DEVELOPMENT OF MILK BASED FUNCTIONAL FLAVOURED DRINK

THESIS SUBMITTED TO THE
ICAR-NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
(DEEMED UNIVERSITY)

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

MASTER OF TECHNOLOGY
IN
DAIRY TECHNOLOGY

By

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B. Tech. (Dairy Technology)

DAIRY TECHNOLOGY SECTION
ICAR-NATIONAL DAIRY RESEARCH INSTITUTE
ADUGODI, BENGALURU- 560 030, INDIA

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Approved By

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CERTIFICATE

This is to certify that the thesis entitled **"DEVELOPMENT OF MILK BASED FUNCTIONAL FLAVOURED DRINK"**, submitted by **Mr. P. ASHOK KUMAR** towards the partial fulfilment 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 guidance and no part of the thesis has been submitted for any other degree or diploma.

(Dr. B.V. Balasubramanyam)
Major Advisor
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THESIS

DEDICATED

TO

MY

BELOVED FAMILY

AND

RESPECTED GUIDE.....

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P. Ashok Kumar

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LIST OF ABBREVIATIONS AND SYMBOLS

°C - Degree Celsius

% - Percentage

min - minute

ml - milliliter

Fig.- figure

mg - milligram

g - gram

wt.- weight

TBC – Total bacterial count

BIS - Bureau of Indian standards

AOAC - Association of official analytical chemists

SMP - skim milk powder

FA - fatty acid

LA - linolenic acid

GC - gas chromatography

ALA- alpha linolenic acid

MUFA- monounsaturated fatty acid

PUFA - polyunsaturated fatty acid

EPA - eicosapentaenoic acid

DHA - docosahexaenoic acid

MF - milk fat

MSNF - milk solids not fat

CO - canola oil

SFM - Sterilized flavoured milk

LDL - low density lipoprotein

FSO - flaxseed oil

ANOVA - analysis of variance

FAME - fatty acid methyl ester

cfu - colony forming unit

cP- centi poise

USDA - United States department of agriculture

NAAC- National Academy of Agricultural Sciences

AI - adequate intakes

AHA - American Heart Association

WHO - World health organization

AACC - American association of cereal chemists

% LA - percent lactic acid

ABSTRACT

Milk is a good source of fat, proteins, minerals and lactose, but poor source of nutraceuticals such as omega 3 fatty acids and soluble fibres. Though the health benefits of omega 3 fatty acids and soluble fibres are well established, their use in dairy foods is very limited. Flavoured milk is one of the most popular health beverages. To improve the functional qualities of flavoured drink, attempts were made to incorporate inulin and replace the milk fat with canola oil and flax seed oil in milk based flavoured drink. The sensory scores of the flavoured drink showed that among the different levels of replacement of milk fat, 50% of milk fat replacement by the oils and their mixture, showed sensory characteristics comparable to those of control flavoured drink. When 75% and above of the milk fat was replaced by canola oil, flax seed oil and their mixture, the drink had typical oily flavour which was not liked by the judges. When different levels of inulin was incorporated in flavoured drink prepared by using optimized levels of oil mixture and milk fat, incorporation of 5% inulin was found to be most acceptable level. Addition of inulin increased the viscosity and sweetness of the drink. The sensory studies showed that, among the different levels of MSNF tried, the use of 9.0 MSNF in experimental drink was comparable to control sample with 9.5% MSNF. Use of 8% sugar in the experimental sample was found to be optimum.The flavoured drink prepared with selected level of canola oil and flaxseed oil, and inulin was found to be stable at sterilization temperature. Among the different flavours tried, the mango flavoured drink was preferred over the banana followed by chocolate flavoured drink. The physico-chemical analysis of the developed flavoured drink showed that the drink had fat, protein, total carbohydrates and ash content of content of 2.9, 3.35, 18.63 and 0.72 per cent respectively and the viscosity, acidity and pH of the sample were 2.40(cP), 0.17% LA and 6.55 respectively. The fatty acid analysis of the control and experimental samples revealed that the control sample had oleic acid and linoleic acid content of 21.3 and 1.8% respectively while the linolenic acid was not detectable. The corresponding values for experimental drink were 23.6, 7.8 and 13.3% on total fat basis. The developed drink had a shelf life of 6 days at refrigerated temperature which was comparable to control flavoured drink.

सारांश

दूध वसा, प्रोटीन, खनिज–लवण व लैक्टोज का अच्छा स्रोत है, परन्तु इसमें न्यूट्रास्यूटिकल जैसे घुलनशील रेशे व ओमेगा–3 फैटी अम्लों का आभाव है। यद्यपि घुलनशील रेशों व ओमेगा–3 फैटी अम्लों के स्वास्थ्यवर्धक गुण विख्यात हैं, फिर भी इनका प्रयोग डेरी खाद्य पदार्थों में बहुत कम हुआ है। सुगंधित दूध एक सुप्रसिद्ध स्वास्थ्यवर्धक पेय है। सुगंधित पेय के विशेष गुणों में बढ़ोत्तरी हेतु, इसमें इन्यूलिन मिश्रित किया गया तथा दूध की वसा को कैनोला व अलसी के तेल द्वारा प्रतिस्थापित किया गया। तेल व तेलों के मिश्रण द्वारा विभिन्न दरों पर वसा प्रतिस्थापन के पश्चात् यह पाया गया कि 50% वसा प्रतिस्थापन के उपरांत संवेदी–गुण, कन्ट्रोल से तुलनीय था। जब दूध की वसा को कैनोला व अलसी के तेल व उनके मिश्रण द्वारा 75% तक प्रतिस्थापित किया गया, तो इसमें एक विशेष तैलीय गंध व स्वाद का आविर्भाव हुआ, जो संवेदी रूप से स्वीकार्य नहीं था। जब दूध की वसा, कैनोला व अलसी के तेल व इनके मिश्रण द्वारा निर्मित सुगंधित पेय में अलग–अलग दरों पर इन्यूलिन संमिश्रित किया गया, तो 5% इन्यूलिन की मात्रा सर्वोत्तम पायी गयी। इन्यूलिन संमिश्रित करने पर पेय की मिठास व श्यानता में वृद्धि पायी गयी। संवेदी–गुणों के मूल्यांकन द्वारा यह पाया गया कि दूध की अवसा–ठोस के विभिन्न दरों में से 9% अवसा –ठोस की मात्रा, 9.5% अवसा–ठोस वाले कन्ट्रोल के साथ तुलनीय था। 8% शर्करा की मात्रा प्रायोगिक नमूने में पर्याप्त पायी गयी। विशेष दर पर इन्यूलिन, कैनोला, अलसी के तेल व उनके मिश्रण द्वारा निर्मित सुगंधित पेय स्टेरिलाइजेशन ताप पर भी स्थायी पाये गये। कई गंधों व स्वादों में आम सर्वोत्तम पाया गया तथा इसके बाद केला व चॉकलेट। विकसित सुगंधित पेय के भौतिक–रासायनिक गुणों के विश्लेषण द्वारा यह पाया गया कि इसमें वसा, प्रोटीन, कार्बोहाईड्रेट व भस्म की मात्रा क्रमशः 2.9, 3.35, 18.63 व 0.72 प्रतिशत थी, परन्तु श्यानता, अम्लीयता व pH क्रमशः 2.40(cP), 0.17% LA व 6.55 पाये गये। कन्ट्रोल व प्रायोगिक नमूनों के वसा–अम्ल–विश्लेषण द्वारा पाया गया कि कन्ट्रोल में ऑलिक अम्ल व लिनॉलिक अम्ल की मात्रा क्रमशः 21.3 व 1.8% पाये गये, जबकि लिनॉलिनिक अम्ल नहीं के बराबर था, परन्तु प्रायोगिक सुगंधित पेय में इनके मान क्रमशः 23.6, 7.8 व 13.3% कुल वसा के आधार पर पाये गये। विकसित सुगंधित पेय का शेल्फ–आयु रेफ्रिजेरेशन ताप पर 6 दिन पाया गया, जो कन्ट्रोल सुगंधित पेय के साथ तुलनीय था।

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

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In recent years, the consumers are more concern about the health which is leading to increase demand for functional foods providing health benefits beyond the nutrition. India's milk production is 155.2 MT (NDDB, 2016). In India, about 45% of total milk production is used for the preparation of traditional milk products. Compelling evidence, however, indicates the greater importance of types of fat, rather than total amount of fat with respect to risk of coronary heart diseases (Hu *et al*., 2001). Polyunsaturated fatty acids (PUFAs) such as omega-3 and omega-6 fatty acids are essential fatty acids which cannot be synthesized in human body and thus must be obtained from foods or supplements.

Omega-3 fatty acids are the essential fatty acids which have gained status as 'functional ingredients' as they possess a wide range of health benefits. It (ALA, DHA and EPA) can lowers the triglycerides and boost the heart health, lowers blood pressure slightly in people with hypertension reduced the risk of high blood pressure and also improves the eye health. Major sources for omega-3 fatty acids are flaxseed oil, soya bean oil, canola oil, olive oil, walnut oil. They are the good source of alpha linolenic acid and fish oils like sardines, salmon oil, algae like marine sources are rich in EPA and DHA type omgega-3 fatty acids (Estrada *et al*., 2011)

Soluble fibre is one type of the dietary fibre which is an important constituent of the balanced diet and soluble in water. It has health benefits such as easy bowel movement, reduction in cholesterol level, control blood sugar levels, helps in digestion process, reduces weight, and cancer preventive action (Tudorica *et al*., 2002). Inulin is one type of soluble fibre which can be used in milk and milk products as fat replacer, low calorie sugar replacer and also provide fibre enrichment to the food products. Major sources of soluble fibre are Oat meal, oat cereal, lentils, apples, chicory root, pears, and carrots.

