DEVELOPMENT OF MILK-FLAXSEED-BASED PROBIOTIC BEVERAGE

THESIS SUBMITTED TO THE

ICAR-NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL

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IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

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IN

DAIRY TECHNOLOGY

BY

RAKESH YADAV

B.Tech (DAIRY AND FOOD TECHNOLOGY)

DAIRY TECHNOLOGY DIVISION

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Approved by:

EXTERNAL EXAMINER

(Dr. Sangita Ganguly)
MAJOR ADVISOR &CHAIRMAN

Members of Advisory Committee

1. **Dr. A. K. Singh**
   Principal Scientist, Head of DT Division
2. **Dr. Latha Sabikhi**
   Principal Scientist, DT Division
3. **Dr. Neelam Upadhyay**
   Scientist (SS), DT Division
4. **Dr. Pradip Behare**
   Scientist (SS), DM Division
   (Joint Director Nominee)

NATIONAL DAIRY RESEARCH INSTITUTE
(Deemed University)
(Indian Council for Agricultural Research)
KARNAL, (Haryana) – 1320

Dr. Sangita Ganguly
Scientist
ICAR,NationalDairyResearch Institute
Karnal, Haryana

CERTIFICATE

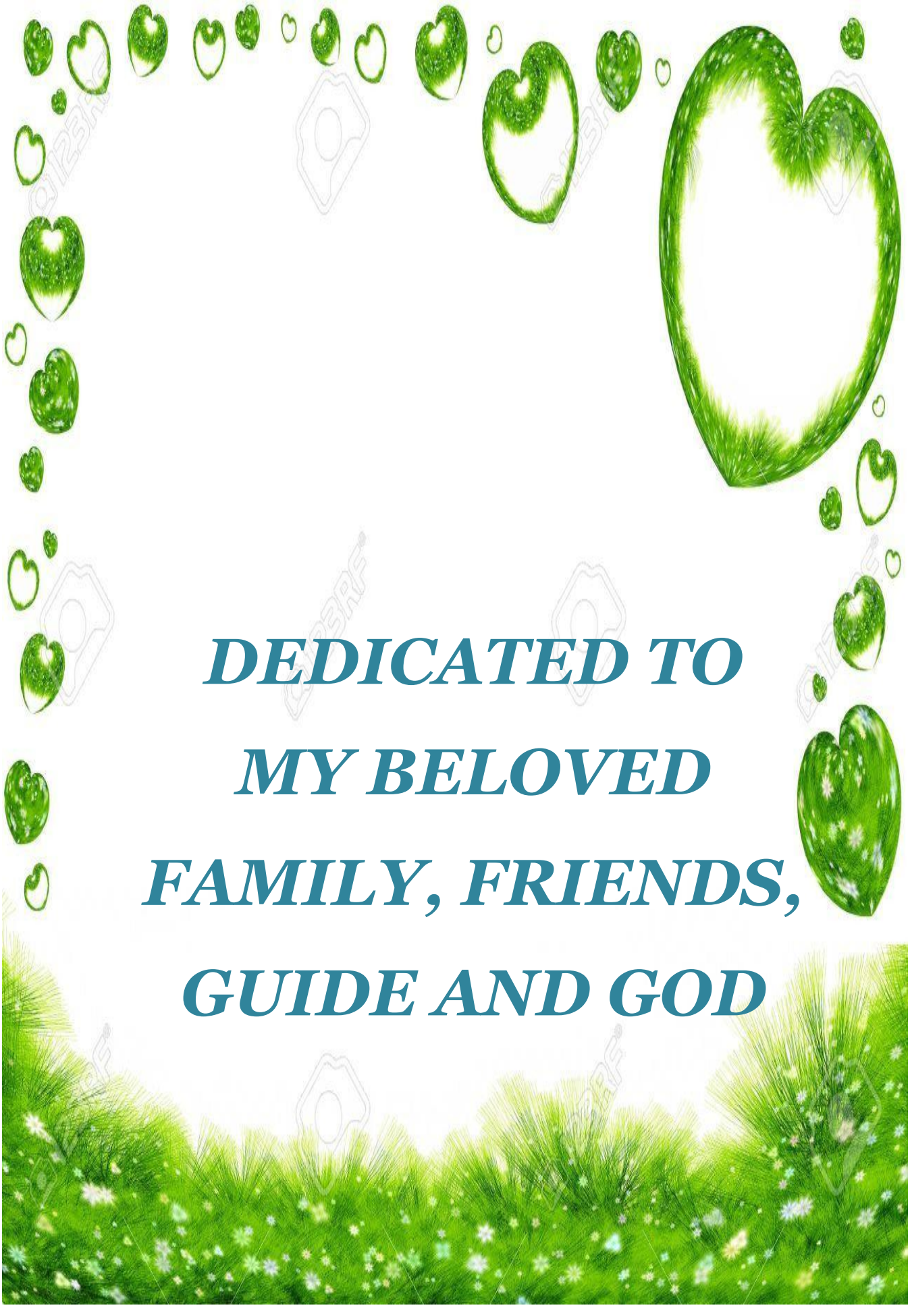
This is to certify that the thesis entitled "***Development of milk-flaxseed- based probiotic beverage***" submitted by **Mr. Rakesh Yadav** towards the partial fulfillment of the requirements for the award of the degree of MASTER OF TECHNOLOGY in DAIRY TECHNOLOGY of the ICAR-NATIONAL DAIRY RESEARCH INSTITUTE(DEEMED UNIVERSITY), Karnal (Haryana), India, is a bonafide research work carried out by him under my supervision and guidance and no part of the thesis has been submitted for any other degree or 'diploma.

Dated: 14.09.2021

S Ganguly

Dr. Sangita Ganguly
(Major Advisor *and* Chairman)

Phone No: +91-184-2259265(work); +91-9050145009 (cell); Fax No: +91-184-2250042
E-mail: sangitandri@gmail.com; sangita.ganguly@icar.gov.in


DEDICATED TO
MY BELOVED
FAMILY, FRIENDS,
GUIDE AND GOD

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Rakesh Yadav

Development of milk-flaxseed-based probiotic beverage

ABSTRACT

The Latin name of flaxseed is *Linum usitatissimum*, which means "very useful". A part of the Linaceae family, and a blue flowering crop. Flaxseed has biologically active components like α-linolenic acid (ALA), lignans, and fiber. Flax lignans are biologically active agents which may protect against estrogen-related disorders in females. Most probiotic foods in the markets worldwide are milk-based and limited work has been carried out regarding the incorporation of flaxseed in dairy products. Hence, the current project was being proposed to development of milk flaxseed-based probiotic beverage. Probiotic organism *Lactobacillus rhamnosus* (RSI 3)was selected based on maximum specific growth rate (k) and minimum generation time (t_g) in the milk medium. The fermentation time was restricted to 10 h by increasing the inoculums level to 4%in order to achieve quick fermentation. The optimized product was having 12% of sugar, 0.2% of stabilizer, and 3.12% of roasted flaxseed flour. Roasting was found to enhance the nutritional attributes of flaxseed by reducing anti-nutritional factors significantly and improve the aroma of the flaxseed flour. The phytic acid content of raw flaxseed was 1.24±0.06 mg/100g which decreased to 1.06±0.07 mg/100g in roasted one. The hydrocyanic acid content in raw flaxseed was 632±5.50 mg/kg which decreased to 588±4.58mg/kg in roasted flaxseed. Whereas oxalate content in raw flaxseed was reduced by 23.65% due to roasting operation. The optimized product had an overall acceptability score of 8.21±0.5. The nutritional composition of the beverage was 2.59% of fat, 4.465% of protein, 0.71% of ash content, and had a probiotic count of 8.18 Log CFU/ml of beverage. The beverage was having a shear thinning behavior. The L*, a*, and b* values of the beverage were 69.87±0.30, 2.06±0.67, and 10.83±0.21, respectively. The anti-nutritional components present in the beverage were Oxalic acid, Phytic acid, and Hydrocyanic acid and their respective amount in 100ml of beverage were 13µg, 32.86mg, and 6.3mg. The anti-nutritional components were below RDA (Recommended Daily Allowance) values in the beverage. Yeast and mold count was zero and the coliform count was not detected in first dilution of the final product.

दूधअलसी आधारित प्रोबायोटिक पेय का विकास-

सारांश

अलसी का लैटिन नाम लिनुमसिटाटिसिमम है, जिसका अर्थ है" बहुत उपयोगी"। लिनासी परिवार का एक हिस्सा, और एक नीले फूल की फसल। अलसीनेα-लिनोलेनिकएसिड) एएलए ,लिग्नान और फाइबर जैसे सक्रिय घटकों सक्रिय किया है। सनलिग्नान जैविक रूप से सक्रिय एजेंट हैं जो महिला में एस्ट्रोजन से संबंधित विकार से रक्षा कर सकते हैं ।दुनिया भर के बाजारों में अधिकांश प्रोबायोटिक खाद्य पदार्थ दूध आधारित हैं और डेयरी उत्पादों में अलसी के समावेश के संबंध में सीमित कार्य किए गए हैं। इसलिए, वर्तमान परियोजना को मिल्क अलसी आधारित प्रोबायोटिक पेय के विकास के लिए प्रस्तावित किया जा रहा था। प्रोबायोटिक ऑर्गेनोजलेक्टोबेसरनस आरएसआई)3) को दुग्ध माध्यम में अधिकतम विशिष्ट विकास दर (के) के आधार पर (टीजी) और न्यूनतम उत्पादन समय चुना गया था।त्वरित किण्वन प्राप्त करने के लिए इनोकुलम स्तर को 4% तक बढ़ाकर किण्वन समय 10 घंटे तक सीमित था। अनुकूलित उत्पाद में 12% चीनी, स्टेबलाइजर का 0.2% और भुना हुआ अलसी आटा का 3.12% था। रोस्टिंग को एंटीपोषणीय कारकों को काफी कम करके अलसी के पोषण गुणों को बढ़ाने और अलसी के आटे की सुगंध में सुधार करने के लिए पाया गया था।कच्चे अलसी की फाइटिक एसिड सामग्री 1.24±0.06 मिलीग्राम/100 ग्राम थी जो भुना हुआ एक में 1.06±0.07mg/100g तक कम हो गई। रॉ अलसी में हाइड्रोसायनिक एसिड की मात्रा 632±5.50 मिलीग्रामकिलोग्राम/ थी जो भुना हुआ अलसी में घटकर 588±4.58 मिलीग्राम प्रति किलोग्राम हो गई। जबकि, रोस्टिंग ऑपरेशन के कारण कच्चे अलसी में ऑक्सलेट सामग्री में 23.65% की कमी आई थी।अनुकूलित उत्पाद में 8.21±0.5 का समग्र स्वीकार्यता स्कोर था। पेय पदार्थ की पोषण संरचना 2.59% वसा, 4.465% प्रोटीन, राख की मात्रा का 0.71% थी और इसमें 8.18 लॉग एफबीयूएमएल पेय की प्रोबायोटिक गिनती थी। पेय / * पदार्थ एक कतरनी पतला व्यवहार कर रहा था । पेय पदार्थों का एल, ए मूल्य क्रमशः * और बी * 69.87±0.30, 2.06±0.67 और 10.83±0.21 था।पेय में मौजूद पोषण विरोधी घटक ऑक्सालिक एसिड, फाइटिक एसिड और हाइड्रोसायनिक एसिड थे और 100 मिलीलीटर पेय में उनकी संबंधित राशि 13µg, 32.86 मिलीग्राम और 6.3 मिलीग्राम थी। पेय पदार्थों में पोषण विरोधी घटक आरडीए (अनुशंसित दैनिक भत्ता) मूल्यों से नीचे थे। खमीर और मोल्ड गिनती शून्य थी और अंतिम उत्पाद में पहले कमजोर पड़ने वाले शून्य में कॉलीफॉर्म काउंट नहीं मिला था।

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percent	KDa	KiloDalton
>	Morethan	KCl	Potassiumchloride
<	Less than	Kg	Kilogram
AAD	Antibiotic associated diarrhoea	LAB	Lactic acid bacteria
a*	Redness	M	Molar
ALA	α-linolenic acid	min	Minimum
ACE	Angio tensin converting enzyme	mL	Millilitre
ANOVA	Analysis of variance	mM	Millimolar
AOAC	Association of Official Analytical Chemists	m2	Squaremeter
AR	Analytical grade	mg	Milligram
ATCC	American type culture collection	PHE	Plate heat exchanger
aw	Water activity	mU	Milliunit
b*	Yellowness	EPA	Eicosapentaenoic acid
PUFA	polyunsaturated fatty acids	N	Normality
CAGR	Compound Annual Growth Rate	NaCl	Sodium chloride
CFU/g	Colony forming units per gram	NCDC	National Collection of Dairy Cultures
˚C	Degree Celsius	NDRI	National Dairy Research Institute
COP	Coefficient of performance	DHA	Docosahexaenoic acid
NCI	National Cancer Institute	SDG	Secoisolariciresinol Diglycoside
DBT	Department of Bio-Technology	SECO	Secoisolariciresinol Diglycoside
K	Specific Growth Rate	t_g	Generation Time
Vf	Rate of Acidification	HCN	Hydrogen Cyanide
TS	Total solid	L*	Perceptual Lightness
MRS	De Man Rogosa and Sharpe agar	ED	Entreo Diol
EL	Entero Lactone	UTI	Urinary Tract infections
w	Weight	VF	Rate of acidification
DNA	Deoxy ribonucleicacid	PPS	Points per second
etal	Andco-workers	Rs	Rupees
FAO	Food and Agricultural Organization	S	Second

FDA	Food and Drug Administration	SMP	Skimmed milk powder
FSSAI	Food Safety and StandardsAuthorityof India	TEAC	Total equivalent antioxidant capacity
GABA	Gamma Amino butyric Acid	TFC	Total fixed cost
G’	Storage modulus	TS	Totalsolids
G”	Lossmo dulus	TVC	Total variable cost
g	Gram	UV	Ultraviolet
GIT	Gastro-intestinal tract	w/v	Weight by volume
ICMR	Indian council of medical research	WHO	World Health Organization
h	Hour	µg	Microgram
H2	Hydrogen	µL	Microliter
IBD	Inflammatory bowel diseases	µM	micromolar
IBS	Irritable Bowel Syndrome		

CHAPTER –1

Introduction

1.1. General Introduction

"Wherever flaxseed becomes a regular food item among the people, there will be better health."

-Mahatma Gandhi

Nowadays, consumers are more concerned about their food; desire to preference testy food has now changed to food that has therapeutic and functional properties. Most of the studies in clinical nutrition have proved that there is a strong interrelation between the type of food intake and human health.

Flaxseed or lens (*Linum usitassimum*), widely known as Alsi, Jawas, Aksebija, and a member of the Lineaceae family, is a blue blooming annual plant that yields tiny flat grains from bright yellow to reddish-brown. The flaxseed has a nutty and crunchy texture (Rubilar *et al.*, 2010). Flaxseed is essential as a functional food in the world food chain. India, China, United States, and Ethiopia are the major flaxseed-producing countries (Oomah and Mazza 1993; Singh *et al.*, 2011). India is first among the world's primary flaxseed producers, with 23.8 percent of global output and third in production, accounting for 10.2 percent of world production (Singh *et al.*, 2011). Flaxseed in India is predominantly grown in Madhya Pradesh, Maharashtra, Chhattisgarh, and Bihar. Flax output was 3.06 million tons per year, and Canada accounts for almost 38 percent of worldwide production (Ganorkae and Jain, 2013). Flaxseed is the greatest vegetarian source of α-linolenic acid and soluble mucilage in the current age. The protein of flaxseeds is high in arginine, aspartic acid, glutamic acid, and lysine limits (Singh *et al.*, 2011 and Chung *et al.*, 2005). High cysteine and methionine levels enhance antioxidant levels such that the risk of cancer is reduced (Oomah, 2001).

Functional food is supposed to provide nutrients and health benefits to the consumers. Available food may contain natural ingredients or components, and they should be part of thedaily diet. Their consumption may reduce the risk of diseases like cancer, diabetes, heart disease, heart-related disease, etc. Probiotic food products are the leading components in the "functional food" sector. The worldwide functional food market has developed from $33 billion in 2000 to $176.7 billion in 2013, and it is poised to

grow $276.68 billion during 2020-2024. It has been predicted that probiotic food comprises about 60-70% of the total functional food market. There had been significant success in producing dairy products containing probiotic bacteria in the past few decapods, such as flavored liquid milk, fermented milk, baby food, milk powder, ice cream, cheese, buttermilk, etc. whey-based beverages, sour and regular cream, because of lactose intolerance in recent years (Rajagopal *et al.*, 2001).

Probiotic food products are a leading component of the functional food market. Probiotics are a live microbial feed supplement that beneficially affects the host animal by improving intestinal microbial balance. Probiotic organisms have been used to required health benefits (Ganguly *et al.*, 2019). Probiotics can be bacteria, molds, or yeasts. However, the most common probiotic organisms are bacteria, particularly lactic acid *bacteria. L. acidophilus, L. casei, L. helveticus, L. Plantarum, L. bulgaricus, L. rhamnosus, L. reuteri, L. fermentum, Bifidobacterium bifidum, B. breve, B. longum,* and the yeast *Saccharomyces bourladi,* etc. (Alard *et al.*, 2018). Indian probiotic market was valued at $2 million as per 2010 estimates. The market value is supposed to increase up to US $522.8 million by 2018.milk and fermented products have 62% of the total market share in probiotic products (Indian consumer survey, 2010).A strategy to increase the nutritional value of food products is by combining milk components with plant components. The combination of cereals/ legume/ oilseed and milk will provide enhanced nutrition and ultimately lead to a value-added functional product. Milk though a good source of nutrients lacks fiber, whereas cereal, legumes, and oilseeds are rich sources of fibre that can provide several health-beneficial effects. The scope for developing processing technologies for the manufacture of novel foods with a combination of milk and plant sources can enhance the nutritional and therapeutic profile of the developed product. It can serve as 'product diversification' the buzzword for sustainability. Exploring the possibilities of combining cereals / legumes / oilseeds with the dairy component may balance nutrition. The primary task that lies ahead would be to design these essential ingredients into a low-cost, tasteful, nutritious that may help in the management of various health disorders.

Although flaxseed is known for multiple health-beneficial effects due to various bioactive components (lignan, omega-3 fatty acids, etc.), limited work has been carried out regarding incorporating flaxseed in dairy products. Studies have not been conducted regarding probiotic fermented products containing milk and flaxseed. Currently, no

similar probiotic milk flaxseed-based product is availableon the market. Estimation of phytoestrogen content in flaxseed incorporated dairy products had not been done before. Milk-flaxseed-based Fermented food products containing probiotic organisms are a desirable option for the overall health management of the female population. Milk can be a source of calcium, whereas flaxseed can provide phytoestrogen, and probiotics can be useful in digestion, nutrient absorption, and overall health management for females. Considering all the aspects, a research is being proposed to ***Development of milk-flaxseed-based probiotic beverage*** with following objectives:

Objective 1: To optimize a milk flaxseed-based probiotic beverage

Objective 2: To study sensory, physico-chemical, rheological, microbiological, and nutritional attributes of the developed product

CHAPTER –2

Review of Literature

2.1 Flaxseed

Flaxseed (*Linum usitassimmum*), popularly known as Alas in Nepali languages, is obtained from the flax plant, a part of the Linaceae family, and a blue flowering crop (Ganorkar *and* Jain, 2013). The flaxseed plant has tiny, delicate leaves that are not as much as an inch long. Stems are divided close to the plant's stand, and the plant's altitude varies from 76.20 to 152.40 centimeters.

Flaxseed is the most basic crop that has been worn mutually for food, and linen fiber is derived from the stem of fiber variety and lubricates from the seed of linseed varieties. The flax is characterized as a flat and oval shape with a pointed tip and varies in color from dark brown to yellow. Flaxseed is primarily grown in America, Argentina, Canada, China, and India (Zhang *et al.*, 2008). Brown, yellow and yellow are the most frequent types of flaxseed variety (Vadukapuram 2009). Today, flaxseed is produced mainly for its oil used in paints, coats, and linoleums (Coşkuner *and* Karababa, 2007). Flaxseed is also utilized as a component in various food preparations like Chutney, ladoo,etc

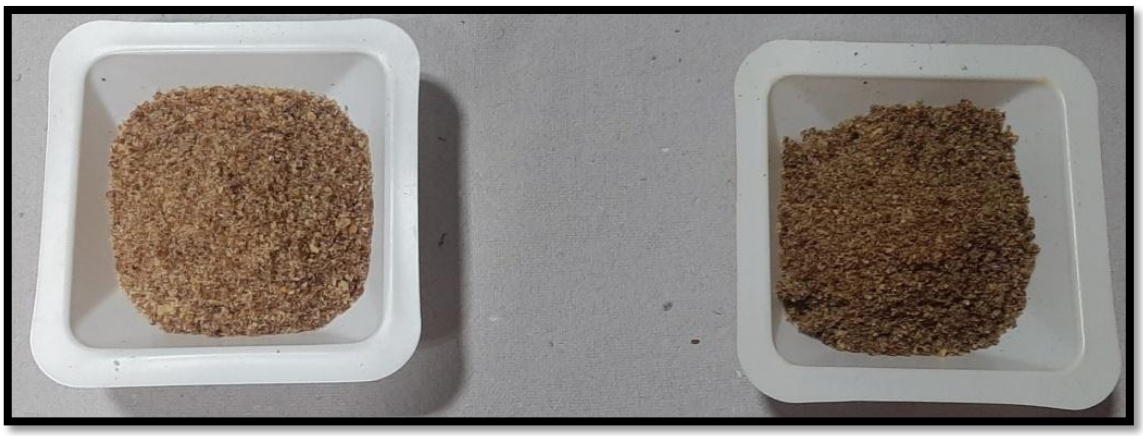

Figure 2.1: Raw and Roasted Flaxseed Flour

Flaxseeds are existing in two fundamental varieties:

a) Brown

b) Yellow or golden.

