from the gel, and as a result, samples have less water to lose during storage. Syneresis is, therefore, directly linked to acidification during storage. Furthermore, an increase in the total solid content, especially protein content, starch, and fiber in sample D which have hydrocolloidal properties, may have conferred a stronger, more homogenous texture to the samples (Peng *et al.,* 2009; Lucey, 2001). Thus, the ability of flaxseed powder to decrease syneresis in freshly prepared sample may be due to its higher hydrocolloid content which favours both probiotic activity (acidification) and enhanced water holding capacity. Further fiber component could also strengthen the yoghurt gel (Bozanic *et al.,* 2001).

The serum expulsion of all the *dahi* samples were significantly affected by the storage time ($P<0.05$), but sample D (FFSFD) had less synersis percent in comparison to the other samples at the end of the storage period. Similar findings were observed by Velez-Ruiz *et al.,* (2011) in flaxseed and calcium fortified yoghurt.

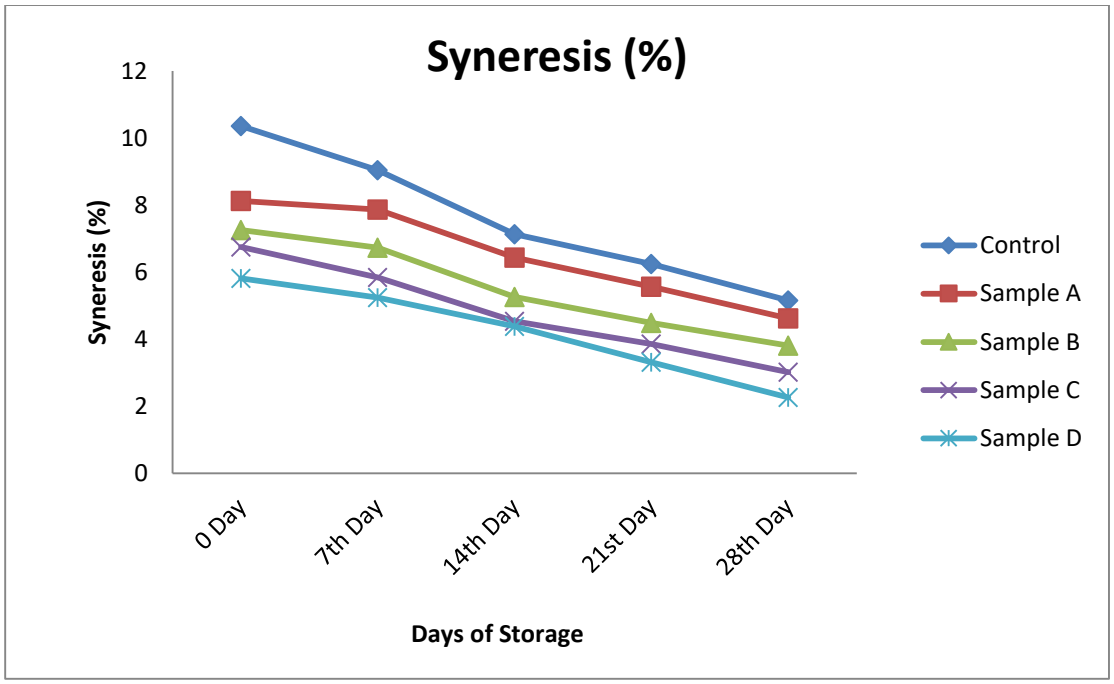


Fig. 4.19 Changes in syneresis of *dahi* samples during storage

Effect of storage on texture profile of FFSFD

4.5.4 Effect of storage period on firmness

The texture profiles of the different *dahi* samples were evaluated at 0 day (after 12 hr of fermentation), 7th, 14th, 21st and 28th days of cold storage and are shown in Table 4.19. Generally, all texture parameters significantly increased ($P<0.05$) during cold storage, being the most marked increase observed after 7 days of cold storage. The texture value of all samples shows a general trend of increase in firmness during the storage time (Seçkin, 2012). The sample B and D (221.92 and 245. 97 g, respectively) with FP shows highest increase in firmness value than the control (177.00g). This observation is supported by many recent studies on effect of fibre on firmness of similar product-yoghurt (Espirito Santo *et al.,* 2012; Staffolo *et al.,* (2004).

Oliveira *et al.,* (2011) concluded that prebiotics could provide additional energy to potentiate exopolysaccharide (EPS) biosynthesis, thus improving firmness and viscosity. Brennan and Tudorica, (2008) indicated that the increased firmness improves texture of yoghurt (*dahi* like product) by reducing susceptibility to rearrangements within its network, shrinkage and whey expulsion. After 12 hr fermentation (0 day), the value of firmness of sample D (245.92g) with FP and encapsulated probiotic bacteria was significantly ($P<0.05$) higher than sample B with free cells of LA. This might be due to the presence of higher protein content in FP and polysaccharides of encapsulant material (alginate which could have lead to the increase in firmness value of sample D in comparison to other samples.

After 7 days of cold storage, in sample B firmness increased rapidly to value of 264.18 g while gradual increase observed in sample D. The possible reason for this could be rapid utilization of soluble fiber content of flaxseed by the free cells LA and BB promoting their growth. On the contrary, in sample D, LA and BB are in encapsulated form with lower metabolic activity which results in gradual utilization of food resources by bacteria.

Increasing of the firmness during storage time may be related to dietary fibers absorbing more moisture because of its higher water-holding capacity (Hashim *et al.,* 2009). However, the fortification types and level of fibers had a significant effect ($P<0.05$) on the yoghurt firmness. Staffolo *et al*., (2004) and Sendra *et al.*, (2010) reported increased firmness and viscosity in fermented milk products with the addition of different fibers.

The samples without flaxseed fibre, sample A and C with free and encapsulated bacteria respectively have higher firmness value than control but lower than sample B and D with FP. This could have resulted by the addition of honey and mango pulp which increases the total solid content, thus increasing firmness value (Tamime, 2006).

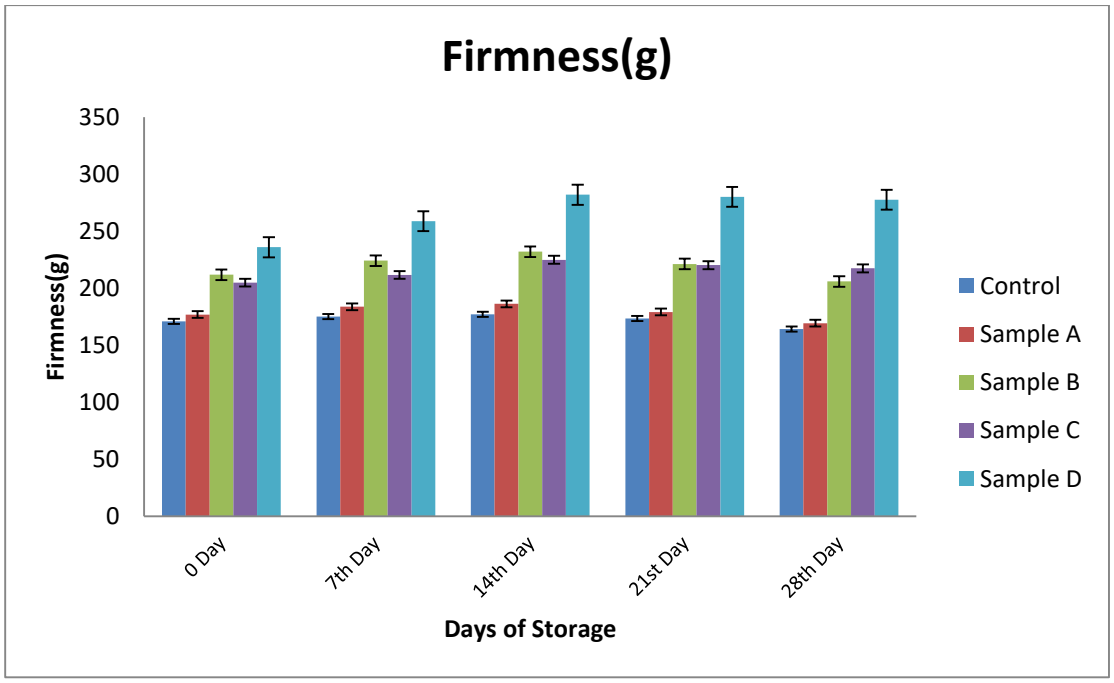


Fig. 4.20 Changes in firmness of *dahi* samples during storage

4.5.5 Effect of storage period on consistency

The most variable parameter was determined to be consistency during storage time. Consistency of sample B and D (3072.46 & 2846.36 gs, respectively) showed the highest significant increase from the control (2222.28 gs), sample A and C

without FP (2475.53 & 2380.50 gs, respectively). The differences in the values of all the samples were significant ($p<0.05$) during storage. The higher values of consistency of sample B and D might be due higher protein content attributed by FP and polysaccharide content of alginate-chitosan microcapsules. After 7 days of storage, there was increase in consistency value in samples with FP and synbiotic microcapsules (sample B, sample C and sample D) while sample A and control showed significant decrease in value. Samples with FP (sample B and D) showed decrease in consistency after 14 days of storage. However, the sample B shows decrease in textural values after 14 days of storage while the sample D values for textural parameters gradually increased or remained constant up till 21 days thereafter decreased during storage time. This observation of Sample D may prolong the shelf life the product FFSFD.

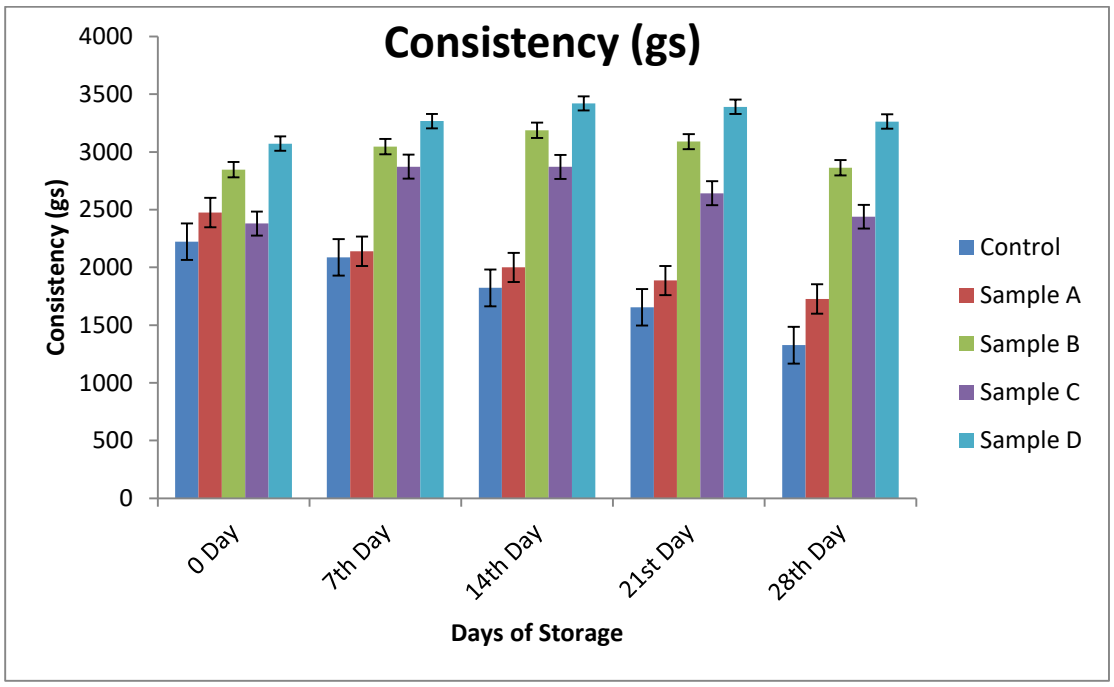


Fig. 4.21 Changes in consistency of *dahi* samples during storage

Similar findings were observed by Rasdhari *et al.,* (2008) in *Hibiscus sabdariffa* fortified yoghurt. Espirito Santo *et al.,* (2012), found that at the end of storage, firmness and consistency in all passion fruit peel powder skim yoghurts were higher than in their respective controls. Consistency increased with addition of

different dietary fibre in yoghurt during storage (Seckin and Baladura, 2012).However, the consistency value decreased after 14th day of storage. The possible reason might be due to increased synersis and acidity due to increased microbial activity. Similar findings were observed by Velez-Ruiz *et al.,* (2011) in flaxseed and calcium fortified yoghurt.

4.5.6 Effect of storage period on cohesiveness

The cohesiveness values of *dahi* samples increased significantly ($p<0.05$) up till 14 days of cold storage, thereafter decreased. This decrease was drastic in control and sample A and sample B. The possible reason might be disruption of gel network by increased metabolic activity. However, in sample C and D with synbiotic microcapsules value remained constant or slight decrease was observed indicating lower metabolic activity of probiotic bacteria and thus enhanced stability of product during storage. The sample B and D with FP had higher cohesiveness values than others. Similar findings were observed by Rasdhari *et al.,* (2008) in *Hibiscus sabdariffa* fortified yoghurt.

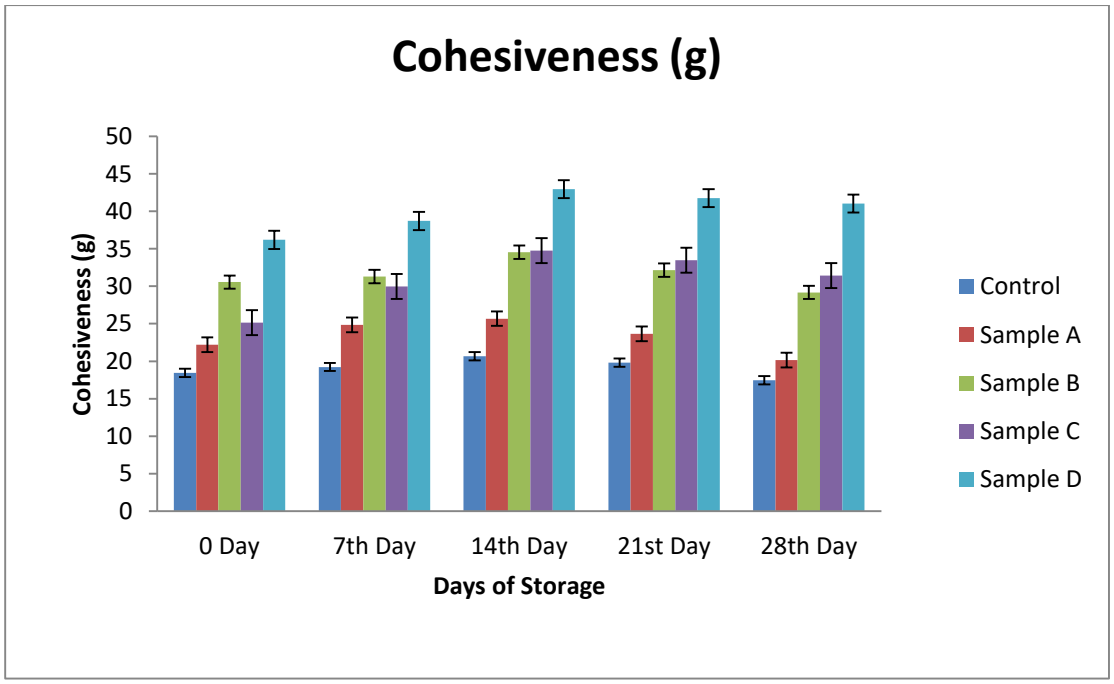


Fig. 4.22 Changes in cohesiveness of *dahi* samples during storage

Espirito Santo *et al.,* (2012), found that at the end of storage, cohesiveness in all passion fruit peel powder skim yoghurts were higher than in their respective controls. Cohesiveness increased with addition of different dietary fibre in yoghurt during storage (Seckin and Baladura, 2012). However, the cohesiveness value decreased after 14th day of storage. The possible reason might be due to increased synersis and acidity due to increased microbial activity. Similar findings were observed by Velez-Ruiz *et al.,* (2011) in flaxseed and calcium fortified yoghurt.

4.5.7 Effect of storage period on index of viscosity

The value of index of viscosity of Sample D (42.15 gs) was significantly higher than control, sample A, B and C (24.57, 35.53 & 28.74 gs). This can be explained by the fact that by incorporating polymer encapsulated bacteria it might be possible to not only increase viability but also improve viscosity/gel properties of yoghurt.

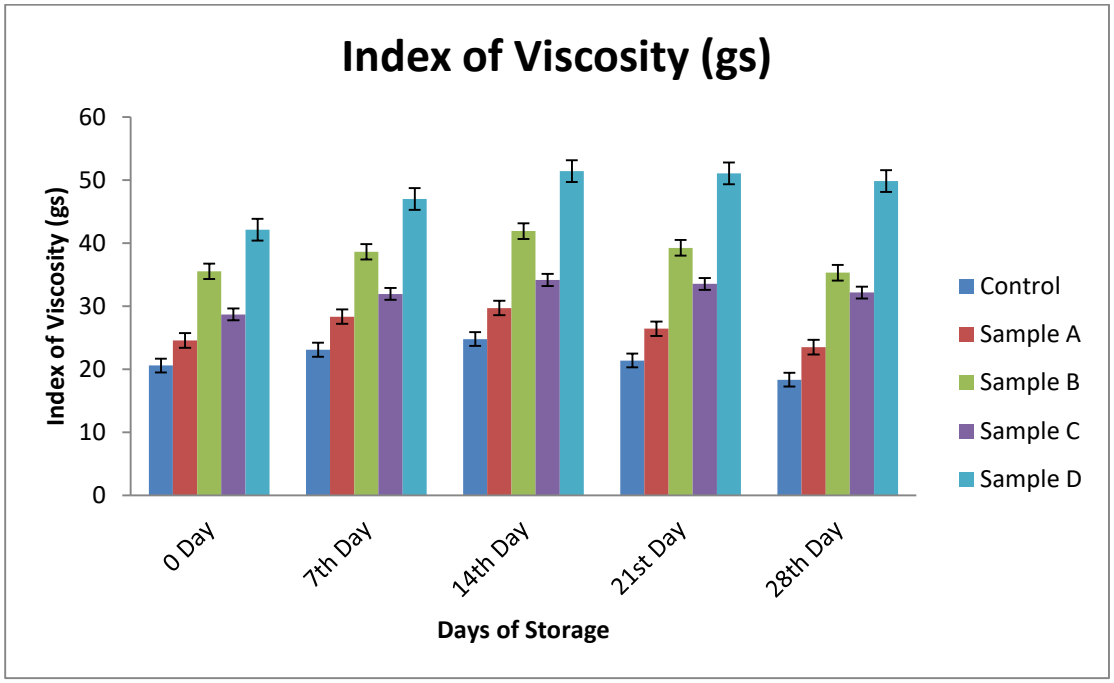


Fig. 4.23 Changes in index of viscosity of *dahi* samples during storage

The cohesiveness, consistency and index of viscosity were observed to be greatly improved by addition of FP in sample B and D. Although, sample B shows deformation of gel structure after 7 days of storage. This can be attributed to the rapid post-acidification change that implies increasing titratable acidity by decreased pH due to active lactose conversion into lactic acid. However, sample D shows least post acidification changes due to lower metabolic activity and slow consumption of lactose which is also enhanced by the buffering capacity of soluble flaxseed fibre (Seçkin *et al.*, 2012).

Adhikari *et al.,* (2000) observed the greater decrease in pH and concurrent increase in acidity, in the nonencapsulated treatment that might be due to metabolic activity of both the bifidobacteria and the yoghurt starter cultures despite their declining populations, which resulted in the production of organic acids from lactose.

4.5.8 Effect of storage on probiotic viable count of *Lactobacillus plantarum*

The *Lactobacillus plantarum* count has been found to increase after 0 day in sample B, C and D while decreased in sample A. Supplementation with 2% flaxseed, in sample B significantly ($P<0.05$) improved the viability of *Lactobacillus plantarum* in FFSFD *dahi* samples during storage (Table 4.11). Addition of cereals could either act as an additional nutrient or modify the unfavourable environmental influences, resulting in improved probiotic viability (Desai *et al.,* 2004; Madhu and Prapulla 2012). In sample B there was increase in viable cell count (log cfu/ml 10.06) till 7^{th} day which may be attributed to the honey as well as the buffering capacity of flaxseed fibre and part of soluble fibre would have promoted the growth of bacteria (Nezhad *et al.,* 2013). While in sample A the increased acidification by DS starter culture and LA may lead to decline in viable cell count of LP (Shah, 2000).

The values presented in the table clearly indicates that as the storage increases the LP count in sample A decreased ($P<0.05$) sharply after 7 day of storage, while in sample B viable cell count decline was observed only after 7^{th} day of cold storage. However, in sample C and D with encapsulated LA and BB, viable count of LP remained nearly contant uptill 14 days of storage.this can be explained by findings of

Shah. 2000b who reported that increased acidity by starter culture and LA results in significant decline in LP population. The viable cell count of LP was least affected in sample D which indicates the buffering capacity and growth promoting substances of flaxseed for probiotic bacteria.

Nezhad *et al.,*(2013) showed that after 21days of cold storage kefir samples with crude flaxseed mucilage, with or without probiotics, showed lower pH values compared to their respective treatments supplemented with pure mucilage. This indicates that LP utilizes flaxseed mucilage as prebiotic for their growth. Prebiotics, stimulate the metabolism of probiotics, by release of increased level of fructose as result of its partial hydrolysis, which gets metabolized as an additional carbon and energy source (Tamime, 2005).

Nevertheless, the Sample D (FFSFD) showed higher cfu/g in comparison to the probiotic yoghurt samples at the end of storage (Table 4.12). The viable cell counts of probiotic bacteria i.e. *Lactobacillus plantarum* by the end of 28 days of storage was above 8 log cfug^{-1}, and thus, the FFSFD developed could be considered as a probiotic product.

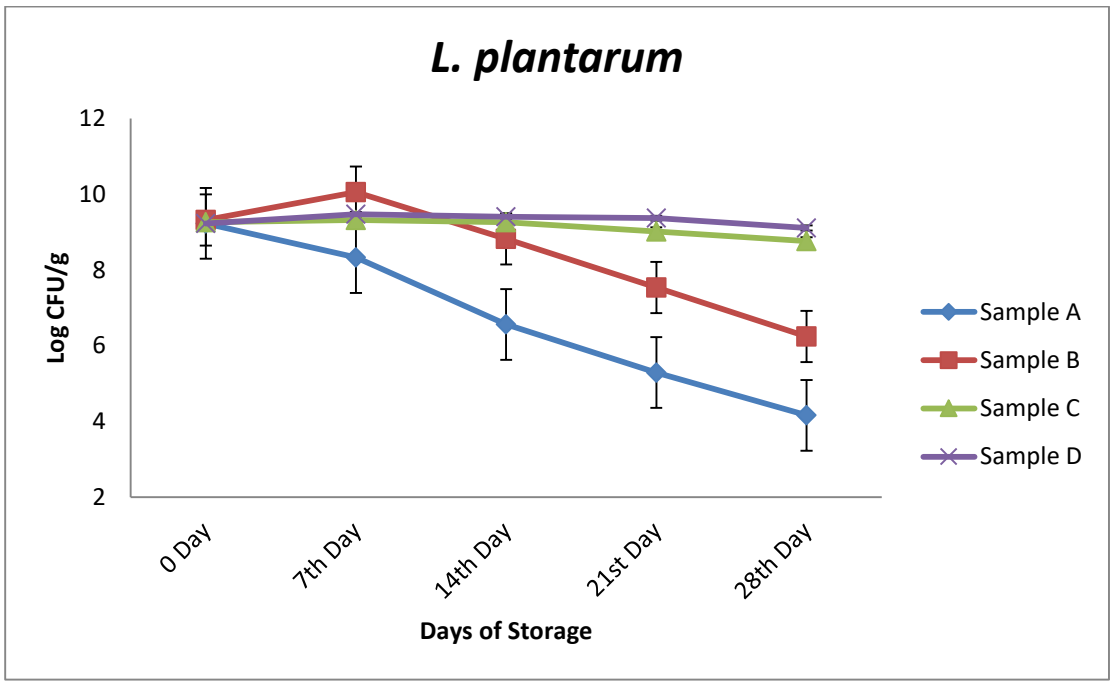


Figure 4.24 Effect of storage on *Lactobacillus plantarum* count of *dahi* samples.

4.5.9 Effect of storage on probiotic viable count of *Lactobacillus acidophilus*

On the 0 day i.e. after 12h of fermentation in freshly prepared *dahi* *Lactobacillus acidophilus* count in all *dahi* samples are presented in Table.4.12. *Lactobacillus acidophilus* count had been found to increase after 0 day in all the samples. There was a marked increase in sample B (10.62) and D (9.61) after 7 days of storage. The possible reason for this increase in cell count might be the supplementation of 2% flaxseed and part of flaxseed soluble fibre would have promoted the growth of bacteria (Nezhad *et al.,* 2013) as well as the buffering capacity of flaxseed fibre would have modified the unfavourable environmental influences, resulting in improved probiotic viability (Desai *et al.,* 2004; Madhu and Prapulla, 2012). Prebiotics, stimulate the metabolism of probiotics, by release of increased level of fructose as result of its partial hydrolysis, which gets metabolized as an additional carbon and energy source (Tamime, 2005).

There was gradual increase in sample C and D which might be due to lower metabolic activity of LA in encapsulated form. The values presented in the table clearly indicates that as the storage time increases the LA count in sample A and B decreased ($P<0.05$) sharply after 7 day of storage, while in sample C and D viable cell count decline was observed only after 14th day of cold storage. In sample D viable cell count remained nearly constant till 21st day of cold storage. Nezhad *et al.*, (2013) showed that after 21days of cold storage kefir samples with crude flaxseed mucilage, with or without probiotics, showed lower pH values compared to their respective treatments supplemented with pure mucilage. This indicates that LA utilizes flaxseed mucilage as prebiotic for their growth. The problem of sensitivity to acidity of the probiotic culture is compounded by the fact that acidity may increase during storage, a phenomenon known as 'over acidification'. This post-acidification, during storage, is due to β-galactosidase which is still active at 0–5 °C. In this case, pH may decrease to less than 4.2, resulting in whey separation and affecting also the LAB viability, due more to hydrogen ions than ions of lactate (Rasic & Kurman, 1978).