Vegetable oils are good sources of PUFAs and MUFAs and among them, the flax seed oil and canola oil are two plant oils that have considerable amount of one of the omega-3 fatty acids i.e. alpha linolenic acid. The alpha linolenic acid accounts for about 52- 62% of total fatty acids in flax seed oil and 10% in canola oil (Daun *et al*., 2003). Due to presence of higher amount of ALA, the flax seed and flax seed oil is utilized as main

food ingredient to enhance the functionality of foods (Oomah and Mazza, 1999).Canola oil i.e. low euricic acid rapeseed oil is now considered to be the best nutritional edible oil available because of its lower content of saturated fatty acids compared to all other competing oils.

The milk and milk products are rich in fat, protein, carbohydrates, minerals and certain vitamins, but not a good source of several micronutrients likes iron, vitamin C and B complex vitamins. It is also deficient in some of the nutraceuticals such as soluble fibres, and omega-3 fatty acids like alpha linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

As per "India Flavoured Milk Market Outlook 2020" the flavoured milk market is anticipated to grow annually at a rate of around 4% during 2015-2020. The global flavored milk industry is at nascent stage as the consumption is still low as compared to other drinks such as carbonated soft drinks. Flavoured milk, by whatever name called, may contain nuts (whole, fragmented or ground) chocolate, coffee or any other edible flavour, edible food colours and cane sugar. It shall be pasteurized, sterilized or boiled. The type of milk shall be mentioned on the label (FSSAI, 2006). The flavored milk is generally available in market in different flavors such as strawberry, chocolate and vanilla, badam flavors in fat free and low fat ranges. In the method of manufacture of chocolate/fruit flavoured milks/ drinks formula may be used (De, 1980).

Flax seed is utilized as main food ingredient to enhance the functional foods, (Oomah and Mazza, 1999). Flaxseed provides essential nutrients such as protein (30-35%) oil (30 – 45%) including omega 3 fatty acids, carbohydrates (30-35%), fibre (10%) and lignan (Bhatty, 1995) flax seed ins known to lower blood cholesterol levels and helps in reducing the cancer risk of heart attacks and stroke, partly through the action of ALA. ALA may be especially important to vegetarians and people with low intake of fatty fish. These characteristics make the flaxseed and its derivatives an attractive source of functional ingredients for preparation of functional foods (Rubilar *et al*., 2010; Dayane *et al*., 2011). Flax seed oil has been proposed to be a valuable ingredient for ice cream products (Hall and Schwarz, 2002). Development and characterization of *dahi* (Indian yoghurt) with omega-3 fatty acids using microencapsulated flaxseed oil. Development of process optimization for production of functional yoghurt by incorporating flaxseed extracts up to 2% and 8% sugar of milk resulted in production of most acceptable yoghurt (Sivakumar, 2014).

Inulin is considered as functional food ingredient, since they affect physiological and biochemical processes, resulting in better health and reduction in the risk of many diseases.

In addition to its prebiotic property, inulin is known to give array of health benefits such as Lowering blood urea and uric-acid levels, lowering of blood serum lipids, reducing the risk of atherosclerosis and reducing the incidences of cardiovascular disease.

By keeping health beneficial factors of omega-3 fatty acids and soluble dietary fibre in consideration, the present work was proposed to develop milk based functional flavoured drink containing functional ingredients. Vegetable oils canola, flax seed oil rich in monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs) and inulin as a source of dietary soluble fibre was selected to improve nutritional profile of flavoured drink. The combination of such ingredients for the preparation of flavoured drink has never tried before. Hence the present study was taken up with following objectives:

1 To optimize the levels of omega-3 rich vegetable oils and inulin for production of milk based functional flavoured drink
2 To analyse the physico-chemical parameters of the flavoured drink
3 To study the shelf life of the developed functional flavoured drink

CHAPTER 2

REVIEW OF LITERATURE

2.0 REVIEW OF LITERATURE

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2.1 OVERVIEW

India's milk production is 155.2 MT in 2016 (NDDB, 2016). In Total milk production of country around 45% of milk is used for the preparation of traditional milk product. As per "India Flavoured Milk Market Outlook 2020" the flavoured milk market is anticipated to grow annually at a rate of around 4% during 2015-2020. The global flavored milk industry is at nascent stage as the consumption is still low as compared to other drinks such as carbonated soft drinks.

Popularity of the Flavoured milk is increased day by day because it is a cheaper cold drink as compared to non-milk based drink and contains more nutritive value (Arora and Kalra, 1984). According to Reiter (1993) chocolate flavoured milk accounts to approximately 98% of flavoured milk sales in USA. Lactose hydrolysed and low sugar chocolate milks are promoted in children's market.

Flavoured milk, by whatever name called, may contain nuts (whole, fragmented or ground) chocolate, coffee or any other edible flavour, edible food colours and cane sugar. Flavoured milk shall be pasteurized, sterilized or boiled. The type of milk shall be mentioned on the label (FSSAI, 2006).When the word 'milk' is used, the product should contain a milk fat percentage at least equal to the minimum legal requirement for market milk and if fat percentage is lower (1 to 2%), then term" Drink" is used (De, 1980). The flavoured milk is generally available in different flavours such as strawberry, chocolate and vanilla flavours. The global flavoured milk industry is at nascent stage as the consumption is still low as compared to other drinks such as carbonated soft drink.

Milk and milk products are rich in fat, protein, carbohydrates, minerals and certain vitamins, but not a good source of several micronutrients likes iron, vitamin C and B complex vitamins. It is also deficient in some of the nutraceuticals such as omega-3 fatty acids like alpha linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and soluble fibres.

Types of flavoured milks:

Chocolate milks/drinks

Fruit flavoured milks/drinks

Sterilized flavoured milks/drinks (De, 1980)

2.2 WORK DONE IN INDIA AND ABROAD

A few studies suggested that flavoured milk may be more easily digested by those who are sensitive to lactose (Dehkordi *et al*., 1995).

Carrapsio *et al*. (2004) conducted studies to determine the effect of fat content on sensory parameters of vanilla flavoured milk and reported that varying fat contents caused significant differerences in perceived viscosity, sweetness and flavour intensity.

Madhusudan (1992) studied the use of nonconventional sweetners in the preparation of flavoured milk.

Jason *et al*. (2006) reported that chocolate milk, with its high carbohydrates and protein content may be considered an effective alternative to commercial fluid replacement drink (CR) for recovery from exhausting, glycogen depending exercise. Strawberry flavoured milk also demonstrates high acceptability with the consumer (Miller *et al*., 2007).The process technology for production of chocolate/fruit flavoured milks/drinks is well documented (De, 1980).

Renner and Zewrmann, (1988) optimized sugar content for the flavoured milk drink on the basis of the sensory scores for four flavours; banana, cocoa, strawberry, and vanilla were optimised to be 3.7, 3.9, 3.6, and 3.9% respectively. Strohman *et al*. (2014) found that in pasteurized flavoured milk at 84°C for 13 sec and storing the bottles of milk immediately in cardboard boxes in a refrigerator (4-7°C), that a great deal of variability of pH between milk samples was present, suggesting microbial growth. They suggested raising the pasteurization temperature and cooling in bottles on ice before transferring it to the refrigerator for reducing this problem.

Yanes *et al*. (2002) found that commercial chocolate milks with different (unspecified) fat levels varied widely in measured viscosity between products and even between lots of the same product.

Hanif *et al*. (1996) studied the acceptability of cow and buffalo flavoured milk prepared by using of different flavours like, chocolate, mango, sweet orange and vanilla contain 1 ml/litre flavour were most acceptable whereas, milk flavoured with mango flavour scored most acceptable at 2ml/ litre. Production and comparison of banana and

chikoo flavoured milk based beverages done by using skimmed buffalo milk and finally concluded chikoo flavoured milk-based beverage was to be more acceptable ($p<0.05$) compared to banana flavoured milk-based beverage studied by (Mangsi *et al.*, 2012).

Repate *et al*. (2010) developed the preparation of flavoured milk from different proportion of cow milk blended milk safflower milk 100:0, 80:20, 70:30, 60:40 and 50:50 was prepared and studied the acceptability. Thus this proved that the cost of flavoured milk could be minimized by using safflower milk and cow milk blended and blending could be done to the maximum proportion of 50:50.

Prakash *et al*. (2010) studied and reported chocolate milk with different carrageenans (kappa and lambda) and sugar concentrations was heat treated indirectly at 145^0C for 6 s using a bench-top UHT plant. During UHT processing, kappa-carrageenan was more effective than lambda-carrageenan in providing stability against fouling during UHT processing. By optimizing concentrations of kappa carrageenan and sugar, fouling could be minimized during UHT processing but the apparent viscosity and sedimentation of UHT-processed chocolate milk increased with increasing concentration of carrageenan and sugar.