They mutually have analogous nutritional characteristics and equivalent numbers of ω-3 fatty acids. Brown flax is superior to an ingredient in paints, varnish, fiber, and

cattle feed (Faintuch *et al.*, 2011).

2.2 Classification and nomenclature of flaxseed

The scientific name of flaxseed is *Linum usitassimum.* Flaxseed is a member of the flax family, and its taxonomic hierarchy is:

Table 2.1: Classification of Flaxseed

Falx Family	Taxonomic Hierarchy
Kingdom	Plantae
Subkingdom	Tracheobionta
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Superorder	Rosidae
Order	Linales
Family	Linaceae
Genus	Linum L.
Species	L. usitatissimum

(USDA 2014)

2.3 Characteristics of flaxseed

The study of the physical characteristics of flaxseed is essential for the equipment design to grip, process, and storage the studies of functional properties are necessary where available properties have a role in food formulation. Bulk density is one of the essential functional properties where higher bulk density is desirable as this property reduces the paste thickness. This is a critical factor for infant formulas where the thickness of solution is an important aspect (Yatnatti *et al.*, 2014).

Flaxseeds are egg-shaped and brown with a glossy appearance. Thousand seeds weight ranged from 5.01 to 6.05 g, and seed length ranged between 4.50 to 5.45 mm per seed in three varieties. The sources were flat and pointed, and the width ranged between 0.90 to 1.45 mm (Arora *and* Rajni, 2006).Coskuner *and* Karababa (2007) analyzed some physical properties of flaxseed (*Linum usitassimum L.*), in which the value of bulk

density was found 0.66 g/cm^3. In the same study, it was reported that the bulk density values of 726.6–555.6 kg/m^{3}for the moisture range of 6.09–16.81% (dry basis), whereas in another study, it was observed that the bulk density of 690.5–545.0 kg/m^3 for the moisture range of 6.09–16.81% (dry basis) for the commercial variety of linseed.

The reduction in bulk density of flaxseed is possibly due to the rise in seed volume with a moisture content, which leads to a decrease in the number of seeds occupying the equal bulk volume (Khan *et al.,* 2016). There was no significant difference observed between full fat roasted and full-fat unroasted flaxseed as bulk density was 0.83 and 0.78 g/m^{3}l, water absorption capacity was 1.83 and 1.48 g/g, and fat absorption capacity of 1.31 and1.20 g/g, respectively. This study showed the higher fat absorption in flax flour of full-fat roasted seed (Hussain *et al.,* 2011).

The coloring magnitude in the flaxseed coat influences the color of the seeds (Coşkuner and Karababa 2007). Flaxseed exists as a whole seed in one form or other of their synthetic forms: powdered flaxseed, flaxseed oil, deforested flaxseed fibre, or lignane extract. Flaxseedcontains about 40-45% oil, including 4% ashes, 23-34% protein, lignan (90-300 mg per 10 g defatted meal), and 5% viscous fibre.

Flaxseed has been measured as oilseed since around 40% oil, over 50 percent of which is α- linolenic acid (Choo *et al.,*2007). The essential oil resource in flaxseed is located in flaxseed cotyledon (75%), the remaining oil in the seed cap (22%), and the embryo/germ (3 percent). Triacylglycerol is the most frequent kind of flaxseed lipid. The four significant triacylglycerols in flaxseed oil are tri linolenoyl glycerol (LnLn) with 30%, 19%, and 70%, dilinolenoyllinoleoylglycerol (LnLnL) with 19%. Fatty acids in flaxseed are mainly composed of unsaturated fatty acids: oleic, linolenic, and linoleic acids. These three unsaturated fatty acids account for almost 70% of the total fatty acid supply (Schorno 2006). The bulk of fatty acids in flaxseed are α-linolenic acid (ALA), which usually account formore than 50% of the total fatty acids (Choo *et al.,* 2007).

2.4 History of consumption of flaxseed

As the sources of fibre (linen) flax were grown as no less than 5000 BC, it is farmed now mainly for oil use (oomah, 2001). For thousands of years, people have been taking flax. Flaxseed nutritional study exceeds its potential as a novel component for bread, buns, muffins,and other bakery items. Ayurveda and the traditional therapeutic framework in China have a long tradition and many recognizable techniques (Patwardhan *et al.,* 2005).

Creative Ayurvedic literature explains more than 200 plants, and skincare lipids, minerals. Flaxseed oil is supposed to transmit intellectual and physical patience by fighting tiredness and controlling the technique of aging. Flaxseed can be helpful in skin problems as it has characteristics similar to Madhura (skin maintenance pH), Balya regarding Ayurveda (enhance tensile strength or elasticity of the skin), Picchaila (TvagdoshahritLubric), Grahi (improves skin moisture-retaining), Vanahrit (wound healing).

2.5 Nutritional composition of flaxseed

Among functional foods, lignans, a high-quality protein, soluble fibre, and phenolic compounds are available food and a good source of alpha-linolenic acid (Oomah, 2001). The flaxseed composition is shown in Table 2.2 below.

Table 2.2: Nutritional Composition of Flaxseed

Nutrients	**Amount Per 100 g of Edibleflaxseed**
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
Potassium	750
Calcium (mg)	170
Iron (mg)	2.7
Vitamin A (µg)	30
Thiamine (B1) (mg)	0.23
Riboflavin (B2) (mg)	0.07
Niacin (mg)	1
Folic acid (µg)	112
Pyridoxine (mg)	0.61

(Morris, 2007; Gopalan *et al.*, 2004; Payne, 2000)

The content of flax changes according to the analysis method, and environmental factors (Daun *et al.*, 2003). Oil, lignin-rich viscous fibres (mucilage), proteins, and minerals are flaxseed's most essential food components. A 100g portion of linseed delivers energy to 534 Kcal and includes around 10% protein, 7% carbohydrates, 53% total fat, and 21% dietary fat. Flaxseed is abundant in most B vitamins, Mg, and Mn, and is low in saturated fatty acids, such as cholesterol. Almost 73% of fatty acids in flaxseed are polyunsaturated fatty acids (PUFA) (Madhusudhan, 2009). Flaxseed is a source of excellent protein, and globulins arethe proteins for storing flaxseed with the highest proportion of globulins (58-66% of the whole protein seed) (Chung *et al.,* 2005). Flaxseed is a multicomponent system that contains bio-active plant components such as oil, protein and dietary fibre, soluble polysaccharides, lignans; vitamins (A, C, and E); and minerals (P, Mg, K, Na, Fe, Cu, Mn, and Zn) (Goyal *et al.,* 2014). 100 g of flaxseed will give around 27.3 g of RDA dietary fibre and 18.3 g protein approximately 10 percent RDA, 30 mg of sodium about 11 percent RDA, 255 mg of calcium approximately 5 percent RDA, 5.73 mg of iron, roughly 16 percent RDA and 813 mg of potassium approximately 89 percent RDA (USDA, National Nutrient Data Base, 2012). The most substantial rise in iron, zinc, and manganese was found in germinated seeds. The germination process decreased cyanogenic glycosides and phytic acid substantially

2.6 Flaxseed as a Source of Omega-3 Fatty Acid

There are two types of omega fats:

a. omega-3 fatty acid

b. omega-6 fatty acids

Linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are three types of omega-3 fatty acids and are nutritionally important. These fatty acids have beenmade known to decrease the threat of cardiovascular illness (Hurteau, 2004).

Flax contains a combination of fatty acids. It is well-off in polyunsaturated fatty acids, predominantly ALA, the crucial omega-3 fatty acid, and linoleic acid (LA), the essential omega-6 fatty acid. These two polyunsaturated fatty acids are essential for humans – that is, the body needs them. Supercritical CO2 extraction gave a higher average ALA content (60.5%) compared to the soxhlet extraction method (56.7%)). ALA and Linoleic acid constitute 57% and 16.0 % of total fatty acids respectively in flax making them the richest source of ALA.

Alpha-linolenic acid from flaxseed gives a helpful cause on blood lipids. It was found to beas effective as oleic acid (18:2η-6) and linoleic acid (18:2η-6) in the drop of, low-density lipoprotein cholesterol, plasma whole cholesterol, and very-low-density lipoprotein (VLLP) cholesterol in 20-34 years old healthy men (Chen *et al.,* 2006).

Three times in a day 12 gram of ALA was given to a group of healthy young women in the flaxseed oil capsules and compared with the group is given in flaxseed flour supplemented products. A notable reduction in blood lipids was found in both cases (Cunnane *et al.,* 1993). Nettleton (2003) summarize the recommendations of leading health organizations about the appropriate ratio of n-6 to n-3 fatty acid intake. Mostly organizations agree that a 5:1 to 10:1 n-6 to n-3 fatty acid ratio is chosen (Institute of Medicine, 2002; WHO/FAO, 2003). But a typical diet has an n-6 to n-3 fatty acid ratio well beyond 10:1; hence flaxseed can be a important lipid source to improve the n-6 to n-3 fatty acid ratio due to the high n-3 content of flaxseed oil. ALA, being the essential fatty acid, the requirement canbe fulfilled by intake of flaxseed products (Morris, 2004).

2.7 Flaxseed as a Source of Protein

Flaxseed is claimed to have 10.5-31 percent of protein (Oomah and Mazza, 1993). Khategaon cultivars cultivated in India offer 21.9 percent protein (Madhusudhan and Singh, 1983). Protein differences can be qualified for genetics as well as for the environment. The hull fraction includes lower protein concentrations, and the flaxseed protein level increases from 19.2% to 21.8%. (Oomah and Mazza, 1997). The main proteins of flaxseed are albumin and globulin proteins. Globulin makes up 73.4%, while albumin makes up roughly 26.6% of total protein (Marcone *et al.,* 1998).

The flaxseed proteins are very high in arginine, aspartic acid, and glutamic acid, but amino acidsthat are limited in flaxseed are lysine, methionine, and cystine. After eight days of germination, the total amino acid content of the flaxseed increased about 15 times with the highest increase (i.e., 200 times) of leucine and glutamine as compared to natural seed (Wanasundara *et al.,* 1999). Oomah and Mazza (1995) evaluated the nutritional value of flaxseed meals to soymeal. They reported that flaxseed meal's net protein utilization and protein efficiency ratio was a bit lower than soy meal. The biological value (BV) of the flaxseed protein was equivalent to the BV of the protein of soya (Frank, 1987). The biological valueof Sakha-1 and Belinka proteins was determined to be 66.43 and 67.70. (El- Kady 2000).

2.8 Dietary Fiber of Flaxseed

Flax is the world's oldest crop of fiber flaxseedand the fibre yield of flax straw is around 20– 25 percent. The primary fibre found in flax is linen. It is employed in northern Europe in - production of fabric. Flax fiber has high mechanical characteristics and low density and is a natural and biodegradable composite. Its mechanical properties are2GPa and 675MPa, respectively, for Young's modulus and failure stress (Charl *et al.,* 2006). Flax fibres aremeltable, flexible and bright; it is more robust than cotton, but less elastic and fuses well withwool, silk, cotton and other materials.

Flaxseeds provide both insoluble and soluble dietary fibre insignificant amounts. The seed coat seems to be mucilage, much of the soluble fibre in flaxseed. It is about 7–10% of the weight of the seed (Mazza *and* Biliaderis, 1989). Flaxseed is unusual among olive sources due to mucilage in the seed external layers (Singh *et al.*, 2011). Flaxseed mucilage has become more popular because of its excellent health advantages and possible functional features (Mazza *and*Biliaderis, 1989; Susheelamma, 1987). It includes 35 – 45% fiber, and 2/3 is insoluble, and 1/3 is soluble. Cellulose, hemicellulose, and lignin are insoluble fibres (Morris, 2007; Oomah, 1993). Flaxseed Mucilageis composed of acidic and neutral polysaccharides. The acidic fraction including L-fucose, L-rhamnose, D-galacturon, and L-galactose,The neutral fraction consists of L-arabinose, D-xylose, and D-galacturonic, and arabinoxylans. These polysaccharides functionally have comparable characteristics to guar gum (Wanasundara Shahidi, 1997). The mucilage is easily removed by water and has high moisturizing qualities (Susheelamma, 1987). Flaxseed mucilage has a water-binding potential of around 1600 to 3000 g water/100 g solids. The great capacity of the water-binding of the flaxseed is related to the presence of polysaccharides in the seed coat.

2.8.1 Metabolism of flax dietary fibre

Dietary fibre from flaxseed reaches the large intestine. It is fermented with the formation of short-chain (SCFA) fatty acids, carbon dioxide, methanol, and biomass by colonic bacteria and shows its laxative properties (Kritchevsky, 1979). Both insoluble, soluble fibres in the large intestine result in an increment of the dry and moist weight of the feces. Soluble fibre improves water binding by raising the weight of the microbial cells by the binding strength of its macromolecules. Compared to insoluble fibre, the role of soluble fibre in fecal bulk was oflittle importance.

2.9 Phytochemicals of flaxseed

Phytochemicals are secondary plant metabolites that are generated to aid protection against infection of bacteria, fungus, and plant viruses and the ingestion by insects and other animals. Phytochemicals are bioactive plant nutrients that may reduce the risks of major chronic illnesses and provide health advantages beyond essential nutrition. Plant Phenols are often classified into several kinds based on their fundamental structure: simple phenols, coumarins, phenolic acids, and isocoumarins, xanthous, naphthoquinones, stillness, flavonoids, anthraquinones, tannins, and lignans. Flavonoids and phenolic acids are more common amongst these (Dykes *and* Rooney, 2007). When combined, phenolics are responsible for the flavor, color, and organoleptic characteristics of foods of vegetable origin (Yanez *et al.,* 2004).

2.9.1 Lignan

Flaxseed is the richest source of lignans, and flax contains about 800 times more lignans than other plant foods, which are diphenolic compounds formed by combining two cinnamic acid residues. Flaxseed lignan is majorly composed of secoisolariciresinol diglucoside(SDG). The amount of SDG varies in the range of 11.7 to 24.1 mg/g for defatted flaxseed flour and 6.1 to 13.3 mg/g for whole flaxseed (Johnsson *et al.*, 2000).

Lignans are considered phytoestrogens. The structural similarity of phytoestrogens to estrogen allow simparting (anti) estrogenic activity by binding to estrogen receptors(ER), Which is the main mechanism by which they exert health benefits. Flaxseed is the richest source of lignans. Epidemiological facts suggest that phytoestrogens defend against hormone-dependent tumors, e.g., breast and prostate cancer. Estrogenic and anti-estrogenic (Welshons 1987), anti-aromatase (Adlercreutz, 1996)(Adlercreutz 1993),and anti-oxidative (Wijewickreme 1999) properties may be accountable for these effects secoisolarisiresinol diglucoside (SDG) (Fig. 1A) as the effectivelignan form. Flaxseed also contains slight amounts of the aglycone form, secoisolariciresinol (SECO) (Fig. 1B) and has around (370 mg/100 g), and other lignans such as metaresinol (1 mg/100 g) and larisiresinol (Metzler 2002). SDG is hydrolyzed to its aglycone, SECO, in the mammalian gastrointestinal tract, possibly through β-glucosidase and β-glucuronidase activity (Thompson 1999). SECO is again changed to the mammalian lignans, enterodiol (ED) (Fig. 1C), and enterolactone (EL) (Figure 2.2) in the presence of colonic gut microflora. (Adlercreutz 2007).

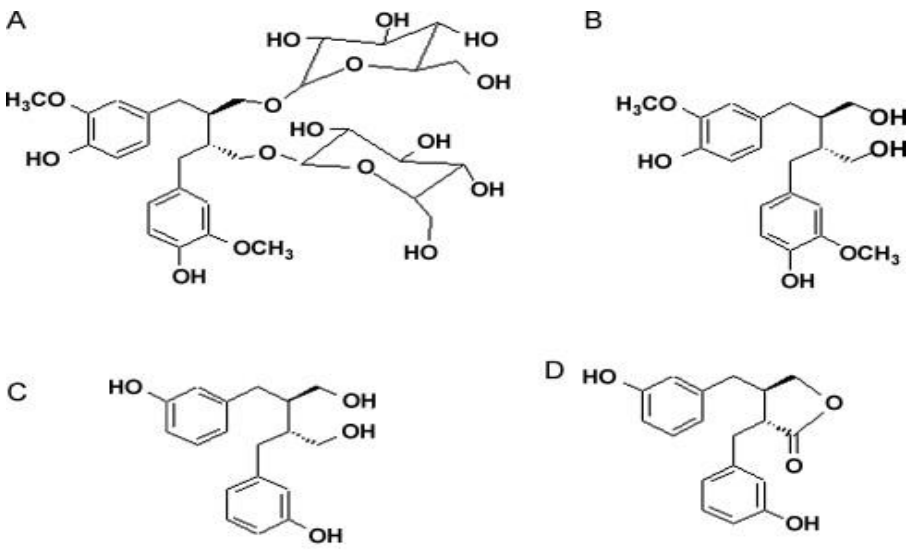

Figure 2.2: Structural representation of Secoisolarisiresinoldiglucoside (SDG) (A), Secoisolarisiresinol (SECO) (B), Enterodiol (ED) (C), and Enterolactone (EL) (D).

Flaxseed lignans have structural and functional similarities with the human estrogen, 17β-estradiol, thus altering hormone metabolism (Ruyter, 2012). Human studies suggested that flax may reduce serum concentrations of 17βestradiol and estrone sulfate and increase prolactin levels in postmenopausal women (Hutchins *et al.*, 2000).Studies indicated that flaxseed could be served as an alternative to traditional estrogen replacement therapy during estrogen imbalance and deficiency in females (Bloedon *and* Szapary, 2004). Literature supports the beneficial effects of flaxseed lignan in reducing atherosclerosis, osteoporosis, angiogenesis, diabetes, and vasomotor effects (hot flushes) during the postmenopausal condition and acting as antioxidants and antineoplastics anti-inflammatories. The mechanism of action is mediated by binding to estrogen receptors, alterations in the concentrations of endogenous estrogen, and antioxidant activity. Lignansafter consuming flaxseed; are converted into mammalian lignans enterodiol and enterolactone by gut florawhich is having a greater antioxidant activity (Morris, 2003).Following flaxseed consumption, secoisolariciresinol (SDG) present in it is converted to the active mammalian lignans, reducing the growth of cancer and tumors, especially hormonal-sensitive tumors like breast cancers, endometrial and prostate tumors (Tour'e *and* Xueming, 2010). It is estimated that lignan, enterodiol, and enterolactone suppress human prostate cancer cells' development.In animal experiments, flaxseed was shown to prevent colon and skin cancer in cell cultures (Thompson, 2003;

Morris, 2003). Research (Danbara *et al.,* 2005) was carried out in which 10 mg/kg enterolactone was injected three times a week. These results indicated that the expression of human colon cancer cells in athymic mice in colon 201 had been decreased.

2.10 Anti-Nutritional Factors

These substances or chemicals either by themselves or through their metabolic products interfere with food digestion or reduce the absorption of nutrients (macro and micronutrients). However, some antinutrients may exert beneficial health effects. They are found in virtually all plants joint in tropical forages. Anti-nutritional factors such as tannin, trypsin inhibitors, oxalates, etc., are found in food grains (Hiremath, 2013).

2.10.1 Hydrocyanic Acid

Flaxseed contains cyanogenic compounds of roughly 264–354 mg per 100 g. Cyanate is naturally present in the plants and, on hydrolysis, convert into hydrogen cyanide. Not clear Cyanide may be toxic to humans as it inhibits the cytochrome C oxidase system involved in respiratory chains (Enneking *and* Wink, 2000). It was found that ingestion of 100 mg/day of cyanide may be lethal to adult individuals.

Food Standards and Safety Authority of India (FSSAI) mentioned the maximum permissible limit of hydrogen cyanide in food grains as 37.5 mg/kg (FSSR 2011). Considering the concentration of cyanogenic compounds in flaxseed as reported in the literature, the daily ingestion of flaxseed indicated (30 g) may contain on average 106 mg of cyanogenic compounds, which is above the tolerable level. But the compounds present in seeds are unstable when subjected to treatments like thermal processing, mechanical processes, including cooking in microwaves, autoclaving, and boiling. Flaxseed contains small amounts of cyanogenic glycosides and linamarin (acetone–cyanohydrinbeta–glucoside C10H17O6N) (Hall *et al.,* 2005).