Table 4.12 Effect of storage on probiotic viable count of *Lactobacillus plantarum* and *Lactobacillus acidophilus* (log 10^8 CFU/g) of different treatments of FFSFD *dahi* samples.

		***Lactobacillus plantarum*(CFU/g)**					***Lactobacillus acidophilus*(CFU/g)**				
S.No.	**Treatment**	**0 d**	**07 d**	**14d**	**21d**	**28d**	**0 d**	**07 d**	**14d**	**21d**	**28d**
1.	Sample A	9.23 ± 0.16^{aE}	8.33 ± 0.04^{aD}	6.56 ± 0.21^{aC}	5.29 ± 0.06^{aB}	4.16 ± 0.24^{aA}	9.5 ± 0.11^{bcE}	9.2 ± 0.12^{aD}	8.23 ± 0.18^{aC}	7.46 ± 0.03^{aB}	6.84 ± 0.05^{aA}
2.	Sample B	9.32 ± 0.14^{bD}	10.06 ± 0.06^{dE}	8.82 ± 0.02^{bC}	7.54 ± 0.09^{bB}	6.24 ± 0.12^{bA}	9.45 ± 0.05^{dD}	10.62 ± 0.13^{dE}	9.19 ± 0.06^{bC}	8.74 ± 0.07^{bB}	7.86 ± 0.06^{bA}
3.	Sample C	9.26 ± 0.12^{aC}	9.32 ± 0.13^{bC}	9.28 ± 0.16^{cC}	9.02 ± 0.17^{cB}	8.76 ± 0.06^{cA}	9.48 ± 0.03^{bD}	9.52 ± 0.16^{cD}	9.36 ± 0.14^{cC}	9.28 ± 0.08^{cB}	9.06 ± 0.10^{cA}
4.	Sample D	9.23 ± 0.11^{aE}	9.47 ± 0.08^{cBC}	9.41 ± 0.15^{dB}	9.37 ± 0.14^{dB}	9.11 ± 0.13^{dA}	9.38 ± 0.15^{aA}	9.61 ± 0.21^{bB}	9.65 ± 0.05^{dB}	9.62 ± 0.13^{dB}	9.37 ± 0.12^{dA}

Values bearing different small superscripts (a, b, c, d, e) in a column differ significantly (Duncan test, $P<0.05$)
Values bearing different capital superscripts (A, B, C, D, E) in between column differ significantly (Duncan test, $P<0.05$)

It was observed that LA was more stable in both the samples (Sample A and Sample B) in comparison to LP during the 28 days of cold storage. This observation was supported by similar findings of Rybka and Kailasapathy, (1995) who demonstrated that *L. acidophilus* could survive in yoghurt better at sufficient levels ($4x10^6$ cfu mL^{-1}) for up to 28 days and *L. acidophilus* was more tolerant of acidic conditions than any other lactic acid bacteria.

The viable cell counts of probiotic bacteria i.e. *Lactobacillus acidophilus* by the end of 28 days of storage was above log 10^8cfug^{-1}, and thus, the FFSFD developed could be considered as a probiotic product.

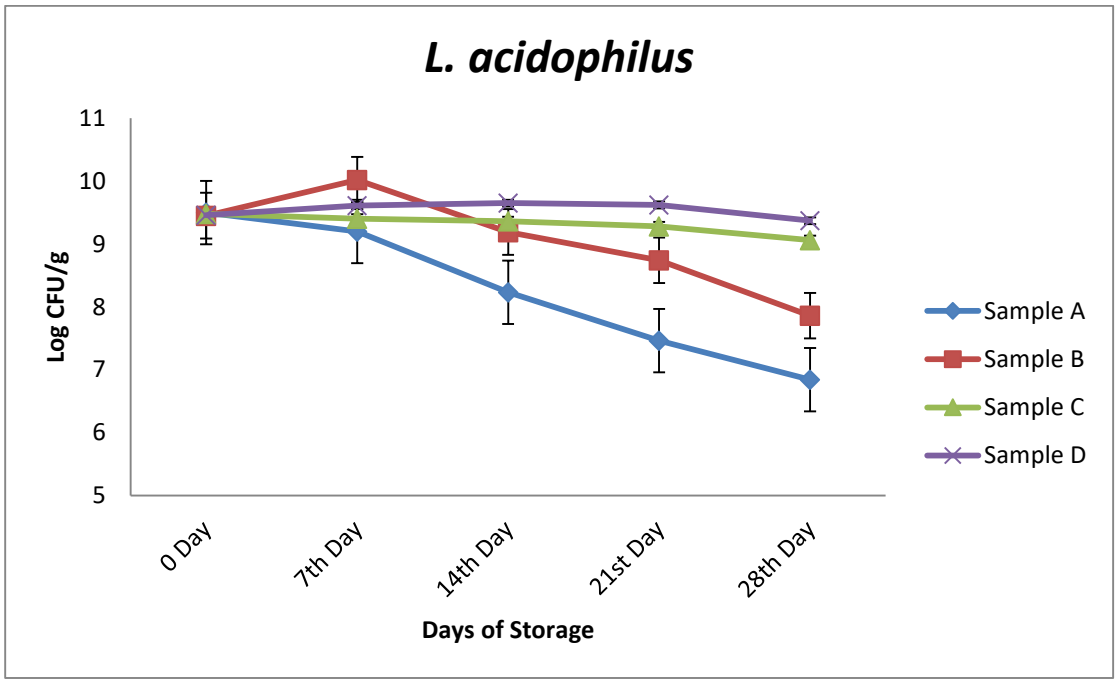


Figure 4.25 Effect of storage on *Lactobacillus acidophilus* count of *dahi* samples.

Effect of acidification on probiotics

Many studies have shown low viability of probiotics in yoghurt (Kailasapathy & Rybka, 1995; Lourens-Hattingh & Viljoen, 2001). The low viability in yoghurt is mainly attributed to the lower pH in yoghurt and further reduction of pH in yoghurt during post-acidification. Microencapsulation seems to be the most promising technology to protect bacterial cells from adverse environment (Kailasapathy, 2002).

The samples C and D with encapsulated LA with FP and without FP have significantly higher viable cell counts of LA which remained constant over the storage time. The probable reason to this might be the increased protection of probiotic bacteria as synbiotic microcapsules of alginate coated with chitosan limits the diffusion of inhibitory substances such as metabolic products from the starter cultures, lactic acid and bacteriocin into the beads (Sun & Griffiths, 2000).

Alginate hydrogels coated with chitosan provides substantial protection for probiotics under aerobic conditions with dissolved oxygen and could therefore be responsible for higher survival rates of encapsulated cells of LA during storage in yogurt (Talwalkar & Kailasapathy, 2004). In addition to this, the sample D supplemented with FP and encapsulated bacteria shows slight insignificant increase ($P<0.05$) in viable cell count after 7 days of storage and remained nearly constant during the storage time. This can be explained by the fact that soluble part of flaxseed fibre enhances the growth of bacteria although the metabolic rate might be slow within the alginate-frutooligosaccharide microcapsules. Thus, indicating the ability of FP selected to stimulate the growth and viability of probiotics. The viable cell counts of sample D with encapsulated bacteria and FP by the end of 28 days of storage was 9 Log CFU ml^{-1}, and thus, the yogurt developed could be considered as a probiotic product. Addition of FP could either act as an additional nutrient or modify the unfavourable environmental influences, resulting in improved probiotic viability.

The results of this study showed that there was an increased survival of 2 and 1 log cell numbers of *L. acidophilus* NCDC 195 due to microencapsulation. Thus, microencapsulation improves viability of probiotic bacteria in acidic foods like yoghurt/*dahi* making it a better probiotic food vehicle. Krasaekoopt *et al.,* (2003) reported that encapsulation facilitates the manufacture of fermented dairy products in which bacteria have constant characteristics and higher stability during storage.

4.6 Changes in sensory and physico-chemical characteristics of flaxseed fortified synbiotic flavoured *Dahi* (FFSFD) during storage

The sensory, physico-chemical characteristics and textural properties of the samples were studied up to 28th day of storage at 4±1^{o}C (Table 4.17, 4.18 & 4.19). The results of the samples in respect of colour, taste, texture, overall acceptability, moisture, fat, protein, DPPH, total phenolic content, free fatty acids and TBA are as follows:

4.6.1 Effect of storage period on colour

The colour score of *dahi* varied from 8.42 ± 0.09 to 7.06 ± 0.05 for FFSFD and from 7.53 ± 0.04 to 6.02 ± 0.03 for CD samples (Table 4.13; Fig. 4.26). The highest colour score for FFSFD sample was 8.42 ± 0.09 followed by 8.06 ± 0.09, 7.62 ± 0.07, 7.28 ± 0.06 and 7.06 ± 0.05 found at 0, 7th, 14th, 21st and 28th days of storage. The values presented in table 4.13 clearly indicates that the highest colour score for CD sample was 7.53 ± 0.04 followed by 7.09± 0.03, 6.75 ± 0.03, 6.53 ± 0.03 and 6.02 ± 0.03 found at 0, 7th, 14th, 21st and 28th days of storage. The values clearly indicates that colour in FFSFD sample was significantly high ($p<0.05$) as compared to CD at all the stages of storage. The high scores of FFSFD may be attributed to the bright yellow colour imparted by mango to FFSFD samples. The values in both the groups significantly ($p<0.05$) decreased as the period of storage prolonged. This may be due to growth of microoganisms responsible for spoilage.

A remarkable decrease in all sensory characteristics was showed during storage periods could be due to proteolytic activity of bacteria and the production of higher acidity (Bakirci and Kavaz, 2008; Foda *et al.,* 2007; Garcia Perez *et al.,* 2005).

Table 4.13 Effect of storage on sensory attributes of flaxseed fortified synbiotic flavoured *dahi* (FFSFD) and control *dahi* (CD) samples

Storage period	Colour		Flavour		Texture		Overall acceptability	
	CD	**FFSFD**	**CD**	**FFSFD**	**CD**	**FFSFD**	**CD**	**FFSFD**
0 Day	7.53 ± 0.04^{eA}	8.42 ± 0.09^{eB}	7.29 ± 0.15^{eA}	8.09 ± 0.06^{eB}	8.53 ± 0.10^{eA}	8.03 ± 0.076^{dB}	7.82 ± 0.15^{eA}	8.37 ± 0.02^{eB}
7th Day	7.09 ± 0.02^{dA}	8.06 ± 0.09^{dB}	6.86 ± 0.18^{dA}	7.75 ± 0.08^{dB}	8.26 ± 0.16^{dA}	7.9 ± 0.072^{dB}	7.72 ± 0.15^{dA}	7.64 ± 0.04^{dB}
14th Day	6.75 ± 0.03^{cA}	7.62 ± 0.07^{cB}	6.78 ± 0.22^{cA}	7.22 ± 0.11^{cB}	8.02 ± 0.19^{cA}	7.43 ± 0.33^{cB}	7.54 ± 0.13^{cA}	7.48 ± 0.11^{cB}
21stDay	6.53 ± 0.01^{bA}	7.28 ± 0.06^{bB}	6.26 ± 0.19^{bA}	6.78 ± 0.12^{bB}	7.62 ± 0.11^{bA}	7.12 ± 0.10^{bB}	7.32 ± 0.14^{bA}	7.22 ± 0.06^{bB}
28thDay	6.02 ± 0.03^{aA}	7.06 ± 0.05^{aB}	6.01 ± 0.19^{aA}	6.34 ± 0.13^{aB}	7.41 ± 0.1^{aA}	6.84 ± 0.12^{aB}	7.14 ± 0.13^{aA}	6.96 ± 0.05^{aB}

Results presented as a mean (n=3). Different small letter superscripts depict the statistical difference ($P<0.05$) between means for the same *dahi*/yogurt batches at different time intervals within a row. Different capital letter superscripts depict the statistical difference $P<0.05$ between means for different *dahi*/yogurt batches within a column.
CD= Control *dahi* samples,
FFSFD= Flaxseed fortified synbiotic flavoured *dahi* samples (optimised level)

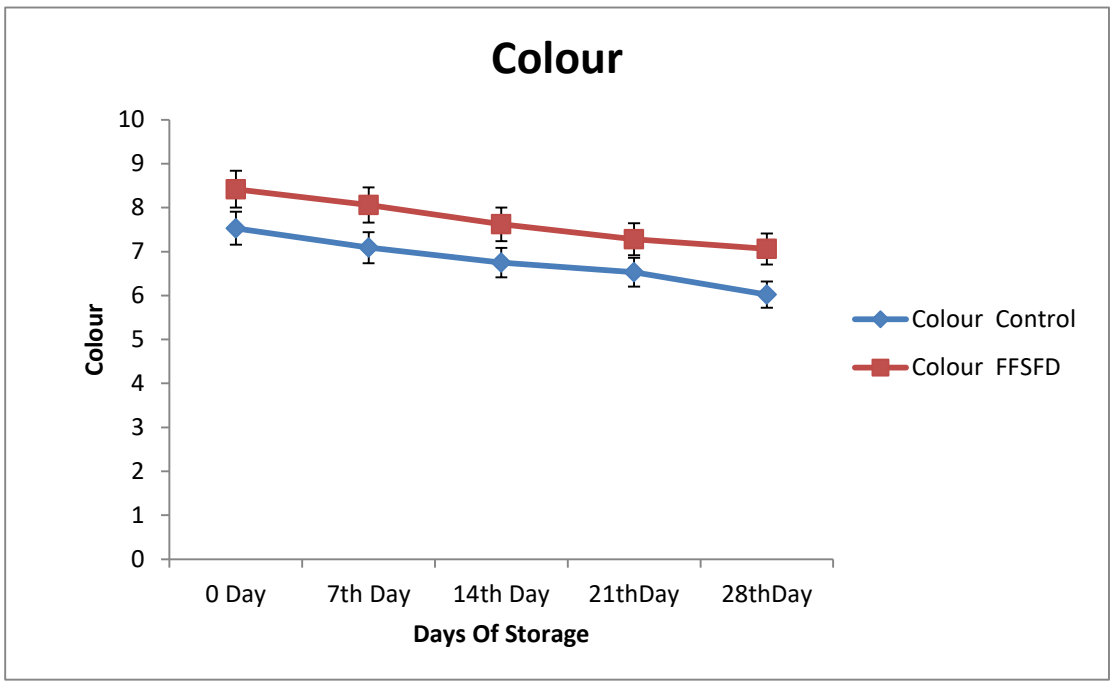


Fig. 4.26 Changes in colour score of *dahi* samples during storage

4.6.2 Effect of storage period on flavour

Flavour (i.e. taste and smell) is the most important factor for the acceptance of the products. The flavour score of FFSFD sample ranged from 8.59±0.06 to 6.34±0.13 and for CD samples from 7.29±0.15 to 6.01±0.19 (Table 4.13; Fig. 4.27). As the storage periods of both (FFSFD and CD) samples increases the flavour score was decreased ($P<0.05$). There was sharp significant ($P<0.05$) decline in the flavour score of FFSFD after 21 days of storage which might be due to increased acidity caused by mango pulp and increase in microbial activity due to the presence of honey. The flavour of FFSFD also decreased sigificantly due to higher oxidation of fat as FFSFD contains flax omega-3 fatty acids which are more susceptible to oxidation.

From the data (Table 4.13) an inverse relationship between the flavour score and storage period were noted. The decrease in flavour score during storage periods may be due to growth of spoilage micro-oganisms resulting which acidity increased and bitter taste developed. The flavour score of FFSFD was not acceptable after 21st day of storage. This may be due to increased acidity and bitter taste. Fernandez-Gracia *et al.,* (1998) reported that fiber addition significantly reduced the flavour scores of yoghurt.

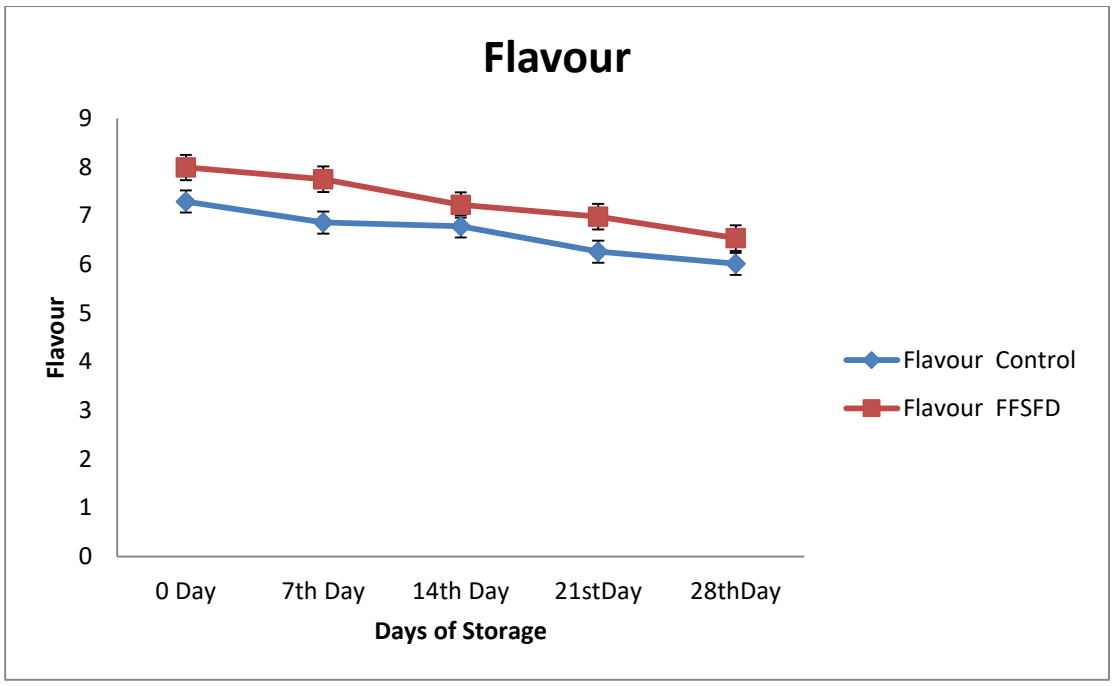


Fig. 4.27 Changes in flavour score of *dahi samples* during storage

4.6.3 Effect of storage period on texture

The effect of different storage periods on texture score of FFSFD against CD samples are clearly depicted in table 4.13. The score for FFSFD samples ranged between 8.53 ± 0.076 and 7.41± 0.12 and for CD between 8.05 ± 0.03 and 6.84 ± 0.12 (Table 4.13; Fig. 4.28). The decrease in texture score was recorded significant ($p<0.05$) up to 14th days of storage in CD samples but in FFSFD sample the change in texture was insignificant uptill 14 days of storage.

The texture score of FFSFD was higher than CD samples during all the storage intervals. This may be due to decrease in moisture content of the samples during storage. Similar findings were reported by Velez-Ruiz, *et al.,*(2011), who reported higher texture attributes of flaxseed yoghurt formulation and concluded the type of fiber favoured a measurable resistance to flow. This flow response could be attributed to major interactions between the fat globules and protein matrix, in which fiber also contributed to those interactions.

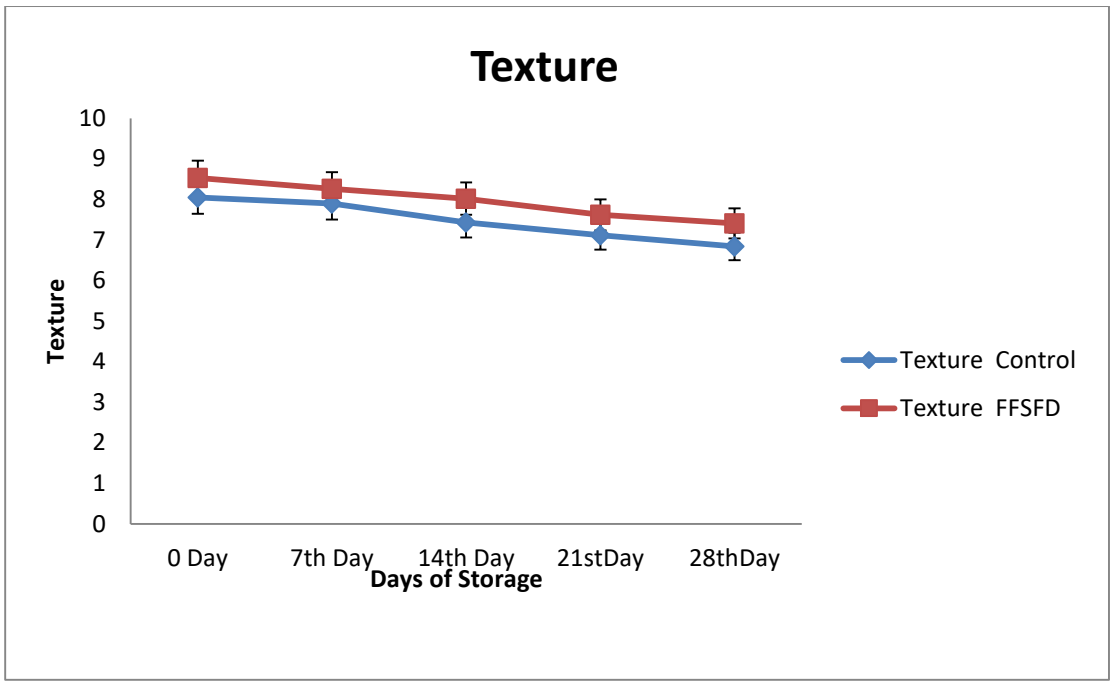


Fig. 4.28 Changes in texture score of *dahi* samples during storage

4.6.4 Effect of storage periods on overall acceptability

The overall acceptability depends on colour & appearance, flavour, texture and sweetness score of the products. The overall acceptability score (Table 4.13; Fig. 4.29) ranged from 8.37±0.02 to 6.96±0.05 for FFSFD and from 7.82±0.15 to 8.37±0.02 for CD samples. As the storage periods of both (FFSFD and CD) samples increases, the overall acceptability score was decreased ($P<0.05$). There was sharp significant ($P<0.05$) decline in the overall acceptability score of control sample after 14th day of storage but was insignificant in FFSFD up till 21 days of storage. After 21 days FFSFD overall acceptability decreased which might be due to increased acidity caused by mango pulp and increase in microbial activity due to the presence of honey. The flavour of FFSFD also decreased sigificantly due to higher oxidation of fat as FFSFD contains pre-cursor of omega-3 fatty acids as ALA which are more susceptible to oxidation.

An inverse relationship ($P<0.05$) between overall acceptability and storage periods were recorded in the samples. This may be due to increased activity of spoilage micro-oganisms in the samples during storage. Kumar *et al.,* (2011),

concluded that there is decline in the sensory parameters when various dairy products were stored for longer duration at refrigerated temperature.

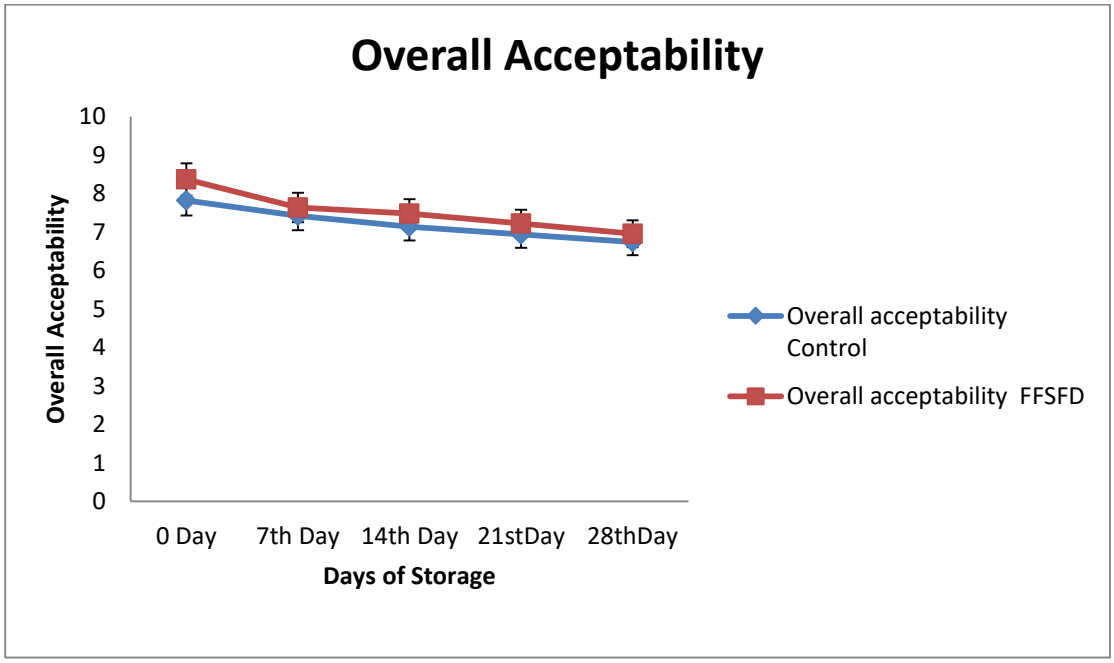


Fig. 4.29 Changes in overall acceptability score of *dahi* samples during storage

4.6.5 Effect of storage on moisture

The moisture content varied from 80.93 ± 0.24 to 75.49 ± 0.25 in FFSFD samples and from 87.75 ± 0.24 to 81.51 ± 0.02 in the CD samples during storage (Table 4.14; Fig. 4.30). The moisture content of FFSFD was the highest at 0 day (80.93 ± 0.24%) followed by 7th (79.61 ± 0.30%), 14th (78.56 ± 0.15%), 21st (76.41 ± 0.17%) and 28th (75.49 ± 0.25 %) days of storage interval, respectively. In CD group, the highest moisture content was 87.75 ± 0.24 followed by 85.93 ± 0.18, 84.57 ± 0.33, 82.52 ± 0.33 and 81.51 ± 0.02 percent found at 0, 7th, 14th, 21st and 28th days, respectively. The moisture content of the CD sample decreases significantly ($p<0.05$) during storage. Fig. 4.24 clearly depicts that the average moisture content was higher in the samples CD than FFSFD. The difference in the values between FFSFD and CD samples were significant ($p<0.05$) throughout the storage. There was an inverse relationship between moisture content and storage period.