Luck, *et al*. (1973) prepared fruit juice flavoured milk from 40% of fruit juice and 60% of milk, 0.25% stabilizer (CMC, alginate) along with 5-10% sugar by weight were added.

Vijayalakshmi and Tamilarasi (2001) studied changes in sensory and microbial qualities of flavoured milk during storage at 6-8°C for up to 6 weeks. Flavoured milk deterioration in appearance was started just after one day. It was observed that the Microorganism counts was increased than expected level in all sample during storage.

Singh *et al*. (2005) developed flavoured milk in combination with the carrot juice in the proportion of 10:90, 20:80 and 30:70 (juice: milk) from three different varieties and analysed for physicochemical and organoleptic characteristics. Combinations of flavoured milk were found acceptable but flavoured milk beverage obtained with incorporation of 20 % carrot juice was highly acceptable.

Kumari *et al*. (2016) studied the stability of aspartame and neotame in pasteurized and in-bottle sterilized flavoured milk tested by HPLC method and found that neotame was more stable than asparatame. Pasteurization (90 ^{0}C/20 min) resulted in approximately 40% loss of aspartame and only 8% of neotame was degraded. During the

storage (4-7^0 C/7 days) aspartame and neotame content decreased significantly ($P < 0.05$) from 59.70% to 44.61% and 91.78% to 87.18%, respectively. Sterilization (121 ^{0}C/15 min) resulted in complete degradation of aspartame; however, 50.50% of neotame remained intact but during storage (30 ^{0}C/60 days) neotame content decreased significantly ($P < 0.05$) from 50.36% to 8.67%.

Kumar (2000) studied and reported that the physico-chemical properties and the storage stability of low fat lassi and flavoured dairy drink and using asparatame and fat replacers gave significant decrease in pH and increase in acidity and viscosity at 25°C than at 8°C. Asparatame losses were more in flavoured dairy drink than in lassi.

Ravindra *et al*. (2014) studied and reported that carbonation at 50 psi for 30 s for 200 ml of pasteurized flavoured dairy drink packaged in glass bottles is recommended. The carbonation of drink had a significant effect on raising the acidity and lowering the pH of system without adversely affecting the sensory quality of product. The carbonation inhibited the growth of microbes, especially psychrotrophs, during storage, nearly doubling the shelf life (up to 30 days vs. 17 days for control) of beverage when stored under refrigeration.

Studies on the development of whey based mango beverages by using whey powder, WPC without any significant changes in constituents in all of the samples after thirty days of storage with slight acidity difference and high nutritional content (Chavan *et al*., 2015). Sameen *et al*. (2013) studied and reported the effect of stabilizers on the quality of carbonated flavoured whey drink was studied and reported carrageenan is best stabilizer than CMC in 30 days refrigerated storage with significant effect on constituents lactose, SNF and viscosity of drink.

Mittal & Bajwa (2012) developed the low calorie functional milk drinks using inulin, sucralose as fat and sugar substitutes respectively. Addition of 4% inulin was found to impart viscosity and sensory properties equivalent to that of control of two percent fat. The cardamom flavoured milk drinks were prepared by replacing sugar and adding 4% inulin in milk of 0.5% fat and 8.5% milk solid-not-fat. The calorific value decreased by 43% in the experimental milk drink compared to control.

Nataraj (2008) developed and reported that sterilized flavoured milk was prepared with 1.5% fat milk added with aspartame 1000ppm plus 8.0% sorbitol, saccharin 75ppm plus 8.5% sorbitol level and sucralose 75ppm plus 8.0% sorbtiol level respectively and

kept for storage studies. Sterilized flavoured milk made with aspartame sweetener was acceptable up to 30 days. Sterilized flavoured milks made with saccharin and sucralose sweeteners were acceptable up to 105 and 120 days of storage at room temperature.

Bhardwaj *et al*. (2002) developed low calorie flavoured drink using saccharin and asparatame combination. Yan cheng (2007) developed chocolate flavoured milk using acesulfame-k and sugar.

Jenner (1989) studied the stability of sucralose in pasteurized flavoured drink which was stored at 4°C and reported that there is no loss of sweetness for 19 days.

Homler (1984) and Newsome (1986) studied the effect of asparatame on the sensory quality of flavoured drink and observed that asparatame cannot be used in the preparation of SFM because at higher temperature of heat treatment it gets destabilized and forms diketopiperazine which resulted in curdling and loss of taste of SFM.

Satyanarayana (2008) developed the sterilized herbal flavoured milk in three flavours of cardamom, sarsaparilla and cinnamom flavoured milk with added three colours of beeet root,carrot and turmeric extracts had a shelf life of 180, 135 days and 3 months at room temperature.

Ramasamy *et al*. (2005) studied that the shelf life of sterilized flavoured milk by incorporation of extracts of beeet root (2%), carrot (5%) and stevia (2%) at 30°C, 5°C temperature and they observed no change in sensory parameters up to 6 months.

Chourasia (2010) prepared sterilized herbal flavoured milk by addition of 2 levels of herbal extract (6 and 12%) and 2 levels of poppy seeds (0.4 and 0.8%) in double toned milk containing 1.5% fat and 9.0% SNF. It has been found that product which contains 12% extract and 0.4% poppy seeds was found to be more acceptable than other treatments with 20 days shelf life with added advantage of higher medicinal and therapeutic values.

2.3 OMEGA-3 FATTY ACIDS AND BENEFITS

Omega-3 fatty acid is an essential fatty acid which is named based on the location of the first double bond, which is between the 3^{rd} and 4^{th} carbon atoms counting from the methyl end of the fatty acid molecule. These are the essential fatty acids which have gained status as ‘functional ingredients’ as they possess a wide range of health benefits. Alpha linolenic acid (ALA), Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) are the three types of omega-3 fatty acids and are nutritionally important which

can lowers the triglycerides and boost the heart health, lowers blood pressure slightly in people with hypertension reduced the risk of high blood pressure and also improves the eye health and reduces the risk of cardiovascular diseases.

Ground flax seed is high in omega-3 fatty acids, which have been shown that to reduce hypertension, cholesterol and triglyceride level (Oomah and Mazza, 1998). Flaxseed acts as good source for lowering cholesterol and improving heart functions due to the eicosanoids derived from omega-3 fatty acids (Simopolous, 1999). It is revealed in many studies that polyunsaturated fatty acids and monounsaturated fatty acids are responsible for lowering the total cholesterol in diet (Zambon *et al*., 2000).

Alpha linolenicacid(ALA) from flax seed exerts positive effects on blood lipids. The dietary alpha linolenic acid was found to be as effective as oleic (18:1n-6) and linoleic acid (18:2n-6) in the reduction of plasma total cholesterol, low density lipoprotein cholesterol and very low density lipo protein cholesterol in 20-34 years old healthy men. In another study, 12 g Alpha linolenic acid (ALA) was taken three times a day by a group of healthy young women in the form of flaxseed oil capsules and compared with other group given the flax seed flour supplemented products. Impressive reduction in blood lipids was observed in both cases (Cunnane *et al*., 1993).

2.3.1 Alpha-linolenic Acid (ALA)-18:3n-3

Takeuchi (2007) reported that the reduced the risk of cardiovascular diseases with alpha linolenic acid may be in part due to an anti-hypertensive effect that can lower both systolic and diastolic pressure. Ogawa (2009) observed that the blood pressure lowering mechanism of dietary alpha linolenic acid may be involved in the reduction of ACE activity and mRNA expression. Bourre (2004) observed that the anti-inflammatory properties of linolenic acid may also extend beyond the cardiovascular system. Preliminary research demonstrates that alpha- linolenic acid can reduce ischemic brain damage, provides neuronalprotection, enhances brain plasticity, and maintains frontal cortex and pituitary gland. Menendez (2006) observed that in postmenopausal patients with primary breast cancer, a diet which rich in linolenic acid shown significant reductions in tumour growth.

2.3.2 Eicosapentaenoic acid (EPA)-20:5n-3

The benefits of EPA rich foods are in large part due to its anti-inflammatory actions. In general eicosanoids derived from EPA are less potent inducers of inflammation, blood vessel constriction, and coagulation than eicosanoids derived from arachidonic acid (Kris-Etherton, *et al*., 2002). Rheumatoid arthritis improvement with EPA may be due to various actions including the production of less inflammatory eicosanoids, the reduction of pro-inflammatory cytokines, and the inhibition of activation of T lymphocytes and of catabolic enzymes (Sales *et al*., 2008). Researchers reported that EPA is a promising treatment for prevention of major coronary events, and especially non-fat coronary events, in hypercholesterolemia patients.

EPA also significantly reduces the levels of plasma triglycerides and may increase the levels of high density lipoproteins, Alzheimers's disease and cognitive decline may be prevented with regular intake of fish oil (Connor *et al*., 2007). Analysis of desaturase enzyme activity and metabolic syndrome reveals that dietary intake of EPA can plays an important role in preventing abdominal obesity and the development of metabolic syndrome.

Other inflammatory and autoimmune diseases in humans that benefit from Supplementation with fish oils include Crohan's disease, ulcerative cholitis, psorias, multiple sclerosis and migraine headaches (Simopoulos, 2002).

Major sources for omega-3 fatty acids are flaxseed oil, soya bean oil, canola oil, olive oil, walnut oil. They are the good source of alpha linolenic acid and fish oils like sardines, salmon oil, algae like marine sources are rich in EPA and DHA type omgega-3 fatty acids (Estrada *et al*., 2011).