Whole flaxseed contains 250-550 mg/100 g cyanogenic glycosides (Mazza 2008). The food containing flaxseeds also includes 10 mg/100 g of linatinegamma-glutamyl-1amino-D- proline) (Mazza, 2008). Linatine (a flaxseed vitamin B6 adversary) did not impact vitaminB6 levels or metabolism in persons who were eaten up to 50 g per day. It was observed that more than 85% of linustatin and neolinustatin were eliminated when flaxseed was roasted at high temperatureslike200°C (Park *et al.,* 2005).

2.10.2 Oxalic Acid

A powerfully oxidized and caustic substance with good chelating activity produced by a wide range of animals, plants, and microbes (Stewart *et al.*, 2004). Oxalic acid and its salts are widely used as the final result of metabolism in many plant tissues. The oxalic acid content of foods has long been a concern for human diets due to the adverse health consequences associated with high oxalic acid intake.

The rise in urine and blood oxalate content causes several illnesses such as vitamin failure and hyperoxaluria. A tiny dosage of oxalate in the body can lead to muscular discomfort, headaches, and twitching. The average lethal dose is around 15 to 30 g, while the lowest deadly amount documented is about 5 g (or 70 mg/kg); (Tsai *et al.*, 2005). Oxalate concentration was reported to vary from 2 to 10 mg/kg in raw flaxseed and less than 2 mg perserving of roasted flaxseed (Hiremath 2013). Hui (1992) ensured that the human consumption of 5 grams or more oxalic acid was deadly, although the adverse impact may be detected at even low levels.

2.10.3 Phytate

Phytic acid is another antinutrient present in flaxseed and ranges from 23 to 33 g/kg of the flaxseed meal (Oomah., 1993). The primary source for inositol and storage of phosphorus in plant seeds ~ 70 percent of all phosphorus is phytic acid or(Phytate; myo-inositol 1,2,3,4,5,6- hexakisphosphate. In the food and animal feed sectors, the significant amount of phytic acidin cereal grains is of major concern, as phosphorus in this form is not accessible to mono- gastric animals due to the absence of endogenous phytase enzymes specialized for phytic acid dephosphorylation. In addition, the high chelating property of phytic acid decreases the bioavailability of other critical dietary elements, including minerals (e.g., Ca^{2+}, Zn^{2+}, Mg^{2+}, Mn^{2+}, $Fe^{2+/3+}$), proteins, and amino acids. The chelating capacity of phytic acid to eliminate iron-mediated oxidation in the colon reduces the risk of colon cancer.Phytic acid reduces calcium, zinc, magnesium, and iron absorption. It is a potent chelator that forms a complex with protein, starch, and minerals their digestibility and bioavailability (Oomah, 1993). In cereals, phytate is located up to 80% in the aleurone layer but is also found in the germ, while the endosperm is almost free of phytate.

2.11 Processing Methods to Reduce Ant nutritional Factors

The anti-nutritional factors plants are generally considered as human food and health concern. So various methods are used to eliminate or decrease their levels to the minimum. Several ways are recommended to reduce the level of anti-nutritional factors in plant foods or seeds like heat treatment, blanching, germination/sprouting, popping, extrusion, and soaking.

Heat treatment is one of the most used and usual methods for food processing. It is very effective because it inactivates the heat liable anti-nutritional factors and improves the protein superiority by inactivating majorly trypsin inhibitors and modifying the protein structure, which leads to a more digestible form of protein (Alegbejo, 2013). But the degree of inactivation or reduction in anti-nutritional factors also depends on heating temperature,heating time, and particle size of the substances.

2.11.1 Blanching

It is suggested that mild heating below the boiling point of water is enough to avoid severe heat treatment and inactivate antinutrients and endogenous enzymes. Still, sometimes gentle heat is required to decrease oxalic acid. Usually, blanching is done by treating the vegetables and seeds with hot water for 1-10min at 75-95ºC. The time/ temperature combination depends upon the types of seeds and vegetables (Cano, 1996)

2.11.2 Sprouting

Sprouting is generally used to enhance the eating quality of legumes and cereals. In this method, grains are shocked in potable or drinkable water, and water is drained out and put ina favorable condition to increase faster sprouting. Due to sprouting, some chemical and physical changes occur in the materials, like the breakdown of materials mainly from endosperm to embryo and the breakdown of carbohydrate and protein. It is reported that germination increases many vitamins (Chen. *et al.*, 1975).

2.12 Effect of roasting on ant nutritional factors present in flaxseed

The dry heat used in the roasting method, whether in an open flame, oven or another heat source can be useful in reducing antinutritional components. Roasting usually causes caramelization or Maillard browning of the surface of the food, which is considered suitable for flavor enhancement. Roasting of flaxseed traditionally been used to prevent gastrointestinal complications (Moknatjou *et al.*, 2015). A study showed that microwave

roasting of flaxseed decreased the HCN level by 83.3 percent owing to glycosidase deactivation or HCN reduction (Feng *et al.,* 2003). Cyanogenic Glycosides are heat-labile and quickly destructed by using processing methods such as autoclaving, microwave roasting, and using some detoxifying enzymes such as β-glycosidases (Cunnane *et al.,* 1993).

2.13 Effect of Processing and Storage on Stability of Flaxseed Lignin Added to Dairy Products

There were no significant effects on the stability of the (Secoisolariciresinol diglucoside) major lignan present in flaxseed. During processing and fermentation. No SDG metabolites were identified, which showed no breakdown of SDG throughout the processing. Fermentation does not seem to convert SDG into an aglycone shape (Hyvarien *et al*., 2006). The effect of pasteurization in whey drinks processing and storage with added SDG was observed and SDG was found to be stable during heat treatment and storage during the study, however, after long storage, the SDG losses were seen.

2.14 Flaxseed as a Functional Food Ingredient

Flaxseed consumption in various forms as a food and for its medicinal properties had been reported from 5000 BC. Flaxseed is the major oilseed mostly studied to date as an important functional food. It is a leading source of omega -3 fatty acid, α-linolenic acid(ALA) (52% of total fatty acid), and phenolic compounds known as lignans >500μg/g). The evidence of the clinical activity linked with flaxseed intake for cancer prevention had been observed by the National Cancer Institute (NCI).

The impact of ALA in the eicosanoids may be beneficially mediated by simply adding flaxseed oil to canola oil with a 1:3 ratio and demonstrating substantial decreases in cardiovascular diseases risk. Flaxseed oil can be used to build innovative anti-inflammatory therapy. Three studies have found that flaxseed intake, raw or defatted, lowers total human and LDL cholesterol. Women's studies demonstrate the crucial function of flaxseed in mediating bone health and its significant phytoestrogen action in reducing hormone-related cancer risk. The protein in flaxseed may potentially impact blood glucose levels due to its increasing insulin release. Combining flaxseed protein with soluble polysaccharide might be crucial for reducing and protecting colon luminal ammonia against recognizedtumor- promoting actions of ammonia (Oomah *et al.,* 2001)

2.15 Value-Added Dairy Products Prepared By Incorporation of Flaxseed

Value-added foods are produced to increase the value of foodstuffs by adding ingredients, processing, aseptic packing, or increasing the product storage time. Wild milk, ice cream, cheese, yogurt, breaking cereals, extruded snacks, and so on is few examples of value-added goods.

The commercial use of flax in preparation of different value-added food products like baked, extruded snacks, and other foods, as a functional food ingredient informs of ground or milled whole seed, flaxseed flour, and flaxseed oil, has been found useful.

2.15.1 Dairy Products

The dairy product functionality may be enhanced and customized simply by changing and enhancing the nutritious quality of the original basis. Milk type and culture, milk fat and non-fat milk solid quantities, fermentation, and temperature influence the cultured milk products' flavor, body, and taste. Milk and milk derivatives have shown beneficial in supplying bioactive substances (Rowan *et al.,* 2005). Valuable milk products include low-lactose or lactose-free products, hydrolyzed protein formulations for milk-hyper-sensitive babies, calcium-enriched milk, vitamins, etc. (Ozer and Kirmaci, 2010).

Dairy-based products are a vital part of functional food. Characteristics of dairy products can be enhanced by enriching and modifying the products with healthy and nutritious substances such as Type of culture and milk, amount of milk fat and solids not fat, and milk not fat solids, fermentation and temperature, flavor and body of cultured dairy products. Studies reported that milk and milk products are successful mediums for providing bioactive compounds. Value-added dairy products include

a. Low-lactose or lactose-free products,

b. Hypoallergenic formulations with hydrolyzed protein for milk-hypersensitive infants,

c. Milk enriched with calcium, vitamins, etc. (Ozer and Kirmaci, 2010).

Goh *et al.*,(2006) studied the characterization of ice cream containing flaxseed oil and determined particle size of fat globules, meltdown rates, the texture of ice cream, and microstructure of ice cream by adding different levels (0%, 3%, 6%,9%, *and* 12% w/w)

of flaxseed oil to the ice cream. It concluded or shown in the study that the higher the portion of flaxseed oil used to replace the ice cream fat resulted in higher meltdown rates and lower the firmness of ice cream.

Giroux *et al* (2010) fortified flaxseed oil @2% concentration in dairy beverages and heated milk protein-sugar blend as antioxidants. It was observed that pre-heated combinations were the most effective in reducing lipid oxidation during the sterilization process.

1. 2% (w/w) flaxseed oil in a 12% (w/ w) ice cream mix can be incorporated without significantly affecting the overall functionality of ice cream.
2. SDG added to yogurt, milk, and cheese were reported to be stable during pasteurization, fermentation, and milk renneting processes (Hyvarinen*et al.*, 2006).
3. Microencapsulated flaxseed oil powder was fortified in Dahi at three diverse formulations as 1, 2, and 3%, which could serve as a potential delivery system of omega-3 fatty acids (Goyal *et al.*, 2016).

Table 2.3: Functional Dairy Products incorporated with Flaxseed components

Product name	Flaxseed form	Processing method	Amount of supplementation	Reference
Dahi (Indian Yogurt)	Microencapsulated flaxseed oil powder (MEFOP)	Fermentation	1–3%	Goyal *et al.*, 2016
Ice cream	Flaxseed oil	Freezing	2-5%	Goh *et al.*, 2006
Cheese	Flaxseed lignan (SDG)	Pasteurisation and fermentation	1 g/10 L	Hyvarinen*et al.*, 2006
Yogurt	Flaxseed lignan (SDG)	Fermentation	100 mg	Hyvarinen*et al.*, 2006
Milk	Flaxseed lignan (SDG)	Heat treatment	1%	Hyvarinen*et al.*, 2006
Whey drinks	Flaxseed lignan (SDG)	Pasteurisation	10 mg/100 ml	Hyvarinen*et al.*, 2006
Butter	Flaxseed lignan (SDG)	-	0.8–1.6%	Ivanov *et al.*, 2011

Table 2.4:Other FoodProducts with flaxseed

Product name	Flaxseed form	Processing method	Amount of supplementation (%)	Reference
Chutney powder	Flaxseed flour	-	50	Rao *et al.*, 2011
Vegetable chilla	Flaxseed flour	Shallow frying	20–40	Rathi and Mogra, 2012
Wheat chips	Flaxseed flour	Frying	10–20	Yuksel*et al.*, 2014
Energy bar	Flaxseed flour	Freezing	0–20	Mridula*et al.*, 2013
Dry-fermented sausages	Flaxseed oil	Fermentation	3.3	Ansorena and Astiasarán, 2004
Tahina	Roasted flaxseed flour	Grinding and mixing	25–100	Ahmed *et al.*, 2010
Manchurian	Flaxseed flour	Shallow frying	20–40	Rathi and Mogra, 2012

Other traditional products are prepared by flaxseed fortification in different forms and at different concentrations.

2.16 Probiotic

Probiotics are a live microbial feed supplement that beneficially affects the host by improving its intestinal microbial balance. The required suggestive concentration of probiotic bacteria is 10^6cfu/g or ml of a product to provide required health benefits. The Indian probiotic market is valued at $12million in 2011, is expected to witness a CAGR of 11% by 2016. Including probiotics into different foods has created a new segment of the functional food sector labeled as 'probiotic foods'. Bacteria from *Lactobacillus* and *Bifidobacterium* genera are commonly considered probiotic (Doron *et al.,* 2006), and they are regarded as safe and don't have any harmful effects on host health. The most commonly used*lactobacilli* are *L. acidophilus, L. casei, L. rhamnosus, L. reuteriL. plantarum, L. johnsonii ,L. brevis, L. cellobiosus, L. fermentum, and L. Gasseri*. The bifidobacterial species used are *Bifidobacterium breve*, B. *Animalissubsp*tactics formerly

known as *B. Lactis* (Masco *et al.,* 2004), and B. *Longumbiotypesinfantis* and *longum* (Masco *et al.,* 2005).

2.16.1 Health benefits and Safety of Probiotic organisms

Most probiotics have been selected as "generally considered as safe (GRAS) based on their long history of use in food fermentation. On the other hand, there have been rare reports of endocarditis and bacteraemias related to *Lactobacillus* spp generally in brutally immune-compromised individuals. This prompted the idea that much surveillance is instituted for probiotics. Salminen and Donahue (1996) reviewed the protection data and found no facts of probiotics being involved in human infections. This is supported by epidemiological data on the safety of dairy products.

Most probiotic food products in international markets are milk-based, and relatively little is done to produce probiotic products with alternative fermentation substrates, such as plant substrate and combinations of milk and plant substrate. Probiotics have been used to enhance immunity, decrease cholesterol, rheumatoid arthritis, prevent cancer, address lactose intolerance and skin problems, constipation or diarrhea, urinary tract infections (UTI), and candidiasis (Ganguly *et al.,* 2019). The main constraints are the lack of information about changes in composition and components generated during probiotic fermentation. In addition, the positive health effects of probiotic composite dairy products in animal and human models are poorly assessed. In this field, a comprehensive and in-depth investigation is needed.

2.16.2 Probiotics Composite Products

The majority of probiotic food items on the market are milk-based. However, recent research has increasingly concentrated on the fermentation of plant substrates. Cereals, legumes, and oilseeds offer more vital vitamins, dietary fibres, and minerals than milk but have less easily fermentable carbohydrates. Combining plant substrate with milk will improve the nutritional value and develop novel functional food products. The composite substrate consists of both milk and plant components. A combination of plant components with milk can provide better nutrition at a reduced cost. This will have a synergistic impact on improving nutrition and ultimately result in value-added food products. Milk lacks fibre, while a valuable source of nutrients, whereas cereals, legumes, and oilseeds are high in fibre. Protein is of high nutritional quality and amino acid profile, whereas some plant proteins are deficient in certain amino acids Milk is

rich in A, B2, B12, Niacin vitamins, whereas plant components are rich in carotene and tocopherol. Milk is an excellent source of calcium, but low in iron, whereas plants substrate isa good source of iron but a poor source of calcium. Plant components are an inexpensive source of nourishment, which ultimately leads to a nutrient-rich product cost- effectively. A composite substrate may also be utilized to make various types of new and economically functional foods. However, the sensory acceptability of these types of food products is a major concern.

2.17 Milk and Barley based Composite Fermented Drink

A milk-barley-based probiotic beverage was prepared using *Lactobacillus Plantarum* and co- culture *Streptococcus thermophilus*. The beverage had a high probiotic count of *L. Plantarum* (8.59 log CFU/ml) and β- glucan content (0.144 g/100 g). Theproduct had detected with ABTS radical scavenging activity of 0.40±0.01 mg TEAC/mL (Ahuja *et al* 2017).

2.17.1 Tarhana

Tarhana is a fermented cereal food that may be characterized as a combination of yogurt, cream, yeast, various vegetables, herbs, and spices. The tarhana dough is fermented for 1 to 5 days after the mixing procedure and promptly dried. Both lactic and yeast fermentationsoccur in tarhana manufacturing concurrently. It has a sour and acidic taste with a yeasty flavor is industrially oven-dried and sun-dried at home. Tarhana can be categorized according to raw materials and processing methods. Tarhana is a good source of B vitamins, minerals, free amino acids, organic acids, and it is a product of LAB and yeast fermentation. It may be considered a functional and probiotic food product.

2.17.2 Cereal Based Fermented Milk Beverage

Gupta *et al* (1992) prepared *rabadi* from barley flour and sour buttermilk, by mixing autoclaved barley flour with fermented buttermilk @ 5% by weight. The mixtures were stirred sufficiently and again fermented at different temperatures and for different fermentation times followed by a salting process. The product fermented at35°C for 18 h had the highest overall acceptability. The technology for the preparation of cereal-based fermented milk beverage using three different kinds of cereal, viz. wheat ,pearl millet (Modha and Pal, 2011), and sorghum (Pintu, 2006) was developed at NDRI (Karnal),

India. The germinated cereal flour was mixed with milk (standardized certain ratio of fat and SNF), pasteurized, cooled, inoculated with a suitable starter culture, and incubated for a suitable time to obtain an acceptable (0.9% to 1%) lactic acidity. The curd thus obtained was mixed with pasteurized water containing stabilizers, spices, and salt. The product had a keeping quality of 7 days at refrigeration temperature.

2.17.3 Whey-cereal based probiotic beverage

The probiotic composite beverage was prepared from underutilized resources, whey-skimmilk (60:40, v/v), germinated pearl millet flour (4.5%, w/v), liquid barley malt extract(3.0%, w/v) and fermented with *Lactobacillus acidophilus* NCDC 13. Probiotic fermentation enhanced nutritional attributes of the product by enhancing the digestibility of protein and starch and reducing anti- nutrients (phytic acid and polyphenol) content (Ganguly *et al* 2013). Further, the probiotic substratewas converted into a sour-spicy beverage and found effective in controlling shigella-induced pathogenicity in the murine model (Ganguly *et al* 2019).In vivo feeding trials on mice further revealed better weight gain and blood hemoglobin level in the probiotic beverage-fed group (P). The probiotic beverage fed group was found to be superior in terms of protein efficiency ratio and apparent protein digestibility to unfermented beverage and casein diet groups (Ganguly *et al.*, 2021). Probiotic beverage scan be a cost-effective source of nutrition for wide segments of the population in the society especially found privileged one.

CHAPTER –3

Materials & Methods

This chapter deals with the materials, experimental methodology, procedure, and technique employed during the present investigation of the development of milk flaxseed-based probiotic beverage and analysis of flaxseed flour. This study comprised the result of milk flaxseed beverage and chemical analysis of raw and roasted flaxseed flour.

3.1 Materials

3.1.1 Milk

Good quality fresh cow and skim milk was collected from Experimental Dairy, NDRI, Karnal. The Fat and TS of cow milk were ranges from 3.5 to 3.8% and 11.5 to 12.2% respectively and the fat and TS of skim milk ranges from 0.1 to 0.3 percent and 8.5 to 9.2% respectively. The titrable acidity of cow milk and skim milk were ranges from 0.14 to 0.17% of lactic acid.

3.1.2 Flaxseed

Superior quality Flaxseed grains of brown variety were purchased in a single lot from the local market of Karnal, Haryana. The grains was free from foreign substances like stones, soil particles, pest infected seeds, soil, weed grains, insects and eggs, larvae or their waste.

3.1.3 Probiotic cultures

Mesosphilic cultures of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* were used for the selection of best probiotic strain.

3.1.3.1 Procurement of probiotic cultures

Four culture strains were collected from National Collection of Dairy Cultures (NCDC), NDRI, Karnal, Haryana.

No of strains	Name of strain	NCDC Number
1	*Lactobacillus rhamnosus*	24
2	*Lactobacillus acidophilus*	195
3	*Lactobacillus acidophilus*	291
4	*Lactobacillus rhamnosus(RSI 3)*	610

3.1.3.2 Activation of Probiotic Culture

First, break the ampoules in round form near the neck of ampoules using an ampoule breaker in aseptic condition and transfer it carefully in 5ml De Man Rogosa Sharp (MRS) broth (sterile) and incubated at 37°C for 16 hours. After completion of the incubation period, these MRS broth tubes should be kept in refrigeration condition.

After homogeneous mixing of this MRS broth tube, 0.1ml of culture were transferred to another MRS broth tubes and again incubated at 37°C for 16 hours. This process is repeated twice or two times sub culturing was needed for activation of these cultures.

3.1.3.3 Preservation of probiotic culture

The freeze-dried cultures were activated in MRS broth (proteose peptone 5 g/l, beef extract 10 g/l, yeast extract 5 g/l, dextrose 20 g/l, polysorbate 80 g/l, ammonium citrate 2 g/l, sodium acetate 5 g/l, magnesium sulphate 0.10 g/l, manganese sulfate 0.05 g/l, di-potassium phosphate 2 g/l, pH at 25 °C 6.5±0.2) at 37°C for 24. Subsequently, the organisms were grown in at least two sub-culturing, before they were used to prepare the 16 h old inoculums (with ~ 8 log CFU/ mL) used in further experiments and product preparation. Stock cultures of *lactobacillus* were maintained at -20°C in MRS containing 20% glycerol. For product preparation the selected organism was grown in milk.