Table 4.14 Effect of storage on chemical attributes of *flaxseed powder fortified synbiotic flavoured dahi* (FFSFD) and control *dahi* (CD) samples

Storage period	Moisture (%)		Protein (%)		Fat (%)	
	CD	FFSFD	CD	FFSFD	CD	FFSFD
0^{th} day	87.75 ± 0.24^{eA}	80.93 ± 0.24^{dB}	3.65 ± 0.07^{dA}	5.26 ± 0.01^{dB}	1.5 ± 0.06^{dA}	4.28 ± 0.04^{dB}
7^{th} day	85.93 ± 0.18^{dC}	79.61 ± 0.30^{dD}	2.81 ± 0.06^{cC}	4.73 ± 0.02^{dD}	1.25 ± 0.06^{cdC}	4.01 ± 0.07^{cdD}
14^{th} day	84.57 ± 0.33^{cE}	78.56 ± 0.15^{cF}	2.65 ± 0.03^{cE}	4.14 ± 0.03^{cF}	0.87 ± 0.14^{bcE}	3.78 ± 0.07^{cF}
21^{st}day	82.52 ± 0.33^{bG}	76.41 ± 0.17^{bH}	2.23 ± 0.09^{bG}	3.79 ± 0.05^{bH}	0.53 ± 0.18^{abG}	3.27 ± 0.07^{bC}
28^{th} day	81.51 ± 0.02^{aI}	75.49 ± 0.25^{aJ}	1.81 ± 0.02^{aI}	3.24 ± 0.07^{aJ}	0.19 ± 0.16^{aH}	2.54 ± 0.03^{aG}

Values are mean ± SE of 3 replicates
Values bearing different small superscripts in a column differ significantly (Duncan test, $p<0.05$)
Values bearing different capital superscripts in between column differ significantly (Duncan test, $p<0.05$)

Table 4.15 Effect of storage on physico-chemical attributes of *flaxseed powder fortified synbiotic flavoured dahi* (FFSFD) and control *dahi* (CD) samples

Storage period	%DPPH Inhibition		Total phenolic content (mg GAE per 100 mL)		Free Fatty Acid %		Thiobarbutric acid (mol of MDA /kg curd)	
	CD	FFSFD	CD	FFSFD	CD	FFSFD	CD	FFSFD
0th day	52.75±0.09^{dA}	83.36±0.2^{dB}	18.07±0.02^{eA}	40.12±0.06^{eB}	0.12±0.01^{aA}	1.25±0.02^{aB}	0.05±0.01^{aA}	0.08±0.02^{aA}
7th days	53.01±1.10^{eA}	85.41±0.4^{eB}	15.39±0.12^{dA}	39.22±0.07^{dB}	0.62±0.02^{bA}	1.63±0.02^{bB}	0.06±0.01^{aA}	0.1±0.01^{aA}
14th days	48.38±0.09^{cA}	80.13±0.6^{cB}	13.78±0.11^{cA}	36.67±0.04^{cB}	1.04±0.01^{cA}	1.99±0.04^{cB}	0.08±0.02^{aA}	0.13±0.01^{abAB}
21st days	36.31±0.07^{bA}	72.64±0.06^{bB}	10.81±0.07^{bA}	29.64±0.03^{bB}	1.56±0.02^{dA}	2.16±0.06^{dB}	0.1±0.03^{abA}	0.16±0.02^{bA}
28th days	30.26±0.06^{aA}	65.15±0.21^{aB}	8.62±0.08^{aA}	25.75±0.10^{aB}	1.92±0.03^{eA}	2.35±0.04^{eB}	0.13±0.01^{bA}	0.19±0.04^{bA}

Values are mean ± SD of three replicates.
Values bearing different small superscripts (a, b, c, d, e) in a column differ significantly (Duncan test, $P<0.05$).
Values bearing different capital superscripts (A, B) in a row differ significantly (Duncan test, $P<0.05$).

Pushkala, (2011) found that moisture content in aloe gel fortified *dahi* was reduced during storage. It was found that there was a gradual decrease in moisture content in all yoghurt samples with the passage of time (Hassan and Amjad, 2010).

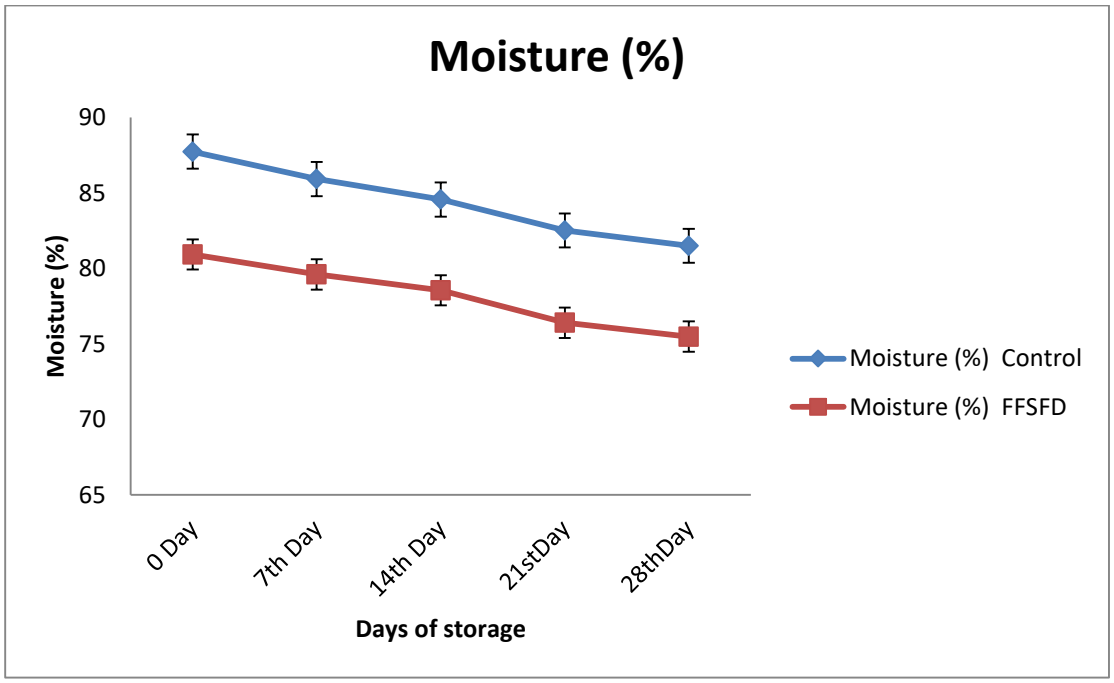


Fig. 4.30 Changes in moisture content of *dahi* during storage

4.6.6 Effect of storage period on protein

The values presented in table 4.14 (Fig. 4.31) clearly depicts that as the storage period increases the protein content decreased ($p<0.05$) in both type of samples. The highest protein content for sample FFSFD was 5.26 ± 0.01 followed by 4.73 ± 0.02, 4.14 ± 0.03, 3.79 ± 0.05 and 3.24 ± 0.07 and for sample CD it was 3.65 ± 0.07 followed by 2.81 ± 0.06, 2.65 ± 0.03, 2.23 ± 0.09 and 1.81 ± 0.02 at 0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th} days of storage, respectively. The protein content was higher in the sample FFSFD than CD (Fig. 4.31), which may be due to presence of higher protein content in flaxseed powder. The results of protein content were similar to those of Morris, (2007).

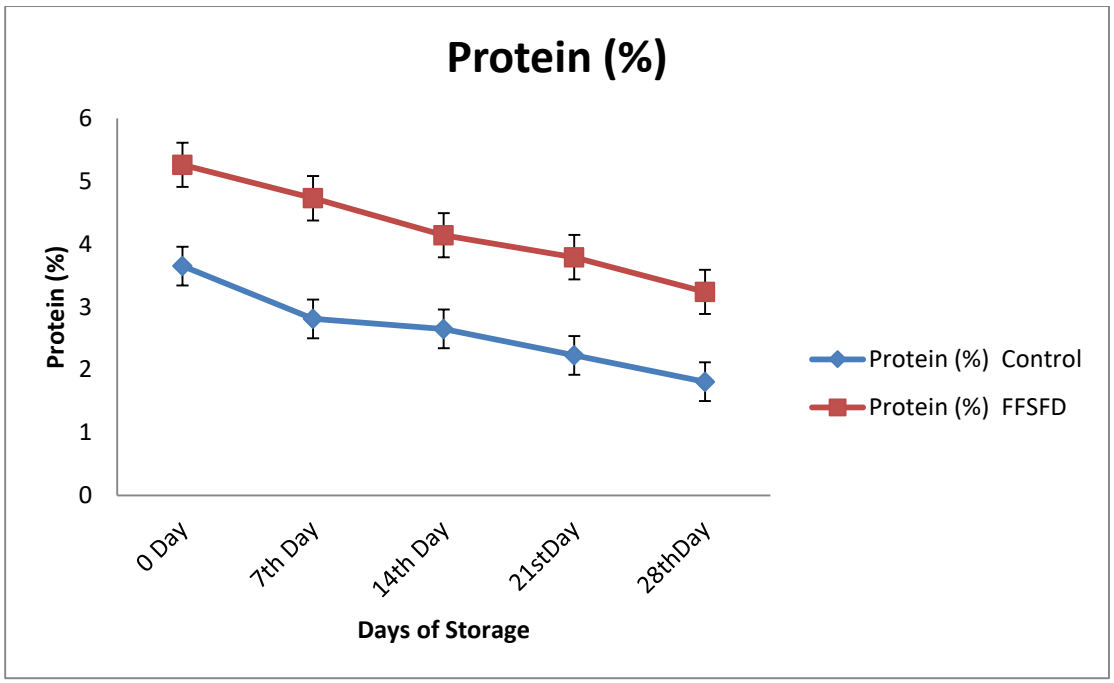


Fig. 4.31 Changes in protein content of *dahi* samples during storage

4.6.7 Effect of storage period on fat

The fat content varied from 4.28 ± 0.04 to 2.54 ± 0.03 in FFSFD samples and from 1.50 ± 0.06 to 0.19 ± 0.16 in CD samples (Table 4.14; Fig. 4.32). Fig. 4.32 clearly indicates that as the storage period increased, the fat content of the FFSFD samples decreased significantly ($p<0.05$). It is clear from the data (Table 4.14) that as the storage period prolonged, the fat content also decreased ($p<0.05$) in the CD samples. These values clearly depicts that the pH values of both product (FFSFD and CD) were decreasing significantly ($p<0.05$) as the storage period increased. The decrease in fat percent of FFSFD was noted much higher than control samples. The decrease in the level of fat may be more in FFSFD due to rapid oxidation of highly susceptible omega-3 fatty acids in the samples during storage. Similar study was found by Soomro *et al.,* (2003). Kumar, (2013) reported that fat content of herbal ice cream decreased during storage.

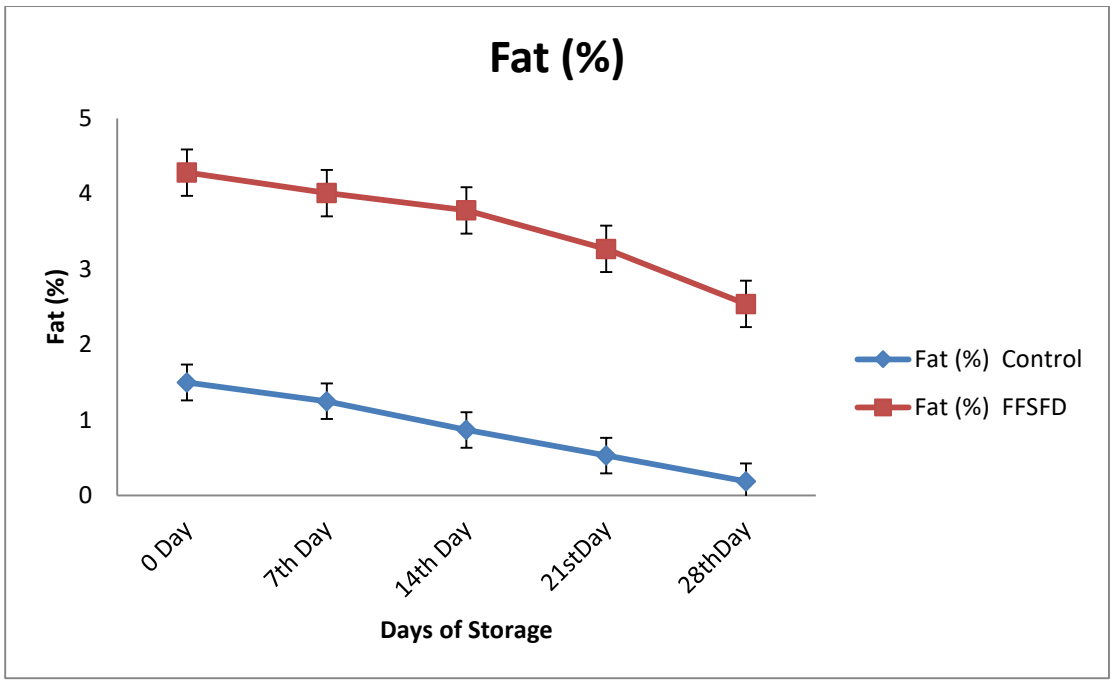


Fig. 4.32 Changes in fat content of *dahi* samples during storage

4.6.8 Effect of storage on antioxidant activity (% DPPH)

The DPPH-scavenging activity of *dahi* samples is shown in figure 4.33. The synbiotic *dahi* FFSFD had significantly ($P<0.05$) higher antioxidant potential compared to control *dahi* sample. On the 0^{st} day i.e. after 12h of fermentation %DPPH radical inhibition in FFSFD sample was 83.36 % when compared to that of control which had DPPH scavenging activity of 52.75%.The difference was found to be significant ($P<0.05$) in between treatments. This is due to reason that flaxseed have been shown to possess antioxidant activity owing to their bound phenolic acids (Hosseinian *et al.,* 2006; Zanwar *et al.,*2010). The higher antioxidant potential of FFSFD also might be due to additional antioxidant activity contributed by mango pulp (Pereira, 2013) honey and LAB. The DPPH scavenging activity of FFSFD (from 83.36±0.6 to 85.4±0.1) and CD (from 52.75±0.9 to 53.84±1.1) increased on the 7^{th} day of storage, however increase in %DPPH scavenging activity of FFSFD was higher than control *dahi*. The possible reason can be given by the findings of Ningegowda *et al.,* (2012) who indicated that the metabolic end products of Lactic acid bacteria, resulting from the utilization of fibres, contribute to the higher antioxidant potential. The antioxidant potential of both the *dahi* samples decreased

during 28 days storage period. There was sharp significant (P<0.05) decline in the %DPPH scavenging activity of FFSFD after 14th day of storage which might be due to increased acidity caused by mango pulp and increase in microbial activity due to the presence of honey.

Rajesha *et al.,*(2006), studied in vivo antioxidant activity of whole flaxseed supplementation by feeding weanling albino rats and indicated that beneficial flaxseed antioxidant components helps to restore the elevated activity of hepatic enzymes at almost normal level i.e. detoxify CCl4 induced free radicals.

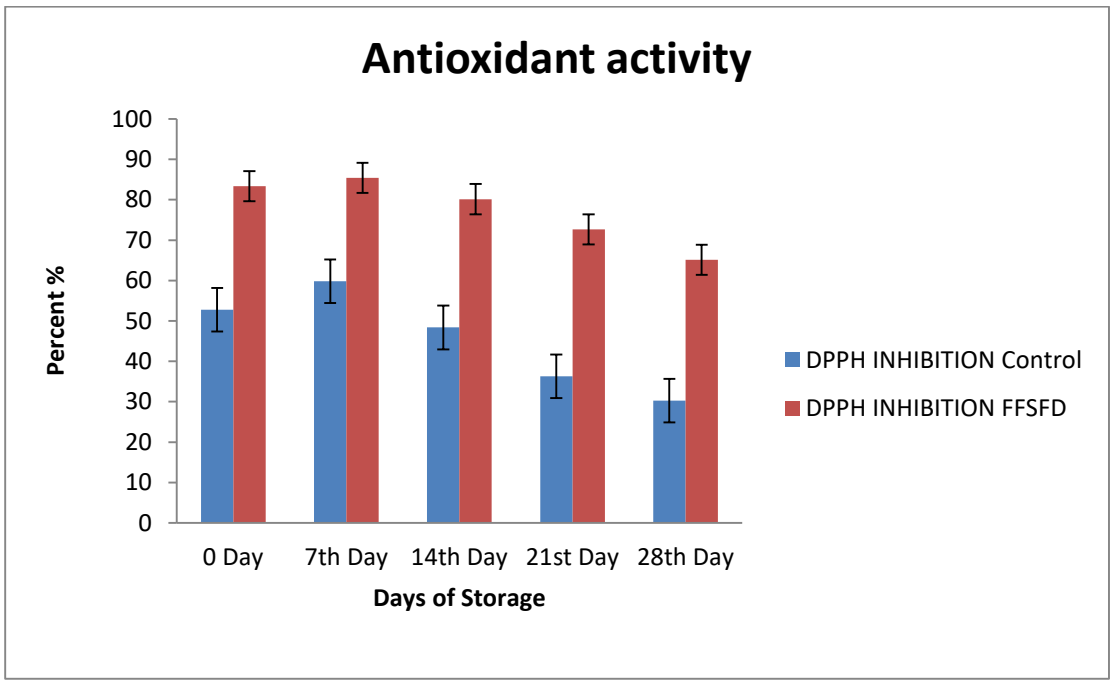


Fig. 4.33 Changes in % DPPH radical scavenging activity of *dahi* samples during storage

4.6.9 Effect of storage on total phenolic content

The total phenolic content of *dahi* samples is shown in Fig 4.33. The synbiotic and probiotic *dahi* had significantly (P<0.05) higher total phenolic content compared to control *dahi* sample. On 0 day total phenolic content in FFSFD sample was 40.12 mg GAE per 100 mL of sample when compared to that of control which had total phenolic content of 18.07 mg GAE per 100 mL of sample. The difference was found to be significant (P<0.05) in between treatments. This is due to reason that flaxseeds

have been shown to possess antioxidant activity owing to their bound phenolic acids (Hosseinian, 2012). Further, incorporation of mango pulp and honey also increase the total phenolic content of FFSFD. Similar findings were reported by Pereira, (2013) in mango *dahi*. The increased total phenolic content in the FFSFD could be also due to the fermentative activity of the probiotics (Ningegowda *et al.,* 2012). Total phenolic content of both the *dahi* samples was found to decrease during 28 days storage period. The total phenolics in control *dahi* sample decreased from 18.07 mg GAE per 100 mL to 8.62 mg GAE per 100 mL of *dahi* by 28 days of storage period and that of probiotic *dahi* was found to decrease from 27.48 mg GAE per 100 mL to 19.21 mg GAE per 100 mL. The total phenolic content of FFSFD decreased from 40.12 mg GAE per 100 mL to 25.75 mg GAE per 100 mL during 28 days of storage period. Ghadge *et al.,* (2010) also reported that total phenols content of yoghurt decrease during storage.

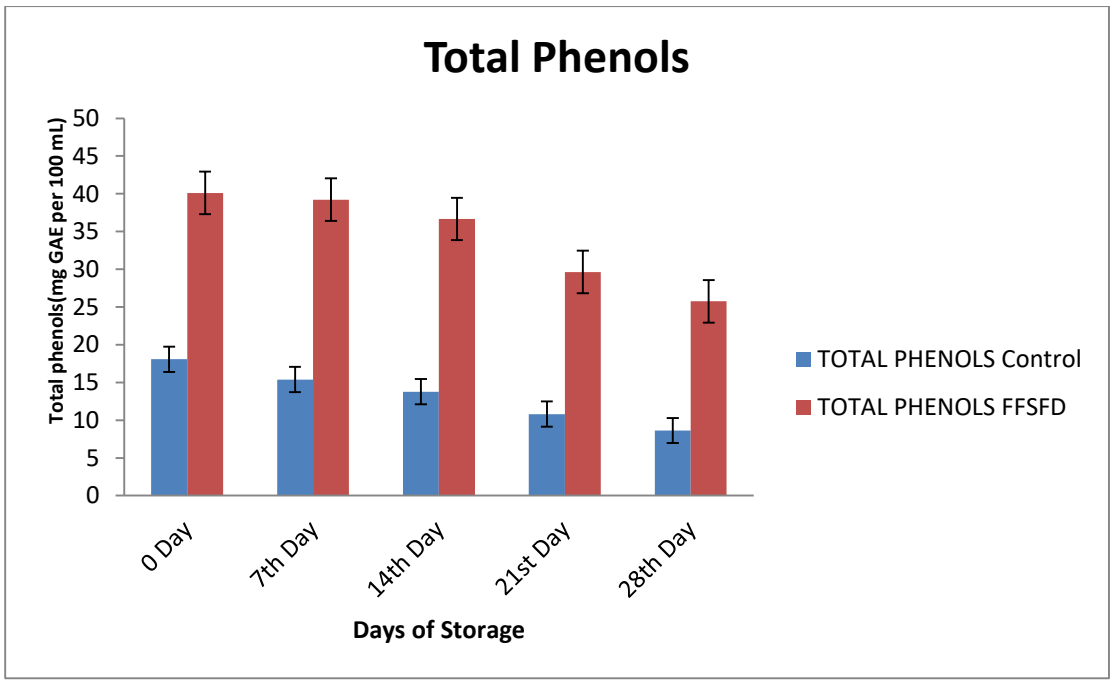


Figure 4.34 Changes in total phenols of *dahi* samples during storage.

4.6.10 Effect of storage period on free fatty acid

The values presented in table 4.15 (Fig. 4.35) clearly depicts that as the storage period increases the free fatty acid also increased ($p<0.05$) in both type of

samples. The lowest free fatty acid for sample FFSFD was 1.25± 0.13 followed by 1.63± 0.10, 1.99± 0.29, 2.16± 0.36 and 2.35± 0.55, for sample CD it was 0.12± 0.53 followed by 0.62± 0.50, 1.04± 0.88, 1.5± 0.29 and 1.9± 0.24 at 0, 7th, 14th, 21st and 28th days of storage, respectively. There was a subsequent increase in the free fatty acid content of *dahi* samples with storage (Estrada *et al.,* 2011). The increase in the level of FFA may be more in FFSFD due to rapid oxidation of highly susceptible omega-3 fatty acids in the samples during storage.

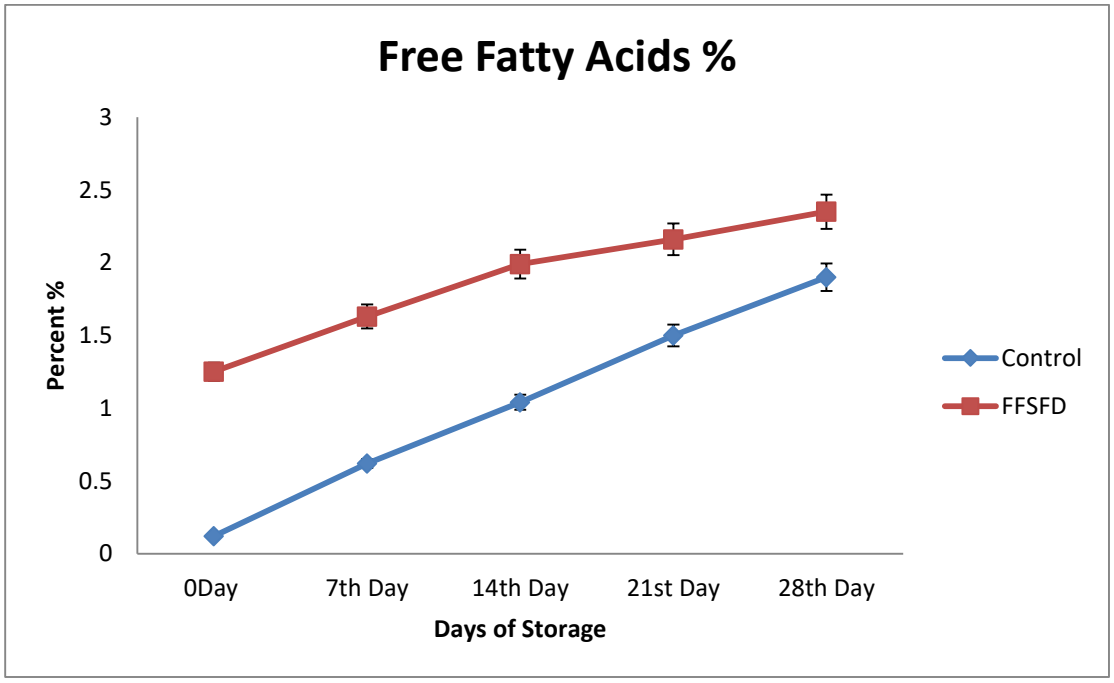


Fig. 4.35 Changes in free fatty acid of *dahi s*amples during storage

4.6.11 Effect of storage period on Thiobarbutric acid

The thiobarbutric acid varied from 0.08 ± 0.003 to 0.19 ± 0.005 in samples of FFSFD and from 0.05 ± 0.003 to 0.19 ± 0.003 in CD samples (Table 4.15; Fig. 4.36). Fig. 4.36 clearly indicates that as the storage periods increased of the samples FFSFD, the thiobarbutric acid increased significantly ($p<0.05$) upto 28th day of storage. It is clear from the data (Table 4.22) that as the storage period prolonged, the thiobarbutric acid also increased ($p<0.05$) in the CD samples. This may be due to oxidation of fat content present in the product. The increase in the level of FFA may be more in FFSFD due to rapid oxidation of highly susceptible omega-3 fatty acids in the

samples during storage. Similar study by Lee *et al*., (2007) reported a slow TBA absorbance increase from 0.083 to 0.10 over the initial 6 d of storage of evening primrose oil (EPO,with high PUFA content) -enriched yogurt, followed by a dramatic increase up to 0.165 after 15 d. The results indicated that lipid oxidation proceeded more rapidly in yogurt with EPO than in that without EPO, is in accordance with the trend observed with CD and FFSFD due to high ALA content of flaxseed powder. Similar findings were made by Estrada *et al.*, (2011) who reported increase in TBA value during storage in strawberry yoghurt fortified with marine fish oil with respect to control sample.