2.3.3 Docosahexaenoic Acid (DHA)-22:6n-3

DHA has unique beneficial effects on cardiovascular health, depression and certain visual dysfunction and other conditions. DHA increase LDL particle size in type 2 diabetic patients which decreases the susceptibility of LDL to glycation, oxidation and lower the progression of endothelial dysfunction in type 2 diabetic patients (Woodman, 2003). Supplementation restores brain DHA levels, enhances learning and memory tasks in aged animals, and significantly reduces beta amyloid, plaques and tau in transgenic AD models (Yurko-Mauro and Curr, 2010).

Depression disorders may be due to relative deficiency of DHA. The principal Omega-3 fatty acid in brain gray matter has neutrophic and neuroprotective properties. Visual processing and optimal retinal function are dependent on DHA content to maintain membrane fluidity and permeability, and the associated enzyme and transport activities (Treen, 1992). Inadequate levels of DHA are associated with alterations in retinal function. Visual processing deficits have been ameliorated with DHA supplementation in some cases (San Giovanni, 2005).

2.3.4 Food sources of omega 3 fatty acids

Omega 3 fatty acids are less ubiquitous and can be found in high amounts in fatty cold water fish, walnuts and oil seeds. ALA is present in high levels (> 50% of fatty acids) in only a limited number of oil seeds such as flax seed, mustard seed and cranberry seed. Walnuts are the only nut source with significant amounts of ALA (Cunnane, 2003). Canola oil and soya bean oil are most widely consumed ALA sources. However they only provide modest levels (5-10% of fatty acids) of ALA. Green vegetables have proportionally high amounts of ALA (30-60%); however, due to low fat in nature, these vegetables are poor sources of ALA. Animals can store ALA in their fat tissue; therefore, grazing animals that consume ALA – containing plants have a high proportion of ALA in their meat carcasses.

Table.2.1 Food sources- Omega 6: Omega 3 (values% of fatty acids)

Linoleic acid (omega-6)	Linolenic acid (omega-3)
Soybean (50-57)	Flax seed (35-56)
Safflower (67-83)	Soybean(5-10)
Sunflower (48-74)	Canola(6-14)
Corn (34-62)	Walnut(13)
Canola (16-25)	Safflower(0.1)
Sesame (35-50)	Olive (0.2-1.5)

(Cunnane, 2003)

2.3.5 Efficiency of conversion of ALA to long chain n-3 fatty acids in human

Brenna *et al*. (2009) reported that alpha linolenic acid (18:3n-3) is the major omega 3 fatty acid in the human diet. It is mainly derived from terrestrial plant consumption and it has long been thought that its major biochemical role is as the principal precursor for long chain polyunsaturated fatty acids (PUFA), of which eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) are the most prevalent. For infants, n-3 long chain polyunsaturated fatty acids are required for the rapid growth of neural tissue in the perinatal period and a nutritional supply is particularly important for development of premature infants. For adults, n-3 long chain PUFA supplementation is implicated in improving a wide range of clinical pathologies involving cardiac, kidney and neural tissues. Studies generally reported that whole body conversion of 18:3n-3 to 22:6n-3 is below 5% in humans, and depends upon the concentration of n-6 fatty acids and long chain polyunsaturated fatty acids in the diet. Oxidation of dietary 18:3n-3 to Co2 accounts for about 25% in the first 24 hours, reaching 60% by 7 days. The remaining 18:3n-3 serves as a source of acetate for synthesis of saturates and monounsaturates, with very little amount stored as 18:3n-3. Studies showed that all the age group of humans can perform the conversion of 18:3n-3 to 22:6n-3.

ALA is considered the essential omega-3 fatty acid because it cannot be synthesized by human body (Giltay *et al*., 2004). The capacity for conversion of ALA to DHA is higher in women than men. Studies of ALA metabolism in healthy young men indicate that approximately 8% of dietary ALA is converted to EPA and 0-4% is converted to DHA. In healthy young women, approximately 21% of dietary ALA is converted to EPA and 9% is converted in to DHA. For the better conversion efficiency in young women compared to men appears to be related the effects of estrogen (Burdge and Wootton, 2002).

2.3.6 Recommended intakes

Although the United States department of agriculture (USDA) has not established recommended intakes for the essential FA, the institute of medicine of the National Academics of Science has reported adequate intakes (AI) for the essential FA (National Research Council, 2005). Adequate intakes represent median taken levels that prevent essential FA deficiency. For men aged 19-50 years, the AI of n-6 FA is 14g/day and for n-3 FA is 1.6g/day is recommended. In men older than 50 years, 17g/ day of n-6

FA and 1.6g/day is recommended. For women aged 19-50 years, the AI for n-6 FA is 11 g/day and for n-3 FA is 1.1 g/day and for women older than 50 years, 12 g/day of n-6 FA and 1.1g/day of n-3 FA are suggested.

More specific n-3 FA recommendations for cardiac health have been reported by professional societies. The American Heart Association (AHA), the American college of cardiology and the European society for cardiology have recommended 0.5 g to 1 g /day EPA+DHA as a secondary prevention measure for cardiovascular diseases (Von Schacky and Harris 2007). An intake of 1 g/day EPA+DHA shows no potential for adverse effects and achievable in the diet (Kris-Etherton *et al*., 2002). In the year 2000, the US food and drug administration (FDA) reported that consumers ingest 3 g of n-3 FA daily with no more than 2 g/day from a dietary supplement (Kris- Etherton *et al*., 2002). Whereas there is little evidence that consumption of <3 g n-3 FA/day has led to any significant bleeding in patients (Kris- Etherton *et al*., 2002). Contrarily Whelan and Rust (2006) reported that consumption of >3 g n-3 FA may lead to excessive bleeding and should only be done under the care of physician. The recommended dietary intake of omega-3 fatty acids vary based upon the source of the recommendation. The WHO and NATO recommended 800 to 1100 mg/day of ALA and 300 to 500 mg/day of EPA+DHA. The AHA scientific statement recommends that supplementation of 0.5-1.8g/day EPA+DHA and 1.5-3.0 g/day of ALA reduces subsequent acute cardiac mortality (Kris Etherton *et al*., 2002).

2.4 FLAX SEED

Flaxseed is a leading a leading source of the omega 3 fatty acid, ALA (52% of total fatty acids), Dietary fibre and other nutrients. The botanical name of flax is *Linum* usitatissimum of the family *linaceae*. The fruit contains a seed known as flax seed or linseed (Pradhan *et al.,* 2010). The seed itself is flat and oval with a pointed tip in colour from deep brown to light yellow. Today it is cultivated more than 50 countries and major of them are in the northern hemisphere. It is an economically important oil seed crop especially in Canada, which produces about 40% of the world's flax seed production, followed by china, United States and India (Rubilar *et al*., 2010). India holds an important position in world vegetable oil economy. The flax seed growing states are Madhya Pradesh, Maharastra, Chattisgarh, Uttar Pradesh, Bihar, Orissa, Karnataka, which

contribute about 83% of area and 80% of the total flax production of the country (Chimmad, 2010).

2.4.1 Composition

As presented in table 2.2 the flaxseed on an average contains approximately 40% lipids, 30% dietary fibre, 20% protein, 7.7% moisture, 3.5% ash, 1% simple sugar. The chemical composition varies considerably among varieties and also depends on the environmental conditions in which plant is grown. The amount of fat in flax seed ranges from 38 to 47%. Flax seed has a unique fatty acid profile, being fairly low in saturated fatty acids and rich in ALA, the essential fatty acid. Of the total fatty acids in flax seed, saturated fatty acids constitute 9%; monounsaturated fatty acids, 18%; and polyunsaturated fatty acids, 73%. Of the unsaturated fatty acids, ALA constitutes the majority at 59% of total fatty acids, making the flaxseed one of the richest source of this fatty acid.

Table.2.2. Composition of flaxseed

Humidity (%)	Protein (%)	Lipids (%)	Fibre (%)	Ash (%)	Reference
7.4	23.4	45.2	-	3.5	Mueller *et al*. (2010)
4-8	20-25	30-40	20-25	3-4	Coskuner and Karabba (2007)

In total unsaturated fatty acids ALA constitute about 59% making flax seed one of the richest sources of omega 3 fatty acid (Bhatty, 1995).

2.4.2 Flax seed oil

Flaxseed oil content in the seeds falls in the range of 38-45% depending upon the location, cultivar, and environmental conditions (Daun *et al*., 2003). Kozlowska (1989) reported an average of 41.4 % oil content for polish cultivars. Flax seed has a high ALA content, generally constituting 50-62% of total fatty acids (Daun *et al*., 2003).

2.4.3 Physico-chemical properties of flax seed oil

The higher specific gravity of 0.935 observed for flax seed oil than other vegetable oils can be directly attributed to the high contributes of linolenic acid listed in table 2.3.