3.1.3.4 Inoculation of culture from MRS broth to milk

The refrigerated MRS broth tubes (15 ml) with activated culture were drawn from the refrigerator and homogeneous mixing was done using vertex mixture about 0.1 ml of this MRS broth with activated culture was transferred to sterile skim milk (10 ml) tube having 11% of TS and homogeneous mixing was done and these tubes were incubated at 37°C for 16 hours. Next day 1 ml from the curd of skim milk having probiotic culture

was transferred to 99 ml of sterile skim milk and incubated at 37°C for 16 hours or up to curd setting time. These probiotic inoculums were used for the product development.

3.1.3.5 Prorogation of cultures

3.1.3.5.1 Stock culture

It is the cultures of a microorganism maintained only to keep the microorganisms in a viable form. It is collected from the NCDC of NDRI karnal.

3.1.3.5.2 Mother culture

It is the first inoculation all cultures were originated from this culture.

3.1.3.5.3 Feeder

It is an intermediate culture. It is used in the preparation of a large volume of prepared culture.

3.1.3.5.4 Bulk culture

This stage is used in dairy products production.

3.1.4 Chemicals

All chemicals used throughout this study were AR grade and purchased from standard suppliers. All operations were performed at room temperature unless otherwise stated.

3.1.4.1Microbiological and biochemical materials

Microbial and biochemical materials were obtained from M/s Himedia , Mumbai.

3.1.5 Glasswares

Glassware made by Borosil was used throughout the study. They were cleaned by using laboratory detergent solution (Teepol) and washed thoroughly with water and rinsed the distillwater and oven dried at 102±2°C for 2 hours before use.

3.1.6 Kits for analysis

Phytic acid (Phytate)/ Total Phosphorus kit and Available Carbohydrates and Dietary Fibre kit were obtained from Megazyme© International Limited, Ireland. IgA estimation kit (K3231081) was obtained from Koma Biotech, USA.

3.2 Methodology

3.2.1 Preparation of roasted flaxseed flour

The flaxseed grains were cleaned by manually picking of foreign matters. Roasting of flaxseed grains was done on open pan roasting for roasting of flaxseed roasting pan was used and flaxseed was roasted up to puffing or to give a nutty flavor after roasting. Then it was transferred to a clean and dry tray and cooled up to room temperature and stored in the colored plastic jar in refrigerated condition and finally grinding of roasted flaxseed was done by using of a mixer and it was packed in PET pouches and sealed by using a hot sealer machine. These roasted flaxseed flour PET pouches were covered by aluminum foil and stored at refrigerated condition.

3.2.2 Screening of the probiotic culture based on growth studies

The four strains of probiotic cultures were screened on the basis of following parameters.

3.2.2.1 Enumeration of probiotic cell count and determination of Specific growth rate (k)

Probiotic organisms were enumerated using MRS agar, and the plates were incubated at 37°Cfor 2-3 days by pour-plate method described by Haughty *et al*. (1993).

The specific growth rate (k) for each substrate was calculated from the following equation:

$k\ (h^{-1}) = 2.303(\log_{10}X2 - \log_{10}X1)/\ (t_2 - t_1)$ where, X1 and X2 were cell counts at times t1 and t2 respectively (Cogan, 1978). The reciprocal of specific growth rate was recorded as generation time (Pelczar *et al.*, 2004).

3.2.2.2 Determination of pH, titratable acidity and calculation of Acidification rate

The pH was determined by using pH meter (Labindia, India). The titratable acidity in form of % lactic acid was determined by modifies the method given by AOAC (1995) methods for cheese. Five grams of sample was mixed uniformly by adding 20 ml hot distilled water (65°C), followed by totaling of 10 ml of 0.1 N NaOH and 1 ml of 0.5% phenolphthalein indicator, previous to titrating against 0.1 N HCl.

The change in pH for the period of fermentation was monitored at 2 h intervals up to

8 hours. The final acidification rate (Vf) was calculated as the variation of pH as a function of time (dpH/dt) and expressed as 10^{-3} pH units/min.

3.2.2.3 Optimization of inoculum level and fermentation time

Different level (1 to 4%v/v) of *lactobacillus rhamnosus(NCDC-24, 610)and Lactobacillus acidophilus (NCDC-195, 291)* were inoculated in sterile skim milk having 11% of TS and fermentation carried out resulting in average initial count of 2.2×10^7, 2.4×10^7, 2.84×10^7 and $7.12.4\times10^6$cfu/ml respectively and fermentation carried out at 37°C for 0-8 hours. The optimum inoculation level and incubation time was selected on the basis of reduced fermentation time (10 hours) to limit the risk of microbial contamination and suitablepH range for the sensory acceptability of the product.

3.2.3 Preparation of milk-flaxseed based probiotic beverage

Milk was initially standardize to 2% of fat by using cow and skim milk and then it was heated at 90°C for 10 minutes and cooled up to 37°C and fermentation applying optimum inoculation level and incubation time. The probiotic curd with sugar syrup, stabilizer was converted to sweet-acidic beverage.100g of curd to 3 to 6 g of roasted flaxseed flour, 15 to 25g of sugar, and 0.15 to 0.45 % of stabilizer on curd basis . Chemical analysis of raw and roasted flaxseed flour

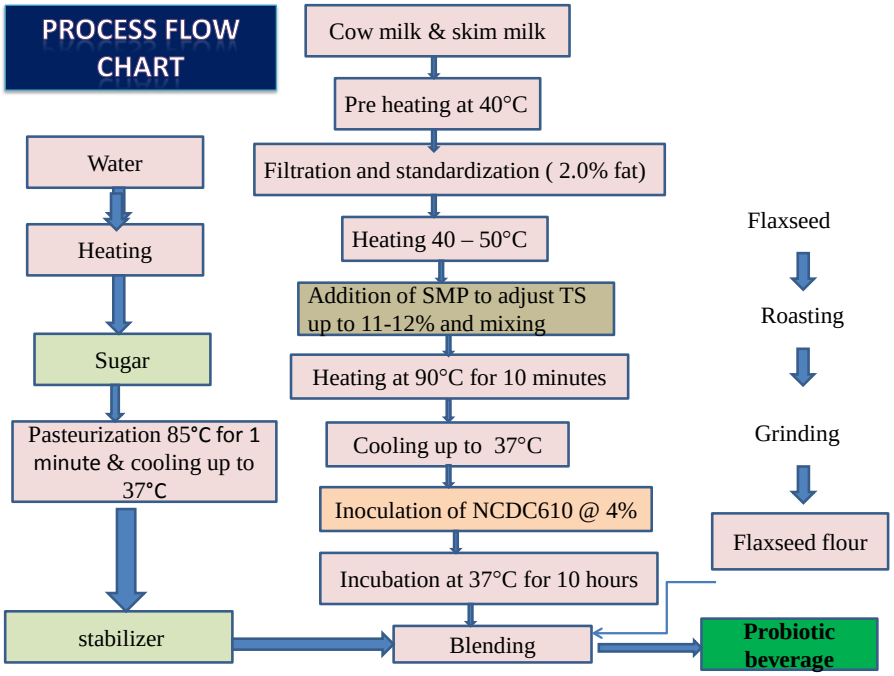

Figure 3.1 Process flow chart for development of milk-flaxseed based probiotic beverage

3.3 Chemical analysis of raw and roasted flaxseed flour

3.3.1 Estimation of Crude Fibre

The crude fibre in the raw and roasted flaxseed flour was estimated as given by IS: SP:18 part IV (1981). About 2.5-3 g sample was weighed accurately and transferred to an extraction apparatus (Soxhlet extractor) and extracted with petroleum ether. Air dried the extracted sample was transferred to a dry one litre conical flask. Two hundred ml of H_2SO_4 (1.25% w/v accurately prepared) was taken in a beaker and brought to boil. The boiling acid was transferred to the flask containing the defatted material. The flask was connected immediately with a water cooled reflux condenser and heated so that the contents of the flask started boiling within one minute. The flask was rotated frequently taking care to keep the material in contact with the acid. Boiling was continued for exactly 30 minutes. The flask was removed and filtered through fine linen (about 18 threads to a cm) held in a funnel and washed with boiling water until the washings were no longer acid to litmus. Some quantity of NaOH solution (1.25% w/v accurately prepared) was brought to boil. Washed residue on the linen was taken into the flask with 200 ml of boiling NaOH solution. The flask was immediately connected to the reflux condenser and boiled for exactly 30 minutes. The flask was removed and contents filtered immediately through the filtering cloth. The residue was thoroughly washed with boiling water and transferred to a Gooch crucible prepared with a thin compact layer of ignited asbestos. The residue was washed thoroughly first with hot water and then with about 15 ml of ethyl alcohol (95% v/v). The Gooch crucible and contents were dried at 105±2℃ in an air oven until constant weight was achieved. The crucible was cooled and weighed. The contents of the Gooch crucible were incinerated in a muffle furnace until all carbonaceous matter was burnt. The Gooch crucible containing ash was cooled in a dessicator and weighed.

Calculation:

$$Crude\ fibre\ (\%by\ Wt) = \frac{W1 - W2}{W} \times 100$$

where,

W1 = weight in gm of Gooch crucible and contents before ashing

W2 = weight in gm of Gooch crucible containing asbestos and ash

W = weight in gm of the dried material taken for the test

Crude fibre content was expressed on the dry weight basis.

3.3.2 Estimation of Protein

The crude protein content of raw and roasted flaxseed flour was determined by Kjeldahl method using factor 6.25 (AOAC, 1995).

The sample was weighted about 0. 5-1 gram and transferred to a 500 ml Kjeldahl flask to check that no part of the sample stuck to the opened neck of the flask. 5 grams of digestive mixture and 25 ml of sulphuric acid (concentrated) was added to it with 2 to 3 glass beads. The flask was placed on the stand in a sloping position in the digestive chamber to digest. The flax was kept for approximately an hour or longer until the digest's color was light blue. Alternatively, only a few droplets of water could be spilled on the flask. The digested samples was cooled and 100ml of distillation watered was added to make the volume 100 ml in Kjeldahl tubes. From this 10ml digested sample was taken tothe distillation device with a practical flashed head and condenser. A delivery tube fits the condenser, which dips just under the surface of the standard acid volume pipetted in a conical flask recipient. (precaution: the solution to received must be under 45°c to prevent ammonia loss. The contents of the digestive flask were mixed till boiling and until 180 ml distillate was received to the receiver. The flask was titrated with 0. 1N sulphuric acid till light red color emerges. A blank titration was performed at the same time.

Calculation:

1 mL of 0.1 N H_2SO_4 = 0.0014gm N

% N_2 = 14.001×(S-B) ×Normality of sulphuric acid/sample weight before drying

% protein = % N_2 ×6.25

3.3.3 Estimation of Moisture and Total Solid

The moisture content of flaxseed powder was determined by heating (Raganna, 2008). The dish was heated in a hot air oven at 102° ± 2°C for about 1 h containing 20 g of the sand with its lid alongside and a stirring rod on top of the lid, in a hot air oven at 102° ± 2°C for about 1 hour. The lid (with the stirring rod on the top) was placed on the dish. The dish was transferred immediately to the desiccators and cooled for at least 45 min,

and the weight of dish with lid and rod recorded to the nearest 0.1mg. About 5 g of the flaxseed flour was takeninto the dish and the lid were replaced with the stirring rod on top and weighing the dish tothe nearest 0.1 mg. the sample was mixed properly and spread evenly with the stirring rodand the stirring rod was left in the dish with the other end resting on rim of the dish. The stirring rod was laid flat inside the dish, and the dish was heated in a hot air over maintainedat 102 ± 2°C for 4 hours. The lid was placed on the dish and dish was allowed to cool in the desiccator and the weight was recorded to the nearest 0.1 mg. The above operations (heating the dish for 1 h) was repeated until the difference in mass between two successive weighing did not exceed 0.5 mg and the lowest mass was recorded.

Moisture % = (W1-W2) ×100/ (W1-W)

Where,

W = weight in gms of the empty dish

W1 = weight in gms of the dish with the material before drying

W2 = weight in gms of the dish with the material after drying.

Total solid = 100- % moisture

3.3.4 Estimation of Total Ash

The ash content of flaxseed was estimated using the method as described in IS: SP: 18 (Part XI) 1981.

New sample (5g) was taken for the ash determination other than the sample was left after moisture determination. The sample was completely charred on an electric heater and then kept in muffle furnace (550°C ± 20°C) for 5 to 6 hours (till gray colored ash obtained). The crucible containing ash was cooled in a desiccator and weighed accurately. The ash content was determined using the following formula.

Note - If ash was still contains black particles add 2-4 drops of previously heated water at 60°C. Smash the ash and evaporate to dryness at 100-110°C. Re-ashing at 550°C. Until ash was white or slightly grey

Calculation

Ash % = (W2- W) ×100/ (W1-W)

Where,

W = weight in gms of the empty dish.

W1 = weight in gms of the dish with the material before ashing.

W2 = weight in gms of the dish with the material after ashing.

3.3.5 Estimation of Fat

The fat content was determined by using standard Soxhlet apparatus using petroleum ether (BP 40-60ºC) as solvent (Raganna, 2008).

The fat content of flaxseed was measured by the use of the soxhlet method of fat estimation. 3 g of sample was weighed in a clean and dried thimble and it was placed in a soxhlet extraction chamber. Petroleum ether was used as a solvent for fat estimation in flaxseed 250 ml of petroleum ether was taken in 500ml rounded bottom distillation flask and fixed on soxhlet assembly heating was done at 50°C for 6 hours and then the evaporation of the solvent was done in drying oven at 102°C for 1 hour. Finally, the changes in the weight of the volumetric flask were measured and calculated by the formula given below

$$\% \text{ Crude fat} = (W2 - W1) \times 100 / S$$

Where

W1 = weight of empty flask (g)

W2 = weight of flask and extracted fat (g)

S = weight of the sample

3.4 Analysis of Beverage

3.4.1 Physical/ chemical

3.4.1.1 pH and Titrable Acidity

The pH meter (Labindia, India) was used for the determination of pH. The titrable acidity of the beverage and the fermented mix was determined in lactic acid by modifying the AOAC (1995) method for cheese. 5g of evenly mixed sample was taken and mixed homogeneously by adding 20ml of distilling water at 65°C. Then add 10 ml of 0.1N NaOH and few drops of 0.5% phenolphthalein indicator. Finally, the mixture was titrated against 0.1N HCl till the pink color disappears completely.

$$TA\ (\%) = \frac{\text{Normality of NaOH} \times (10 - \text{titrate value}) \times DF}{\text{Weight of sample} \times 10}$$

Where

DF = Dilution Factor

DF = ml of water Sample in gm

3.4.1.2 Viscosity

The viscosity of the beverage was determined at 20±0.1°C using viscosity Anton paar rheometer. The attachment used was the AMP assembly of coaxial cylinders (spindle TL-5). The sample volume was 8-10 ml, and the shear rate was obtained by multiplying round per minute by 1.32. The relationship between shear stress (τ) Pa s and shear rate (γ) s^{-1} can be expressed as:

$$\tau = k\gamma^n$$

Where

k - Consistency index (2.72±0.38)

n- Flow behavior index (0.519±0.03)

3.4.1.3 Wheying off

The wheying off the beverage value was determined by following the modification in procedure of (IS: SP: 18 - Part XI, 1981). 20ml of the product was centrifugated at 2500rpm for 10 minutes in 30 ml centrifuge tubes, and results were expressed as water separated per 20ml.

3.4.1.4 Color measurement

The color of beverage and flaxseed flour was measured by reflectance spectroscopy technique employing reflectance meter, color flex (Hunter lab, Reston, Virginia, USA). Before the tasting instrument is calibrated with standard black glass and white glass tiles supplied by the producer. The light sources were dual-beam xenon flash lamp, and the data was received from software in terms of L*(Lightness ranges 0 for black to 100 for white), a* (Redness range from +60 for red to -60 for green), and b*(Yellowness range from +60 yellowto -60 for blue). In values of the international color system.

Whiteness index (WI) = $100 - \sqrt{(100-L^*)^2 + a^{*2} + b^{*2}}$

3.4.2 Sensory parameters

The samples with different treatments as well as final products were drawn from the refrigerator just before sensory evaluation. All samples were evaluated for sensory attributes like colour and appearance, body and texture, flavour and overall acceptability using a suitable sensory scorecard was developed by Dr. Kaushik Khamrui PS, DT NDRI, Karnal. The sensory panel comprising of 12 discriminative and communicative judges from the faculty, staff and research scholars of Dairy Technology Division of NDRI, Karnal. The sensory panelists were have adequate knowledge about product.

Randomly, one sample container (PET bottle) 50 ml of each treatment was drawn from the refrigerator just before serving and was served to each panelist for judging.

3.4.3 Microbiological analysis

3.4.3.1 Preparation of Diluents

Salt saline (0.85 to 0.90% of NaCl solution) was prepared using distilled water (DW). 9 ml of this saline solution was filled into 15 ml test tubes using hand hold 10ml micropipette and plugged using cotton or plastic caps. The plugged saline test tubes were autoclaved at 121°C for 15 to 20 minutes.

3.4.3.2 Preparation of Samples

The probiotic beverage is homogeneously mixed by using a sterile glass rod. After this, 11ml of the sample was taken in a sterile container in aseptic condition (Laminar air flaw), transferred in 99 ml of saline solution, and dissolved appropriately by vigorously shaking. This is taken as 1:10 dilution. Further dilution was created by transferring 1ml o sample from this dilution to 9 ml sterile diluents. These dilutions were used for plating. The maintenance and preparation of microbiological media used for the entire investigation followed the method suggested by Marshall (1993). All records were done by the pour plate method described by Haughty *et al*(1993). Results were shown in log CFU/ml.

3.4.3.3 Probiotic Count

Probiotic organisms were enumerated using MRS agar, and the plates were incubated at 37°C for 2-3 days.

3.4.3.4 Coliform Count

Coliform count in the sample was determined using Violet Red Bile Agar (VRBA), and plates were incubated at 37°C for 24 hours.

3.4.3.5 Yeast and Mold Count

Yeast and mold count was determined using potato dextrose agar (PDA) and decreased pH up to 3.5 using 10% tartaric acid (2ml of 10% tartaric acid in 100 ml of PDA). Plates were incubated at 25°C for 3-5 days.

3.4.4 Chemical Analysis

3.4.4.1 Total Solid

The gravimetric method estimated the total solids of beverage as per the procedure outlined in (IS: SP: 18 - Part XI, 1981).

3.4.4.2 Fat

The crude fat content in flaxseed flour and beverage was done by ether extraction method in flour (AOAC, 1995) in a soxhlet apparatus.

3.4.4.3 Protein

The protein content in flaxseed flour and beverage was estimated by the micro-Kjeldahl method as per the method of AOAC (1995).

3.4.4.4 Ash

The ash content of flour and probiotic flaxseed lassi was estimated by the gravimetric method as per the procedure outlined in IS: SP: 18 - Part XI, 1981.

3.4.4.5 Carbohydrate

The content of the available carbohydrate in flaxseed flour was determined by difference. % carbohydrate = 100- (%moisture +% protein +% fat + %ash +%crude fiber).

3.4.5 Anti-nutritional Properties

3.4.5.1 Estimation of Phytic Acid

The phytic acid and phosphorus contents of raw and roasted flaxseed flour were estimatedusing the phytic acid estimation kit (Megazyme International Ireland Limited).

Chemicals used for phytic acid estimation: The chemicals readily available with kit were sodium acetate buffer (25 ml, 200 mM, pH 5.5) and sodium azide (0.02% w/v) as a preservative, phytase suspension (1.2 ml, 12,000 U/ml), glycine buffer (25 ml, 400 mM, pH 10.4), plus $MgCl_2$ (4 mM), $ZnSO_4$ (0.4 mM) and sodium azide (0.02% w/v) as a preservative, alkaline phosphatase suspension (1.2 ml, 80 U/ml), phosphorus standard solution (24 ml, 50 µg/ml) and sodium azide (0.02% w/v) as a preservative and oat flour control powder (5 g phosphorus content).

Reagents prepared:

1. Colour reagent:

   Solution A. Ascorbic acid (10% w/v) / Sulphuric acid (1 M):

   Solution B. Ammonium molybdate (5% w/v): 25 ml

   Solution B added to Solution A. Mixed one part Solution B with five parts Solution A

2. Trichloroacetic acid (50% w/v): 100 ml – add 50g of trichloroacetic acid to 60 ml of distilled water and dissolve make the volume 100ml using distill water.

3. Hydrochloric acid (0.66 M): 1 L –add 54.5 ml of hydrochloric acid to 945.5 ml of distil water and mix.

4. Sodium hydroxide (0.75 M): 200 ml –Add 6 g of sodium hydroxide pellets in 80 ml of distill water and make volume 200 ml by using distill water.

5. Phytic acid- provided by (sigma cat. No. P5681).

Standard assay procedure:

A. ***Sample extraction****:* One g of sample material was accurately weighed into a 75 ml glass beaker. Twenty ml of HCl (0.66 M) was added, the beaker was covered with foil and stirred vigorously for a minimum of 3 h at room temperature (preferably overnight for convenience).

One ml of extract was transferred to a 1.5 ml microfuge tube and centrifuged at 13,000 rpm for 10 min. The resulting extract supernatant (0.5 ml) was immediately transferred to a fresh 1.5 ml microfuge tube and neutralized by addition of 0.5 ml of NaOH solution (0.75 M). The neutralized sample extract was used in the enzymatic dephosphorylation reaction procedure as described below.