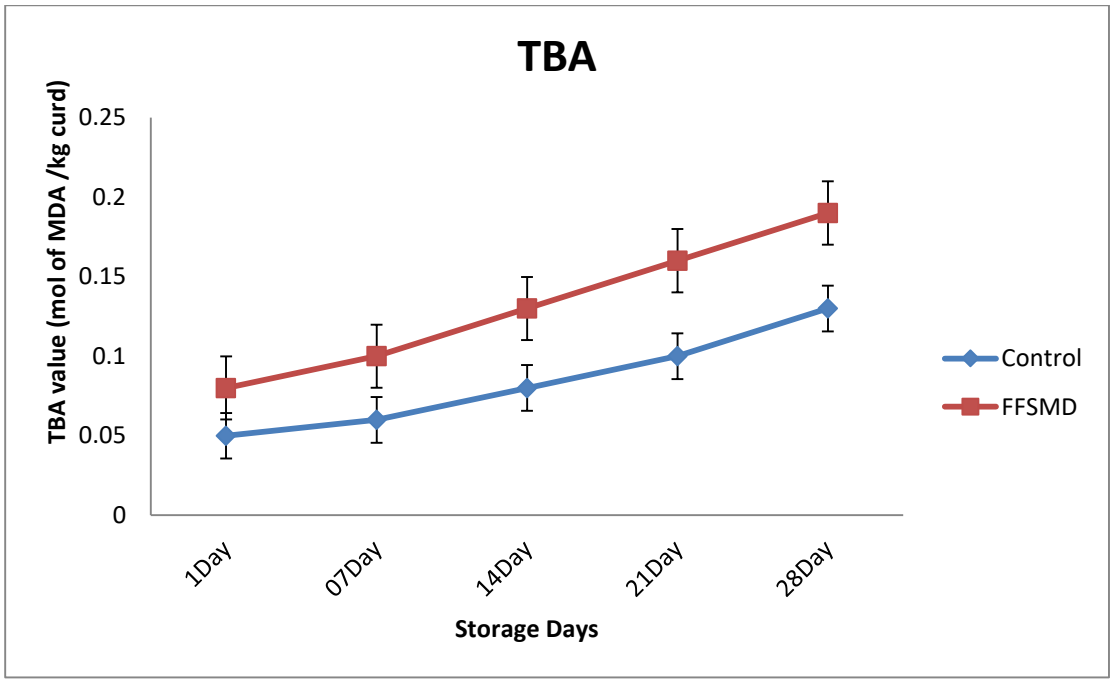


Fig. 4.36 Changes in thiobarbituric acid of *dahi* during storage

4.7 Changes in microbial characteristics of flaxseed fortified synbiotic flavoured *Dahi* during storage

The microbial count of the samples stored up to 28th days of storage at 4±1°C in respect of total plate count (TPC), yeast and mould count (YMC) and coliform count were studied and results are discussed as follows:

4.7.1 Effect of storage period on total plate count (TPC)

During storage, one or more food characteristics can reach an undesirable state and as a consequence the consumer may reject the product or it can even cause detrimental health. At this moment, it is considered that the food has reached the end of its shelf life (Singh *et al.,* 2011). The effect of storage on TPC values (10^7cfu/ml) for FFSFD and CD samples are depicted in Table 4.16. The TPC of yoghurt samples varied from 9.05 ± 0.03×10^7/ml to 4.97 ± 0.02 cfu/ml in FFSFD and from 10.12 ± 0.04×10^7cfu 8.6 ± 0.13×10^7cfu/ml to 8.6 ± 0.10×10^7cfu/ml in CD sample during storage (Table 4.16; Fig. 4.37).

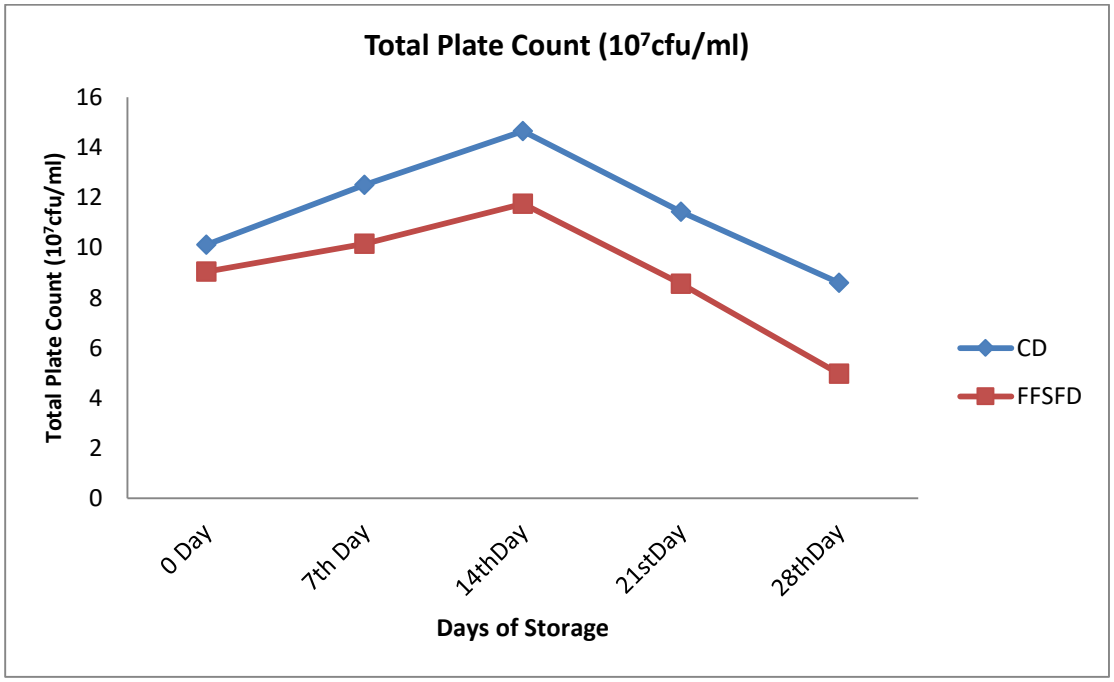


Fig. 4.37 Changes in TPC of *dahi* samples during storage

The values presented in the table 4.16 (Fig. 4.37) clearly indicates that the highest TPC (10^7cfu/ml) was recorded at 14th day of storage for both (FFSFD and CD) samples. As the storage increases the TPC count also increased ($p<0.05$) upto 14th days but thereafter decreased up to 28th days for both (FFSFD and CD) samples. This decrease may be due to increased competition for food within the microbes. The increase in TPC count may be due to growth of undesirable microorganisms during storage.

Total plate count showed a reduction after 21 days of storage in control and water melon yoghurt. This may be due to the depletion of nutrients and death of some survivors of the products (Warakaulle *et al.,* 2014). Mbaeyi and Anyanwu, (2010) also observed that drastic reduction of total viable count of the yogurt samples formulated with solar-dried bush mango pulp during storage.

4.7.2 Effect of storage period on yeast and mould (YMC) and coliform count

Table 4.16 (Fig. 4.38) clearly depicts that up to 7th day the YMC was absent but it augmented to 5.21 ± 0.04×10^{1}cfu/ml for CD and 4.52 ± 0.01×10^{1}cfu/ml for FFSFD sample upto 28th day of storage. The differences in the value of YMC in both FFSFD and CD samples were non-significantly increased ($p>0.05$) with increase in storage period upto 14th days, but further enhancement in storage period indicates increased ($p<0.05$) in YMC. The coliform count was absent during storage in both (FFSFD and CD) samples. Nigam *et al.,* (2009), Bhat *et al.,* (2010) and Kumar *et al.,* (2011); Al-Kadamany *et al.,* (2003) also reported similar decline in the YMC of various dairy products during refrigerated storage.

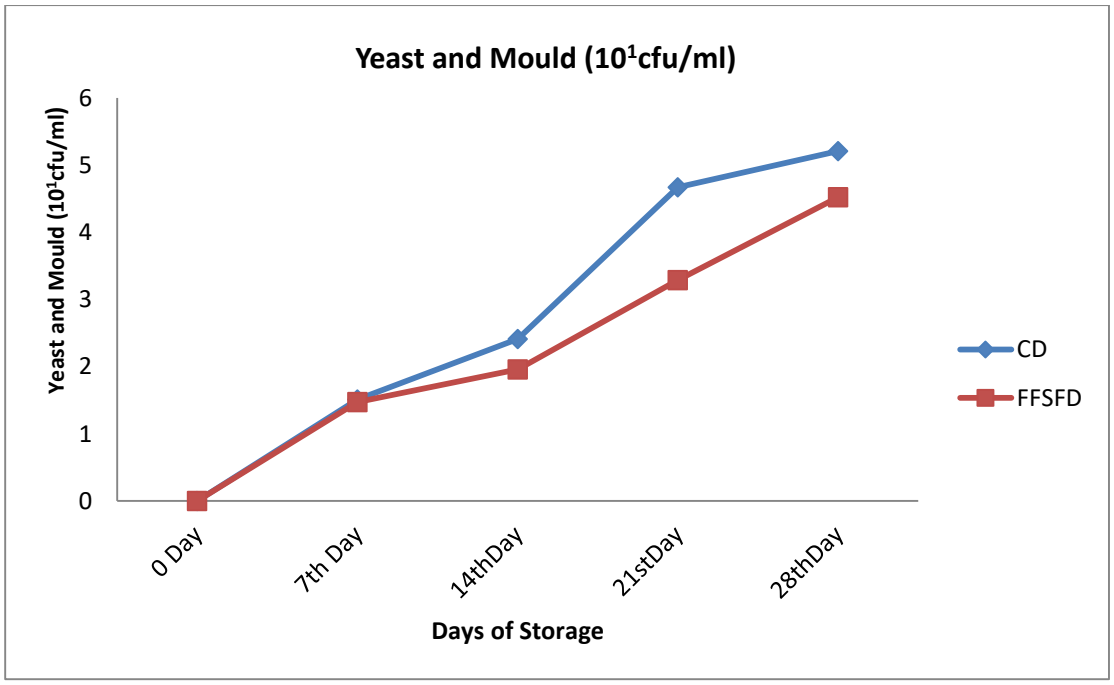


Fig. 4.38 Changes in yeast and mould count of *dahi* samples during storage

Table 4.16 Effect of storage on microbial characteristics of flaxseed fortified synbiotic flavoured *dahi* (FFSFD) and *control dahi* (CD) samples

Storage period	Total plate count (10^7cfu/ml)		Yeast and Mould (10^1cfu/ml)		Coliform count	
	CD	FFSFD	CD	FFSFD	CD	FFSFD
0 Day	10.12 ± 0.03^{bB}	9.05 ± 0.04^{cA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	ND	ND
7th Day	12.50 ± 0.04^{dB}	10.16 ± 0.03^{dA}	1.51 ± 0.04^{bA}	1.47 ± 0.11^{bA}	ND	ND
14thDay	14.65 ± 0.12^{eB}	11.75 ± 0.04^{eA}	2.41 ± 0.11^{cB}	1.96 ± 0.08^{cA}	ND	ND
21stDay	11.43 ± 0.18^{cB}	8.56 ± 0.01^{bA}	4.67 ± 0.23^{dB}	3.29 ± 0.04^{dA}	ND	ND
28thDay	8.6 ± 0.13^{aB}	4.97 ± 0.02^{aA}	5.21 ± 0.04^{eB}	4.52 ± 0.01^{eA}	ND	ND

Values bearing different small superscripts in a column differ significantly (Duncan test, P<0.05)
Values bearing different capital superscripts in a row differ significantly (Duncan test, P<0.05)
CD=Control *dahi* samples,
FFSFD= *Flaxseed fortified synbiotic flavoured dahi* sample (optimised level)
ND is abbreviated as Not Detected

PHASE IV: NUTRITIONAL IMPACT OF FLAXSEED FORTIFIED SYNBIOTIC FLAVOURED *DAHI* ON THE PERFORMANCE OF SWISS MICE

Nutritional impact of Flaxseed Fortified Synbiotic Flavoured *Dahi* on the performance of Swiss mice was studied under the following heads:

4.8 Impact of dietary treatments on the feed intake and body weight gain of mice

The average feed intake per mice per week (Table 4.17; Fig. 4.39) in descending orders were 31.36±0.028, 30.25±0.045, 27.56±0.037, and 28.68±0.019 gm in the groups G_1, G_2, G_3 and G_4, respectively. These values clearly indicate that the feed intake decreased ($P<0.05$) when FFSFD was added in the feed. The difference in feed intake between control *dahi* and *flaxseed fortified synbiotic flavoured dahi* was also significant ($P<0.05$). This lower food intake of flaxseed (maybe due to the fact that flaxseed has high level of fat and fiber) fortified synbiotic flavoured *dahi* was reflected by a significantly lower mean body weight gain.

A diet which is high in fat and dietary fiber is generally bulky and is less likely to cause overeating and weight gain. The experimental mice seemed to eat less after eating high satiety diets (fiber containing diets) than after eating low satiety diet (fiber free diet). Dietary fiber has a high water holding capacity (Eastwood, 1973) and it is likely that mice fed with flaxseed fibers, in spite of the lower energy in their diet compared to those fed fiber- free diet, did not eat more because the fiber swells in their intestine and gives satiety sense. As a result mice fed with fiber rich diets utilized more fats from their food, while the control mice deposited it in their tissues.

Irrespective of the groups, the feed intake per mice per week was significantly ($P<0.05$) increasing with the week. The experimental mice weight positively increased during the treatment period in all groups with the average percentage of 5.61 ± 0.013gm. This was only due to biological demand for physiological functions of the body for growing mice. In conformity with the present findings, Kumar *et al.,* (2010) reported similar decrease in feed intake when yoghurt was fed to the rats.

The average body weight gain (Table 4.18; Fig. 4.40) per mice per week was 6.4±0.021, 6.1±0.025, 5.3±0.024 and 5.6±0.038 gm recorded in the groups G_1, G_2, G_3 and G_4 respectively. The body weight gain was directly proportional to the intake of control diet (CD), high cholesterol diet (HCD) and *flaxseed fortified synbiotic flavoured dahi* (FFSFD) fed groups. The values in the table 4.18 indicates that the differences among all the groups for body weight gain were recorded significant ($P<0.05$). The intensity of increase in body weight gain was significantly ($P<0.05$) high in groups with high cholesterol diet than other groups. This may be due to deposition of fat in their tissues. In conformity with the present findings, Yadav *et al.,* (2006) reported similar decrease in body weight gain when high fructose *dahi* was fed to the albino rats.

Although, differences among all the groups for body weight gain were recorded significant ($P<0.05$), diet type had no significant ($P<0.05$) effect on the percents of weight of liver, heart, kidneys and of spleen to final body weight (Table.4.19). The present findings were supported by similar findings of Lucas *et al.,* (2004) and Prasad, (1997).

4.9 Impact of dietary treatments on biochemical characteristics of blood

4.9.1 Glucose

The levels of glucose in blood were 126.27±1.76, 140.33±2.96, 119±0.58, and 122±1.15 mg/dl in groups G_1, G_2, G_3 and G_4 respectively (Table 4.19; Fig. 4.40). These values shows that the serum glucose in group G_2 (HCD) was significantly high ($P<0.05$) than rest of the treatment groups. The administration of flaxseed in FFSFD to mice in G_3 and G_4 resulted in the significant decrease of glucose concentration in comparison to the result obtained from the G_2 (HCD) group .The relationship between the levels of glucose in the blood and concentration of flaxseed in the feed was positive and the difference in the values in all the groups was very high ($P<0.05$). The value recorded in all the groups were within the normal range (50 to 160 g/dl) as reported in mice blood.

Table 4.17 Impact of feeding *flaxseed fortified synbiotic flavoured dahi* on the feed intake/mice/week (gm)

Groups	Weeks					
	W1	**W2**	**W3**	**W4**	**Total**	**Average**
G1	28.23 ±0.024^{dA}	29.93 ±0.010^{bB}	32.08 ±0.023^{bC}	35.23 ±0.026^{bD}	125.47	31.367±0.028d
G2	26.25 ±0.022^{cA}	28.87 ±0.022^{cB}	31.45 ±0.046^{cC}	34.44 ±0.057^{cD}	121.01	30.252±0.045c
G3	23.65 ±0.019^{aA}	26.50 ±0.057^{eB}	28.89 ±0.047^{eC}	31.23 ±0.054^{eD}	110.27	27.567±0.037a
G4	24.83 ±0.019^{bA}	27.88 ±0.014^{dB}	29.91 ±0.042^{dC}	32.12 ±0.093^{dD}	114.74	28.685±0.019b

Values bearing different small superscripts (a, b, c) in a column differ significantly (Duncan test, P<0.05)
Values bearing different capital superscripts (A, B, C) in between column differ significantly (Duncan test, P<0.05)

Table 4.18 Impact of feeding *flaxseed fortified synbiotic flavoured dahi* on the body weight gain/mice/week (gm)

Groups	Weeks					
	W_1	**W_2**	**W_3**	**W_4**	**Total**	**Average**
G_1	6.12 ±0.034^{dA}	6.48 ±0.041^{dB}	6.50 ±0.024^{dC}	6.79 ±0.018^{cD}	25.89	6.42 ±0.030^{d}
G_2	5.85 ±0.028^{cA}	6.10 ±0.024^{cB}	6.26 ±0.067^{cC}	6.67 ±0.031^{bD}	24.88	6.22 ±0.038^{c}
G_3	4.32 ±0.018^{aA}	5.12 ±0.043^{aB}	5.29 ±0.020^{aC}	5.85 ±0.032^{aD}	20.58	5.14 ±0.025^{a}
G_4	5.41±0.025^{bA}	5.65 ±0.011^{bB}	5. 68 ±0.023^{bC}	5.89 ±0.038^{aD}	22.93	5.65 ±0.024^{b}

Values bearing different small superscripts (a, b, c) in a column differ significantly (Duncan test, P<0.05)
Values bearing different capital superscripts (A, B, C) in between column differ significantly (Duncan test, P<0.05)

Table 4.19 Impact of feeding CD, HCD and FFSFD on % of weight of liver, heart, kidneys and spleen to final total body weight (FW) of mice at the end of experiment

Groups	Liver /FW*	Heart/FW*	Kidney/FW*	Spleen/FW*
G_1	2.74±0.019	0.26±0.021	0.69±0.013	0.19±0.01
G_2	2.79±0.016	0.28±0.014	0.72±0.023	0.21±0.03
G_3	2.74±0.012	0.24±0.023	0.68±0.017	0.16±0.03
G_4	2.76±0.025	0.24±0.015	0.69±0.014	0.18±0.02

*No significant differences in data. Values are mean±SD.

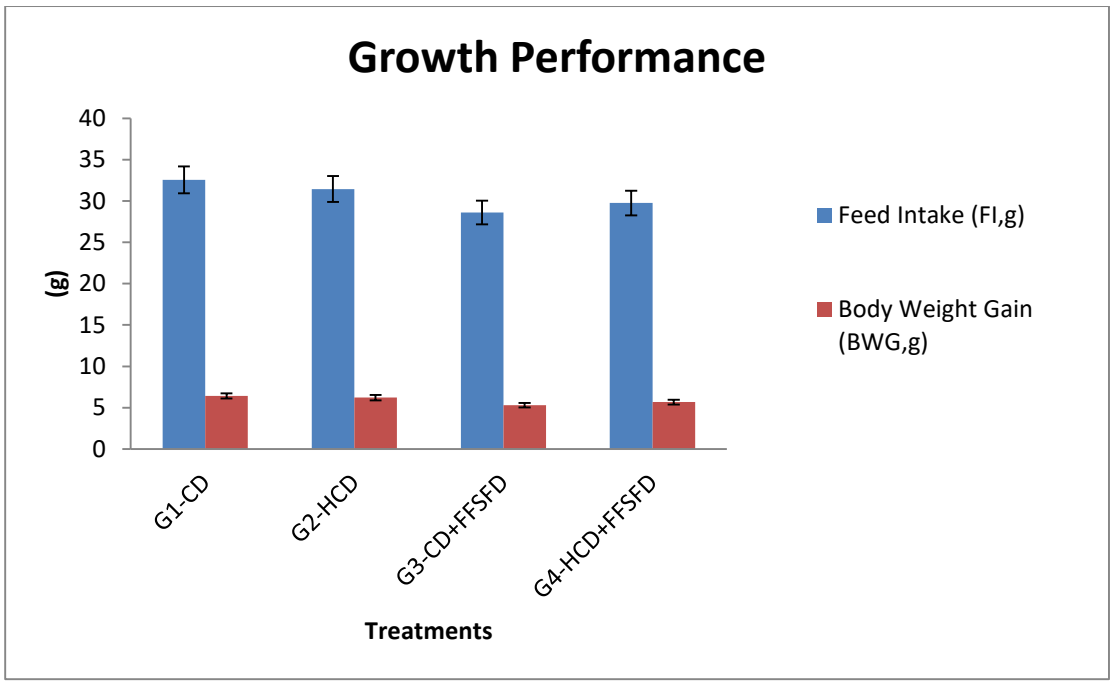


Fig. 4.39 Impact on the FI and BWG of swiss mice fed with Control diet (CD), high cholesterol diet (HCD) and *flaxseed fortified synbiotic flavoured dahi* (FFSFD)

The finding of Makni *et al.,* (2011) revealed that the administration of flax and pumpkin seed mixture attenuated the increased levels of the plasma enzymes produced by the induction of diabetes and found helpful in preventing diabetic complications in adult rats. Flaxseed fiber plays an important role in lowering the blood glucose levels. Studies demonstrated that insoluble fiber slowsdown the release of sugar in the blood and thus help in reducing blood glucose levels to great extent (Thakur *et al.,* 2009; Kapoor *et al.,* 2011).

4.9.2 Cholesterol

The effect of dietary feed on cholesterol levels are given in table 4.20 (Fig. 4.40). The highest serum cholesterol was 85.13±0.47mg/dl in group G_2 followed by 77.27±0.37, 61.57±0.38 and 46.13±0.35 mg/dl in groups G_1, G_4 and G_3 respectively. The levels of cholesterol decreased ($P<0.05$) in the blood in the presence of FFSFD in the diet. The cholesterol level in the blood was significantly high ($P<0.05$) in group G_2 as compared to G_1, G_4 and G_3 due to HCD. The all the values recorded in present study were within the normal range (40 to 130 mg/dl). The reduction in the levels of

cholesterol with the consumption of flaxseed can be attributed to the presence of components like fiber, lignans and alpha linolenic acid that may bind cholesterol in the intestine. Ratnayake *et al.,* (1992) have shown that a 20% and higher flax seed diet given for 90 days in rats decreased serum total cholesterol. Cunnane *et al.,* (1993) reported a decrease of 9% in total cholesterol after 4 weeks of flax seed (50g/daily) diet in human volunteers. Prasad, (1997) reported flax seed is effective in reducing hypercholesterolemic atherosclerosis by 46% without lowering the serum cholesterol in rabbits. Lucas *et al.,* (2004) found that flaxseed is beneficial in reducing plasma cholesterol and plaque formation induced by ovarian hormone deficiency in hamsters. Khalesi *et al*.,(2011) concluded that 30 days consumption of flaxseed in rats may significantly reduce total cholesterol and increase high density lipoprotein cholesterol in blood.

4.9.3 Triglyceride

The levels of serum triglyceride in the blood (Table 4.20; Fig. 4.40) was maximum ($P<0.05$) in group G_2 (126.1±0.47 mg/dl) than rest of the groups i.e., G_1 (114.43 ±0.37 mg/dl), G_3 (96.6 ±0.38 mg/dl) and G_3 (88.63±0.35 mg/dl). The values in the HCD group G_2 was significantly high ($P<0.05$) than *flaxseed fortified synbiotic flavoured dahi* groups, G_3 & G_4 and control diet group G_1. The values shown in the table (Table 4.20) also depicts that FFSFD decreases the levels of blood triglyceride significantly ($P<0.05$) inspite the presence of higher cholesterol in diet G_4. The triglyceride levels in rat blood found in present study were within the normal range (45 to 110 mg/dl, www.ratfanclub.org). Similar to present study, Makni *et al.,* (2011) found decrease ($P<0.05$) in plasma triglyceride, when supplemented with flaxseed and pumpkin seed mixture to diabetic rats. However, contrary to the present investigation Lucas *et al.,* (2004) found that triglyceride concentrations were significantly higher in the flax-fed hamsters.

Table 4.20 Impact of feeding *flaxseed fortified synbiotic flavoured dahi* on the biochemical parameters of the mice.