Table.2.3 Physico-chemical properties of flax seed oil

Parameter	Flaxseed oil
Relative density (20˚C/ water at 4˚C)	0.925-0.935
Refractive index (nD at 4˚C)	1.475
Melting point (˚C)	20-24
Flash point (˚C)	120-135
Viscosity (cP)	46.4-46.8
Iodine value (g I2/ 100g oil)	182-203
Unsafoniable matter (%)	0.1-1.7
Safonification value (%)	0.1-2.0
Triglycerides (%)	94-98

(Frankel, 1993; Green and Dribnenki, 1994; Gunstone *et al*., 1994; Eskin *et al*., 1996)

2.4.4 Health benefits of flax seed

Flaxseed consumption of 50g per day or 20g/day flax seed oil for several weeks has resulted in the reduction in 6-9% of serum total cholesterol and 9-18% of low density lipoprotein cholesterol (Cunnane *et al*., 1995). The ingestion of 10 g of flax seed can reduce breast cancer and prostate cancer risk.

Studies have found that the soluble fibre in the flax seed in the flax seed like that found in oat bran and fruit pectin can help to lower cholesterol. Soluble fibre also has been found to regulate blood sugar levels. Insoluble fibre aids digestion by increasing bulk, reducing the time that waste remains in the body and preventing constipation. These characteristics seem to have a role in protecting against cancer (Simmons *et al*., 2010; Lakka *et al*., 2002).

2.4.5 Utilization of flax seed in Dairy and Food products

Milk has proven as a successful raw material, carrier and matrix for developing health promoting functional foods. An increasing variety of probiotic products, such as yoghurt, milk drinks, and cheese, have been developed and launched on the market. Dairy products are also supplemented with other bioactive components, such as plant sterols, peptides and omega -3 fatty acids have been introduced commercially.

Flax seed is utilized as main food ingredient to enhance the functional foods (Oomah and Mazza, 1999). Lau (2007) developed a novel flax seed enriched milk based beverage with improved health benefits and studied on physico-chemical and sensory analysis. Abou- Zeid (2016) studied that PUFA fortification in milk and dairy products made them with improved neutraceutical benefits but these foods are susceptible to oxidation. Whole or ground flax seed can be used in various food products, such as bread(Carter, 1993; Garden, 1993), pasta (Lee *et al*., 2003; 2004; Manthey *et al*., 2000, 2002), candy, chocolate bar, chocolate (Kozlowska,1989), salad toppings (Carter,1993), cake (Lee *et al*., 2004), tortilla (Ghosh *et al*., 2004).Flax seed oil has been proposed to be a valuable ingredient for ice cream products (Hall and Schwarz, 2002).Flax seed oil replaced between 10% and 25% of the milk fat in ice cream formula has been investigated. Replacement of milk fat by up to 25% flax seed product exhibited an oil like mouth feel, however the presence of the oil in the product could not be detected by 60% of the panellists using an informal sensory evaluation. Flax seed oil incorporation in to ice cream showed no effects on physico chemical properties of the ice-creams. However, it increased the colour of ice cream towards yellowness, decreased the sweetness, smoothness and creaminess. Flax seed oil incorporation also slightly ($P<0.05$) decreased the acceptance of aroma, flavour, texture and overall acceptability of formulated ice creams. Most acceptable level of flax seed oil substitution is up to 2.5%.

Development and characterization of *dahi* (Indian yoghurt) with omega-3 fatty acids using microencapsulated flaxseed oil microcapsules and fortified at 2 % level was observed comparable to control, which was further studied for titratable acidity, syneresis, firmness, stickiness, oxidative stability (peroxide value), α-linolenic acid (ALA, ω-3) content and sensory attributes during 15days of storage (Goyal *et al*., 2016). Hall *et al.* (2004) studied the stability of lignan in yoghurt and reported that flax seed extracts addition did not have negative effects on the fermentation.

Development of process optimization for production of functional yoghurt by incorporating flaxseed extracts up to 2% and 8% sugar of milk resulted in production of most acceptable yoghurt (Sivakumar, 2014).

2.5 CANOLA OIL

Canola, known in Europe as rapeseed, is the major oil seed crop in Canada of several cultivars of rapeseed bred to be low in erucic acid from

the *Brassicaceae* family of plants. Wild rapeseed oil contains large amounts of erucic acid, which is known to cause health problems, so the canola plant was developed from rapeseed in order to use it to produce a food grade canola oil with lower erucic acid levels.

2.5.1 Composition

It contains on average 6% saturated of palmitic (16:0) 4%,Stearic (18:0) 2% and 92% Unsaturated of 56% oleic 18:1 n-9, 26% linoleic 18:2 n-6 and linolenic 18:3 n-3, 2% other fatty acids (Gunstone, 1996).The conversion from high erucic acid rapeseed to canola resulted in an oil with very low levels of saturated fatty acids (6%), high levels of the monounsaturated fatty acid, oleic acid (58%) and moderate levels of polyunsaturated fatty acids (36%) compared to other edible vegetable oils. The fatty acid composition of canola oil is similar to that of olive oils with respect to the high level of oleic acid hut is much lower in palmitic acid and higher in polyunsaturates, particularly linolenic acid (10%). Because of presence of unsaturated fatty acid that renders canola oil susceptible to oxidative rancidity. The most valuable component of canola seed is the oil although the meal is used in animal feed (Daun *et al*., 1983).

2.5.1.1 Fatty acids composition**:** Mag (1990) studied and reported the fatty acid composition of canola oil which are listed in table 2.4.

Table.2.4 Fatty acids composition of canola oil

Components	Canola	Rape seed
Triglycerides (%)	94.4 - 99.1	91.8 - 99.0
Phospholipids (%)		
Crude Oil	up to 2.5	up to 3.5
Water-degummed	up to 0.6	up to 0.8
Acid-degummed	up to 0.1	-
Free Fatty Acids (%)	0.4 - 1.2	0.5 - 1.8
Unsaponifiables (%)	0.5 - 1.2	0.5 - 1.2
Tocopherols (ppm)	700 – 1200	700 – 1000
Chlorophylls (ppm)	5 – 35	5 – 35
Sulphur (ppm)	3 – 15	5 – 25

(Mag, 1990)

2.5.2 Physico-chemical properties: Przybylski *et al*. (2011) observed and reported the following parameters presented table in 2.5

Table.2.5. Physico-chemical properties of canola oil

Parameter	Value
Relative Density (g/cm3; 20˚C/water at 20˚C)	0.914 - 0.917
Refractive Index (nD 40˚C)	1.465 - 1.467
Crismer value (CV)	67 – 70
Viscosity (Kinematic at 20˚C, mm2/sec)	78.2
Cold Test (15 Hrs at 4˚C)	Passed
Smoke Point (˚C)	220 – 230
Flash Point, Open cup (˚C)	275 – 290
Specific Heat (J/g at 20˚C)	1.910 - 1.916
Thermal Conductivity (W/m K)	0.179 - 0.188

(Przybylski *et al*., 2011)

2.5.3 Utilization of canola oil

This study has showed that cold spredable butter of butterfat- canola oil (80%-20%) spreads can be substantially improved via both chemical and enzymatic inter-esterification; however, butter flavour degradation must be minimized before inter-esterification (either chemical or enzymatic) can be commercially used to produce cold-spreadable butter or a butter-based spread (Rousseau and Marangoni, 1998).

The feeding to dairy cows of canola seed protected from ruminal metabolism by emulsification and encapsulation in a matrix of aldehyde-treated protein resulted in a 10% increase in milk fat and no change in milk yield or protein content. Feeding the protected canola supplement significantly reduced the proportions of saturated fatty acids (C16:0, C 14:0 and C12:0) in milk fat; there were corresponding increases in proportions of C18:0, C18:1, C18:2, and C18:3. Yield of C18:0 monounsaturated and polyunsaturated fatty acids increased by 54%, which is equivalent to 143 g/d (Ashes *et al*., 1992).

Gulati *et al*. (2002) studied and reported that feeding cows with rumen protected n-3 fatty acids derived from canola/soybean (70/30,w/w) and soybean/linseed

oil (70/30) supplements increases the proportions of these acids C18:3 from 0.8 to 2.49 and 0.64 to 8.45% in milk fat respectively.

Jenab *et al*. (2013) developed the base stock production by Lipase-catalysed interesterification between canola oil and fully hydrogenated canola oil in contact with supercritical carbon dioxide at optimal reaction conditions to obtain the maximum degree of interesterification were 65°C, 10 MPa and 2 h of reaction time with an immobilised enzyme concentration of 6% (w/v) of initial substrate and which can be used for the production of margarine products with good TG composition, melting and crystallisation behaviour.

Rezaei and Temelli (2000) studied and analysed the effect of pressure, temperature, and CO_2 flow rate on the extent of conversion and the product composition in the enzyme-catalyzed hydrolysis of canola oil in supercritical carbon dioxide ($SCCO_2$) was investigated using lipase from *Mucormiehei* immobilized on macroporous anionic resin (Lipozyme IM). Reactions were carried out in a continuous flow reactor at 10, 24, and 38 MPa and 35 and 55°C. Supercritical fluid chromatography was used to analyze the reaction products. A conversion of 63–67% (triglyceride disappearance) was obtained at24–38 MPa.

Lobato-Calleros *et al*. (2002) studied the effect of lipophilic and hydrophilic low-molecular weight emulsifiers on microstructure and textural characteristics of Manchego cheese-like products (MCLP), which are made from canola oil skim milk emulsion of nine emulsifier blends comprised of different proportions of polyoxyethylene sorbitan monostearate (P), sorbitan monostearate **(S)** and glycerol monostearate (G) were used to prepare the emulsions. High proportions of P in the emulsifier blend resulted in MCLP cheeses having smaller and individual oil droplets surrounded by a dense protein structure, which exhibited elevated values of hardness, springiness and chewiness, but low values of adhesiveness and cohesiveness when comparing with control cheese (MCFF) sample made from whole milk in Texture profile analysis (TPA).