B. Enzymatic dephosphorylation reaction: Reaction tube: microfuge tube (1.5 ml), Temperature: ~ 40, Final volume: 1.39 ml

Pipetted into 1.5 ml microfuge tubes	Free Phosphorus	Total Phosphorus
distilled water	0.62 ml	0.60 ml
sodium acetate buffer	0.20 ml	0.20 ml
sample extract	0.05 ml	0.05 ml
Phytase	-	0.02 ml
Mixed by vortex and incubated in a water bath set at 40°C for 10 min. After 10 min, started the next reaction by addition of:		
distilled water	0.02 ml	-
glycine buffer	0.20 ml	0.20 ml
ALP	-	0.02 ml
Mixed by vortex and incubated in a water bath set at 40℃ for 15 min. After 15 min, stopped the reaction by addition of:		
trichloroacetic acid (50 % w/v)	0.30 ml	0.30 ml
Centrifuged the terminated reaction at 13,000 rpm for 10 min (do not mix the tube after centrifugation). Carefully pipetted the supernatant for colorimetric determination of phosphorus		

C. Colorimetric determination of phosphorus: Wavelength: 655 nm, Cuvette: 1 cm light path (glass or plastic; 1.5 ml semi-micro), Temperature: ~ 40℃, Final volume: 1.5 ml, Sample solution: 0.5-7.5 µg of phosphorus in a (1.0 ml sample volume), Read against air (without a cuvette in the light path) or against water.

Pipetted into a 1.5 ml microfuge tube	Sample
sample or phosphorus standard	1.00 ml
colour reagent	0.50 ml
Mixed by vortex and incubated in a water bath set at 40℃ for one h. After one h, mixed by vortex and transferred one ml to a semi-micro cuvette and read the absorbance at 655 nm (A655) within 3 h.	

D. Preparation of phosphorus calibration curve: The standard phosphorus solutions were prepared as described in the table below and treated as samples for the colorimetric determination of phosphorus (see section C above).

Pipetted into 13 ml polypropylene tubes	STD 0 (0 µg)	STD 1 (0.5 µg)	STD 2 (2.5 µg)	STD 3 (5 µg)	STD 4 (7.5 µg)
distilled water	5.00 ml	4.95 ml	4.75 ml	4.50 ml	4.25 ml
phosphorus standard soln.	-	0.05 ml	0.25 ml	0.50 ml	0.75 ml
total volume	5.00 ml	5.00 ml	5.00 ml	5.00 ml	5.00 ml

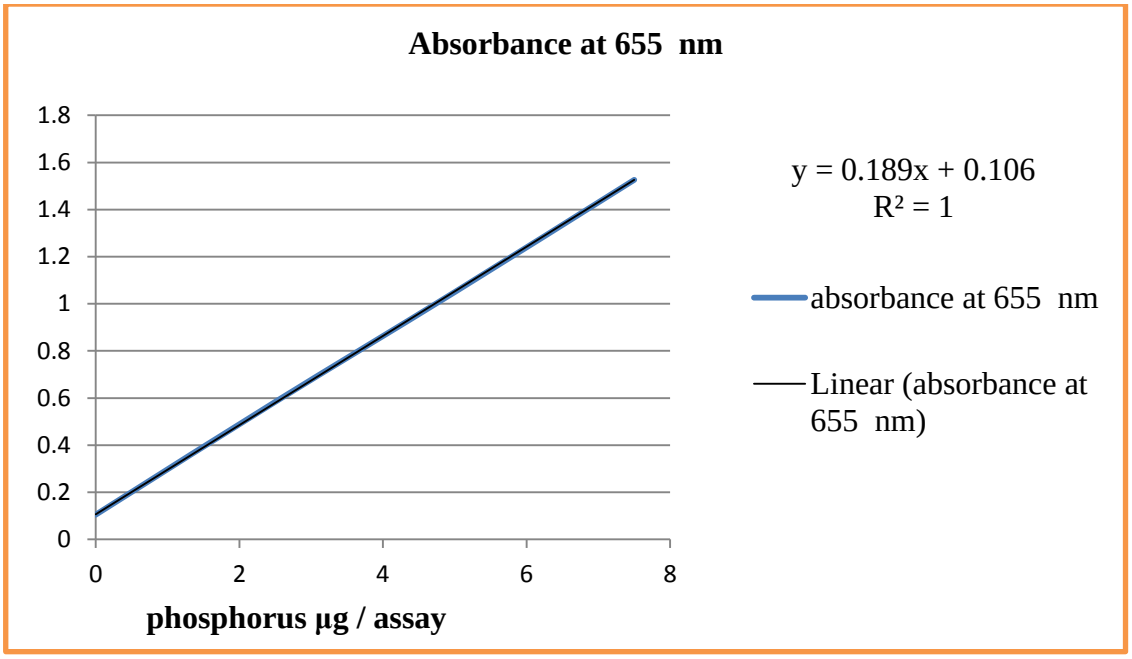

Figure 3.2: Phosphorus Standard Curve for Flaxseed Powder

Calculation:

A. Phosphorus calibration curve: The absorbance (A655) of each phosphorus standard (STD0-4) was determined. The absorbance of STD 0 was subtracted from the absorbance of the other standards (STD 1- 4), thereby leading to ΔA phosphorus.

Calculated M as follows, for each standard (STD 1 - 4):M = P (µg) [µg/ΔA phosphorus]

ΔA phosphorus

3. Calculated the mean M as follows:

Mean, M= (MSTD1 + MSTD2 + MSTD3 + MSTD4 [µg/ΔA phosphorus]

4 'Mean M' was used to calculate the phosphorus content of the test samples in section B.

B. Phosphorus / phytic acid content: The absorbance (A655) of 'Free Phosphorus' and the 'Total Phosphorus' of the sample was determined. The absorbance of the 'Free Phosphorus' sample was subtracted from the absorbance of the 'Total Phosphorus' sample, thereby givingΔA phosphorus.

The concentration of phosphorus was calculated as follows:

C = mean M x 20 x Fx ΔA phosphorus[g/100 g] 10,000 x 1.0 x v

where,

Mean, M = mean value of phosphorus standards [µg/ΔAphosphorus]

20 = original sample extract volume [ml]

F = dilution factor

ΔA = absorbance change of sample 10,000 = conversion from µg/g to g/100 g

1.0 = weight of original sample material [g]

v = sample volume (used in the colorimetric determination step)

It followed for phosphorus:

c = mean M x 20 x 55.6 x ΔAphosphorus [g/100 g]

10,000 x 1.0 x 1.0

= mean M x 0.1112 x ΔAphosphorus [g/100 g]It followed for phytic acid:

c = phosphorus [g/100 g] [g/100 g] 0.282

Note- When the absorbance (A_{655}) obtained for a sample is below 0.100 absorbance units or above that of STD4 refers to "Modified Sample Extraction"

The calculation of the phytic acid content assumes that the amount of phosphorus measured is exclusively released from phytic acid and this is comprises 28.2% of phytic acid.

MODIFIED SAMPLE EXTRACTION

a) Sample with A_{655} above that of STD4: Where the absorbance A_{655} obtained for a sample above that of STD4, repeat the standard assay procedure using 1 g of sample material in 100 ml of 0.66M hydrochloric acid. In this instant the original sample extract volume becomes 100 ml.

c = mean M x **100** x 55.6 x ΔAphosphorus [g/100 g]

10,000 x 1.0 x 1.0

Conversion to phytic acid content (g /100 g) was the same as described above.

b) Sample with A_{655} below0.100: Where the absorbance A_{655} obtained for a sample below 0.100 absorbance repeat the standard assay procedure using appropriate amount of sample material in the sample extraction step as given below Extraction table

A_{655} generated phytic acid (g/L)	Recommended amount of sample material (g)
0.03-0.05	4
0.05-0.10	2.5

3.4.5.2 Estimation of Oxalic Acid

The titration method as described by Day and Underwood (1986) was followed. 1g of sample was weighed into 100 ml conical flask and adds 75 ml 3M H_2SO_4, and stirred for one h with a magnetic stirrer. This was filtered using a What man No one filter paper and take 25 ml of the filtrate and titrated as hot in opposition to 0.05 M $KMnO_4$ solution until a faint pink color persisted for at least 30 s. The oxalate content was calculated by taking 1ml of 0.05 m KMnO4 as equivalent to 2.2 mg oxalate (Chinma and Igyor, 2007).

3.4.5.3 Estimation of Hydrogen Cyanide

The alkaline AOAC titration technique (1984) was used for oxalic acid measurement. The 100 cc sample was distilled by steam into a NaOH solution. Dilute KI solution was used for the distillation. This was then titrated against a solution of 0.02 M $AgNO_3$. The endpoint was reached when a shift from a clear to a faint but permanently murky solution occurred.

The cyanide concentration of hydrogen was measured by using 1ml of 0.02 m $AgNO_3$, equal to 1.08 milligrams of cyanide hydrogen (HCN).

3.5 Statistical Analysis

All the experiments were carried out in triplicate for optimization. Results are expressed as mean ± standard deviation (SD). The data obtained during optimization was analyzed for one-way ANOVA using IBM SPSS Statistics 25 software as a function of multiple comparison Tukey Test ($p<0.05$).

CHAPTER –4

Results and Discussion

4.0 Introduction

A general variety of flaxseed (*Linum usitatissimum*) was collected from the local market of Karnal (Haryana), India. Roasting was done on an open pan up to puffing of flaxseed. Then it is grinded into powdered form and the powdered samples were analyzed for the effect of roasting on nutritional and anti-nutritional components.

The development of milk flaxseed–based probiotic beverage was conducted in different phases. In the first phase, four probiotic organisms were screened for their fermentation dynamics like rate of acidification, specific growth rate (k), Generation time (t_g), etc. The particular selected probiotic microorganisms were incorporated in milk for curd making and this curd was used for making milk flaxseed-based beverage. The milk flaxseed-based probiotic beverage was characterized in terms of sensory, chemical, and rheological aspects. The results obtained during this work are discussed in this section.

4.1 Development of milk-flaxseed based probiotic beverages

4.1.1 Collection of probiotic strains

Four probiotic organisms of ***Lactobacillus rhamnosus*** (NCDC-24, NCDC-610) and ***Lactobacillus acidophilus*** (NCDC-195, NCDC-291) were collected from the National Collection of Dairy Cultures (NCDC) microbiology division of National Dairy Research Institute, Karnal (Haryana), India.

4.1.2 Screening of probiotic strain

For screening of probiotic strain initially sterile skim milk has 11% of total solid were prepared and1 percent of inoculation level was fixed to study the fermentation dynamics of different probiotic organisms. The changes in pH, probiotic count, and acidity was monitored at 2 hours interval up to 8 hours and specific growth rate (K) iis the rate of increase of biomass of a cell population per unit of biomass concentration, Generation time (t_g) Reciprocal of specific growth rate, Rate of acidification expressed as 10^{-3} pH units per minute were calculated and the selection of probiotic strain was done based on maximum specific growth rate and minimum generation time.

Table 4.1: pH, Acidity and Probiotic Counts in Sterilized Skim Milk after Eight Hoursof Fermentation

Probiotic culture	pH	Acidity (% of lactic acid)	Probiotic count
NCDC 24	6.00±0.03^{b}	0.261±0.009^{ab}	8.18±0.52^{a}
NCDC 195	5.95±0.02^{b}	0.276±0.005^{a}	7.96±0.34^{a}
NCDC 610	**5.98±0.03^{b}**	**0.270±0.009^{a}**	**8.24±0.01^{a}**
NCDC 291	6.16±0.02^{a}	0.246±0.010^{c}	8.04±0.02^{a}

Mean ±SD (n=3); Mean values with different superscripts are significantly different fromeach other ($p<0.05$).

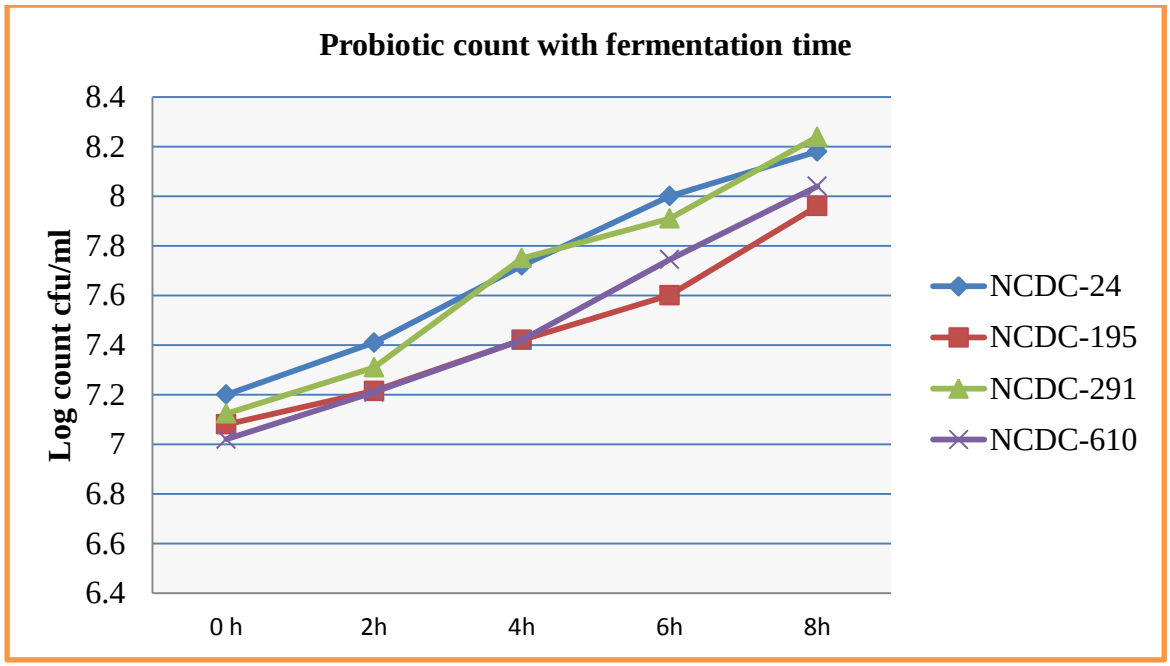

Figure 4.1: Probiotic Count (Log CFU/ml) During Eight Hours of Fermentation inSterile Milk

After eight hours of fermentation, it was observed that maximum probiotic count was foundin the case of NCDC-610 (8.24 Log CFU/ ml) and minimum in case of NCDC-195 (7.96 LogCFU/ ml).

The growing need for *lactobacillus* bacteria uses in industry and the expending growth probiotic market led to a search for which has a high yield of these bacteria after same

fermentation time. The all selected *lactobacillus* strains showed the ability to grow in sterile skim milk media and maximum probiotic count was obtained in the case of NCDC-610. It was may be due to its higher specific growth rate and less doubling time. It was reported that every strains of probiotics have different growth rate and the growth of the probiotis was also affected by the cultural medium (Blandino *et al* 2003).

Table 4.2: Fermentation dynamics of different probiotic strains in sterilized skim milk during fermentation

Probiotic culture	Specific growth rate k (h^{-1})	Generation time t_g(h)	Rate of acidification V_f ($X10^{-3}$ pH units/min)
NCDC24	0.273±0.14[a]	5.065±0.39[a]	1.229±0.04[a]
NCDC195	**0.228±0.11[a]**	**4.38±0.39[a]**	1.305±0.05[a]
NCDC610	**0.302±0.01[a]**	**3.307±0.06[a]**	**1.264±0.04[a]**
NCDC291	0.267±0.01[a]	3.740±0.18[a]	0.910±0.05[b]

Mean ±SD (n=3); Mean values with different superscripts are significantly different fromeach other ($p<0.05$).

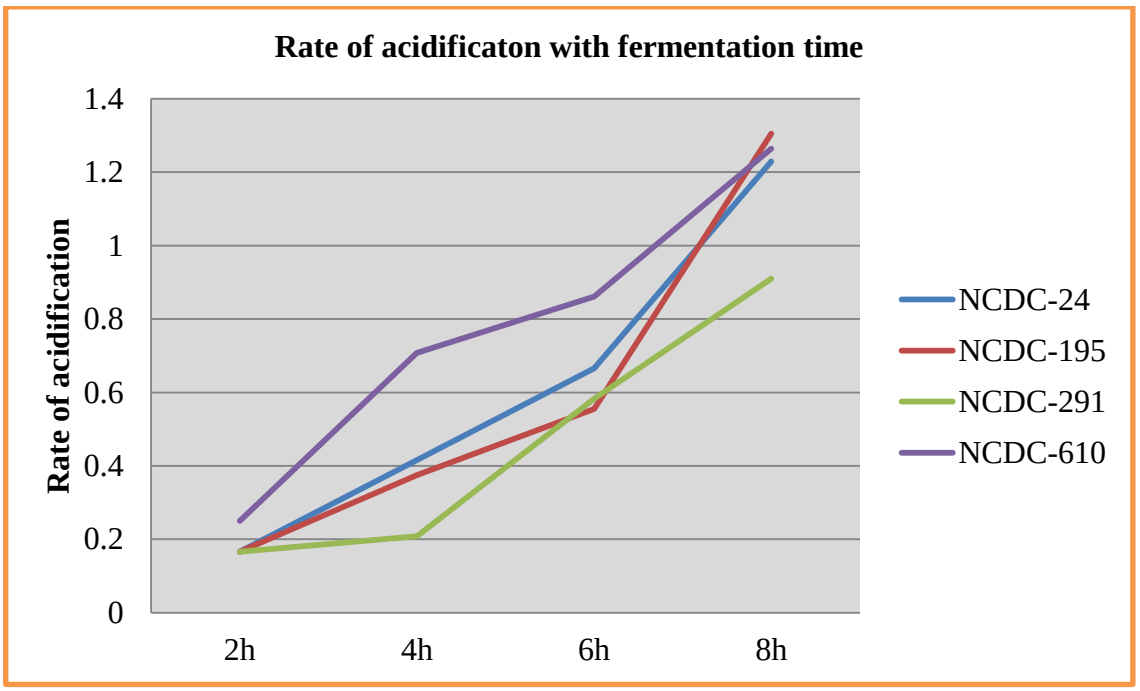

Figure 4.2: Rate of Acidification during Eight Hours of Fermentation in Sterile Skim Milk

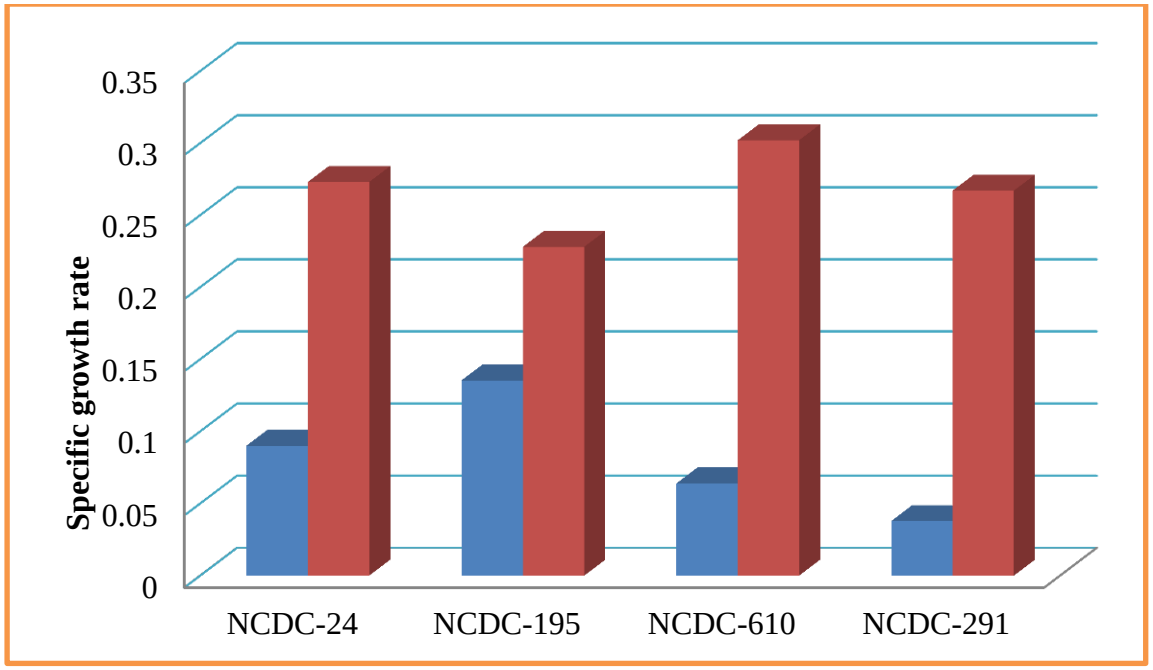

Figure 4.3: Specific Growth Rate during Eight Hours of Fermentation in Sterile Skim Milk

The specific growth rate was found maximum in the case of NCDC-610 around 0.302±0.01

/hour and minimum generation time (t_g) of 3.07±0.06 hours minimum specific growth rate k (h-1) were found in the case of NCDC-195 and generation time was 5.715±0.390 hours and the specific growth rate was found 0.267±0.01 and 0.273±0.14 (h-1) in case of NCDC-291 and NCDC-24 respectively so we can conclude that NCDC-610 gives the best growth in sterile skim milk.