Groups	Glucose (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
G_1	126.27±1.76^{c}	114.43±0.37^{c}	77.27±0.19^{c}	34.83±0.033^{a}	36.33±0.01^{c}	9.13±0.02^{c}
G_2	140.33±2.96^{d}	126.1±0.47^{d}	85.13±0.15^{d}	36.13±0.067^{b}	49.19±0.033^{d}	11.33±0.067^{b}
G_3	119± 0.58^{a}	88.63±0.35^{a}	46.13±0.49^{a}	42.20±0.012^{d}	30.83±0.033^{a}	8.37±0.03^{a}
G_4	122±1.15^{bc}	96.6±0.38^{b}	61.57±0.5^{b}	39.53±0.033^{c}	33.12±0^{b}	8.37±0.013^{a}

Values bearing different small superscripts (a, b, c) in a column differ significantly (Duncan test, $P<0.05$)

Table 4.21 Impact on the total lipids and total cholesterol of endogenous organs of mice fed with Control diet (CD), high cholesterol diet (HCD) and *flaxseed fortified synbiotic flavoured dahi* (FFSFD).

Total Lipids(mg/g dry wt.)					
Groups	**Liver**	**Heart**	**Kidney**	**Spleen**	**Faeces**
G_1	52.7±0.34^{c}	19.2±0.35^{c}	21.3±0.33^{c}	9.2±0.37^{c}	63.25±0.55^{a}
G_2	56.32±0.47^{d}	21.65±0.37^{d}	25.42±0.38^{d}	10.56±0.35^{d}	70.06±0.35^{d}
G_3	44.26±0.45^{a}	14.67±0.25^{a}	17.55±0.35^{a}	7.8±0.38^{a}	68.3±0.53^{c}
G_4	48.69±0.35^{b}	17.04±0.22^{c}	20.34±0.24^{b}	8.3±0.31^{b}	67.89±0.45^{b}
Total Cholesterol(mg/ g dry wt.)					
Groups	**Liver**	**Heart**	**Kidney**	**Spleen**	**Faeces**
G_1	4.7±0.05^{c}	2.1±0.12^{c}	3.9±0.16^{c}	2.9±0.03^{c}	4.5±0.13^{b}
G_2	5.3±0.16^{d}	3.2±0.35^{d}	4.2±0.19^{d}	3.6±0.02^{d}	4.11±0.12^{a}
G_3	3.4±0.19^{a}	1.7±0.08^{a}	2.1±0.13^{a}	1.8±0.01^{a}	6.4±0.03^{d}
G_4	4.1±0.23^{b}	1.9±0.02^{b}	2.8±0.15^{b}	2.3±0.06^{b}	5.7±0.06^{c}

Values bearing different small superscripts (a, b, c) in a column differ significantly (Duncan test, $P<0.05$)

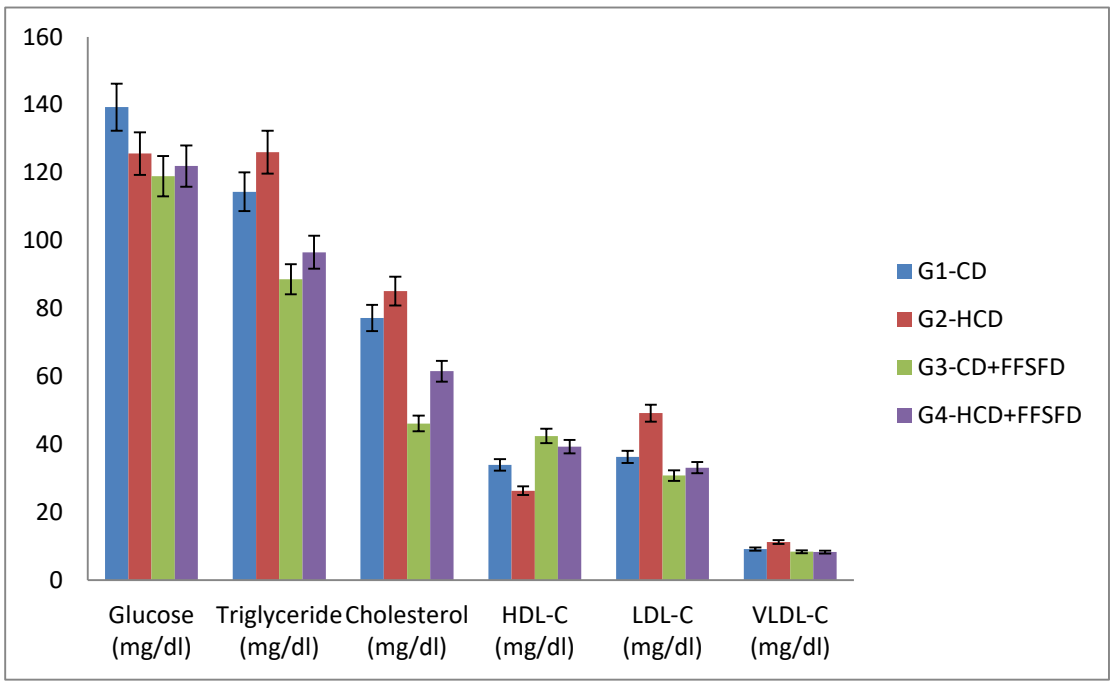


Fig. 4.40 Impact on the biochemical parameters of different groups of swiss mice.

4.9.4 HDL-C, LDL-C, and VLDL-C

The levels of serum HDL-C, LDL-C and VLDL-C values in the blood are given in Table 4.20; Fig. 4.40.The HDL was the maximum ($P<0.05$) in group G_3 (39.47±0.19 mg/dl rest of the groups i.e., G_4 (41.47 ±0.15 mg/dl), G_2 (36.37 ±0.49 mg/dl) and G_1 (34.63±0.5 mg/dl). The values in the FFSFD group G_3 and G_4 was significantly high ($P<0.05$) than HCD and CD groups, G_2 & G_1 group. The values shown in Table 4.20; Fig. 4.40. depicts that the LDL and VLDL values were lower in the FFSFD groups G_3 and G_4 than in HCD and CD group G2 and G1.The values shown in the table (Table 4.20) also depicts that FFSFD increases the levels of HDL blood triglyceride significantly ($P<0.05$) inspite the presence of higher cholesterol in diet G_4. Flaxseed lignans have been shown to effectively lower serum total- and LDL-cholesterol while increasing HDL-cholesterol concentrations (Renaud *et al.,* 2001). Similar to present study, Prasad, (2005) found that flaxseed lignan complex increased the levels of serum HDL-C by 24% and LDL-C decreased by 34-39% in rabbits on regular chow diet. The results of present study, was in line with findings of Yamashita *et al.,* 2003 and Zhang *et al.,* 2007 who observed dietary flaxseed lignan extract

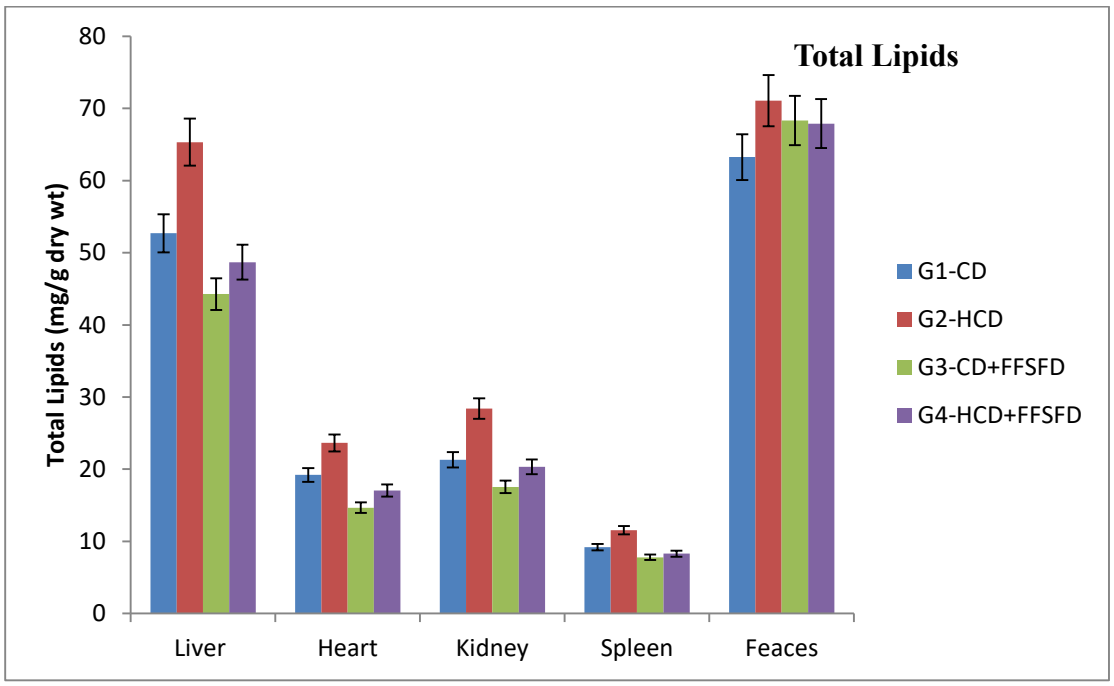
lowers plasma cholesterol and glucose concentrations in hypercholesterolaemic subjects.

4.9.5 Effect on total lipids and total cholesterol on various body organs of mice

The effect of dietary feed on total lipids of various endogenous organs of mice viz. liver, heart kidney, spleen and faeces are given in table 4.15 (Fig. 4.49). The total lipid level was significantly high ($P<0.05$) in G_2 group with HCD in liver, heart kidney, spleen and faeces (56.32±0.47; 21.65±0.37, 25.42±0.38, 10.56±0.35 and 70.06±0.35 mg/ g dry wt). The levels of total lipids decreased in ($P<0.05$) G_4 and G_3 blood in the presence of FFSFD in the diet. As depicted in the table 4.20, same observation is with the levels of total cholesterol in various organs of mice. The level of total cholesterol in liver, heart kidney, spleen and faeces was significantly high ($P<0.05$) in G_2 (5.3, 3.2, 4.2, 3.6 and 4.11(mg/ g dry wt.), respectively) than in FFSFD groups G_3 & G_4 and control group G_1. The levels of TL and TC in liver, heart, kidney and spleen (mg/ g dry wt.) of mice fed with diet containing FFSFD were significantly ($P<0.05$) lower than those found in mice fed with high cholesterol diet and control diet.

Triglycerides deposited as stored energy of the organism, while PL and cholesterol act as essential building blocks in the cell structures (Rotenberg and Jakobsen, 1978; Rolandelli *et al.*, 1989). In G_3 and G_4 rat fed with FFSFD, the serum and liver lipids were significantly decreased compared to HCD group G_2 and control G_1 this may due to the increased TG catabolism. Prasad, (2005) reported that flaxseed lignan complex decreased the TC by 20%, LDL-C by 14%, TC/HDL-C by 34–39% and increased the HDL-C by 30% at the end of 2 months in high cholesterol fed rabbits.

(a)



(b)

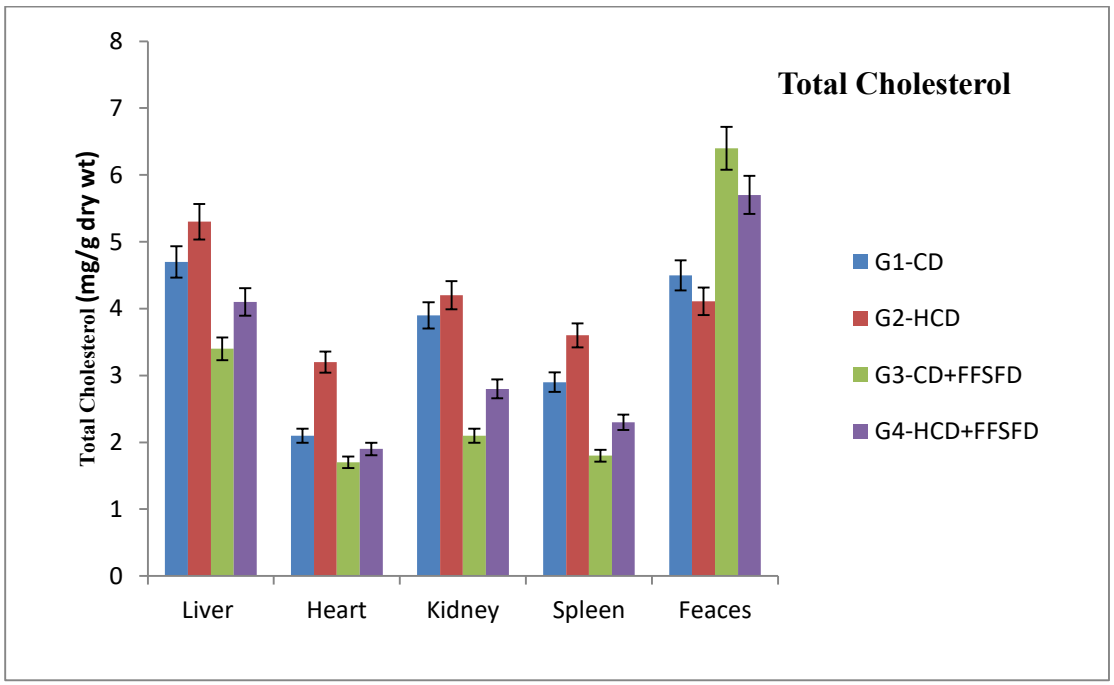


Fig 4.41 Impact on the (a) total lipids and (b) total cholesterol of endogenous organs of mice in different test groups.

Many earlier studies (Jenkins *et al.*, 1999; Shinnick *et al.,*, 1990; Arjmandi *et al.,* 1998) supported the view that dietary fibre of flaxseed and other cereals have significant impact on cholesterol-lowering properties. Shinnick *et al.,* (1990) reported rats fed 1% cholesterol had elevated serum and liver cholesterol concentrations, which were decreased by 25 and 39%, respectively, by 10% dietary fiber in the form of oat bran flour.

Most carefully controlled studies indicated that there are several mechanisms for the hypocholesterolemic effect of dietary fibers. Soluble fiber may bind bile acids or cholesterol in the intestine, preventing their reabsorption into the body. The liver responds by taking up more LDL-c from the blood stream thereby lowering the concentration of LDL-c in the blood. Short Chain Fatty Acids (SCFA) products of fermentation from soluble fiber in the gut, may inhibit synthesis of cholesterol by the liver, reducing the concentration of blood cholesterol (Andersson *et al.*, 2002).

Soluble gum of the flaxseed may be helpful in the prevention of cardiovascular diseases by exhibiting hypocholesterolemic effect (Pan *et al.,* 2007, 2009). However, the faecal total lipid and total cholesterol level increased in groups fed with FFSFD, G_3 and G_4. This might be due to the reduction in absorption of plasma cholesterol also lessen the absorption of dietary fat. Kristensen *et al.,* (2012) studied the effect of differently processed flax fibers on the fat excretion and energy balance in rats and observed higher values for fat excretion through faeces in flax supplemented groups.

Flaxseed fortified synbiotic flavoured *Dahi*

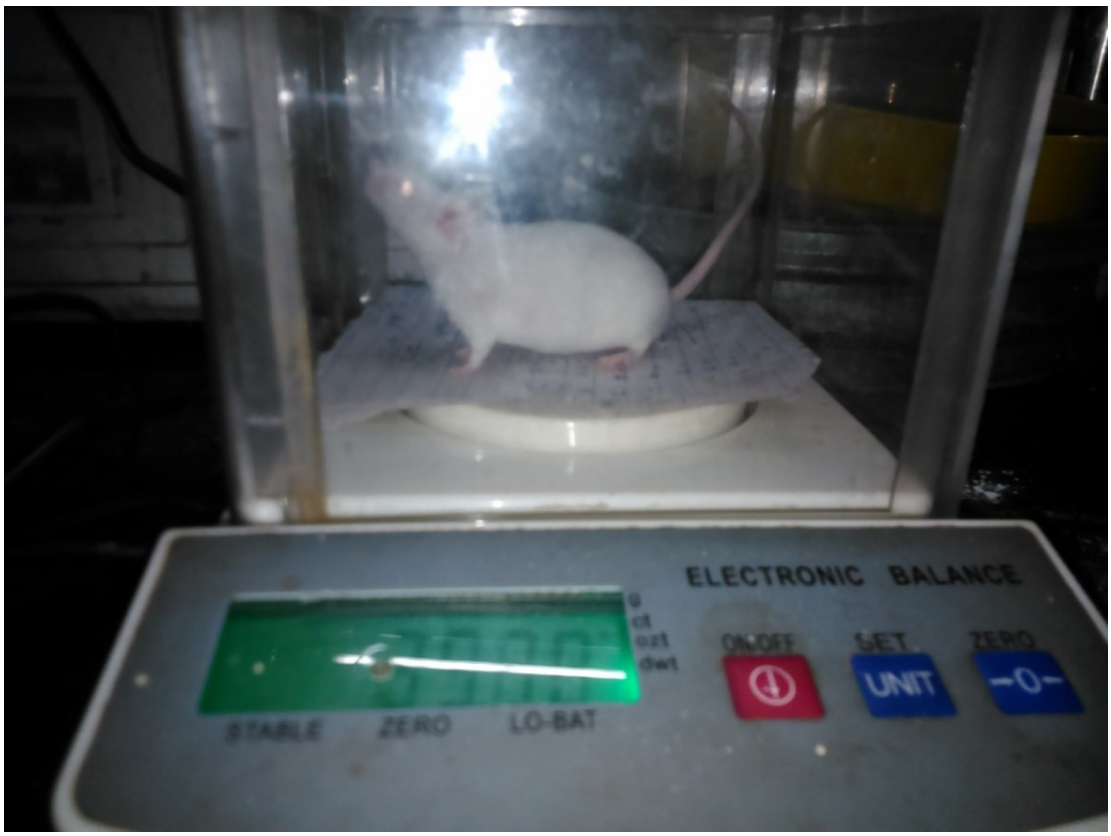
Control and different treatment samples of Flaxseed fortified synbiotic flavoured *Dahi*

Finely ground flaxseed powder

Flaxseed powder

Synbiotic microcapsules of *Lactobacillus acidophilus*

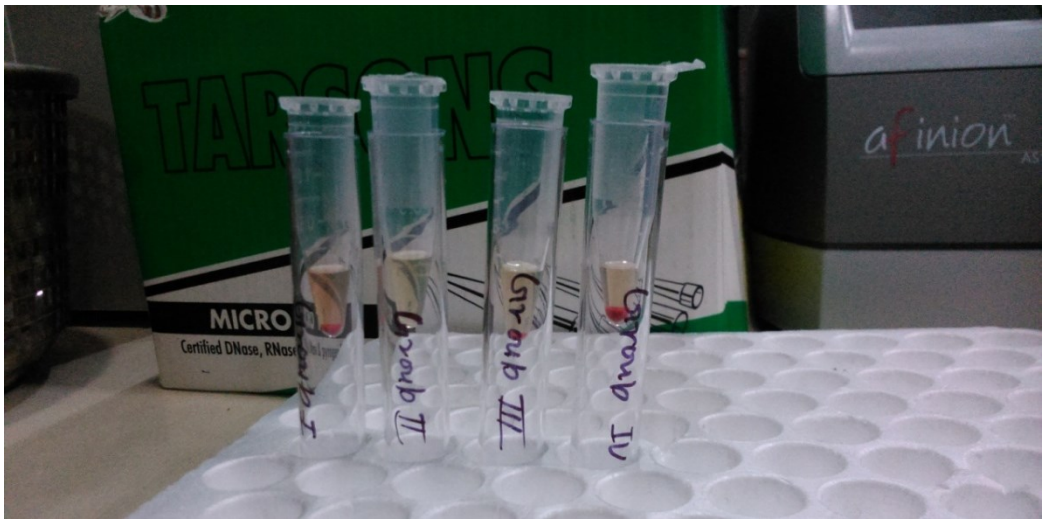
Feeding of mice in groups



Weighing of mice

Impact of Flaxseed fortified synbiotic flavoured *dahi* on cholesterol fed mice

Excised liver, heart, kidneys and spleen of mice



Collection of serum samples

Chapter V

SUMMARY AND CONCLUSION

In recent years, there is significant increase in cardiovascular diseases in developing countries which can be prevented and combat by nutritional interventions. The increased use of omega-3 fatty acids is a powerful example of one such nutritional intervention that may produce significant cardiovascular benefits. Flaxseeds have been known to possess cholesterol lowering properties and effective in prevention of CVD risks. To increase its consumption in daily life, it can be incorporated as an ingredient in the preparation of dairy products.

Aiming to this, the present investigation entitled "Development of Flaxseed Fortified Synbiotic Flavoured *Dahi* and Its Impact on the Cholesterol-fed Mice" are summarised in the following four phases:

PHASE I: Optimization of Flaxseed Fortified Synbiotic Flavoured *Dahi*

Optimization of yoghurt and biscuits has been performed using Central Composite Design (CCD) of Response Surface Methodology (RSM) Minitab version 17. The independent variables for *dahi* were in the proportions of full fat flaxseed powder (FFP) (1-3g/100g), mango pulp (3-7%), and honey (2-6%). The formulation with 2g flaxseed powder, mango pulp 5% and honey 4% was considered to be the most appropriate for producing the flaxseed fortified synbiotic flavoured *dahi* (FFSFD). The predicted scores for the formulation of this product were given as sensory, textural, antioxidant activity, probiotic viable count and whey separation scores. Different sensory responses were studied on 9 point hedonic scale and found to be 8.43 for taste, 8.26 for colour and 8.35 for overall acceptability. Antioxidant activity as % DPPH inhibition was found to be 85.04%.Textural properties were found to be 245.78g for firmness and 36.69gs for cohesiveness. Probiotic viable count of *Lactobacillus acidophilus* was found to be 9.38 10^8 cfu/g and whey separation was 5.17 ml/100g for optimized FFSFD. The interaction effects of flaxseed powder with mango pulp and honey on all the responses was significant ($p<0.05$). The desirability of the product was found to be nearly 81.7%. Thus, the optimized FFSFD was found

high in protein, fat, total solids, dietary fibre, antioxidant activity and total phenolic content compared to control samples.

PHASE II: Physico-chemical and acidification kinetics of *flaxseed fortified synbiotic flavoured dahi*

The moisture content of the *dahi* samples ranged between 80.03 to 87.42%. Moisture content of FFSFD was lower than control due to incorporation of flaxseed powder, mango pulp and honey which lead to the reduction of moisture content. The protein content was higher in FFSFD (5.26%) than control *dahi* (3.65%). The protein content increased as the proportion of the flaxseed powder increased in the *dahi*. The fat content ranged between 4.28 to 1.5% in FFSFD and control *dahi* samples, respectively. Fat content of FFSFD increased due to incorporation of flaxseed powder which have high omega-3 fatty acid content. The ash content in the FFSFD *dahi* (3.42%) also increased as compared to control *dahi* samples (0.64%). This could be due to the fact that ingredients of FFSFD *viz.* flaxseed powder, mango pulp and honey are high in mineral content. The carbohydrate content of the FFSFD *dahi* samples increased with flaxseed powder, mango pulp and honey fortification from 16.11% in FFSFD to 6.07% in control *dahi*. The pH values of the *dahi* samples ranged from 4.65 to 4.73. FFSFD sample had the lowest value, when compared with the control sample. The titratable acidity also ranged from 0.75 to 0.68% in the *dahi* samples. The control *dahi* samples had lower acidity values (0.68%) than FFSFD (0.75%). This could be due to availability of more of sugars provided by honey to the fermenting microbes in FFSFD. There was increased dietary fibre content in the FFSFD as compared to control *dahi* (0.00%). It may be due to high amount of dietary fibre present in flaxseed powder. There was increase in antioxidant activity and phenolic content in the FFSFD compared to control *dahi*. The increase in antioxidant activity and total phenolic content was contributed by flaxseed powder, the probiotic bacteria, mango pulp and honey which are known to be rich in antioxidant activity and total phenolic content. There was decrease in syneresis due to hight total solid content, in the FFSFD *dahi* as compared to control *dahi* (10.36%).

The acidification kinetics of FFSFD *dahi* was characterized by V_{max}, t_{Vmax}, pH_{Vmax}, $t_{pH\ 5.0}$ and $t_{pH\ 4.5}$. The maximum rate of acidification (V_{max}) was significantly

reduced ($P < 0.05$) by the addition of flaxseed powder in samples (Sample B and D) in comparison to their control sample (sample A and C) which can probably be ascribed to the presence of substances with buffering capacity in FP, such as organic acids and phenolic compounds. The V_{max} value of FFSFD (23.25×10^{-3}pHunits/min) was significantly high in comparison to control *dahi* sample (25.73×10^{-3}pH units/min). This indicates that flaxseed powder significantly reduces the V_{max} higher in respect to the samples without FP when all other factors are same. Flaxseed mucilage acts as a good source of prebiotic, enhancing lactic acid bacteria growth and the ability of *L. acidophilus* to ferment FOS more effectively than other microorganisms.

Furthermore, it was observed that samples with free cells of probiotic LA had lower V_{max} value than samples with encapsulated LA (sample C and D). This indicates profuse consumption of growth promoting substances by probiotic bacteria in free state, while encapsulated LA exhibited controlled steady fermentation. This might be the primary reason for the higher acidification rate of FFSFD (Sample D), than sample B with LA in free state. The pH_{Vmax} i.e. pH at maximum rate of acidification was recorded to be 5.17 of sample B while that of sample D was 5.47 the encapsulated bacteria leads to slower rate of acidification than in free state.

The t_{Vmax} value for *dahi* samples ranged from 2.33 to 3.13 h. Nevertheless, the time to reach the maximum acidification rate (t_{Vmax}) was significantly ($P<0.05$) reduced by the presence of the FP in FFSFD sample D (3.05 h) than the control sample (3.32 h) without FP. Same trend is shown in FFSFD sample for time to reach pH 5.0 ($T_{pH5.0}$) and end of fermentation pH4.5 ($T_{pH4.5}$). The t_{Vmax} value of *dahi* samples was significantly affected by the state of probiotic bacteria. The content of total solids of synbiotic flavoured *dahi* fortified with flaxseed powder (FP) was found to be 25.11 ±0.25 and control *dahi* as 10.16± 0.48 g 100 g^{-1}. As expected, total solid content of *dahi* increased significantly ($P < 0.05$) in the presence of FFP, mango pulp and honey.