Andrew *et al.* (1996) developed and reported monounsaturated filled skim milk dairy product including, an emulsifier (mono-glycerides) and a vegetable oil (canola oil)contains at least 70% by weight of monounsaturated oleic acid, not more than 12% by weight of polyunsaturated linoleic acid and not more than 0.5% by weight of polyunsaturated linolenic acid. The filled dairy product may also include a polysaccharide

or oligosaccharide modifier and a carbohydrate gel stabiliser with good functional properties for human health because the developed product rich in MUFA and PUFA.

Kaufmann *et al*. (2012) studied the anhydrous milk fat and blends containing up to 40% (w/w) rapeseed oil (RO) were crystallized using a slow (0.05 °C/m or fast (5 °C/min) cooling rate. Fast cooling of the blends decreased the peak melting temperature of both high and low melting fractions of milk fat as the content of RO increased, while increasing RO combined with slow cooling did not affect melting behaviour. Solvent effects dominating in fast cooled samples are suggested to be responsible for the change in melting behaviour. The texture of fast cooled samples correlated with solid fat content. In slow cooled samples addition of RO decreased the hardness, which was ascribed to an increase in crystal cluster size. In addition, our study demonstrates that it is possible to produce blends with similar SFC, which differed in textural properties, by combining the effects of cooling rate and oil content.

2.6 INULIN

Inulin has been defined as a poly disperse carbohydrate material consisting mainly, if not exclusively, of beta (2-1) fructosyl-fructose links ranging from 2 to 60 units long and it belongs to a class of fructans (Kaur and Gupta, 2002). Native chicory inulin has an average degree of polymerization (DP) of 10 to 20, whereas oligofructose contains chains of DP 2 to 10, with an average DP of 4. It is used for a variety of purposes, including as a replacement for fat and sugar (Kocer *et al*., 2007), low-caloric bulking agent and a texturizing agent.

It is also used for its physiological features as a soluble dietary fibre and for its prebiotic properties (Tungland and Meyer,2002). The prebiotic concept is defined as "the selective stimulation of growth of one or a limited number of microbial species in the gut microbial that confers health benefits to the host" (Gibson and Roberforid, 2008). Inulin, oligofructose, galacto-oligosaccharides (GOS) and synthetic fructo-oligosaccharides (FOS) are examples of such prebiotics (Revathy *et al*., 2011). Inulin is considered as functional food ingredient, since they affect physiological and biochemical processes, resulting in better health and reduction in the risk of many diseases. It has been reported that the caloric value of inulin is 1.0 kcal/g (Gibson and Roberforid, 1995). Inulin is also used as a texturizer, thereby enhancing sensory properties, and is useful inrecipes that aim for a low glycemic index of 46 (Chawla and Patil, 2010).

2.6.1 Sources of inulin

Inulin is a naturally occurring non-structural, storage carbohydrate, found in leeks, onions,wheat, asparagus (*Asparagus officianalis*) garlic, Jerusalem artichoke (*Helianthus tuberosus*) and chicory (*Cichorium intybus*) root.

2.6.2 Composition

Inulin comes under both soluble as well as insoluble fiber categories depending on the degree of polymerization (DP). Delzenne (2003) and Gibson(1999) observed and reported inulin and short-chain FOS are compounds with unique D-fructofuranose polymers linked by a $\beta 2 \rightarrow 1$ bond at the anomeric C2, and are accumulated in the tissues of many plant species with varying degrees of polymerization ranging from 2 to 60 (inulin) or 2 to 20 (oligofructose).

2.6.3 Physico chemical characteristics of chicory root inulin

Franck (2002) studied and reported the following physical properties of standard inulin and high performance inulin presented in table 2.6

Table.2.6. Physico- chemical characteristics of chicory root inulin

Attributes	Standard inulin	High performance inulin
Chemical structure	GFn ($2 \leq n \leq 60$)	GFn ($10 \leq n \leq 60$)
Average degree of polymerization	12	25
Dry matter (%)	95	95
Inulin content (% on d.m.)	92	99.5
Sugars content (% on d.m.)	8	0.5
pH	5-7	5-7
Heavy metals (ppm on d.m.)	< 0.2	<0.2
Appearance	White powder	White powder
Taste	Neutral	Neutral
Sweetness (sucrose = 100%)	10%	None
Solubility in water at 25°C (g/l)	120	25
Viscosity in water (5%) at 10°C (MPa.s)	1.6	2.4

(Franck, 2002)

G = glucosyl unit; F = fructosyl unit; and d.m. = dry matter.

2.6.4 Health benefits of the inulin: Karimi *et al*. (2015) reported the following health benefits of inulin in table 2.7

Table 2.7 Health benefits of inulin

Application Area	Effects
Blood	• Lowering blood urea and uric-acid levels • Lowering blood-serum lipids
Cardiovascular system	• Reducing the risk of atherosclerosis • Reducing the incidence of cardiovascular disease
Intestine	• Lowering blood-serum lipids stimulating the body's immune system • Decreasing the levels of pathogenic bacteria • Relieving constipation • Reducing the incidence of colon cancer • Decreasing the symptom of irritable bowel syndrome • Increasing the stool frequency
Bone	• Decreasing the risk of osteoporosis • Increasing the calcium, magnesium, copper, iron & zinc absorption
Skin	• Improving the severity of atopic dermatitis
Weight management	• Increasing feelings of satiety • Smaller increase in body mass index

(Karimi *et al*., 2015)

2.6.5 Inulin as a dietary fibre

According to the American Association of Cereal Chemists (AACC, 2001) Dietary Fibre Definition Committee, dietary fibre is defined as the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin, and associated plant substances which are promote beneficial physiological effects including laxation, blood cholesterol attenuation and blood glucose attenuation.

Kalyani *et al*. (2010) reported that dietary fibres may be classified as water-soluble or gel forming viscous fibres and water insoluble fibres. Insoluble fibres consist mainly of cell wall components such as cellulose, lignin, and hemicelluloses present mainly in wheat, most grain products, and vegetables. Soluble fibre consists of non-cellulose polysaccharide such as pectin, gums and mucilage found in fruits, oat, barley, dried beans and legumes.

Elleuch *et al*. (2011) reported with higher dietary fibre intake stool weight tends to be higher and transit time enhances which may contribute to the prevention of large bowel disorders such as constipation, diverticulitis and large bowel cancers. Alles *et al.* (1996) found that inulin and oligo-fructose markedly enhance colonic fermentation and increases breath hydrogen excretion in humans.

Ghoddusi *et al*. (2007) reported inulin decreases the amount of generated gas while enhancing or maintaining its prebiotic effect. Microencapsulation of the probiotic microorganisms in addition to the prebiotic has been used to increase the probiotics survival in the developed symbiotic food (Aqilah, 2010).

Babu and Nithyalakshmi (2011) studied inulin has been used as a thermal protective agent in the form of symbiotic microcapsules to improve the thermal resistivity of prebiotics on probiotics. FOS and inulin can inhibit the growth of harmful bacteria and the generation of potentially harmful metabolites by releasing of short-chain fatty acids that reduces the pH, and there by prevent the growth of pathogenic bacteria.

2.6.6 Inulin as a fat replacer

Barclay *et al*. (2010) observed the presence of fat in dairy products plays an important role in their physical, rheological and textural properties. Fat, apart from its nutritional significance in cheese, contributes to the sensory and functional properties of dairy products (Miocinovic *et al*., 2011). Carmichael *et al*. (1998) studied and reported the low-fat food plans have been recommended for weight loss and maintenance.

Meyer *et al.* (2011) observed and reported inulin is particularly suitable for fat replacement in low fat cheeses to improve mouth feel. The fat-substituting property of inulin is based on its ability to stabilize the structure of the aqueous phase, which creates an improved creaminess (Ibrahim *et al*., 2004). High performance (HP) inulin with long chain and high molecular weight is the most desirable as a fat replacer by reducing the

formation of inulin microcrystals when mixed with water or milk; these microcrystals are not discretely perceptible and have a smooth, creamy mouth feel and it has no sweetness. Since inulin has been used successfully to replace fat in dairy products (Kaur and Gupta, 2002).

2.6.7 Inulin as texturizer

Highly soluble fibres, those that are highly branched and those that are relatively short-chain polymers, such as inulin, have low viscosities. They are generally used to modify texture or manage water migration, influence the colligative properties of the food system and enhance the food product's taste, mouth feel and shelf life without significantly altering its specific application characteristics and improve its marketability as a health-promoting or functional food product (Tungland and Meyer, 2002). Phillips and Williams (2000) studied the concentration of inulin increases to more than 15%, it can form a stable gel or cream which acts as mimics a fat-like texture. Akin *et al*. (2007) observed that the viscosity of the products increases with increasing levels of inulin and that the interaction of hydrocolloids with milk proteins may result in either improvement or deterioration of textural properties (Donkor *et al*., 2007).The dairy industry is very healthy and its basic commodities are maintaining relatively stable consumption. But the milk products are though rich source of fat and proteins lacks in soluble fibres and essential fatty acids such as omega 3 fatty acids. The review of available literature shows the importance of the omega 3 and omega 6 fatty acids and soluble fibres on the human health. However, no information is available on the incorporation of canola oil and flaxseed oil as source of omega 3 fatty acids and inulin as source of soluble fibre in milk based beverages. Hence this investigation was taken upto incorporate these ingredients in milk based flavoured drinks to enhance the functional qualities of the drink.