Table 4.3: Lactic Acid Bacteria Count of Different Probiotic Strains in Sterilized Skim Milk-Flaxseed Based Substrate (2%) During Eight Hours of Fermentation

Probiotic Culture	Specific growth rate k (h^{-1})	Generation time t_g(h)	Probiotic Count
NCDC24	0.140 ± 0.02^{a}	7.277 ± 1.29^{a}	7.70 ± 0.52^{b}
NCDC195	0.206 ± 0.01^{b}	4.861 ± 0.27^{b}	8.02 ± 0.34^{a}
NCDC291	0.176 ± 0.02^{a}	5.721 ± 0.09^{b}	7.67 ± 0.02^{b}
NCDC610	0.359 ± 0.02^{c}	2.784 ± 0.64^{c}	8.21 ± 0.01^{a}

Mean ±SD (n=3); Mean values with different superscripts are significantly different from each other ($p<0.05$).

Table 4.2 and 4.3 illustrates the effect of Flaxseed flour on specific growth rates (k) and generation time (t_g) as well as viable count.

The specific growth rates (k) after 8 h for control (Sterile Skim milk without flaxseed flour) and skim milk containing @ 2% flaxseed flour for NCDC-24 were 0.273±0.14 hours and 0.140±0.02 hours respectively, for NCDC-195 were 0.228±0.11 and 0.206±0.01 per hours respectively. The specific growth rates (k) for NCDC-291 were 0.267±0.1 and 0.176±0.02 per hour respectively and k for NCDC-610 were 0.302±0.01 and 0.359±0.01 per hour respectively.

It was indicating that specific growth rates (k) for NCDC-195 and NCDC-610 have increasedsignificantly in presence of flaxseed in milk The increase in growth rate due to the addition ofRFSF(Roasted flaxseed flour) in milk substrate may be due to an increase in the availability of different nutrient contents such as different sugars like galactose, xylose, arabinose and rhamnose or minerals such as manganese, iron, zinc, etc., which is an important growth factor of the organism (Blandino *et al* 2003). These increases in specific growth rates may be caused by the presence of unsaturated fatty acid acids such as linoleic acid and ALA. The fatty acid content in the cultural medium highly affects the composition of the cell membrane of *Lactobacillus sp*. (Florence *et al* 2016). On the other hand, unsaturated fatty acids are prone to fast oxidation due to unsaturated bonds in their molecules. ALA most commonly undergoes auto-oxidation caused by atmospheric oxygen and oxidation caused by hydrogen peroxide which is produced naturally in foodstuffs.

Oxidation products are generally aldehydes that are known to cause unpleasant sensoryproperties in fermented products. Flaxseed is a richer source of polysaccharides and proteins.

Specific growth rates (k) for NCDC-24 and NCDC-291 have decreased significantly in presence of flaxseed in milk The decrease in growth rate due to the addition of Flaxseed flourin milk substrate may be due to the presence of different anti-nutritional factors in flaxseed such as phytic acid, oxalate and hydrocyanic acid. A study was carried out in which wheat flour, maize flour, barley and rye flour was added in the growth medium of *lactobacillus* strains and it was found that growth rate was increase with compared to control cultural medium.

A another study was carried out regarding influence on the growth of probiotic microorganisms in milk by variety of flaxseed composition *lactobacillus acidophilus, lactobacillus helveticus* and *bifidobacterium sp.* In this study it was found that *lactobacillus helveticus* exhibited growth in the presence of unstable growth in the presence of different flaxseed varieties and more repetition of the experiment required. *Bifidobacterium Sp.is* sensitive to environmental conditionsand usually required an addition of more nutrients compared to milk (Florence *et al.*, 2016).

The generation time (t_g) increase for NCDC-24 from 5.065 hours to 7.277 hours in presence of flaxseed in sterile skim milk and generation time (t_g) were also increased for NCDC-291 from 3.704 to 5.71 hours and the generation time (t_g) was reduced for the NCDC-195 and NCDC-610 were 5.71 to 4.861 and 3.302 to 2.78 hours respectively. The higher reduction was recorded for (NCDC-610) 15.80 percent with the addition of 2% flaxseed flour in sterile milk –flaxseed based substrate.

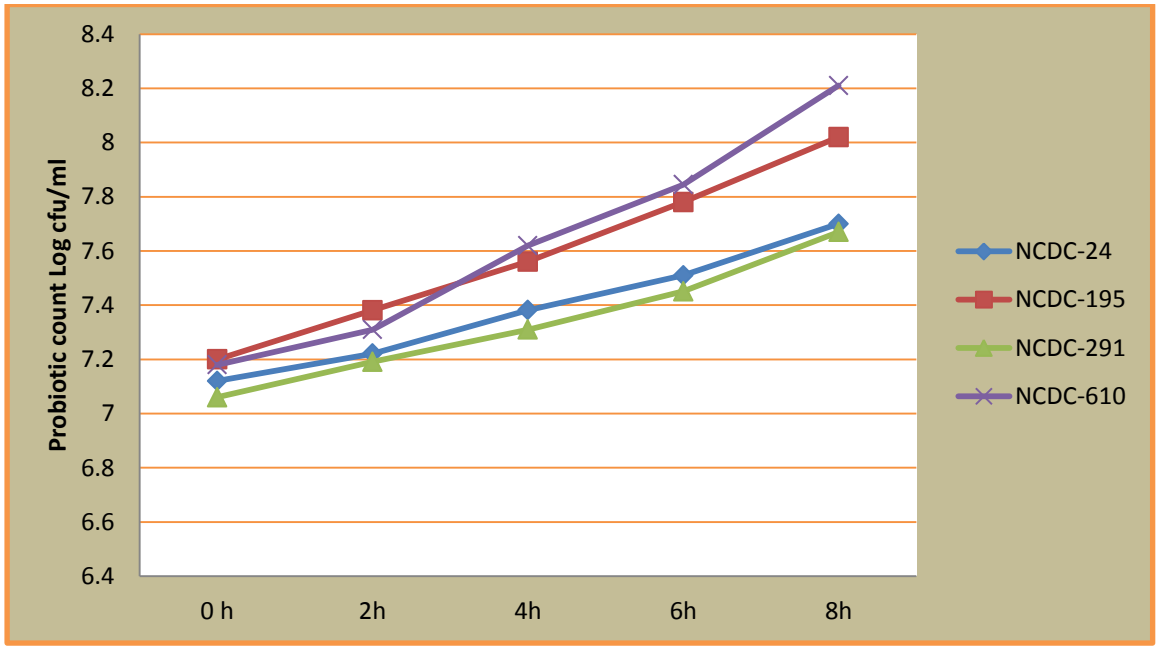

Figure 4.4: Probiotic Count during Eight Hours of Fermentation in Milk Flaxseed (2%)Based Substrate

It was found that flaxseed significantly affects the growth of probiotic organisms in presence of flaxseed in milk during the fermentation process. It adversely affects the growth of NCDC- 24 as well as NCDC-291 and significantly increases its generation time. It is maybe due to the presence of anti-nutritional factors in flaxseed (Smolova *et al.*, 2017).

Phytic acid has ability to bind trace metals like iron and copper which are the important growth factors for the probiotics (Steer and Gibson, 2002). Some strains of *lactobacillus* have ability to degradation of phytic acid. In a study twelve strains of lactic acid bacteria were examined for their ability to degrade phytic acid when its degradation was observed and results were founds that some strains have ability to degradation of phytic acid and some strains were show no effect on phytic acid degradation (Shirani *et al.*, 2008).

It was found that flaxseed significantly decreases the Generation time of NCDC-195 and NCDC- 610.

4.1.3 Effect of Inoculation level of Lactobacillus rhamnosus (RSI 3) on pH, acidity, and probiotic count in sterilized skim milk

Table 4.4: Inoculation Level

Inoculation level	pH	Acidity (%lactic acid)	Probiotic count
NCDC 610-1%	6.29±0.030^{a}	0.186±0.005^{d}	8.30±0.005^{c}
NCDC 610-2%	6.13±0.005^{b}	0.219±0.005^{c}	8.50±0.094^{b}
NCDC 610-3%	5.99±0.005^{c}	0.249±0.005^{b}	8.58±0.039^{b}
NCDC 610-4%	5.89±0.015^{d}	0.276±0.005^{a}	9.00±0.343^{a}

Mean ±SD (n=3); Mean values with different superscripts within a column are significantly different from each other ($p<0.05$).

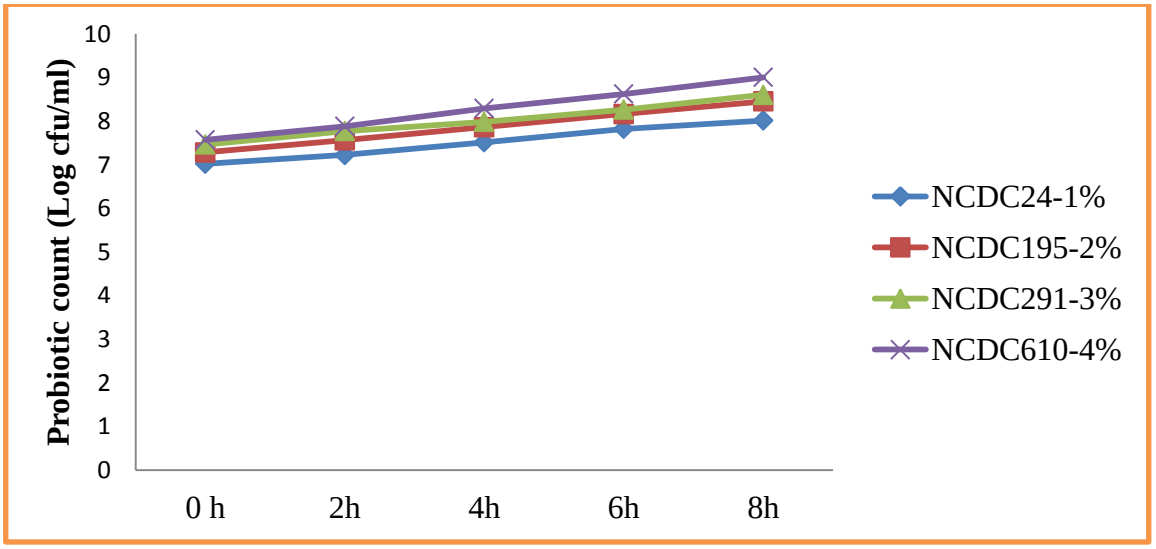

Figure 4.5: Effect of Inoculation Level on LAB Count during Eight Hours of Fermentation in Sterile Skim Milk

It was found that when the level of inoculation increased from 1 to 4% the viable counts as well as the rate of acidification were increased during 8 hours of incubation time at 37°C the viable counts were 8.30, 8.50, 8.58, and 9.0±0.343 Log10(CFU/ml) for the level of inoculation 1%,2%, 3%, and 4% respectively. Because the level of inoculation increases than the higher initial probiotic count in the milk or fermentation medium.

It was found that as the level of inoculation increased the rate of acidification also increases. Due to the higher number of starter culture organisms in the fermentation medium.

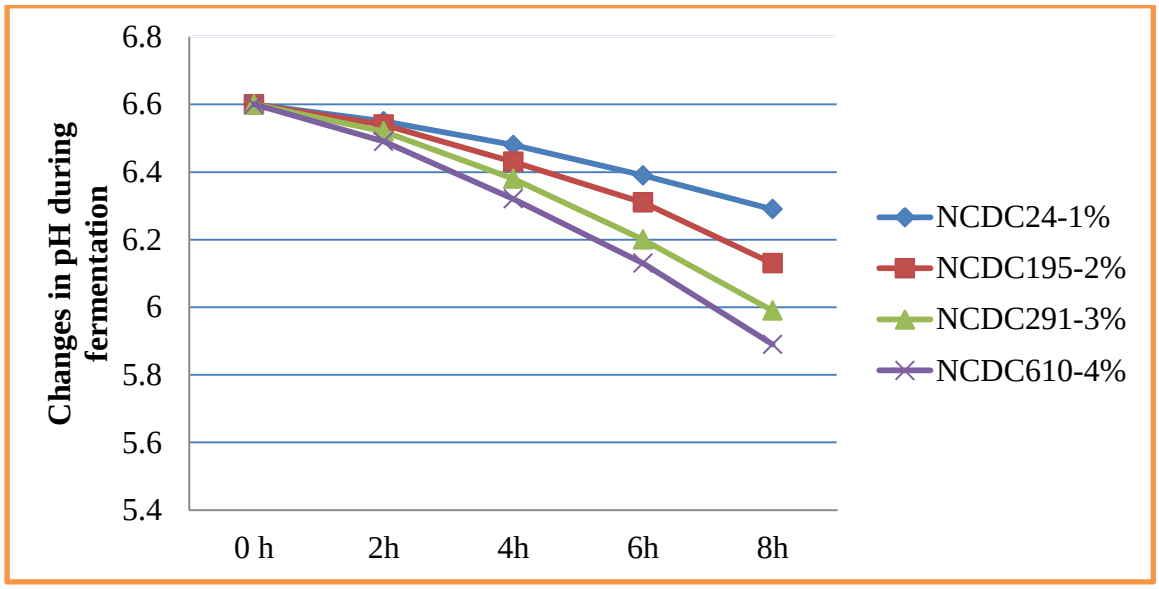

Figure 4.6: Effect of Inoculation Level on pH during Eight Hours of Fermentation inSterile Skim Milk

It was found that when the level of inoculation increases from 1 to 4% in cultural medium after a fix time of fermentation(8h) the maxim mum decreases in the pH was found in case of 4% inoculation level and minimum in case of 1% inoculation level I was due to the higher probiotic count in 4% cultural medium than 15 cultural medium.

4.1.4 Final optimization of fermentation condition for Lactobacillus rhamnosus (RSI 3) based on fermentation time.

Table 4.5 indicates that as the level of inoculation increased from 1 to 4 % in milk ther the curd setting time decreased significantly from 13.5±0.58 hours to 10.5±0.41 hours respectively.

Table 4.5: Level of Inoculation

Level of inoculation (%)	Curd setting time in hours (Final pH between 5.0 to 5.1)	Probiotic count
1	13.50±0.58[a]	8.30±.005[c]
2	12.63±0.48[a]	8.50±.094[b]
3	11.69±0.37[b]	8.58±.039[b]
4	10.50±0.41[b]	9.00±0.343[a]

Mean ±SD (n=3); Mean values with different superscripts within a column are significantly different from each other ($p<0.05$)

It was found that as per the level of inoculation increased from 1 to 4% than considering 1% level of inoculation for curd setting time as control than an increase in the level of inoculation to 2%, 3% and 4% than % reduction in curd setting time were 7.51%, 13.49%, and 22.23 % respectively. Maximum reduction in curd setting time was found 22.23% in case of 4% level of inoculation. Fermentation time was restricted to 10 hours for quick fermentation so a 4 percent level of inoculation was selected for the product development.

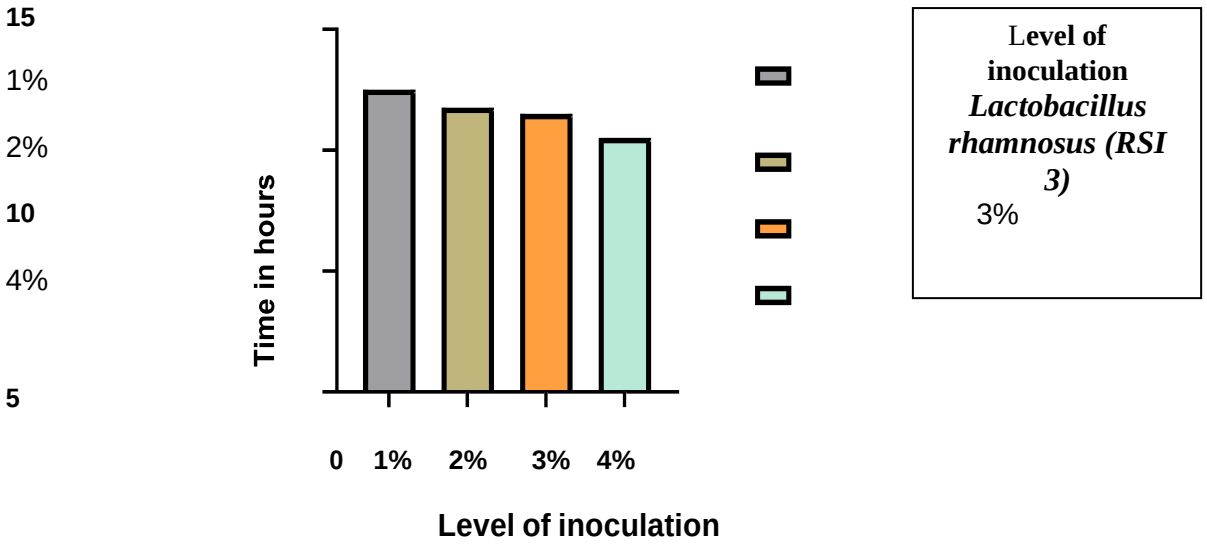

Figure 4.7: Effect of Inoculation Level on Fermentation Time during Eight Hours ofFermentation in Sterile Skim Milk

4.1.5 Preparation of Probiotic curd for product development

Probiotic inoculam was prepared by culturing @ 1-2% of culture in sterile skim milk having 11 to 12 percent of total solid and it was incubated at 37°C for 16 hours and it was kept in refrigerated condition. For preparation of probiotic curd for product making cow and skim milk were standardized up to 2% of fat and 12 % of total solid by adding skim milk powder after than standardized milk has been treated at 90°C for 10 minutes and cooled up to 37°C after cooling of milk @4% of culture was inoculated and incubation was done at 37°C for 10 hours. After 10 hours of incubation probiotic curd was analyzed for its acidity, probiotic count, and total solid. The characteristic of the probiotic curd which was mixed with flaxseed flour, sugar syrup, and stabilizer are given below

- Acidity = 0.65±0.02% lactic acid
- Total solid = 14.12±0.06%
- Probiotic count = 10.32±0.03 log cfu/ ml.

4.2 Analysis of raw and roasted flaxseed flour

Table 4.6: Composition of Raw and Roasted Flaxseed Flour

Parameter	Raw flaxseed flour	Roasted flaxseed flour
Moisture (%)	7.95 ± 0.17^{a}	1.93 ± 0.07^{b}
TS (%)	91.95±0.48a	98.04 ± 0.19^{b}
Ash content (%)	2.54 ± 0.09^{a}	2.83 ± 0.07^{b}
Fat (%)	43.07 ± 0.50^{a}	48.92 ± 1.52^{b}
Protein (%)	24.95 ± 0.63^{a}	26.34 ± 0.46^{b}
Crude fibre (%)	5.33 ± 0.08^{a}	6.14 ± 0.14^{b}
Oxalate content(mg/Kg)	5.14 ± 0.71^{a}	4.13 ± 0.21^{b}
Hydrocyanic acid(mg/100g)	632 ± 5.50^{a}	588 ± 4.58^{b}
Phytic acid(g/100g)	1.24 ± 0.06^{a}	1.06 ± 0.07^{b}

Mean ±SD (n=3); Mean values with different superscripts aresignificantly different from each other ($p<0.05$) between rows.

The fat content of raw flaxseed was found to be 43.07±0.50% while that roasted flaxseed was48.92±1.52 percent.

It was reported that depending on the cultivar and growing conditions, flaxseed contains 40- 50% oil and meal of which above 50% is α-linolenic acid (Choo et al., 2007). The key resource of oil in flaxseed is found in the flaxseed cotyledon (75%) and the leftover oil is found in the seed coat/hull (22%) and the embryo/germ (3%). Triacylglycerol is the mainly common lipid type found in flaxseed. The four leading triacylglycerols in flaxseed oil are trilinolenoylglycerol (LnLnLn) at 30% dilinolenoyllinoleoylglycerol (LnLnL) at 19%, and dilinolenoylpalmitoylglycerol (LnLnP) at 7%. The fatty acid supply of flaxseed is principally calm of unsaturated fatty acids: oleic, linolenic, and linoleic acids. These 3 unsaturated fatty acids report for above 70% of the entire fatty acid supply (Schorno, 2006). The majority of fatty acid found in flaxseed is α-linolenic acid (ALA).

By means of oil content of flaxseed significant difference ($P<0.05$) was found between roasted and raw flaxseed, roasted flaxseed have higher amount of oil content it seems that thisfact could be due to loss of moisture at elevated temperature.

In the present study moisture content of the raw and roasted flaxseed was found to be 7.95±0.17 and 1.93±0.07% respectively, while the Total solid content was 91.95±0.48 and 98.04±0.19 percent respectively, ash content of raw and roasted flaxseed was found to be 2.54±0.09 and 2.83±0.07 percent. It is reported that flaxseed contains 7.7% moisture and 3.4% ash, which is the mineral-rich residue left after samples are burned (Morris, 2003). From the results it was concluded that the chemical, minerals and nutritional properties of flaxseed were significantly improved by the roasting process.