Thus, the present study of correlation analysis related to kinetic parameters indicates that multiple factors such as culture composition and presence of growth

promoting substances such as, FP, oligosachharides, higher TS and state of probiotic bacteria can affect acidification parameters of synbiotic *dahi*/yoghurt.

PHASE III: Shelf life study of *flaxseed fortified synbiotic flavoured dahi*

The optimized FFSFD and control *dahi* samples were stored plastic cups at 4 ± 1°C for shelf life determination and observations were carried out at 0, 7th, 14th, 21st and 28th days intervals. Results showed FFSFD was stable upto 14th day of cold storage, thereafter onset of deterioration of FFSFD was detected. The FFSFD was significantly ($P<0.05$) high in protein, fat, total solid, acidity, antioxidant activity and total phenolic content but lower ($P<0.05$) in moisture than control *dahi.* The protein, fat, moisture, pH, titratable acidity, antioxidant activity and total phenolic content and syneresis showed significant difference ($p>0.05$) throughout the storage period. As the storage periods prolonged, the protein, fat, total solids, antioxidant activity, total phenolic content and pH value decreased while titratable acidity increased ($p<0.05$) in control and types of *dahi* sample. However, it was observed that in (sample D) FFSFD, pH and titratable acidity was stable up to 21 days of storage and had minimal changes in texture and sensory attributes in comparison to other samples. The free fatty acid and TBA value of FFSFD was significantly ($p<0.05$) high than the control *dahi* samples, which may be due to higher oxidation of alpha linolenic acid of flaxseed during storage.

During storage, sensory attribute score depreciated considerably in both the samples. The colour score of *dahi* varied from 8.42 ± 0.09 to 7.06 ± 0.05 for FFSFD and from 7.53 ± 0.04 to 6.02 ± 0.03 for CD samples. The flavour score of FFSFD sample ranged from 8.59±0.06 to 6.34±0.13 and for CD samples from 7.29±0.15 to 6.01±0.19. The texture score for FFSFD samples ranged between 8.53 ± 0.076 and 7.41± 0.12 and for CD between 8.05 ± 0.03 and 6.84 ± 0.12. The overall acceptability score ranged from 8.37±0.02 to 6.96±0.05 for FFSFD and from 7.82±0.15 to 8.37±0.02 for CD samples. The colour, flavour, texture & overall acceptability score of FFSFD was significantly high ($p<0.05$) as compared to control *dahi* CD.

During storage, textural properties of FFSFD (sample D) increased significantly upto 14th day of storage, thereafter decreased significantly ($P<0.05$).However, the CD sample showed increase in texture values up till 7th day of storage and thereafter, decreased drastically. There was slight increase in the firmness, cohesiveness, consistency and index of viscosity of FFSFD samples during storage.

During entire storage periods, the counts of LP (10^8cfu/g) and LA (10^8cfu/g) were significantly high ($P<0.05$) in (sample D) FFSFD than its control (sample B). These counts increased ($P<0.05$) in all the samples up to 21st day of storage, but thereafter it decreased.

The highest total plate count (TPC) (10^7cfu/ml) was recorded at 14th day of storage for both (FFSFD and CD) samples. As the storage increases the TPC count also increased ($p<0.05$) upto 14th days but thereafter decreased up to 28th days for both (FFSFD and CD) samples. Yeast and Mould counts (YMC) were not observed up to 7th day, but at 14th day increased significantly ($p<0.05$) in both the groups (FFSFD and CD). The coliform counts (10^1cfu/g) were not observed in any sample during storage.

The product was quite acceptable up to 21 days. After that the sensory, textural and chemical properties of the product started deteriorating.

PHASE IV: Nutritional impact on Cholesterol-fed swiss mice

Four groups of swiss mice were maintained on basal or control diet, basal diet with control *dahi* at 30% level (G_1), basal diet with 1g cholesterol (i.e. high cholesterol diet-HCD) (G_2), basal diet with FFSFD *dahi* at 30% level(G_3) and basal diet with HCD along with FFSFD *dahi* at 30% level (G_4). The overall weekly feed intake/rat was recorded to be 125.47, 121.01, 110.27 and 114.74 gm in groups G_1, G_2, G_3 and G_4 respectively. The difference in feed intake between CD and FFSFD groups were high ($P<0.05$). The intensity of increase in body weight gain of mice were significantly ($P<0.05$) high in treatment groups G_1 and G_2 than FFSFD groups G_3 and G_4.

The levels of glucose in blood were 126.27±1.76, 140.33±2.96, 119±0.58, and 122±1.15 mg/dl in groups G_1, G_2, G_3 and G_4 respectively. These values shows that the serum glucose in group G_2 (HCD) was significantly high ($P<0.05$) than rest of the treatment groups. The administration of flaxseed in FFSFD to mice in G_3 and G_4 resulted in the significant decrease of glucose concentration in comparison to the result obtained from the G_2 (HCD) group.

The highest serum cholesterol was 85.13±0.47mg/dl in group G_2 followed by 77.27±0.37, 61.57±0.38 and 46.13±0.35 mg/dl in groups G_1, G_4 and G_3 respectively. The levels of serum cholesterol decreased ($P<0.05$) in the blood in the presence of FFSFD in G_3 and G_4 group as compared to control and high cholesterol diet group G_1 and G_2 group. The blood triglyceride decreased ($P<0.05$) in the presence of FFSFD in G_3 and G_4 group in comparison to control and high cholesterol diet group G_1 and G_2 group group. The level of HDL-C significantly ($P<0.05$) increased in G_3 and G_4 group in comparison to control and high cholesterol diet group G_1 and G_2. However, the levels of LDL-C and VLDL-C decreased significantly ($P<0.05$) in FFSFD G_3 and G_4 groups.

The levels of total lipids and total cholesterol in liver, heart, kidney and spleen (mg/ g dry wt.) of mice fed with diet containing FFSFD, G_3 & G_4 were significantly ($P<0.05$) lower than those found in mice fed with high cholesterol diet and control diet G_2 & G_1 group.

Thus, the results from present investigation, suggests that diet-induced hypercholesterolemia can be effectively prevented and combat by FFSFD in daily diet. It also suggests that flaxseed ALA increases the HDL-C while lowers the LDL-C. In conclusion, *flaxseed fortified synbiotic flavoured dahi* is beneficial in reducing hypercholesterolemia as well as has high physiochemical and functional properties while acceptable sensory, textural and storage qualities for consumer acceptance.

REFERENCES

Adhikari, K, Mustapha, A, Grun, IU, & Fernando, L, Viability of microencapsulated bifidobacteria in set yoghurt during refrigerated storage, Journal of Dairy Science, 83, 9:1946–1951, 2000.

Adlercreutz, H, Bannwart, C, Wahala, K, Makela, T and Brunow, G, Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens, Journal of Steroid Biochemistry and Molecular Biology, 42:147–153, 1993.

Adlercreutz, H, Western diet and western diseases: some hormonal and biochemical mechanisms and associations, Scandinavian Journal of Clinical and Laboratory Investigation Supplementum, 201:3–23, 1990.

Ahmad, AL, Ismail, S and Bhatia, S, Optimization of coagulation-flocculation process for palm oil mill effluent using response surface methodology, Environmental Science and Technology, 39, 2828-2834, 2005.

Akalin, AS, Erisir, D, Effects of inulin and oligofructose on the rheological characteristics and probiotic culture survival in low-fat probiotic ice cream, Journal of Food Science, 73(4): M184-8, 2008.

Akande, KE, Doma, UD, Agu, HO and Adamu, HM, Major anti nutrients found in plant protein sources: their effect on nutrition, Pakistan Journal of Nutrition, 9:827–832, 2010.

Alakali, F, Miranda, G, Le Bars, D and Monnet, V, Milk fermentation by Lactococcus lactis with modified Proteolytic systems to accumulate potentially bioactive peptides, Le Lait, INRA Editions, 84, (1-2), 115-123, 2004.

Al-Kadamany, E, Medhat, K, Tharwat, H and Imad, T, Estimation of shelf-life of concentrated yoghurt by monitoring selected microbiological and physicochemical changes during storage, LWT- Food Science and Technology, 36, 407–414, 2003.

Al-Okbi, SY, Highlights on functional foods, with special reference to flaxseed, Journal of Natural Fibers, 2, 3: 63–68, 2005.

Al-Waili, NS, Natural honey lowers plasma glucose, creactive protein, homocysteine, and blood lipids in healthy, diabetic, and hyperlipidemic subjects: comparison with dextrose and sucrose, Journal of Medicinal Food, 7:100–107, 2004.

Amerine, MA, Pongborn, RH and Roescler, EB, Principles of sensory evaluation of food, Academic Press, New York, 338-339,1965.

Anandharaj, M, Sivasankari B and Parveen RR, Effects of Probiotics, Prebiotics, and Synbiotics on Hypercholesterolemia: A Review, Chinese Journal of Biology, 2014, 1-7, 2014.

Andersson, M, Ellegard L and Andersson, H, Oat bran stimulates bile acid synthesis within 8 h as measured by 7-hydroxy-4-cholesten-3-one, American Journal of Clinical Nutrition, 76:1111-1116, 2002.

AOAC, Official methods of analysis of AOAC International, Vol. II, 16th Ed. Virginia, USA, 1995.

AOAC, Official Methods of Analysis of Association of AOAC International, 16th Ed., Vol. 01, Method 985.29. Washington DC, 2008.

AOAC, Official methods of analysis of the association of official agricultural chemists, Association of Official Analytical Chemists, Washington, DC, 125-139, 2000.

Aportela-Palacios, A, Sosa-Morales, ME and Velez-Ruiz, JF, Rheological and Physicochemical Behaviour of Fortified Yogurt, with Fiber and Calcium, Journal of Texture Studies, 36: 333-349, 2005.

Arend, WP, Dayer, JM, Inhibition of the production and effects of interleukin-1 and tumor necrosis factor α in rheumatoid arthritis, Arthritis Rheumatoid, 38:151–160, 1995.

Arjmandi, B, Khan, D and Juma S, Whole flaxseed consumption lowers serum LDL - cholesterol and lipoprotein (a) concentrations in postmenopausal women, Nutrition Research, 18, 7: 1203-1214, 1998.

Ashar, MN and Chand, R, Fermented milk containing ACE-nhibitory peptides reduces blood pressure in middle aged hypertensive subjects, Milchwissenschaft, 59:363–366, 2004.

Astwood, K, Lee, B and Manley-Harris, M, Oligosaccharides in New Zealand honey dew honey, Journal of Agricultural and Food Chemistry, 46, 4958−4962, 1998.

Bakirci, I and Kavaz, A, An investigation of some properties of banana yogurts made with commercial ABT-2 starter culture during storage, International Journal of Dairy Technology, 61, 270-276, 2008.

Barcelo-Coblijn, G and Murphy, EJ, Alpha-linolenic acid and its conversion to longer chain n3 fatty acids: benefits for human health and a role in maintaining tissue n3 fatty acid levels, Progress Lipid Research, 48:355–374, 2009.

Barrangou, R, Altermann, E, Hutkins, R, Cano, R and Klaenhammer, RT, Functional and comparative genome analyses of an operon involved in fructooligosaccharide utilization by *Lactobacillus acidophilus*, Proceedings of

the National Academy of Sciences of the United States of America, 100, 8957–8962, 2003.

Basel, Karger: World Review of Nutrition Dietetics, 74–8.

Beejmohun,V, Microwave-assisted extraction of the main phenolic compounds in flaxseed, Phytochemical Analysis, 18:275–282, 2007.

Bhat, ZF, Pathak, V, Bukhari, SAA and Ahmad, SR, Development of kashmiri saffron phirni from reconstituted skim milk, Beverage Food World, 37, 881-883, 2010.

Bhathena, SJ, Ali, AA, Haudenschild, C, Latham, P, Ranich, T, Mohamed, AI, Hansen, CT and Velasquez, MT, Dietary flaxseed meal is more protective than soy protein concentrate against hypertriglycerdemia and steatosis of the liver in an animal model of obesity, Journal of American College of Nutrition, 22:157–164, 2003.

Bhatty, RS, Further compositional analyses of flax: mucilage, trypsin inhibitors and hydrocyanic acid, Journal of American Oil Chemistry Society, 70:899–904, 1993.

Bille, P and Keya E, A Comparison of Some Properties of Vat-Heated and Dry Skim Milk Powder Fortified Set Yoghurts. The Journal of Food Technology in Africa, 7, 1: 21-23, 2002.

Bille, P, and Keya, E, A Comparison of Some Properties of Vat-Heated and Dry Skim Milk Powder Fortified Set Yoghurts, Journal of Food Technology in Africa, 7, 1: 21-23, 2002.

Bloedon, LT, and Szapary, PO, Flaxseed and cardiovascular risk, Nutrition Revision, 62, 18-27, 2004.

Bloedon, LT, Balikai, S, Chittams, J, Cunnane, SC, Berlin, JA, Rader, DJ and Szapary, PO, Flaxseed and cardiovascular risk Factors: Results from a double blind, randomized, controlled clinical trial, Journal of American College of Nutrition, 27,1: 67–74, 2008.

Bozanic, R, Rogelj I and Tratnik, U, Fermented acidophilus goat's milk supplemented with inulin: comparison with cow's milk, Milchwissenschaft, 56, 11:618-22, 2001.

Brennan, CS and Tudorica, M, Carbohydrate-based fat replacers in the modification of the rheological, textural and sensory quality of yoghurt: comparative study of the utilisation of barley beta-glucan, guar gum and inulin, International Journal Food Science and Technology, 43, 824-833, 2008.

Brown, GD and Kosikowski, FV, How to make honey yoghurt, American Dairy Review, 32 60–62, 1970.

Bujalance, C, Jimenez-Valera, M, Moreno, E and Ruiz-Bravo, A, A selective differential medium for *Lactobacillus plantarum*, Journal of Microbiological Methods, 66, 3: 572–575, 2006.

Cann, PA, Read, NW and Holdsworth, CD, What is the benefit of coarse wheat bran in patients with irritable bowel syndrome?, Gut, 24:168–173, 1984.

Cao, YH and Cao, RH, Angiogenesis inhibited by drinking tea, Nature, 398, 6726:381, 1999.

Carter, JF, Potential of flaxseeds and flaxseed oil in baked goods and other products in human nutrition, Cereal Foods World, 38:754–759, 1993.

Champagne, CP, Gardner, NJ, Soulignac, L, and Innocent, JP, The production of freeze-dried immobilized cultures of *Streptococcus thermophilus* and their acidification properties in milk, Journal of Applied Microbiology, 88, 124–131, 2000.

Champagne, CP, Green-Johson, J, Raymond, Y, Barrete, J, and Buckley, N, Selection of probiotic bacteria for the fermentation of a soy beverage in combination with *Streptococcus thermophilus*, Food Research International, 42, 612–621, 2009.

Chandan, RC, White, CH, Kilara, A and Hui, YH, Manufacturing Yogurt and Fermented Milks, Blackwell Publishing, 2006.

Chandramouli, V, Kailasapathy, K, Peiris, P, Jones, M, An improved method of microencapsulation and its evaluation to protect *Lactobacillus spp*. in simulated gastric conditions, Journal of Microbiological Methods, 56, 1:27–35, 2004.

Chen, HH, Xui, S. and Wang, Z, Interaction between flaxseed gum and meat protein, Journal of Food Engineering, 80:1051-1059, 2007.

Chen, J, Liu, X, Shi, Y, Ma, C, Determination of the lignin secoisolariciresinol diglucoside from flaxseed (*Linum usitatissimum*) by HPLC, Journal of Liquid Chromatography and Related Technologies, 30:533–544, 2007.

Chen, MJ, Chen, KN and Kuo, YT, Optimal thermotolerance of *Bifidobacterium bifidum* in gellan-alginate microparticles, Biotechnology Bioengineering, 98, 2:411–419, 2007.

Chen, ZY, Chan, PT, Ho, KY, Fung, KP and Wang, J, Antioxidative activity of natural flavonoids is governed by number and location of their aromatic hydroxyl groups, Chemistry and Physics of Lipids, 79:157–63, 1996.

Chung, M, Lei, B and Li-Chan, E, Isolation and structural characterization of the major protein fraction from NorMan flaxseed (*Linum usitatissimumL*.), Food Chemistry, 90:271–279, 2005.

Clark, WF, Muir, AD, Westcott, ND, and Parbtani, A, A novel treatment for lupus nephritis: lignan precursor derived from flax, Lupus, 9: 429–436, 2000.

Clavel, T, Borrmann, D, Braune, A, Dore, J, Blaut, M, Occurrence and activity of human intestinal bacteria involved in the conversion of dietary lignans, Anaerobe, 12:140–147, 2006.

Cliffe, S, Fawer, MS, Maier, G, Takata, K and Ritter, G, Enzyme assays for the phenolic content of natural juices, Journal of Agriculture and Food Chemistry, 42, 1824–1828, 1994.

Codex, A, Milk and Milk Products. Codex Standard for Milk fat Products, Codex Stan A-2-1973. Rome, Italy: FAO and WHO, 2007.

Cunnane, SC and Thompson, LU (eds) Flaxseed in human nutrition, Champaign, IL: AOCS Press, 1995.

Cunnane, SC, Ganguli, S, Menard, C, Liede, AC Hamadeh, MJ, Chen, ZY, Wolever, TMS, and Jenkins, DJA, Highα–linolenic acid flaxseed (*Linum usitatissimum*): some nutritional properties in humans, British Journal of Nutrition, 49: 443–453, 1993.

Cunnane, SC, Ganguli, S, Menard, C, Liede, AC Hamadeh, MJ, Chen, ZY, Wolever, TMS, and Jenkins, DJA, Nutritional attributes of traditional flaxseed in healthy young adults, American Journal of Clinical Nutrition, 61:62–8, 1995.

Dave, RI and Shah, NP, Evaluation of media for selective enumeration of *Streptococcus thermophilus, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus acidophilus* and *bifidobacteria*, Journal of Dairy Science, 79, 1529–1536, 1996.

de Lorgeril, M, Salen, P, Laporte, F and de Leiris, J, Alpha-linolenic acid in the prevention and treatment of coronary heart disease, European Heart Journal Supplements, D3:D26–D32, 2001.

Deeth, HC, Fitz-Gerald, CH and Wood, AF, A Convenient method for determining the extent of Lipolysis in milk, Australian Journal of Dairy Technology, 30, 109-111, 1975.

Del Piano, M, Morelli, L, Strozzi, GP, Allesina, S, Barba, M, Deidda, F, Lorenzini, P, Ballarè, M, Montino, F, Orsello, M, Sartori, M, Garello, E, Carmagnola, S, Pagliarulo M, Capurso, L, Probiotics: from research to consumer, Digest Liver Disease, 38, 2: 48–55, 2006.

Desai, AR, Powell, IB and Shah, NP, Survival and activity of probiotic lactobacilli in skim milk containing prebiotics, Journal of Food Science, 69, 57–60, 2004.

Dianawati, D and Shah, NP, Survival, acid and bile tolerance and surface hydrophobicity of microencapsulated *B. animalis* ssp. *lactis* Bb 12 during

storage at room temperature, Journal of Food Science, 76, 9: M592-M599, 2011.

Dieken, H, Use of flaxseed as a source of omega-3 fatty acids in human nutrition, Proceedings of Flax Institute, 54:1–4, 1992.

Ding, WK and Shah, NP, Acid, bile and heat tolerance of free and microencapsulated probiotic bacteria, Journal of Food Science, 72, 9:446-450, 2007.

Ding, WK. and Shah, NP, An improve method of microencapsulation of probiotic bacteria for their stability in acidic and bile conditions during storage, Journal of Food Science, 74, M53–M61, 2009.

Dubois, V, Breton, S, Linder, M, Fanni, J and Parmentier, M, Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential, European Journal of Lipid Science and Technology, 109:710–732, 2007.

Eastwood, MA, Vegetable fiber: Its physical properties, Proceedings of the Nutrition Society, 32: 137-143, 1973.

Eke, MO, Olaitan, NI and Sule, HI, Nutritional evaluation of yoghurt-like product from baobab (Adansonia digitata) fruit pulp emulsion and the micronutrient content of baobab leaves, Advance Journal of Food Science and Technology, 5, 10: 1266-1270, 2013.

El Bakri, JM and Zubeir, EM, Chemical and microbiological evaluation of plain and fruit yoghurt in khartoum state sudan, International Journal of Dairy Science, 4, 1: 1-7, 2009.

Eliana, P, Lillian, B and Isabel, CFR, Relevance of the Mention of Antioxidant Properties in Yogurt Labels: In Vitro Evaluation and Chromatographic Analysis, Antioxidants, 2, 62-76, 2013.

Espírito Santo, AP, Perego, P, Converti, A, Oliveira, MN, Influence of food matrices on probiotic viability – a review focusing on the fruit bases. Trends in Food Science and Technology, 22, 377–385, 2011.

Estrada, JD, Boeneke, C, Bechtel, P and Sathivel, S, Developing a strawberry yogurt fortified with marine fish oil, Journal of Dairy Science, 94: 5760–5769, 2011.

FAO/WHO, Report of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria, Córdoba, Argentina, 2001.

Farinde, OE, Adesetan, T, Obatolu, V and Oladapo, M, Chemical and microbial properties of yogurt processed from cow's milk and soymilk, Journal of Food Processing and Preservation, 33, 245–254, 2009.

Fedenuik, RW and Biliaderis, CG, Composition and physiochemical properties of linseed (*Linum usitatissimum*) mucilage, Journal of Agricultural Food Chemistry, 42:240–247, 1994.

Fernandez-Garcia, E, and McGregor, JU, Fortification of sweetened plain yogurt with insoluble dietary fiber, European Food Research Technology, 204, 433–437, 1997.

Fernandez-Gracia, E, McGregor, JU, and Traylor, S, The addition of oat fiber and natural alternative sweeteners in the manufacture of plain yogurt, Journal of Dairy Science, 81, 655–663, 1998.

Figueroa-Gonzáles, I, Quijano, G, Ramírez G and Cruz-Guerrero, A, Probiotics and prebiotics- perspectives and challenges, Journal of the Science of Food and Agriculture, 91, 8:1341, 2011.

Fodje, AML, Chang, PR, and Leterme, P, In vitro bile acid binding and short chain fatty acid profile of flax fiber and ethanol co-products, Journal of Medicinal Food, 12, 1065-1073, 2009.

Fonseca, F, Beal, C, and Corrieu, G, Method of quantifying the loss of acidification activity of lactic acid starters during freezing and frozen storage, The Journal of Dairy Research, 67, 83–90, 2000.

Franck, A, Inulin. In: Stephen, AM, Phillips, GO, Williams PA (eds) Food polysaccharides and their applications, CRC Press Taylor and Francis Press, Boca Raton, 335–351, 2006.

FSSAI, Food Safety Standards Authority of India, FDA Bhawan near Bal Bhavan, Kotla Road, New Delhi, 29-30, 2011.

Fukumitsu, S, Aida, K, Shimizu, H and Toyoda, K, Flaxseed lignin lowers blood cholesterol and decreases liver disease risk factors in moderately hypercholesterolemic men, Nutrition Research, 30:441–446, 2010.

Funk, CD, Prostaglandlins and leukotrienes: advances in eicasanoid biology, Science, 294:1871–1875, 2001.

Gabor, H and Abraham, S, Effect of dietary menhaden oil on tumor cell loss and the accumulation of mass of a transplantable mammary adenocarcinoma in BALB/c mice, Journal of National Cancer Institute, 76:1223–1231, 1986.

Ganorkar, PM and Jain, RK, Flaxseed—a nutritional punch, International Food Research Journal, 20:519–525, 2013.

Garcia-Perez, FJ, Lario, Y, Fernandez-Lopez, J, Sayas E, Perez-Alvarez, JA and Sendra, E, Effect of orange fiber addition on yoghurt color during fermentation and cold storage, Colour Research Application, 30, 6: 457–463, 2005.

Gebara, C, Chaves, KS, Ribeiro, MCE, Souza, FN, Grosso, CR and Gigante, ML, Viability of *Lactobacillus acidophilus* La5 in pectin-whey protein microparticles during exposure to simulated gastrointestinal conditions, Food Research International, 51, 2: 872–878, 2013.

Ghadge, PN, Prasad, K and Kadam, PS, Effect of fortification on the physico-chemical and sensory properties of buffalo milk yoghurt, Electronic Journal of Environmental Agricultural and Food Chemistry, 7, 5, 2890-2899, 2008.

Gheldof, N and Engeseth, NJ, Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of *in vitro* lipoprotein oxidation in human serum samples, Journal of Agricultural and Food Chemistry, 50, 3050–3055, 2002a.

Gheldof, N, Wang, XH and Engeseth NJ, Identification and quantification of antioxidant components of honeys from various floral sources, Journal of Agricultural and Food Chemistry, 50 5870–5877, 2002b.

Gibson, G and Roberfroid, M (eds) Colonic microbiota, nutrition and health. Dordrecht, The Netherlands: Kluwer Academic, 1999.

Gibson, GR, and Roberfroid, B, Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics, Journal of Nutrition, 125, 6: 1401-12,1995.

Gibson, GR, Beatty, ER, Wang, X and Cumming, JH, Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin, Gastroenterology, 108, 4: 975-982, 1995.

Gmeiner, M, Kneifel, W, Kulbe, KD, Wouters, R, De Boever, P, Nollet, L and Verstrate, W, Influence of a synbiotic mixture consisting of *Lactobacillus acidophilus* 74-2 and a fructooligosaccharides preparation on the microbial ecology sustained in a simulation of the human intestinal microbial ecosystem (SHIME reactor), Applied Microbiology and Biotechnology, 532: 219-23, 2000.

Gonzalez, MJ, Schemmel, RA, Gray, J, Dugan, L, Sheffield, LG and Welsch, CW, Effect of dietary fat on growth of MCF-7 and MDAMB231 human breast carcinomas in athymic nude mice: relationship between carcinoma growth and lipid peroxidation product levels, Carcinogenesis, 12:1231–1235, 1991.