2.6.8 Recommended intake of dietary fibre

For a healthy diet the recommended daily dose of dietary fibre is 25g for persons consuming two thousand kcal daily and 30 g per day for those consuming 2500 kcal. World Health Organization (WHO) recommends 16-24g/d of non-starch polysaccharides or 27-40g/d of total dietary fibre.

CHAPTER 3

SCOPE AND PLAN OF WORK

3. SCOPE AND PLAN OF WORK

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3.1 SCOPE

With the increase in awareness among the consumers, the demand for heath foods with health benefits in increasing steadily. The importance of omega 3 fatty acids and soluble solids on the human health is well documented. The milk is considered as one of the carriers for such functional ingredients. Considering the popularity of the flavoured milk and importance of omega 3 fatty acids and soluble solids, the present investigation was taken up with the following objectives:

1 To optimize the levels of omega-3 rich vegetable oils and inulin for production of milk based functional flavoured drink
2 To analyse the physico-chemical parameters of the flavoured drink
3 To study the shelf life of the developed functional flavoured drink

3.2 PLAN OF WORK

3.2.1 Process optimization for functional flavoured drink

Type of heat treatment: Pasteurization

3.2.1.1 Milk fat

Toned milk used as a source of milk fat from experimental dairy plant, ICAR- National Dairy Research Institute, Bengaluru and standardized to 3% fat, 9.5% MSNF.

3.2.1.2 Selection of oil for blending with milk fat

Canola oil and flaxseed oil are selected for blending with milk fat which are rich in omega 3 fatty acids.

Canola oil, flax seed oil and their combination (1:1) were used for blending with milk fat.

3.2.1.2.1 Optimization of milk fat with vegetable oils

Various proportions of milk fat (MF) and selected vegetable oils i.e. canola and flaxseed oil

Were selected based on the preliminary trials and effect of selected proportions on sensory attributes.

- 100:0, 75: 25, 50:50, 25:75, 0:100

3.2.1.3 Optimization of level of inulin, MSNF and Sugar

Based on preliminary trials, inulin level selected were 2, 3, 4, 5, 6% for preparation of flavoured milk by using blend of milk fat, vegetable fat: 50:50

Milk SNF selected for optimization: 8.5, 9.0 and 9.5%

Sugar levels selected for optimization were 6, 7, and 8%

3.2.1.4 Selection of colour and flavour for preparation of functional flavoured drink

Following flavours were selected to study the liking

Mango, Chocolate, Banana

3.2.2 Study of stability of the developed flavoured drink to sterilization

To study the stability of developed flavoured drink to sterilization, flavoured drink was sterilized at 121^0C for 15 min.

3.2.3Analysis of developed flavoured drink

Developed flavoured drink was analysed for compositional, sensory evaluation by using 9- point hedonic scale and physico-chemical which are listed below.

3.2.3.1 Proximate analysis

Moisture

Fat

Protein

Total carbohydrate content

Ash

Total solids

Fatty acid analysis

3.2.3.2 Physico- chemical analysis

pH,

Titratable acidity,

Viscosity

3.2.3.3 Sensory analysis

Developed flavoured drink was analysed for sensory attributes such as colour& appearance, body and texture, flavour, sweetness and overall acceptability of flavoured drink by using 9 point hedonic scale at refrigerated conditions.

3.2.4 Storage studies of developed flavoured drink

Developed flavoured drink was stored at refrigerated temperature and checked for changes in physico-chemical, sensory, and microbiological for regular interval of 2 days.

3.2.4.1 Sensory evaluation

Sensory parameters such as colour& appearance, body and texture, flavour, sweetness and overall acceptability of flavoured drink at refrigerated conditions were checked by 9 point hedonic scale.

3.2.4.2 Physico-chemical analysis

pH, Acidity, Viscosity.

3.2.4.3 Microbial analysis

Total bacterial count (TBC)

Coliform

CHAPTER 4

MATERIALS AND METHODS

4.0 MATERIALS AND METHODS

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With increase in consumer awareness regarding health, focus of the food technologists has shifted to produce healthy and convenience foods by blending of the benefits of various food raw materials to provide maximum health benefits to the consumers beyond nutrition. In present study, attempts were made to blend milk fat, vegetable oils which are rich in polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA), and soluble dietary fiber (Inulin) to develop functional flavoured drink with balanced fatty acid profile and soluble fibre. The materials used in experimental trials and methods to optimize and analyse the product are presented in the chapter.

4.1 MATERIALS

4.1.1 Ingredients

The types and sources of various ingredients used during present investigation are presented here:

4.1.1.1 Skim milk

Raw skim milk taken from the Experimental Dairy of ICAR-National Dairy Research Institute, Bengaluru was used for flavoured drink preparation with canola oil, flaxseed oil and mixture of two oils.

4.1.1.2 Pasteurized milk

Pasteurized milk taken from the Experimental Dairy of ICAR-National Dairy Research Institute, Bengaluru was used for preparation of control and flavoured drink with different levels of selected vegetable oils.

4.1.1. 3 Canola oil

Canola oil of Jivo brand was purchased from Nilgiris Market, Bengaluru, India.

4.1.1.4 Flax seed Oil

Flax seed Oil of Prano brand was procured from Nilgiris market Bengaluru, India.

4.1.1. 5 Skim milk powder

Spray dried skim milk powder (Nandini brand) manufactured by Mother Dairy, Yehlanka, Karnataka Milk Federation, Bengaluru was used to increase the milk solids not fat (MSNF) content of flavoured drink.

4.1.1.6 Inulin

Inulin was procured from M/s DKSH India, Pvt. Ltd for the preparation of flavoured drink preparation.

4.1.1.7 Sugar

Refined crystalline cane sugar was purchased from local market, Bengaluru, India for its use in flavoured milk preparation.

4.1.1.8 Emulsifier

Glycerol mono stearate (GMS) procured from local market, Bengaluru, India was used as emulsifier in the preparation of flavoured drink.

4.1.1. 9 Flavours

Banana and chocolate flavours of Aromatic Bangalore Pvt. Ltd procured from local market, Bengaluru, India and Mango flavour of Dohler India Private Ltd procured from Sri Durga agencies, Bengaluru, India were used as flavouring agents in preparation of flavoured drink.

4.1.1.10 Colours

Kesar badam colour and chocolate brown colour of IFF brand purchased from local market, Bengaluru, India were used as colouring agents.

4.1.2 Reagents for analysis

4.1.2.1 Chemicals

Analytical grade chemicals from reputed pharma companies were used for chemical analysis.

4.1.2.2 Media

The dehydrated media were obtained from M/S Hindustan dehydrated Media (Hi Media) Laboratories Ltd., Mumbai for microbiological analysis of the samples.

4.1.2.3 Glass wares

Glass wares used were of Schott & Duran and Borosil brand and were thoroughly cleaned by soap solution, dried & used. For microbiological analysis the glassware were sterilized as per standard practice before they were used.

4.1.2.4 Fatty Acids Standard

Fatty acids standard was purchased from Supelco Scientific with total 37 fatty acids present in it by the name SUPELCO-37.

4.1.3 Equipments

4.1.3.1 Analytical weighing balance

A weighing balance supplied by Spark instruments, Bengaluru was used for weighing the raw materials.

Sartorius Analytical weighing balance of 0.1 mg accuracy was used for weighing minor ingredients like emulsifiers and samples for analysis.

4.1.3.2 Homogenize*r*

Two stage homogenizer manufactured by Goma, India was used to homogenize the control milk and the mixture of oils and skim milk for development of functional flavoured drink.

4.1.3.3 Hot air oven

Hot air oven (Apollo Scientific Surgical Co., Bangalore.) it was used for sterilization of glassware.

4.1.3.4 Vortex mixer

Vortex mixer of Matrix Mix Company was used. The purpose is to mix the mixture of milk sample and added chemicals extraction mixture before the mixture was centrifuged for of fatty acid profile analysis of fat.

4.1.3.5 R & C laboratory centrifuge

The centrifuge sold and serviced by Vijay enterprises was used to spin the extracted sample at 3000 rpm for 5 minute for separation of hexane layer containing the fat/oil.

4.1.3.6 Super Mixer Grinder

Panasonic MXC300S, was used for mixing the mixture of oil, skim milk and emulsifier before the mixture was homogenized.

4.1.3.7 Sealing machine

Sealing machine supplied by Hitech Pack, Bengaluru was used for the sealing of LDPE pouches after filling of pasteurized flavoured milk for storage study.

4.1.3.8 pH meter

The electrode assembly of a digital pH meter (Digisun Electronics, Hyderabad, Model: DI 707) calibrated against standard buffer of pH 7.0 and 4.0 (Qualigens Fine Chemicals) was used for estimation pH of flavoured drink samples.

4.1.3.9 Ostwald viscometer

The kinematic viscosity of flavoured drink samples were determined using Ostwald viscometer (Vensil Glassware, Chennai). The viscosity measurements were carried out at 40°C.