Moisture content of flaxseed was decreases by roasting because of evaporation of moisture at higher temperature. The total solid content of flaxseed was increases due to loss of moisture during roasting of flaxseed.

It was found that non roasted flaxseed contain 5.33±0.08 % of crude fibre while roasted flaxseed was contain 6.14±0.14% of crude fibre. Results reported are in close agreement with these finding of (Pant and Awasthi, 2015).

4% ash, 5% viscous fibre (mucilage). Flaxseeds give out as a greater source of both insoluble and soluble dietary fibre. Most of the soluble fiber of flaxseed appears to be the mucilage of the seed coat. It is around 7 – 10 % of seed weight (Mazza *and* Biliaderis, 1989).

The protein content was found 24.95±0.63 and 26.34±0.46 percent in raw and roasted flaxseed flour respectively.

It was reported that flaxseed contains around 10.5 -31% of protein content (Oomah and Mazza, 1993). Differences in protein can be quantified to both genetics and the environment. The nearby protein content of dehulled and defatted flaxseed wide-ranging considerably depends upon cultivar growth location and seed processing. Hull fraction contains lower protein levels and that dehulling increases the protein level of flaxseed protein level from 19.2% to 21.8% (Oomah and Mazza, 1997). Albumin and globulin kind proteins are the key proteins in flaxseed.

The hydrocyanic acid content in raw flaxseed was found to be 632±5.50 mg/kg which decreased to 588±4.58mg/kg on open pan roasting up to puffing or 30 seconds. The hydrocyanic acid content in raw flaxseed was reduced by 6.96%, on 30 seconds of roasting.

It was found that HCN was reduced up to 85% when it was heated for 2hours at 200 °C (Park *et al.,* 2005). Feng *et al.,* 2003 reported that Microwave roasting of flaxseed reduced 83% of HCN content due to the deactivation of glycoside and evaporation of HCN. Cyanogenic glycosides are heat liable and easily by different processing methods like roasting, Microwave roasting, and certain detoxifying enzymes like β-glycosidases, releasing hydrogen cyanide which can be evaporated by using steam (Cunnane *et al.*, 1993).

The phytic acid content in raw flaxseed was found to be 1.24±0.06mg/100g which decreased to 1.06±0.07mg/100g in an open pan roasting up to puffing or 30 seconds. The phytic acid content in raw flaxseed was reduced by 14.51%, on 30 seconds roasting time.

It was reported that the amount of phytic acid in flaxseed varies from 0.80- 1.50 % of the dry seed weight (Oomah *et al.*, 1996). It was found that Germination may reduce phytic acid up to 40% in flaxseed. It was reported that open pan roasting of flaxseed for 4 minutes was result in decrease of phytic acid from 2.03g/100g to 1.51g/100g.

Oxalate content in raw flaxseed was found to be 5.41±0.71mg/kg which decreased to 4.13±0.21mg/kg in an open pan roasting up to puffing or 30 seconds. Oxalate content in raw flaxseed was reduced by 23.65%, on 30 seconds roasting time.

In a study, it was reported that oxalate content in raw flaxseed range from 2 to 10 mg/kg (Hiremath, 2013). The sample was contained 5.41±0.71mg/kg oxalate; hence it may be

considered a low oxalate food. Hui (1992) stated that intake of 5g or more oxalic acid would be fatal to humans but the negative effect can be seen at even low values.

4.3 Optimization of beverage formulation

The ratio of roasted flaxseed flour, sugar, and stabilizer was optimized through one-way ANOVA. The level of probiotic culture and fermentation time was kept constant as optimizedin preliminary studies.

For optimization of beverage 15 treatments were taken in which two variables were fixed and one variable was changed to study its effect on sensory and physicochemical properties of thebeverage.

Variables:-

I. Roasted flaxseed flour (4- 6% curd basis).
II. Sugar (15 -25 % curd basis)
III. Stabilizer (Pectin 0.15-0.45% curd basis)

Responses observed were

- Sensory Parameters (Consistency, sweetness, particulate matters, rancid, oxidized andoverall acceptability,etc
- Probiotic Count
- pH
- Viscosity

Table 4.7 was indicating that as the level of roasted flaxseed flour increased and the level of stabilizer was constant then there was a significant increase in the particulate matters in the beverage due to the higher flaxseed particles in the beverage and the color of the beverage turns to off white when the leveled of roasted flaxseed flour was increasing in the beverage. Particulate matters were slightly increases in the beverage if level of stabilizer was constant and increases in the quantity of RFSF because flaxseed was contain more than 50% of oil content so during grinding of flaxseed it was not possible to ground flaxseed in too much smaller particles. If flaxseed was converted into too much small particles then oil separation occurred which leads to oxidative changes inthe product. Particulate matters were not increases too much with increase in the level of flaxseed flour due to the presence of soluble fibre mucilage which has ability to bind

1500 g of water per 100 g of solids so its increases its viscosity result in lesser particulate matter were observed. The color of the beverage was going to off white with increases in flaxseed flourdue to the blackish-brown color of flaxseed flour.

Table 4.7: Treatment Codes

Treatment	Flour CB	StabilizerCB	Sugar CB
T1	4%	0.15%	15%
T2	4%	0.15%	20%
T3	4%	0.15%	25%
T4	4%	0.30%	15%
T5	4%	0.30%	20%
T6	4%	0.30%	25%
T7	4%	0.45%	15%
T8	4%	0.45%	20%
T9	4%	0.45%	25%
T10	5%	0.15%	20%
T11	5%	0.30%	20%
T12	5%	0.45%	20%
T13	6%	0.15%	20%
T14	6%	0.30%	20%
T15	6%	0.45%	20%

Where CB- Curd basis,

Table 4.8: Effect of Different Treatments on Particulate Matters and Color of Beverage

Treatment	Particulate matters	color
T1	8.17 ± 0.76^{de}	4.17 ± 0.289^{abc}
T2	7.67 ± 0.58^{cde}	4.33 ± 0.284^{abc}
T3	7.66 ± 0.29^{cde}	4.16 ± 0.312^{abc}
T4	6.5 ± 0.5^{abc}	4.34 ± 0.281^{abc}
T5	6.66 ± 0.58^{abcd}	4.26 ± 0.5^{abc}
T6	6.5 ± 0.5^{abc}	4.27 ± 0.288^{abc}
T7	5.83 ± 0.29^{ab}	4.31 ± 0.115^{abc}
T8	5.67 ± 0.29^{a}	4.33 ± 0.278^{abc}
T9	6.2 ± 0.20^{ab}	4.21 ± 0.5^{abc}
T10	8.20 ± 0.28^{cde}	3.76 ± 0.212^{ab}
T11	7.17 ± 0.24^{bcde}	3.81 ± 0.305^{ab}
T12	6.83 ± 0.32^{abcd}	3.8 ± 0.264^{ab}
T13	8.29 ± 0.29^{cde}	3.6 ± 0.114^{a}
T14	6.82 ± 0.12^{abcd}	3.52 ± 0.115^{a}
T15	6.67 ± 0.32^{abcd}	3.57 ± 0.157^{a}

Table 4.9: Effect of Different Treatments on Sweetness, Acidic and Free Whey of Beverage

Treatment	Free whey/ Oxidized/ Rancid/Bitter/Ropiness	Acidic (Nil-Pronounced)	Sweet Nil-pronounced
T1	0±00	4.17 ± 0.24^{bc}	6.17 ± 0.28^{a}
T2	0±00	4.33 ± 0.28^{cd}	$6.83\pm.31^{ab}$
T3	0±00	3.6 ± 0.20^{ab}	8.67 ± 0.29^{c}
T4	0±00	4.12 ± 0.31^{cde}	6.33 ± 0.26^{ab}
T5	0±00	4.63 ± 0.15^{cde}	6.77 ± 0.25^{ab}
T6	0±00	3.26 ± 0.28^{a}	8.33 ± 0.57^{c}
T7	0±00	4.73 ± 0.27^{cde}	6.6 ± 0.18^{ab}
T8	0±00	4.9 ± 0.12^{de}	7.08 ± 0.12^{b}
T9	0±00	3.4 ± 0.20^{a}	8.16 ± 0.29^{c}
T10	0±00	4.33 ± 0.28^{cd}	6.67 ± 0.27^{ab}
T11	0±00	4.5 ± 0.10^{cde}	6.6 ± 0.18^{ab}
T12	0±00	4.43 ± 0.21^{cde}	6.83 ± 0.15^{ab}
T13	0±00	4.56 ± 0.11^{cde}	6.73 ± 0.25^{ab}
T14	0±00	4.93 ± 0.15^{e}	6.36 ± 0.16^{ab}
T15	0±00	4.87 ± 0.17^{de}	6.46 ± 0.21^{ab}

Table 4.9 was indicating that as per the level of RFSF was increases then there was no effect on oxidized, rancid flavor off beverage may be due to the absence of any kind of off flavoring

Component in the product and any oxidative substances in the flaxseed flour and other ingredients and it were also found that no significant effect on the acidity of beverage but when the level of sugar increased from 15 to 25% it sweetness increases significantly

Table 4.10: Effect of Different Treatments on Consistency, Granularity and Overall Acceptability of Beverage.

Treatment	Consistency (Too thin-too thick)	Granularity (Nil- Extreme)	OA (Dislike extremely –Like extremely)
T1	$5.170.29^{ab}$	6.13 ± 0.23^{d}	6.83 ± 0.29^{abc}
T2	5.06 ± 0.12^{a}	6.17 ± 0.28^{d}	8.16 ± 0.15^{de}
T3	5.2 ± 0.20^{ab}	6.2 ± 0.15^{de}	6.16 ± 0.30^{a}
T4	6.33 ± 0.32^{cd}	5.23 ± 0.06^{abc}	6.66 ± 0.57^{abc}
T5	6.53 ± 0.18^{de}	5.03 ± 0.15^{abc}	8.13 ± 0.23^{de}
T6	6.56 ± 0.35^{def}	5.13 ± 0.12^{abc}	6.33 ± 0.57^{a}
T7	7.66 ± 0.20^{f}	5.1 ± 0.18^{abc}	7.17 ± 0.28^{abcd}
T8	7.56 ± 0.32^{f}	4.9 ± 0.20^{ab}	8.33 ± 0.28^{de}
T9	7.3 ± 0.34^{ef}	4.9 ± 0.24^{a}	6.5 ± 0.5^{ab}
T10	5.97 ± 0.45^{bcd}	6.22 ± 0.17^{ef}	7.34 ± 0.57^{abcde}
T11	6.5 ± 0.10^{de}	5.43 ± 0.21^{bc}	7.66 ± 0.46^{bcde}
T12	7.17 ± 0.29^{ef}	5.37 ± 0.16^{bc}	7.33 ± 0.51^{abcde}
T13	5.66 ± 0.28^{abc}	6.48 ± 0.15^{f}	6.83 ± 0.38^{abc}
T14	6.76 ± 0.25^{def}	5.62 ± 0.06^{c}	8.21 ± 0.5^{e}
T15	7.23 ± 0.24^{ef}	5.53 ± 0.12^{c}	7.91 ± 0.21^{cde}

Table 4.10 was showing that when the level of RFSF was increasing its consistency was increases significantly due to the presence of water-soluble fibre mucilage in the flaxseed flour which had watered bound capacity and decreases granularity by increasing consistency. When the level of sugar increased from 15 to 20 percent then its overall acceptability was increasing but above 20 percent of the sugar level, its overall acceptability was decreasing significantly. Overall acceptability was also decreased with an increase in roasted flaxseed flour when the stabilizer level was constant due to the increases in the granularity of the beverage.

It was reported that flaxseed proteins show a good water absorption capacity than soy protein, which is the most frequent plant-based protein utilized in the food industry. As reported by (Madhusudhan and Singh, 1985a, b), the water absorption capacity of ground flaxseed, boiledground flaxseed, and soy meal were 717, 897, and 630 g/100g protein, respectively.

It was also reported that that the flaxseed mucilage can alter viscosity. Flaxseed mucilage exhibits shear thinning with improved shear rate, i.e. Non-Newtonian behavior, at a concentration above 0.2%, whereas at a lower concentration it exhibits constant viscositywith increased shear rate, i.e. Newtonian behavior (Mazza and Biliaderis, 1989).

Overall acceptability of product was 6.83, 8.16, and 6.16 when sugar levels were 10, 12, and 14 percent respectively. Overall acceptability was found maximum in the case of 12% of the level of sugar in the product.

When the level of stabilizer was increased from 0.1, 0.2, and 0.3 percent in the product then the consistency of the product increased because pectin can form a mess that trips liquid.

Table 4.11: Effect of Different Treatments on pH, wheying off and Probiotic Count ofBeverage

Treatment	pH	Probiotic Count (Log cfu/ml)	Wheying off (ml)
T1	5.33 ± 0.042^{ab}	7.67 ± 0.072^{a}	4.5 ± 0.1^{f}
T2	5.28 ± 0.02^{abc}	7.65 ± 0.147^{a}	4.53 ± 0.05^{f}
T3	5.27 ± 0.005^{abc}	7.65 ± 0.081^{a}	4.52 ± 0.06^{f}
T4	5.31 ± 0.01^{abc}	7.70 ± 0.102^{a}	3.7 ± 0.1^{de}
T5	5.31 ± 0.043^{abc}	7.64 ± 0.130^{a}	3.73 ± 0.12^{de}
T6	5.28 ± 0.047^{abc}	7.70 ± 0.102^{a}	3.76 ± 0.15^{e}
T7	5.25 ± 0.012^{c}	7.62 ± 0.066^{a}	3.47 ± 0.06^{bcd}
T8	5.24 ± 0.005^{c}	7.85 ± 0.066^{a}	3.57 ± 0.05^{cde}
T9	5.25 ± 0.015^{abc}	7.74 ± 0.219^{a}	3.6 ± 0.1^{cde}
T10	5.32 ± 0.04^{abc}	7.69 ± 0.207^{a}	4.36 ± 0.08^{f}
T11	5.29 ± 0.015^{abc}	8.01 ± 0.091^{a}	3.56 ± 0.09^{cde}
T12	5.24 ± 0.012^{c}	8.03 ± 0.056^{a}	3.33 ± 0.05^{abc}
T13	5.33 ± 0.02^{a}	8.07 ± 0.05^{a}	4.23 ± 0.07^{f}
T14	5.28 ± 0.02^{abc}	8.18 ± 0.047^{a}	3.23 ± 0.17^{ab}
T15	5.26 ± 0.026^{bc}	8.03 ± 0.05^{a}	3.18 ± 0.12^{ab}

Table 4.11 was showing that there is no significant effect on pH and probiotic count of beverage when the level of RFSF and stabilizer and sugar were increases. When the level of RFSF was increases and the level of stabilizer is constant then wheying off was decreased due to the presence of soluble fibre mucilage in the flaxseed. When the level of RFSF was 6 percent in beverage and level of stabilizer in beverage were 0.30 and 0.45 percent then there was no significant effect on wheying off of

beverage so 0.30 percent level of stabilizer on curd basis was optimized in the final beverage when the level of roasted flaxseed flour up to 6% on curd basis if level of flour were increases more than 6% than it may change.

The probiotic count in the product was found 8.18±0.047 log CFU/ml in treatment-14. This was meeting the minimum legal requirement of probiotic count as given by the ICMR-DBT (Indian Council of Medical Research and Department of Biotechnology) in any probiotic product.

4.4 Optimized formulation of milk- flaxseed based probiotic beverage

4.4.1 Preparation of probiotic curd

Cow milk and skim milk were taken for standardization of milk fat up to 2% in milk and the standardization of milk was done by using the Pearson's square formula. After the standardization of milk it was heated up to 40°C and skim milk powder was added to adjust 12 percent of solid-not-fat content in the milk to prevent whey separation after that milk was treated at 90°C for 10 minutes and cooled up to 37°C after than @4% on milk basis of selected culture was inoculated and it was incubated for at least 10 hours at 37°C after setting of curd it was tested for acidity, total solid and probiotic count. Roasted flaxseed flour was used for the preparation of the beverage.

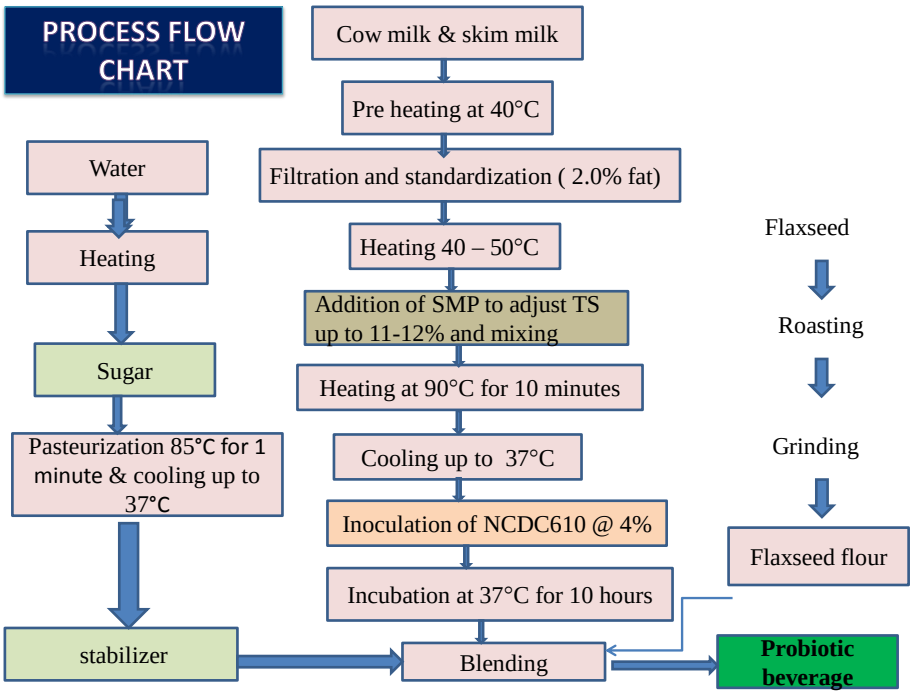

Figure 4.8 Flow Diagram of optimized Milk-Flaxseed Based Probiotic Beverage

4.4.2 Preparation of sugar syrup

For preparation of sugar syrup RO water was used the RO water was preheated up to 40°C then calculated quantity of grinded powdered sugar was added and pasteurization of sugar syrup was did by giving the heat treatment at 85°C for 1 minute and cooled up to 37°C.

Note- Losses in water by evaporation during pasteurization of sugar syrup was maintained by adding of boiled water in sugar syrup.

4.4.3 Preparation of stabilizer solution

For preparation of stabilizer solution RO water was heated up to 71°C and calculated amount of high methoxy pectin was dissolved by using a sterile glass rod this stabilizer solution was cooled up to 37°C.

4.4.4 Levels for the optimized formulation

One kg of curd having 14% of total solid and acidity of 0.65% of lactic acid was blended with 60 g of roasted flaxseed flour, 200 g of powdered sugar, and 3 g of stabilizer (520 to 530 ml of RO water was used to make sugar syrup and stabilizer solution). The final product was having 3.1 to 3.3% of roasted flaxseed flour, 11.2 to 11.4 % of sugar and 0.17 to 0.18% of stabilizer and 22 to 23% of total solids.

4.5 Analysis of Beverage

4.5.1 Chemical/ physical parameters

Table 4.12: Rheological/ Physical Parameters of Beverage

Chemical/ physical parameters	Values
pH	5.29±0.02
Wheying off (ml per 20ml)	3±0.2

N=3 mean ± SD

The pH of the beverage was found 5.29±0.02 and wheying off was 3±0.2 ml/20ml. As per thelevel of flaxseed flour were increased in milk wheying off will decreases due to the presence of soluble fibre in flaxseed.

Table 4.13: Colour Values of Beverage

Color properties	L* (Lightness)	A* (Redness)	B* (Yellowness)
Mean with Standard Deviation	69.87±0.30	2.06±0.67	10.83±0.21

Colour is considered an important parameter for the sensory acceptability of the product. In color measurement L* value indicates lightness (100) to blackness (0), a* indicates redness (+60) to greenness (-60) hues, where b* indicates yellowness (+60) to blueness (-60) hues.

The L* value for the optimized beverage was 69.87±0.30 it is indicated that the beverage waslight in color. Thea* value of the probiotic beverage was 2.06±0.67 and the b* value was 10.83±0.21 which indicates that the values of b* are positive due to the addition of cow milk during the preparation of beverage and cow milk containing carotene which imparts the yellow color of cow milk.

4.5.2 Rheological parameters

4.5.2.1 Viscosity- Viscosity of the beverage was an important parameter. Viscosity of the optimized beverage was measured by using Anton paar rheomter. The viscosity of a fluid is an measure of its resistance to deformation at a given rate and it was describes the internal friction of a moving fluid to identify that the product was thin or thick.