Gopalan, C, Sastri, R and Balasubramanian, SC, Nutritive value of Indian foods, National Institute of Nutrition, ICMR, Hyderabad, 52, 2004.

Gorder, DD, Dolecek, TA, Coleman, GG, Tillotson, JL, Brown, HB, Lenz-Litzow, K, Bartsch, GE, and Grandits, G, Dietary Intake in the Multiple Risk Factor Intervention Trial (MRFIT): Nutrient and food group changes over 6 years, Journal of American Dietetic Association, 86: 744–751, 1986.

Guven, M, Yasar, K, Karaca, OB and Hayaloglu, AA, The effect of inulin as a fat replacer on the quality of set-type low-fat yogurt manufacture, International Journal of Dairy Technology, 58, 180-184, 2005.

Halliwell, B and Gutteridge, JM, Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts, Archives of Biochemistry and Biophysics, 246:501–514, 1986.

Halliwell, B, Gutteridge, JM and Cross, CE, Free radicals, antioxidants, and human disease:where are we now?, Journal of Laboratory and Clinical Medicine, 119, 6:598–620, 1992.

Hanif, MS, Zahoor, T, Iqbal, Z, Ihsan-ul Haq and Arif, AM, Effect of storage on rheological and sensory characteristics of cow and buffalo milk yoghurt, Pakistan Journal of Food Science, 22, 2: 61-70, 2012.

Harper, CR, Edwards, MJ, DeFilipis, AP and Jacobson, TA, Flaxseed oil increases the plasma concentrations of cardioprotective (n-3) fatty acids in humans, Journal of Nutrition, 136:83-7, 2006.

Hashim, IB, Khalil, AH and Afifi, HS, Quality characteristics and consumer acceptance of yogurt fortified with date fibre, Journal of Dairy Science, 92, 11: 5403 – 5407, 2009.

Hassan, A and Amjad, I, Nutritional evaluation of yoghurt prepared by different starter cultures and their physiochemical analysis during storage, African Journal of Biotechnology, 9, 20: 2913-2917, 2010.

Heidebach, T, Först, P and Kulozik, U, Microencapsulation of probiotic cells for food applications, Critical Reviews of Food Science and Nutrition, 52, 4:291–311, 2012.

Hernández-Ledesma, B, Contreras, MM and Recio, I, Antihypertensive peptides: Production, bioavailability and incorporation into foods, Advances in Colloid and Interface Science, 165, 23–35, 2011.

Hijova, E, Chmelarova, A and Bomba, A, Improved efficacy of prebiotic by flaxseed oil and horse chestnut in experimental colon cancer, Bratislava Medical Journal, 112, 161-164, 2011.

Ho, C, Cacace, J and Mazza, G, Extraction of lignans, proteins and carbohydrates fromflaxseed meal withpressurized low polarity water, LWT - Food Science and Technology, 4, 1637-1647, 2007.

Hossain, MN, Fakruddin, Md., and Islam MN, Development of fruit dahi (yoghurt) fortified with strawberry, orange and grapes juice, American Journal of Food Technology, 7, 9: 562-570, 2012.

Hosseinian, FS and Beta, T, Patented techniques for the extraction and isolation of secoisolariciresinol diglucoside from flaxseed, Recent Patents Food Nutrition and Agriculture, 25:25–31, 2009.

Hosseinian, FS, Muir, AD, Westcott, ND and Krol, ES, Antioxidant capacity of flaxseed lignans in two model systems, Journal of the American Oil Chemists' Society, 83: 835-840, 2006.

Hrabova, H and Hylmar, B, Dairy Science Abstracts, 49, 22, 1987.

Hu, C, Yuan, YV and Kitts, DD, Antioxidant activities of the flaxseed lignan secoisolariciresinol diglucoside, its aglycone secoisolariciresinol and the mammalian lignans enterodiol and enterolactone in vitro, Food and Chemical Toxicology, 45: 2219–2227, 2007.

Hussain, S, Anjum, FM, Butt, MS, Khan, MI, and Asghar, A, Physical and sensoric attributes of flaxseed flour supplemented cookies, Turkish Journal of Biology, 30: 87–92, 2006.

Hutchins, AM and Slavin, JL, Effects of flaxseed on sex hormone metabolism, In: Thompson LU, Cunnane SC (eds) Flaxseed in human nutrition, 2nd Ed. AOCS Press, Champaign, 126–149, 2003.

IDF, General Standard of Identity for Fermented Milks, International Standard 163, Brussels: International Dairy Federation, 1992.

Imele, H and Atemnkeng, A, Preliminary study of the utilisation of coconut in yoghurt production, The Journal of Food Technology in Africa, 6, 1: 11-12, 2001.

Institute of Medicine, Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids, Washington, DC: Food and Nutrition Board, 8-97, 2002.

Jenkins, DJ, Kendall, CW, Vidgen, E, Agarwal, S, Rao, AV, Rosenberg, RS, Diamandis, EP, Novokmet, R., Mehling, CC, Perera, T, Griffin, LC and Cunnane, SC, Health aspects of partially defatted flaxseed, including effects on serum lipids, oxidative measures, and ex vivo androgen and progestin activity: a controlled crossover trial, American Journal of Clinical Nutrition, 69, 3, 395-402, 1999.

Jenkins, DJ, Kendall, CW, Vidgen, Mehling, CC, Vuksan, V, Ransom, TPP, Rao, AV, Rosenberg-Zqnd, R, Tariq, N, Corey, P, Jones, PJ, Raeini, M, Story, JA, Furumoto, EJ, Illingworth, DR, Pappu, AS and Connelly, PW, Effect of a diet high in vegetables, fruits and nuts on serum lipids and colonic function, Metabolism Clinical and Experimental, 46:530-537, 1997.

Jin, JS, Kakiuchi, J and Hattori, M, Enantioselective oxidation of enterodiol to enterolactone by human intestinal bacteria, Biological Pharmaceutical Bulletin, 30:2204–2206, 2007.

Johnsson, P, Kamal-Eldin, A, Lundgren, LN and Aaman, P, HPLC method for analysis of secoisolariciresinol diglucoside in flaxseeds, Journal of Agricultural Food Chemistry, 48:5216–5219, 2000.

Kabir, MA, Rashid, MH, Hassan, MN, Afroz, MF and Miraz, FH, Manufacture of dahi from skim milk adding mango juice, Bangladesh Journal of Animal Science, 43, 2: 128-131, 2014.

Kagan, J, Yogurt-a rising star in the dairy industry, Cultured Dairy Products Journal, 20 -24, 1985.

Kailasapathy, K, Polysaccharide ingredients in dairy product applications: Increase in cheese yield Food Australia, 48, 458–461, 1996.

Kailasapathy, K and Chin, JC, Survival and therapeutic potential of probiotics organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp., Immunology and Cell Biology, 78, 80-88, 2000.

Kailasapathy, K and Rybka, S, *Lactobacillus acidophilus* and *Bifidobacterium* spp.: Their therapeutic potential and survival in yogurt, The Australian Journal Dairy Technology, 52, 28–35, 1997.

Kailasapathy, K, Microencapsulation of probiotic bacteria: technology and potential applications, Current Issues in Intestinal Microbiology, 3, 39–48, 2002.

Kajiwara, S, Gandhi, H and Ustunol, Z, Effect of honey on the growth of and acid production by human intestinal *Bifidobacterium spp.*: an *in vitro* comparison with commercial oligosaccharides and inulin, Journal of Food Protection, *65*, 214−218, 2002.

Kang, JX, Fat-1 transgenic mice: a new model for omega-3 research, Prostaglandins Leukotrienes Essential Fatty Acids, 77:263–267, 2007.

Kapoor, S, Sachdeva, R and Kochhar, A, Flaxseed: a potential treatment of lowering blood glucose and lipid profile among diabetic females, Indian Journal Nutrition Dietetics, 48:529–536, 2011.

Kaur, N, Chugh, V and Gupta, AK, Essential fatty acids as functional components of foods—a review, Journal of Food Science Technology, 51, 10:2298-2303, 2012.

Kawase, M, Hashimoto, H, Hosoda, M, Morita, H and Hosono, A, Effect of administration of fermented milk containing whey protein concentrate to rats and healthy men on serum lipids and blood pressure, Journal of Dairy Science, 83:255-263, 2000.

Khalesi, S, Jamaluddin, R and Ismail, A, Effect of Raw and Heated Flaxseed (Linum Usitatissimum L.) on Blood Lipid Profiles in Rats, International Journal of Applied Science and Technology, 1, 4, 2011.

Khuri, KH and Cornell, JA, Response surface designs and analysis, Marcek Decker, New York, 1987.

Kim, Y, Brecht, JK and Talcott, ST, Antioxidant phytochemical and fruit quality changes in (*Mangifera indica L.*) following hot water immersion and controlled atmosphere storage, Food Chemistry, 105:1327–34, 2007.

Krajcova, A, Schulzova, V, Hajslova, J and Bjelkova, M, Lignans in flaxseed, Czech Journal of Food Sciences, 27, 2: S252–S255, 2009.

Krasaekoopt, W, Bhandari, B and Deeth, H, The influence of coating materials on some properties of alginate beads and survivability of microencapsulated probiotic bacteria, International Dairy Journal, 14, 8:737–743, 2004.

Kremer, JM, n-3 fatty acid supplements in rheumatoid arthritis, American Journal of Clinical Nutrition, 71:349–351, 2000.

Kristensen, M, Jensen, MG, Aarestrup, J, Petersen, KEN, Sondergaard, L, Mikkelsen MS and Astrup, A, Flaxseed dietary fibers lower cholesterol and increase fecal fat excretion, but magnitude of effect depends on food type, Nutrition and Metabolism, 9:8, 2012.

Kritchevsky, D, Metabolic effects of dietary fiber, Western Journal of Medicine, 130, 2:123–127, 1979.

Kumar, A, Singh, NK and Sinha, PR, Inhibition of 1,2-dimethylhydrazine induced colon genotoxicity in rats by the administration of probiotic curd, Molecular Biology Reports, 37:1373–1376, 2010.

Kumar, P and Mishra HN, Storage stability of mango soy fortified yoghurt powder in two different packaging materials: HDPP and ALP, Journal of Food Engineering, 65, 569–576, 2003.

Kumar, P and Mishra, HN, Optimization of Mango Soy Fortified Yoghurt Formulation Using Response Surface Methodology, International Journal of Food Properties, 6, 3: 499-517, 2003.

Kumar, P, and Mishra, HN, Mango soy fortified set yoghurt: effect of stabilizer addition on physicochemical, sensory and textural properties, Food Chemistry, 87, 501–507, 2004.

Kumar, S, Bhat, ZF and Kumar, P, Effect of Apple Pulp and Celosia and Argentea on the Quality Characteristics of *Shrikhand*, American Journal of Food Technology, 6, 9: 817-826, 2011.

Kumar, S, Process standardization for Herbal ice cream, Ph.D. Thesis, AHD, IAgSc, Banaras Hindu University, Varanasi, UP, India, 2013.

Lankaputhra, WEV and Shah, NP, Survival of *L. acidophilus* and *Bifidobacterium* spp. in the presence of acid and bile salts, Cultured Dairy Products Journal, 30, 8, 2–7, 1995.

Larisch, BC, Poncelet, D, Champagne, CP and Neufeld, RJ, Microencapsulation of *Lactococcus lactis* subsp. *cremoris*, Journal of Microencapsulation, 11, 2:189–193, 1994.

Law, MR, Wald, NJ, Wu, T, Hackshaw, A and Bailey, A, Systematic underestimation of association between serum cholesterol concentration and ischaemic heart disease in observational studies: data from BUPA study, British Medical Journal, 308, 6925, 363–366, 1994.

Li, D, Sinclair, A, Wilson, A, Nakkote, S, Kelly, F, Abedin, L, Mann, N and Turner, A, Effect of dietary alpha-linolenic acid on thrombotic risk factors in vegetarian men, The American Journal of Clinical Nutrition, 69, 5: 872–882, 1999.

Lisere, AM, Ré, MI, Franco, BDGM, Microencapsulation of *Bifidobacterium animalis* subsp. *lactis* in modified alginate-chitosan beads and evaluation of survival in simulated gastrointestinal conditions, Food Biotechnology, 21, 1: 1-16, 2007.

Liu, S, Buring, JE, Sesso, HD, Rimm, EB, Willett, WC and Manson, JE, A prospective study of dietary fiber intake and risk of cardiovascular disease among women., Journal of the American College of Cardiology, 39, 1:49–56, 2002.

Locke, CA and Stoll, AL, Omega-3 fatty acid in major depression, World Review of Nutrition Diet, 89:173–185, 2001.

Lorri, W and Svanberg, U, Lactic fermented gruels with improved in vitro protein digestibility, Intentional Journal of Food and Nutrition, 44, 29-36, 1993.

Lourens-Hattingh, A, and Viljoen, BC, Review: Yoghurt as probiotic carrier food, International Journal of Dairy*,* 11, 1–17, 2001.

Lucas, EA, Lightfoot, SA and Hammond, LJ, Flaxseed reduces plasma cholesterol and anthosclerotic lesion formation in Soy isoflavones prevent ovariectomized Golden Syrian hamsters, Anthosclerosis, 173:223-229, 2004.

Lucey, AJ, The relationship between rheological parameters and whey separation in milk gel, Food Hydrocolloids*,* 15, 603-608, 2001.

Lucey, JA, Tamehana, M, Singh, H and Munro, PA, Effect of interactions between denatured whey proteins and casein micelles on the formation and rheological

properties of acid skim milk gels, Journal of Dairy Research, 65, 555–567, 1998.

Lunn, J and Theobald, HE, The health effects of dietary unsaturated fatty acids, British Nutrition Foundation, Nutrition Bulletin, 31, 178–224, 2006.

Luz Sanz, M, Polemis , N, Morales , V, Corzo , N, Drakoularakou, A, Gibson , GR and Rastall, RA, In Vitro Investigation into the Potential Prebiotic Activity of Honey Oligosaccharides, Journal of Agricultural Food Chemistry, 53, 8, 2914–2921, 2005.

Lyer, C and Kailasapathy, K, Effect of co-encapsulation of probiotics with prebiotics on increasing the viability of encapsulated bacteria under in vitro acidic and bile salt conditions and in yogurt, Journal of Food Science, 70, 1: 18–23, 2005.

Maassen, CB, Van Holten-Neelen, C, Balk, F, den BakGlashouwer, MJ, Leer, RJ, Laman, JD, Boersma, WJ and Claassen, E, Strain-dependent induction of cytokine profiles in the gut by orally administered Lactobacillus strains, Vaccine 18: 2613-2623, 2000.

Macmohan, B and Godson, C, Lipoxins: endogenous regulators of inflammation, American Journal of Physiology: Renal Physiology, 286, 189–201, 2004.

Madhu, AN, Amrutha, N and Prapulla, SG, Characterization and Antioxidant Property of Probiotic and Synbiotic Yogurts, Probiotics and Antimicrobial Properties, 4, 90–97, 2012.

Madhusudan, KT, Singh, N, Isolation and characterization of major protein fraction (12 S) of flaxseed proteins, Journal of Agricultural Food Chemistry, 33: 673–677, 1985.

Maes, M, Smith, R, Christophe, A, Cosyns, P, Desnydes R and Meltzer, H, Fatty acid composition in major depression: decreased omega 3 fractions in cholesteroyl esters and increased C20:4 omega-6/C20:5 omega-3 ratio in cholesteroyl esters and phospholipids, Journal of Affective Disorders, 38, 35–46, 1996.

Makni, M, Sefi, M, Fetoui, H, Garoui, M, Garouri, NK, Bou-dawara, T and Zeghal, N, Flax and pumpkin seeds mixture ameliorates diabetic nephropathy in rats, Food Chemistry of Toxicology, 48:2407–2412, 2010.

Manson, E, Tosteson, H, Ridker, PM, Satterfield, S, Hebert, P and O'Connor, GT, The primary prevention of myocardial infarction, New England Journal of Medicine, 326, 21, 1406-1416, 1992.

Marinescu, A and Pop, F, Variation in physico-chemical parameters of probiotic yogurt during refrigeration storage, Carpathian Journal of Food Science and Technology, 1, 2:18-26, 2009.

Mason, RL, Gunst, RF and Hess, JL, Statistical Design and Analysis of Experiments, Eighth applications to engineering and science, 2nd ed., Wiley, New York, 2003.

Mataragas, M, Dimitriou, V, Skandamis, PN, Drosinos, EH, Quantifying the spoilage and shelf-life of yoghurt with fruits, Food Microbiology, 28, 611-616, 2011.

Mazur, W, Fotsis, T, Wahala, K, Ojala, S, Salakka, A and Adlercreutz, H, Isotope dilution gas chromatographic-mass spectrometric method for the determination of isoflavonoids, coumestrol, and lignans in food samples, Analytical Biochemistry, 233: 169–180, 1996.

Mazza, G and Biliaderis, CG, Functional properties of flaxseed mucilage, Journal of Food Science, 54:1302–1307, 1989.

Mazza, G, Production, Processing and Uses of Canadian Flax, First CGNA International Workshop, Temuco, Chile, August 3–6, 2008.

Mbaeyi, IE and Anyanwu, LN, Production and evaluation of yogurt flavored with solar-dried bush mango (irvingia gabonensis) pulp, Journal Tropical Agriculture, Food, Environment and Extension, 9, 2: 137-146, 2010.

McKinley, MC, The nutrition and health benefits of yoghurt – review, Society of Dairy Technology, 58, 1– 12, 2005.

Mishra, PS, and Mishra, HN, Optimization of the prebiotic & probiotic concentration and incubation temperature for the preparation of synbiotic soy yoghurt using response surface methodology, LWT - Food Science and Technology, xxx 1-10, 2014.

Mohania, D, Kansal, VK, Shah, D, Nagpal, R, Kumar, M, Gautam, SK, Singh B, and Behare, PV, Therapeutic effect of probiotic dahi on plasma, aortic, and hepatic lipid profile of hypercholesterolemic rats, Journal of Cardiovascular Pharmacology and Therapeutics, 18, 5:490-497, 2013.

Mokarram, RR, Mortazavi, SA, Habibi, NMB and Shahidi, F, The influence of multi stage alginate coating on survivability of potential probiotic bacteria in simulated gastric and intestinal juice, Food Research International, 42, 8:1040–1045, 2009.

Molan, PC, Cooper, RA and Harding, KG, Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds, Journal of the Royal Society of Medicine , 92, 283−285, 1999.

Molan, PC, The antibacterial activity of honey: 1. The nature of antibacterial activity, Bee World, 73, 5−28, 1992.

Montgomery, DC, Design and Analysis of Experiments, 4th edition, John Wiley & Sons, New York, 1997.

Moreno Rojas, R, Sanchez-Segarra, PJ, Martinez, MG, Otero, MJG and Lopez, MAA, Mineral composition of skimmed milk fruit added yoghurts—nutritional assessment, Milchwissenschaft, 55,9, 510–512, 2000.

Morris, DH, Flax—a health and nutrition primer, (4th edn.) Available from: www.flaxcouncil.ca, 2007.

Morris, MC, Evans, DA and Tangney, CC, Relation of the tocopherol forms to incident alzheimer disease and to cognitive change, American Journal of Clinical Nutrition, 81:508–514, 2005.

Mortazavian, AM, Azizi, A, Ehsani, MR, Razavi, SH, Mousavi, SM, Sohrabvandi, S, and Reinheimer, JA, Survival of encapsulated probiotic bacteria in Iranian yogurt drink (doogh) after the product exposure to simulated gastrointestinal conditions, Milchwissenschaft, 63, 4:427–429, 2008.

Mortazavian, AM, Razavi, SH, Ehsani, MR and Sohrabvandi, S, Principles and methods of microencapsulation of probiotic microorganisms, Iranian Journal Biotechnology, 5,1: 1–17, 2007.

Mosilhey, SH, Influence of different capsule materials on the physiological properties of microencapsulated Lactobacillus acidophilus, Thesis PhD-Ingeneur, Institute of Food Technology, Faculty of Agriculture, University of Bonn, Germany, 2003.

Mueller, K, Eisner, P, Yoshie-Stark, Y, Nakada, R and Kirchoff, E, Functional properties and chemical composition of fractionated brown and yellow linseed meal (Linum usitatissimum L.), Journal of Food Engineering, 98:453–460, 2010.

Murata, Y, Toniwa S, Miyamoto, E, Kawashima, S, Preparation of alginate gel beads containing chitosan salt and their function, International Journal of Pharmacology, 176, 2:265–268, 1999.

Murphy, PA and Hendrich, S, Phytoestrogens in foods, Advances in Food and Nutrition Research, 44:195–246, 2002.

Muruganandan, S, Gupta. S, Kataria M, Lal. J and Gupta PK, Mangiferin protects the streptozotocin-induced oxidative damage to cardiac and renal tissues in rats, Toxicology and Pharmacology, 176:165–73, 2002.

Nazzaro, F, Fratianni, F, Coppola, R, Sada, A and Orlando, P, Fermentative ability of alginate-prebiotic encapsulated *Lactobacillus acidophilus* and survival under simulated gastrointestinal conditions, Journal of Functional Foods, 1, 319–323, 2009.

Nezhad, MH, Duc, C, Han, N and Hosseinian, F, Flaxseed soluble dietary fibre enhances lactic acid bacterial survival and growth in kefir and possesses high antioxidant capacity, Journal of Food Research, 2, 5: 0887-0895, 2013.

Nigam, N, Singh, R and Upadhayay, PK, Incorporation of chakka by papaya pulp in the manufacture of *shrikhand*, Journal of Dairying, Foods and Home Sciences, 28, 2, 115-118, 2009.

Ningegowada, AM and Prapulla, SG, In vitro fermentation of prebiotics by *Lactobacillus plantarum* CFR 2194: selectivity, viability and effect of metabolites on β-glucuronidase activity, World Journal of Microbiology and Biotechnology, 28, 901–908, 2012.

Nishino, T, Shibahara-Sone, H, Kikuchi-Hayakawa, H, Lshikawa, F, Transit of radical scavenging activity of milk products prepared by Millard reaction and *Lactobacillus caseii* strain shirota fermentation through the hamster intestine, Journal of Dairy Science, 83, 915-922, 2000.

O'Rell, KR, and Chandan, RC, Yogurt: Fruit preparations and flavoring materials. In Manufacturing Yogurt and Fermented Milks, Chandan, RC (Ed.) Blackwell Publishing: Iowa, U. S. A. 151-166, 2006.

Oakenfull, D, Physicochemical properties of dietary fiber: Overview. In Handbook of Dietary Fiber (Cho, SS and Dreher, ML, eds.), Marcel Dekker Inc, New York, NY, 95–206, 2001.

Oliveira, SPR, Florence, RCA, Perego, P, Oliveira, NM and Converti, A, Use of lactulose as prebiotic and its influence on the growth, acidification profile and viable counts of different probiotics in fermented skim milk, International Journal of Food Microbiology, 145, 1: 22-27, 2011.

Ooi, LG, Bhat, R., Rosma, A., Yuen, KH and Liong MT, A synbiotic containing *Lactobacillus acidophilus* CHO-220 and inulin improves irregularity of red blood cells, Journal of Dairy Science, 93, 10: 4535–4544, 2010.

Oomah, BD and Mazza, G, Compositional changes during commercial processing of flaxseed, Industrial Crops and Products, 9:29–37, 1998.

Oomah, BD, Flaxseed as a functional food source, Journal of the Science of Food and Agriculture, 81: 889–894, 2001.

Oomah, BD, Kenaschuk, EO and Mazza, G, Phytic acid content of flaxseed as influenced by cultivar, growing season and location, Journal of Agricultural Food Chemistry, 44: 2663–2666, 1996a.

Oomah, BD, Mazza, G and Kenaschuk EO, Cyanogenic compounds in flaxseed, Journal of Agricultural Food Chemistry, 40:346–348, 1992.

Oomah, BD, Mazza, G and Kenaschuk, EO, Dehulling characteristics of flaxseed, Lebensmittel- Wissenschaft and Technologie, 29:245–250, 1996b.

Ozer, BH and Kirmaci, HA, Functional milks and dairy beverages, International Journal of Dairy Technology, 63, 1: 1-15, 2010.

Pan, A, Sun, J, Chen, Y, Ye, X, Li, H, Yu, Z, Wang, Y, Gu, W, Zhang, X, Chen, X, Demark-Wahnefried, W, Liu, Y and Lin, X, Effects of a flaxseed-derived lignan supplement in type 2 diabetic patients: a randomized, double-blind, cross-over trial, PLoS One, 2:e1148, 2007.

Pan, A, Yu, D, Demark-Wahnefried, W, Franco, OH and Lin, X, Meta-analysis of the effects of flaxseed interventions on blood lipids, American Journal of Clinical Nutrition, 90, 2:288–297, 2009.

Payne, TJ, Promoting better health with flaxseed in bread, Cereal Foods World, 45, 3:102–104, 2000.

Pella, D, Dubnov, G, Singh, RB, Sharma, R and Berry, EM, Effects of an Indo-Mediterranean diet on the omega-6/ omega-3 ratio in patients at high risk of coronary artery disease, The Indian Paradox, 92, 74-80, 2003.

Peng, Y, Serra, M, Horne, DS and Lucey, JA, Effect of fortification with various types of milk protein on the rheological properties and permeability of non-fat set yogurt, Journal of Food Science, 74, 9: 666-673, 2009.

Pereira, MA, O'Reilly, E, Augustsson, K, Fraser, GE, Goldbourt, U, Heitmann, BL, Hallmans, G, Knekt, P, Liu S, Pietinen, P, Spiegelman, D, Stevens, J, Virtamo, J, Willett, WC and Ascherio, A. Dietary fiber and risk of coronary heart disease: a pooled analysis of cohort studies, Archives of Internal Medicine, 164, 4:370–376, 2004.

Perez, E, Rodriguez-Malaver, A and Vir, P, Antioxidant capacity of the Venezuelan pure honey, Free Radical Biology Medicine, *35*, 120, 2003.