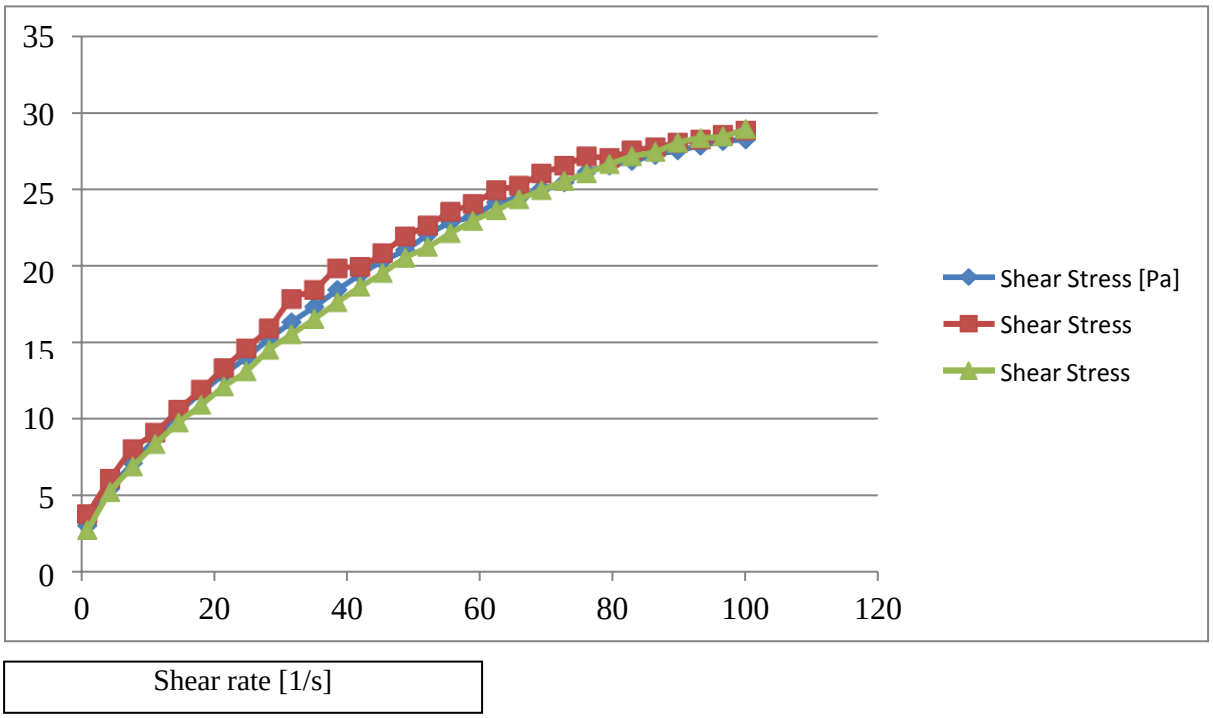

Figure 4.9: Relationship between Shear Stress Vs Shear Rate of optimized beverage

Product	Consistency index (k)	Flow behavior index (n)
Optimized beverage	2.72±0.38	0.519±0.03 (n<1)

The model fitted was the Power-law equation (Oswald-de Waele model, t=kgn)

The optimized product was showing shear thinning (is the non-Newtonian behavior) of fluid whose viscosity decreases under shear strain.

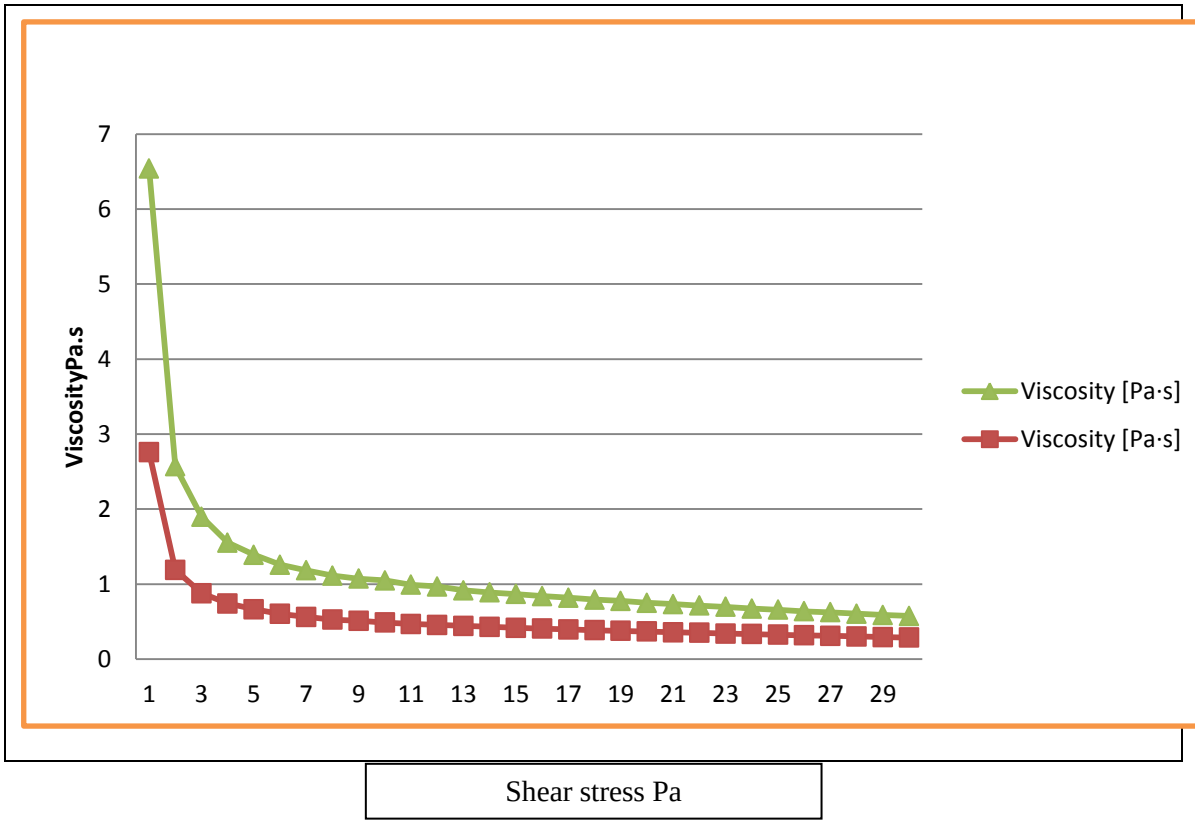

Figure 4.10 Viscosity v/s Shear stress for optimized product

It was reported that flaxseed mucilage can change viscosity. Flaxseed mucilage exhibits shearthinning with amplified shear rate, i.e.non-Newtonian behavior, at a concentration above 0.2%, (Mazza *and* Biliaderis, 1989).

This behavior was caused by the existence of two fractions that possess dissimilar viscosity behavior in flaxseed mucilage. The neutral fraction of flaxseed mucilage exhibited shear thinning. An acidic portion of flaxseed mucilage exhibited Newtonian fluid behavior at every concentration (Cui *et al.*, 1994). The dissimilarity in viscosity actions between the twofractions of the mucilage is primarily caused by the dissimilarity in their molecular size.

4.5.3 Sensory parameters

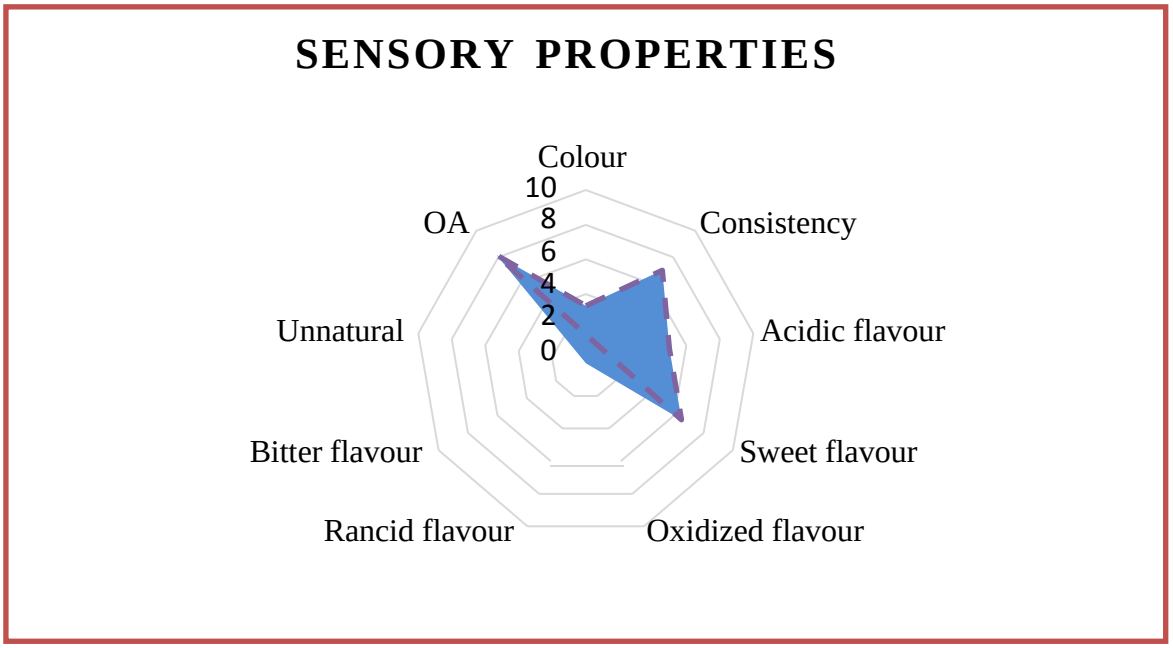

Figure 4.11: Sensory Parameters of Beverage

Figure 4.21 was about the sensory properties of the developed beverage. It is showing that the developed beverage was does not have any kind of unnatural, oxidized, rancid, and bitter flavor. The beverage was have overall good acceptability score. The developed beverage was having medium consistency and medium acidity so it was giving acidic with sweet taste. The absence of any kind of off flavor in the beverage was indicating that there is no oxidation of linolic and ALA was present in the roasted flaxseed flour. The overall acceptability score of developed beverage was high due to the medium consistency, good appearance, low granularity and acidic with sweet taste.

4.5.4 Microbiological parameters (fermented beverage only):

Probiotic count was found 8.41±0.11 Log cfu/ml, coliform and yeast and mold count was not detected in initial dilution at the final product.

Table 4.14: Microbiological Parameters of Optimized Beverage

Microbiologicalparameters	Count (LogCFU/ml)
Probiotic count	8.41±0.11
Coliform	ND*
Yeast *and mold*	ND*

N=3 Mean ± SD *Not Detected in first dilution

4.5.5 Chemical analysis

Table 4.15: Composition of Beverage

Parameters	Per 100ml of beverage
Total solid	22.61±0.12
Fat	2.59±0.05
Protein	4.46±0.08
Carbohydrate	11.24±0.10
Ash	0.71±0.01

Table 4.15 showing that the final product was contained 22.61±0.12% of total solid and 2.59±0.05 percent of fat, 4.46±0.08 percent of protein, 11.24±0.10 percent of carbohydrate, and 0.71±0.01 percent of ash content. protein and fat content in beverages were due to the addition of roasted flaxseed flour in beverage flaxseed flour was contains about 48% of fat and 36% of protein.Increase in

Sensory score card of the optimized beverage

Table 4.16 Sensory parameters of the optimized beverage

Parameters	colour	Free whey	Acidic	Sweet	Oxidized	Unnatural	Consistency	Granularity	Overall acceptability
Score obtained		0	4.93±0.15	6.36±0.16	0	0	6.76±0.25	5.62±0.06	8.20±0.5

4.5.6 Anti-nutritional properties

Table 4.17: Anti-Nutritional Factors Present in Beverage

Ant nutritional factors	Quantity in100ml ofbeverage
Oxalic acid (mg)	0.013±.0.001
Phytic acid (mg)	32.86±.01
Hydrocyanic acid (mg)	6.03±.01

Table 4.16 is indicating the anti-nutritional factors present in the product. The beverage was contained 0.013±.0.001 mg of oxalate per 100ml of product, 32.86±.01mg of phytic acid, and 6.03±0.01mg of HCN per 100ml of product.

It was reported that the mean fatal dose of oxalic acid for an adult is about 15 to 30 g, but the lowest reported fatal dose is merely 5 g (or about 70 mg/kg) (Tsai *et al.*, 2005). Food Safety and Standards Authority of India (FSSAI) mentioned the maximum permissible limit of hydrogen cyanide in food grains as 37.5 mg/kg (FSSR, 2011).

CHAPTER –5

Summary and Conclusions

SUMMARY AND CONCLUSION

To fulfill the objectives, the development of milk-flaxseed- based probiotic beverages was conducted in various phases. Fermentation studies of the selected organisms. Growth of the selected probiotic organism will be observed on.

a) Milk-flaxseed-based composite substrate (0-2%) to select the level of roasted flaxseed flour. optimization of fermentation condition. The level of probiotic culture (1-5%) and fermentation time will be optimized considering minimum curd setting time and suitable probiotic count and acceptable pH. Optimization of Beverage formulation. The ratio ofroasted flaxseed flour, sugar, and stabilizer will be optimized through one-way ANOVA. Thelevel of probiotic culture and fermentation time will be kept constant as optimized in preliminary studies.

5.0 Development of Milk-flaxseed-based probiotic beverage

5.1 Collection of Probiotic Strains

Four indigenious probiotic strains were collected from National Collection of Dairy Cultures(NCDC), NDRI, Karnal, Haryana. They are

1. *Lactobacillus acidophilus* (NCDC-195)
2. *Lactobacillus acidophilus* (NCDC-291)
3. *Lactobacillus rhamnosus* (NCDC-24)
4. *Lactobacillus rhamnosus* (NCDC-610).

5.1.1 Selection of probiotic strains

The specific growth rate (K) and Generation time (tg) was checked for different probiotic strains in sterile skim milk and milk-flaxseed based substrate. The culture(NCDC-610)- *Lactobacillus rhamnosus* has shown minimum generation time (3.307±0.06 hours) and maximum specific growth rate 0.302±0.01 h^{-1}. Therefore NCDC-610 was selected for product development.

5.1.2 Optimization of Level of Inoculation

Different levels (1 to 4%v/v) of *lactobacillus rhamnosus* (NCDC- 610)was inoculated in standardized milk having 2% of fat and 12% of TS and the fermentation was carried out

at 37°C for 10 hours. The level of inoculation @ 4% was optimized on the basis of 10 hours fermentation time.

5.1.3 Development of Probiotic Beverage

For preparation of probiotic beverage cow milk and skim milk were taken and standardized to2% of fat. The standardized milk was heated at 90°C for 10 minutes and cooled up to 37°C and 4% of NCDC-610 was inoculated and incubation was done at 37°C for 10 hours to make probiotic curd. This probiotic curd was taken and blended with roasted flaxseed flour, sugar syrup and stabilizer. The optimization of beverage was done on the basis of sensory, physical and rheological attributes of the beverage.

5.2 Characterization of Milk-Flaxseed- based probiotic Beverage

5.2.1 Composition of Beverage

The Water content, total solid, fat, protein, carbohydrate, sugar and ash content of the optimized beverage was 77-78%, and 22.61%, 2.59%, 4.46%, 11.2-11.4% and 0.71% respectively. The pH of the beverage was found 5.29±0.02 and wheying off was 3±0.2 ml/20 ml.

5.2.2 Level of anti-nutrients and microbial quality of beverage

The major anti-nutrients were present in the beverage are phytic acid, oxalic acid and hydrocyanic acid. The level of phytic acid, oxalic acid and HCN were 32.86±0.01mg, 13±0.001µg, and 16.74±0.01mg in per 100ml of beverage respectively. Food Safety and Standards Authority of India (FSSAI) mentioned the maximum permissible limit of hydrogen cyanide in food grains as 37.5 mg/kg(FSSR 2011) .While the lowest lethal amount of oxalic acid documented is about 5 g (or 70 mg/kg). (Tsai *et al.*, 2005).

5.2.3 Rheological attributes of the beverage

The beverage was non-Newtonian fluid and showing shear-thinning flow properties. The model fitted was power law equation (Oswald-de Waele model, $\tau = k\gamma^n$) and the consistency index (k) was 2.72±0.38 and flow behavior index (n) was estimated as 0.519±0.03.

5.2.4 Color profile of Beverage

The L* value for the optimized beverage was 69.87±0.30 the a* value of the probiotic beverage was 2.06±0.67 and the b* value was 10.83±0.21.

5.2.5 Sensory prfile of beverage

The overall acceptability score of the beverage was 8.21±0.5. The beverage was free fromany kind of off flavor, oxidized and rancid flavor.

5.3 Future Recommendation

Based on the current study the following recommendations are made for future work in thearea of the probiotic flaxseed-based beverage.

5.3.1 Estimation of phytoestrogen content in the product

In the present study phytoestrogen content in the product as well as in the raw and roasted flaxseed flour was not measured the phytoestrogen content can be measured by using HPLC with gas chromatography method. The phytoestrogen was very important from the health management in females.

5.3.2 Storage study of product

Storage study of the developed product was more important to identify its shelf life and physic-chemical changes during storage period as well as the effect on the numbers of probiotic counts in the product with time.

5.3.3 Consumers' acceptability study of product

Consumer acceptability study of the product was necessary for the acceptability of the product for further modifications and for commercial production of the product.

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Annexures

ANNEXURE - 1

Dairy Technology Division, ICAR-NDRI, Karnal
Quantitative Descriptive Sensory Analysis Card

Product: Milk-flaxseed based probiotic beverage **Panelist:** _____

Sample Codes: _____ **Date:** _____

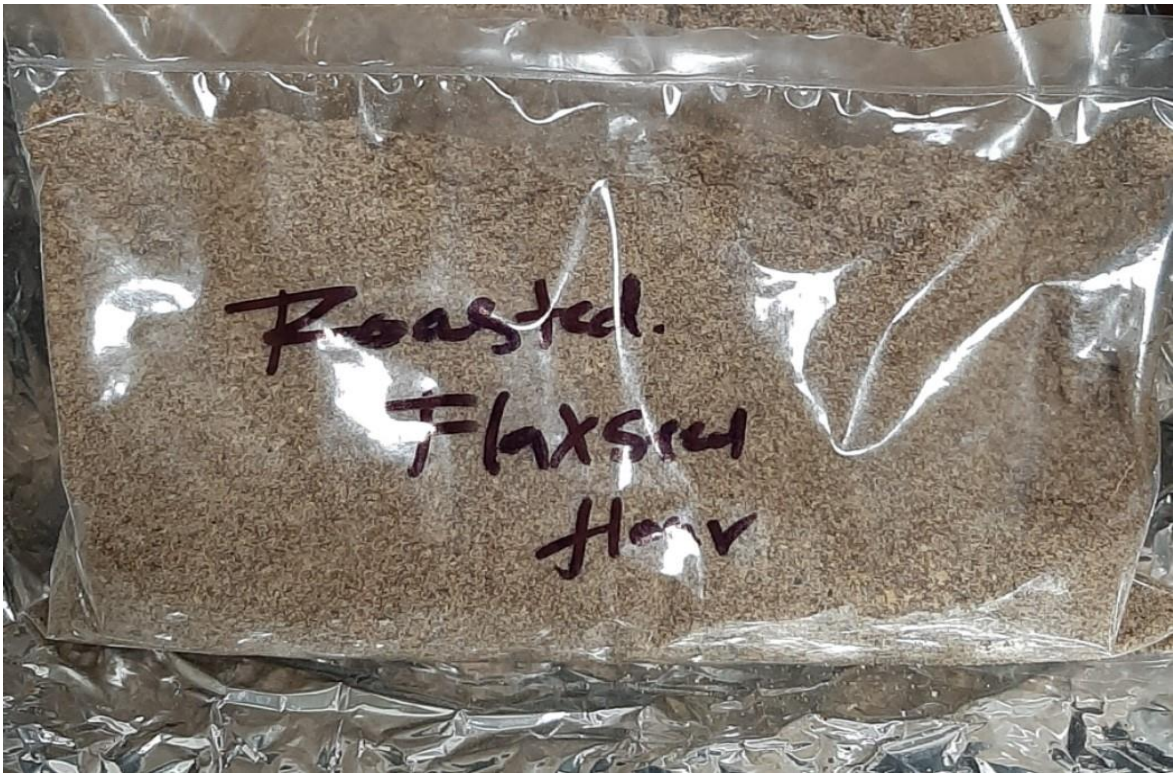
Characteristics	Low end	Intensity score 0 1 2 3 4 5 6 7 8 9 10	High end
Appearance			
Colour	Off-white		White
Free whey	Nil		Pronounced
Particulate Matters	Nil		Numerous
Flavour			
Acidic	Nil		Pronounced
Sweet	Nil		Pronounced
Oxidized/Rancid	Nil		Pronounced
Bitter	Nil		Pronounced
Un-natural/Stale	Nil		Pronounced
Texture			
Consistency	Too Thin		Too Thick
Ropiness	Nil		Pronounced
Granularity	Nil		Extreme
Overall acceptability	Disliked Extremely		Liked Extremely

Remarks, if any: _____

Name and Signature of Panelist: _____

ANNEXURE - 2

1.0 Roasting of flaxseed



2.0 Packaging of roasted flaxseed flour

ANNEXURE - 3

3.0 Milk flaxseed based probiotic beverage

4.0 Milk flaxseed based probiotic beverage with seven kinds of flavours