Pérez-Jiménez, J, Neveu, V, Vos, F and Scalbert, A, Identification of the 100 richest dietary sources of polyphenols: an application of the phenol-explorer database, European Journal of Clinical Nutrition, 64: 112-120, 2010.

Prakash, S, Tomaro-Duchesneau, C, Saha, S and Cantor, A, The gut microbiota and human health an emphasis on the use of microencapsulated bacterial cells, Journal of Biomedical Biotechnology, 2–12, 2011.

Prasad, K, Hypocholesterolemic and antiatherosclerotic effect of flax lignan complex isolated from flaxseed, Atherosclerosis, 179, 2: 269-275, 2005.

Prasad, K, Antihypertensive activity of Secoisolariciresinol diglucoside (SDG) isolated from flaxseed: role of guanylatecyclase, International Journal of Angiology, 13:7–14, 2004.

Prasad, K, Antioxidant activity of secoisolariciresinol diglucosidederived metabolites, secoisolariciresinol, enterodiol, and enterolactone, International Journal of Angiology, 9:220–225, 2000.

Prasad, K, Antioxidant activity of secoisolariciresinol diglucosidederived metabolites, secoisolariciresinol, enterodiol, and enterolactone, International Journal of Angiology, 9: 220–225, 2000b.

Prasad, K, Flaxseed: a source of hypocholesterolemic and antiatherogenic agents, Drug News Perspective, 13, 2: 99-104, 2000.

Prasad, K, Hydroxyl radical-scavenging property of secoisolariciresinol diglucoside (SDG) isolated from flax-seed, Molecular Cell Biochemistry, 168:117–123, 1997.

Prasad, K, Mantha, SV, Muir, AD, and Westcott, ND, Reduction of hypercholesterolemic atherosclerosis by CDC-flaxseed with very low alpha-linolenic acid, Atherosclerosis, 136: 367–375, 1998.

Prasad, K, Regression of hypercholesterolemic atherosclerosis in rabbits by secoisolariciresinol diglucoside isolated from flaxseed, Atherosclerosis, 197, 1:34–42, 2008.

Priya, AJ, Vijayalakshmi, SP and Raichur, AM, Enhanced survival of probiotic *Lactobacillus acidophilus* by encapsulation with nanostructured polyelectrolyte layers through layer-by-layer approach, Journal of Agricultural Food Chemistry 59, 21:11838–45, 2011.

Prodaniuc, N, Study regarding some physical chemical characteristics of the yoghurt with red beetroot juice, Carpathian Journal of Food Science and Technology, I, 2: 44-49, 2009.

Pushkala, R and Srividya, N, Influence of Aloe Gel Enrichment on the Physicochemical, Textural and Sensory Characteristics of Dahi, Journal of Food Science and Engineering 1, 141-153, 2011.

Rabetafika, HN, Remoortel, VV, Danthine, S, Paquot, M and Blecker, C, Flaxseed proteins: food uses and health benefits, International Journal of Food Science and Technology, 46:221–228, 2011.

Rajesha, J, Murthy, CKN, Kumar, MK, Madhusudhan, B and Ravishankar, G. Antioxidant potentials of flaxseed by in vivo model, Journal of Agricultural and Food Chemistry, 54: 3794-3799, 2006.

Raju, PN and Pal, D, Effect of dietary fibers on physico-chemical, sensory and textural properties of Misti Dahi, Journal of Food Science and Technology, 51, 11: 3124–3133, 2014.

Ramanathan, A and Sivakumar K, Probiotic foods evaluation of fibre enriched and vitamin - c fortified sweetened probiotic dahi, Probiotics in Sustainable Food Production: Current Status and Future Prospects, 72-76, 2013.

Ranganna, S, Handbook of analysis and quality control for fruit and vegetable products, 2nd Ed. Tata-McGraw-Hill Education Pvt. Ltd, New Delhi, 2001.

Rasdhari, M, Parekh, T, Dave, N, Patel, V and Subhash, R, Evaluation of various physic-chemical properties of *Hibiscus sabdariffa* and *L casei* incorporated probiotic yoghurt, Pakistan Journal of Biological Sciences, 11(7):2101-2108, 2008.

Rasic, J and Kurmann, A, Probiotic dairy products and Dairy Products in Human Nutrition, 95-97, 1978.

Ratnayake, W, Behrens, W, Fischer, P, L'Abbé, M, Mongeau, R and Beare-Rogers J, Chemical and nutritional studies of flaxseed (variety Linott) in rats, The Journal of Nutritional Biochemistry, 3,5: 232-240, 1992.

Ratnayake, WMN, Behrens, WA, Fischer, PWF, L'Abbe, MR, Mongeau, R, Beare-Rogers, JL, Flaxseed: chemical stability and nutritional properties, Proceedings of Flax Institute, 54:37, 1992.

Rebole, A, Rodriguez, ML, Ortiz, LT, Alzueta, C, Centeno, C, Trevino, J, Mucilage in linseed: effects on the intestinal viscosity and nutrient digestion in broiler chicks, Journal Science of Food Agriculture, 82:1171–1176, 2002.

Renaud, SC and Lanzmann-Petithory, D, Alpha-linolenic acid in the prevention of cardiovascular diseases, World Reviews of Nutrition Diet, 88:79–85, 2001.

Riaz, QUA and Masud, T, Recent trends and applications of encapsulating materials for probiotic stability, Critical Reviews of Food Science and Nutrition, 3:231–44, 2013.

Ridges, L, Sunderland, R, Moerman, K, Meyer, B, Astheimer, L and Howe, P, Cholesterol lowering benefits of soy and linseed enriched foods. Asia Pacific Journal of Clinical Nutrition, 10:204–211, 2001.

Riediger, ND, Othman, R, Fitz, E, Pierce, GN, Suh, M and Moghadasian, MH, Low n6:n3 fatty acid ratio, with fish or flaxseed oil, in highfat diet improves plasma lipids and beneficially alters tissue fattyacid composition in mice, European Journal of Nutrition, 47:153–160, 2009.

Roberfroid, MB and Delzenne, NM, Dietary fructans, Annual Reviews of Nutrition, 18: 117–143, 1998.

Rolandelli, R, Koruda, M, Settle, R, Leskiw, M, Stein, T and Rombeau, J, The effect of pectin on hepatic lipogenesis in the enterally-fed rat, Journal of Nutrition, 119: 89-93, 1989.

Rotenberg, S and Jakobsen, P, The effect of dietary pectin on lipid composition of blood, skeletal muscle and internal organs of rats, Journal Nutrition, 108: 1384-1392, 1978 .

Rouillard, LF, Thiais, AJ and Robin, JR, Cosmetic or pharmaceutical composition containing, as active ingredient, mangiferin or its derivatives, in pure or in plant extracts, U.S. Patent, 5, 824:320, 1998.

Roumyan, N, Zapryanov, P and Kondareva, S, On some aspects of a new fermented milk product medina, Biotechnology and Biotechnological Equipment, 10 86–89, 1996.

Rubilar, M, Gutiérrez, C, Verdugo, M, Shene, C and Sineiro, J, Flaxseed as a source of functional ingredients, Journal of Soil Science and Plant Nutrition, 10: 373–377, 2010.

Rybaka, S, and Kailasapthy, K, The survival of culture bacteria in fresh and freeze dried AB yoghurt, Australian Journal of Dairy Technology, 50, 51-57, 1995.

Rybka, S, The enumeration of Lactobacillus, Streptococcus and Bifidobacterium species in yoghurt, B.Sc. Dissertation, University of New South Wales, Sydney. 1994.

Saini, A, Harjai, K, Mohan, H, Punia, RPS and Chhibber, S, Long-term flaxseed oil supplementation diet protects BALB/c mice against *Streptococcus* pneumonia infection, Medical Microbiology Immunology, 199:27–34, 2010.

Saint-Eve, A, Levy, C, Le Moigne, M, Ducruet, V and Souchon, I, Quality changes in yogurt during storage in different packaging materials, Food Chemistry, 110, 285–293, 2008.

Saint-Eve, A, Levy, C, Le Moigne, M, Ducruet, V and Souchon, I, Quality changes in yogurt during storage in different packaging materials, Food Chemistry, 110, 285–293, 2008.

Sakai, K, Mishima, C, Tachiki, T, Kumagi, H and Tochikura, T, Mortality of bifidobacteria in boiled yogurt. Journal of Fermentation Technology, 65, 2: 215–220, 1997.

Saleh, NAM and El-Ansari, MAI, Polyphenolics of twenty local verities of *Mangifera indica*, Planta Medica, 28:124–30, 1975.

Salvador, A and Fiszman, SM, Textural and sensory characteristics of whole and skimmed flavored set-type yogurt during long storage, Journal of Dairy Science, 87, 4033-4041, 2004.

Sanchez, GM, Re, L, Giuliani, A, Nunez-Selles, AJ, Daviso, GP and Leon-Fernandez, O, Protective effects of *Mangifera indica L.* extract, mangiferin and selected

antioxidants against TPA-induced biomolecules oxidation and peritoneal macrophage activation in mice, Pharmacological Research, 42:565–573,2000.

Sanchez-Segarra, PJ, Martinez, MG, Otero, MJG, Valverda, AD and Lopez, MA, Influence of the addition of fruit on the mineral content of yoghurts: nutritional assessment, Food Chemistry, 20, 85–89, 2000.

Santos-Buelga, C and Scalbert, A, Proanthocyanidins and tannin-like compounds: nature, occurrence, dietary intake and effects on nutrition and health, Journal of Science of Food and Agriculture, 80:1094 –1117, 2000.

Sanz, ML, Gonzalez, M, de Lorenzo, C, Sanz, J and Martínez-Castro, I, Carbohydrate composition and physicochemical parameter values of artisanal honeys from Madrid (Spain), Journal of Science of Food Agriculture, 84, 1577−1584, 2004.

Sanz, ML, Sanz, J and Martínez-Castro, I, Gas chromatographic−mass spectrometric method for the qualitative and quantitative determination of disaccharides and trisaccharides in honey, Journal of Chromatography, A, 1059, 143−148, 2004.

Sanz, T, Salvador, A, Jimenez, A and Fiszman, S, Yogurt enhancement with functional asparagus fibre, effect of fibre extraction method on rheological properties, colour and sensory acceptance, European Food Research Technology, 227, 1515 – 1521, 2008.

Sartor, RB, Probiotic therapy of intestinal inflammation and infections, Current Opinion of Gastroenterology, 21, 44-50, 2005.

Scalbert, A and Williamson, G, Dietary intake and bioavailability of polyphenols, Journal of Nutrition, 130: 2073–2085, 2000.

Schaafsma, G, Meuling, WJA, Van, Dokkum, W and Bouley, C, Effects of a milk product, fermented by Lactobacillus acidophilus and with fructo-oligosaccharides added, on blood lipids in male volunteers, European Journal of Clinical Nutrition, 52, 6: 436-440, 1998.

Schieber, A, Ullrich, W and Carle, R, Characterization of polyphenols in mango puree concentrate by HPLC with diode array and mass spectrometric detection, Innovative Food Science and Emerging Technology, 1:161–166, 2000.

Schramm, DD, Karim, M., Schrader, HR, Holt, RR, Cardetti, M and Keen, CL, Honey with high levels of antioxidants can provide protection to healthy human subjects, Journal of Agricultural Food Chemistry, 51, 1732−1735, 2003.

Seckin, AK and Baladura, Emrah, Effect of using some dietary fibers on colour, texture and sensory properties of strained yogurt, GIDA, 37, 2: 63-69, 2012.

Sendra, E, Fayos, P, Lario, Y, Fernandez-Lopez, J, Sayas-Barbera, E, and Perez-Alvarez, JA, Incorporation of citrus fibers in fermented milk containing probiotic bacteria, Food Microbiology, 25, 13–21, 2008.

Shah, NP, Probiotic bacteria: selective enumeration and survival in dairy foods, Journal of Dairy Science, 83, 4: 894–907, 2000.

Shahidi, F, Janitha, PK and Wanasundara, PD, Phenolic antioxidants, Critical Reviews of Food Science and Nutrition, 32: 67–103, 1992.

Shakir, KAF and Madhusudan, B, Hypocholesterolemic and hepatoprotective effects of flaxseed chutney: evidence from animal studies, International Journal of Clinical Biochemistry, 22: 117–121, 2007.

Shamala, TR, Shri Jyothi, Y, Saibaba, P, Stimulatory effect of honey on multiplication of lactic acid bacteria under *in vitro* and *in vivo* conditions, Letters of Applied Microbiology, 2000, *30*, 453−455.

Sheu, TY and Marshall, RT, Micro-encapsulation tobacilli in calcium alginate gels, Journal of Food Science, 54, 3, 557–561, 1993.

Shinnick, FL, Ink, SL and Marie, JA, Dose response to a dietary oat bran fraction in cholesterol-fed rats, Journal of Nutrition, 120: 561-568, 1990.

Simopoulos, AP, Essential fatty acids in health and chronic diseases, American Journal of Clinical Nutrition, 70: 560–569, 1999.

Simopoulos, AP, Omega-6/omega-3 essential fatty acid ratio and chronic diseases, Food Reviews International, 20:77–90, 2011.

Singh, KK, Jhamb, SA and Kumar, R, Effect of pre-treatments on performance of screw pressing for flaxseed, Journal of Food Process Engineering, 35, 4: 543-556, 2011a.

Singh, KK, Mridula, D, Rehal, J and Barnwal, P, Flaxseed: a potential source of food, feed and fiber, Critical Reviews of Food Science and Nutrition, 51, 210–222, 2011b.

Sodini, S and Martin, JH, Effect of pH on survival of Bifidobacterium bifidum and Lactobacillus acidophilus in frozen fermented dairy desserts, Cultured Dairy Products Journal, 2, 13–21, 2004.

Sok, D, Cui, HS and Kim, MR, Isolation and bioactivities of furfuran type lignan compounds from edible plants, Recent Patents Food Nutrition and Agriculture, 1:87–95, 2009.

SooMro, HA and Masud, T, Probiotic Characteristics of Lactobacillus spp. Isolated from Fermented Milk Product Dahi, Journal of Food Science and Technology Research, 18, 1, 91 – 98, 2012.

Soukoulis, C, Panagiotidis, P, Koureli, R and Tzia, C, Industrial yogurt manufacture: monitoring of fermentation process and improvement of final product quality, Journal of Dairy Science, 90, 2641–2654, 2007.

Spence, JD, Thornton, T, Muir, AD, and Westcott, ND, The effect of flax seed cultivars with differing content of alpha-linolenic acid and lignans on responses to mental stress, Journal of American College of Nutrition, 22: 494–501, 2003.

Spiller, RC, Pharmacology of dietary fiber, Pharmacology and Therapeutics, 62:407–427, 1994.

Staffolo, MD, Bertola, N, Martino, M, Bevilacqua, YA, Influence of dietary fibre addition on sensory and rheological properties of yogurt, International Dairy Journal, 14, 3: 263 – 268, 2004.

Strange, ED, Benedict, RC, Smith, JL, Swift, CE, Evaluation of rapid tests for monitoring alternations in meat during storage, Journal of Food Protection, 10, 843-847, 1977.

Struijs, K, Vincken, JP and Gruppen, H, Bacterial conversion of secoisolariciresinol and anhydrosecoisolariciresinol, Journal of Applied Microbiology, 107:308–317, 2009.

Sturgeon, SR, Heersinka, JL, Volpeb SL, Bertone-Johnsona, ER, Puleoa, E, Stanczykc, FZ, Sabelawskid, S, Wahalae, K, Kurzerf ,MS and Bigelowa, C, Effect of dietary flaxseed on serum levels of estrogens and androgens in postmenopausal women, Nutrition and Cancer, 60:612–618, 2008

Sultana, K, Godward, G, Reynolds, N, Arumugaswamy, R, Peiris, P and Kailasapathy, K, Encapsulation of probiotic bacteria with alginate–starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt, International Journal of Food Microbiology, 62, 47–55, 2000.

Sun, W and Griffths, MW, Survival of bifidobacteria in yogurt and simulated gastric juice following immobilization in gellan-xanthan beads, International Journal of Food Microbiology, 61, 17-25, 2000.

Susheelamma, NS, Isolation and properties of linseed mucilage, Journal of Food Science and Technology, 24:103–106, 1987.

Sykora, J, Valeckova, K, Amlerova, J, Siala, K, Dedek, P, Watkins, S, Varvarovska, J, Stozicky, F, Pazdiora, P and Schwarz, J, Effects of a specially designed fermented milk product containing probiotic *Lactobacillus casei* DN-114001 and the eradication of *H. pylori* in children: a prospective randomized double-blind study, Journal of Clinical Gastroenterology, 39, 692-698, 2005.

Talwalkar, A, and Kailasapathy, K, Comparative studies of selective and differential media for the accurate enumeration of strains of *Lactobacillus acidophilus,*

Bifidobacterium spp. and *L. casei* complex from commercial yoghurts, International Dairy Journal, 14(2), 143–149, 2004.

Tamime, AY and Deeth, HC, Yoghurt: technology and biochemistry, Journal of Food Properties, 151-166, 1980.

Tamime, AY and Marshall, VME, Physiology and Biochemistry of fermented milks, In: Low B.A. (ed.): Microbiology and Biochemistry of Cheese and Fermented Milk. Elsevier Applied Science Publishers, London and New York, 153-192, 1997.

Tamime, AY and Robinson, RK, Yoghurt science and technology Boca Raton, FL: CRC Press, 326–333, 1999.

Tamime, AY, Fermented milks, Oxford: Blackwell, 2006.

Tamime, Y, Probiotic dairy products (Vol. 1), Oxford: Blackwell, 2005.

Tarpila, A, Wennberg, T and Tarpila, S, Flaxseed as a functional food, Current Topics of Nutraceutical Research, 3:167–188, 2005.

Thakur, G, Mitra, A, Pal, K and Rousseau, D, Effect of flaxseed gum onreduction of blood glucose & cholesterol in type 2 diabetic patients, International Journal of Food Science and Technology, 60:126–136, 2009.

Thompson, LU, Seidl, MM, Rickard, SE, Orcheson, LJ, and Fong, HH, Antitumorigenic effect of a mammalian lignan precursor from Flaxseed, Nutrition and Cancer, 26:159–165, 1996.

Tolkachev, ON and Zhuchenko, AA, Biologically active substances of flax: medicinal and nutritional properties, Pharmaceutical Chemistry Journal, 34:360–367, 2000.

Toure, A and Xueming, X, Flaxseed lignans: source, biosynthesis, metabolism, antioxidant activity, bio-active components and health benefits, Comprehensive Reviews of Food Science Food Safety, 9:261–269, 2010.

Varghese, KS, and Mishra, HN, Modelling of acidification kinetics and textural properties in dahi (Indian yogurt) made from buffalo milk using response surface methodology, International Journal of Dairy Technology, 61, 3: 284–289, 2008.

Vedamuthu, ER, The yogurt story-past, present and future, Dairy Food Environment and Sanitation, 7, 371-374, 1991.

Velez-Ruiz, JF, Rheological properties of yogurt, In Progress in Food Engineering Research and Development (Carrod, JM, ed.), Nova Science Publishers, Inc., New York, NY, 223–242, 2008.

Voss, A, Reinhart, M, Sankarappa, S and Sprecher, H, The metabolism of 7,10,13,16, 19-docosapentaenoic acid to 4, 7,10,13,16,19-docosahexaenoic acid in rat liver is independent of a 4-desaturase, Journal of Biological Chemistry, 266, 19995-20000, 1991.

Walstra, P and Jenness, R, Dairy Chemistry and Physics, A Wiley-Interscience Publication, NewYork, Chichester, Brisbane, Toronto, Singapore, 162-185, 1984.

Wanasundara, JP and Shahidi, F, Functional properties and amino acidcomposition of solvent extracted flaxseeds meals, Food Chemistry, 49:45–51, 1994.

Wanasundara, JP and Shahidi, F, Removal of flaxseed mucilage by chemical and enzymatic treatments, Food Chemistry, 59:47–55, 1997.

Wang C, Makela T, Hase T, Adlercreutz H, Kurzer MS (1994) Lignans and flavonoids inhibit aromatase enzyme in human preadipocytes, Journal of Steroid Biochemisrty and Molecular Biology, 50:205–212

Wang, LQ, Meselhy, MR, Li, Y, Qin, GW and Hattori, M, Human intestinal bacteria capable of transforming secoisolariciresinol diglucoside to mammalian lignans, enterodiol and enterolactone, Chemical and Pharmaceutical Bulletin, 48:1606–161, 2000.

Warakaulle, STSK, Weerathilake, WADV and Abeynayake, NR, Production and Evaluation of Set type Yogurt Incorporated with Water melon (Citrallus lanatus), International Journal of Scientific and Research Publications, 4,10: 1-4, 2014.

Westcott, ND and Muir, AD, Chemical studies on the constituents of Linum spp. In: Muir AD, Westcott ND (eds) Flax: the genus Linum, Taylor & Francis, London, pp 55–73, 2003.

White, JW, Detection of honey adulteration by carbohydrate analysis, Journal – Association of Official Analytical Chemists, 6311–18, 1980.

WHO, Diet, nutrition and the prevention of chronic diseases. WHO Technical Report, Series, 916, 2003.

Xu, Y, Hall, C and Wolf-Hall, C, Antifungal activity stability of flaxseed protein extracts using response surface methodology, Journal of Food Science, 73:M9–M14, 2008.

Yaakob, H, Rashidah, AN, Daud, KS, Malek, AR and Rahman, AR, Optimization of ingredient and processing levels for the production of coconut yoghurt using response surface methodology, Food Science and Biotechnology, 21, 4: 933-940, 2012.

Yadav, H, Jain, S and Sinha, PR, Evaluation of changes during storage of probiotic dahi at 7°C, International Journal of Dairy Technology, 60, 205-210, 2007.

Yadav, H, Jain, S, and Sinha PR, Antidiabetic effect of probiotic dahi containing *Lactobacillus acidophilus* and *Lactobacillus casei* in high fructose fed rats, Nutrition, 23,1: 62-8, 2007.

Yadav, H, Jain, S, and Sinha PR, Effect of skim milk and dahi (yogurt) on blood glucose, insulin, and lipid profile in rats fed with high fructose diet, Journal of Medicinal Food, 9, 3: 328-35, 2006.

Yaghoobi, N, Al-Waili, N and Ghayour-Mobarhan, M, Natural honey and cardiovascular risk factors; effects on blood glucose, cholesterol, triacylglycerole, CRP, and body weight compared with sucrose, The Scientific World Journal, 20: 463–469, 2008.

Yamashita, K, Ikeda, S and Obayashi, M. Comparative effects of flaxseed and sesame seed on vitamin E and cholesterol levels in rats, Lipids, 38, 12:1249–1255, 2003.

Yamashita, T, Sano, T, Hashimoto, T and Kanazawa, K, Development of a method to remove cyanogen glycosides from flaxseed meal, International Journal of Food Science and Technology, 42:70–75, 2007.

Yaniv, Z and Rudich, M, Medicinal herbs as a potential source of high quality honeys, In *Bee Products*, Mizrahi, A and Lensky, Y, (eds.) Plenum Press: New York, 77−81, 1996.

Zanwar, AA, Hegde, MV and Bodhankar, SL, In vitro antioxidant activity of ethanolic extract of *Linum usitatissimum*, Pharmacologyonline, 1: 683-696, 2010.

Zhang, W, Wang, X, Liu, Y, Tian, H, Flickinger, B and Empie, M, Dietary flaxseed lignan extract lowers plasma cholesterol and glucose concentrations in hypercholesterolaemic subjects, British Journal of Nutrition, 99, 6:1301-1309, 2007.

Zheng, MS and Lu, ZY, Antiviral effect of mangiferin and isomangiferin on herpes simplex virus, Chinese Medical Journal, 103:160–165, 1990.

Zibadi, S and Watson, RR, Passion fruit (*Passiflora edulis*): composition, efficacy and safety, Evidence Based Integrative Medicine, 1:183–187, 2004.

APPENDICES

7.1 Mineral Mixture

Nutritional value (mg/kg of diet)	
CaHPO4	17.200
KCl,	4000
NaCl,	4000
MgO,	420
MgSO4,	2000
Fe2O3,	120
FeSO4·7H2O,	200
trace elements,	400
MnSO4·H2O,	98
CuSO4·5H2O,	20
ZnSO4·7H2O,	80
CoSO4·7H2O,	0.16
Kl,	0.32
Sufficient starch to bring to 40 g (per kg of diet).	

7.2 Vitamin mixture

Nutritional value (mg/kg of diet)	
Retinol	12
Cholecalciferol	0.125
Thiamine	40
Riboflavin	30
Pantothenic acid	140
Pyridoxine	20
Inositol	300
Cyanocobalamin	0.1
Menadione	80
Nicotinic acid	200
Choline	2720
Folic acid	10
P-aminobenzoic acid	100
Biotin	0.6
Sufficient starch to bring to 20 g (per kg of diet)	

7.3 Sensory Evaluation Card for Judging of Flaxseed Fortified Synbiotic Flavoured *Dahi*

Name of the student	:	ID No.	:
Batch No.	:	Date preparation	:
Name of Judge	:	Date of Judging	:

Please rate the sample for the quality attributes according to 9-POINT HEDONIC SCALE given below as guide points on back.

Grade	Score	Sample No.				
		1	2	3	4	5
Liked extremely	9					
Liked very much	8					
Liked moderately	7					
Liked slightly	6					
Neither like nor disliked	5					
Disliked slightly	4					
Disliked moderately	3					
Disliked very much	2					
Disliked extremely	1					

Score Card

Sensory profile in terms of characteristics	Sample No.				
	1	2	3	4	5
Body & texture					
Colour & Appearance					
Sweetness					
Flavour					
Mouthfeel					
Overall Acceptability					

Remarks, if any (please point out the principal defects).

Sample No.

Signature of Judge **Name of Judge** **Date**