4.1.3.10 Muffle furnace

Muffle furnace (Murhopye Scientific Company, Mysore, India) maintained at 550±10ºC was used for the determination of the ash content of the flavoured drink.

4.1.3.11 Autoclave

Autoclave (Appollo Scientific Surgical Co., Bengaluru) was used for the sterilization of microbiological media and preparation of sterilized functional flavoured drink SAMPLES.

4.1.3.12 Gas chromatography Unit

Gas chromatographic system Model 7890A GC (Agilent) with Flame Ionization Detector (FID) and DB-WAX, 30m*0.25mm i.d.*0.25 m column was used for fatty acid profile analysis of the flavoured drink.

4.2 METHODOLOGY

4.2.1. Method of preparation of flavoured drink

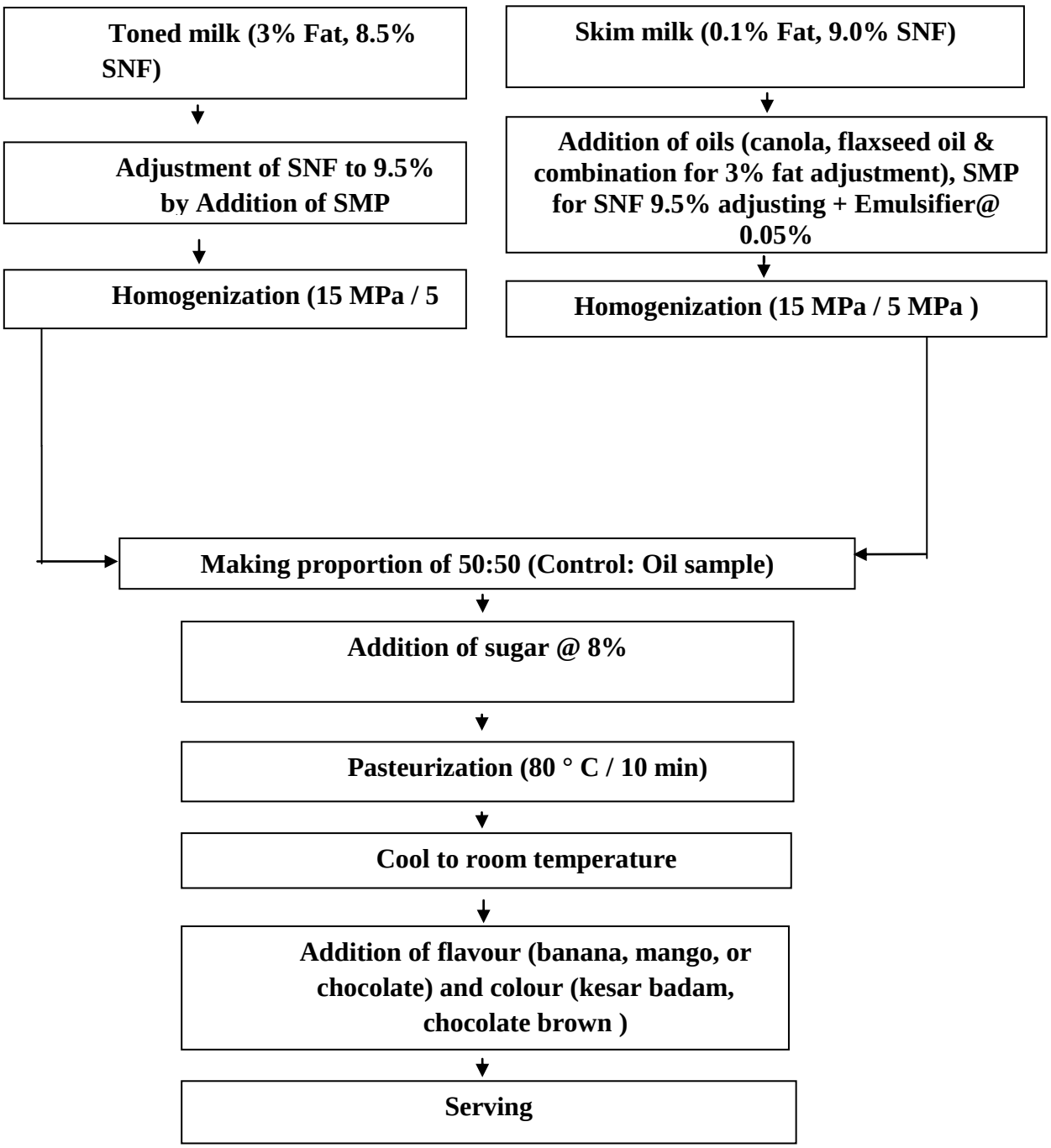

Fig. 4.1 Method of preparation for flavoured drink

The flow diagram for the preparation of flavoured drink is shown in fig. 4.1.

4.2.2 Preparation of experimental samples

As shown in the fig. 4.1 for the preparation of experimental flavoured drink the canola oil was added to skim milk to get 3.0% fat in the milk. The emulsifier (GMS) was added at 0.05% of the milk volume. The Milk SNF was adjusted to 9.5% and the cane sugar was added at 8% (w/v) of the milk total milk volume. The mixture was then filtered through muslin cloth and pre heated to about 60^0C and homogenized at 2500psi in 1^{st} stage and 500 psi for 2^{nd} stage by using 2 stage homogenizer. The homogenized milk was then given a final heat treatment of 80^0C for 10 minute and cooled to room temperature. The selected colour and flavour was added to the cooled milk and packed into 200 mL pouches before it was stored at refrigerated temperature for further analysis.

Similarly flavoured milk with flax seed oil was prepared by replacing the canola oil with flax seed oil and following the procedure as described above.

To study the effect of blend of oils, the flax seed oil and canola oil were mixed in 1: 1 ratio and then the mixture was added to skim milk before the skim milk and oil blend were homogenized, to give total fat content of milk of 3.0%.

To prepare the experimental flavoured milk with different proportions of vegetable oil and cow milk with 3.0% fat and 9.5% MSNF were mixed in 0:100, 25:75, 50:50, 75:25 and 100:0 ratio to give the oil: milk fat ratio of 0:100, 25:75, 50:50, 75:25 and 100:0 in the milk. Flavoured milk with only milk fat was served as control. The blending of milk was done before giving final heat treatment of 80°C for 10 min.

4.2.3 MSNF content

To optimize the level of Milk SNF in the flavoured drink, the MSNF of the milk was adjusted to 8.5%, 9%, and 9.5% before homogenization of the mixture of oil and skim milk.

4.2.4 Inulin

To optimize the level of inulin incorporation in the flavoured drink, inulin was added at 2, 3, 4, 5, and 6% of the milk taken to study the effect of inulin on sensory and viscosity of flavoured drink.

4.2.5 Sugar content

For optimization of sugar level in flavoured drink preparation, sugar was added at 6, 7 and 8% level to study its effect on sensory properties and viscosity of the flavoured drink. However, the sugar content in control sample was maintained at 8%.

4.2.6 Selection of flavours

Mango, Chocolate, Banana flavours and corresponding colours were added to the optimized flavoured drink to select the colour and flavour done for preparation of functional flavoured drink. The liking of flavour was decided based on the sensory evaluation.

4.2.7 Heat treatment

Pasteurization: the control and experimental flavoured drink samples were heat treated at 80°C for 10 min, cooled to room temperature, colour& flavour were added and stored under refrigerated conditions before it was analysed for various parameters.

After blending the cow milk and milk with vegetable oil to the optimized level, colour and flavour were added, filled in bottles, crown corked and sterilized at 121°C for 15 min to know the stability of the developed flavoured drink for sterilization conditions.

4.3 PHYSICO-CHEMICAL ANALYSIS:

4.3.1 pH

The pH of pasteurized flavoured drink sample was measured by using a pH meter by the method described in IS: SP: 18(Part XI, 1981). The pH meter was first calibrated by using standard buffer of pH 4.0 and 9.0 and then using pH 7.0 at 20.0 ± 0.1°C.

4.3.2 Titratable acidity

The titratable acidity of flavoured drink samples were determined according to the procedure given in BIS (1981). The flavoured dink samples were well mixed and 10 ml of the sample was taken in a beaker. A few drops of phenolphthalein indicator was added as indicator and titrated against 0.1N NaOH solution to persistent pale pink colour end point. The acidity was expressed in percent lactic acid.

Calculation:

Acidity (% LA) = (V/W) × 0.9

Where,

V= volume of NaOH used (ml) and

N = normality of NaOH

W= volume of flavoured milk sample taken (ml).

4.3.3 Viscosity

Viscosity of flavoured drink was measured by the capillary viscometer (Ostwald viscometer). Thirteen millilitre of distilled water was tempered to a temperature of 40^0C and filled into the arm A. then time for flowing of distilled water through capillary of arm B was calculated. Similarly time for flow of equal volume of flavoured drink was calculated at the same temperature of viscosity was calculated by the formula:

$\eta = k \times t$

(k = $\frac{\eta}{t}$ of water)

Where,

η = viscosity,

k = conversion factor,

t = time (seconds)

4.3.4 Determination of fat content:

Fat in flavoured drink samples were determined by Mojonnier- gravimetric method as per the method ISI (1981b).

First extraction: Approximately 10 g of flavoured drink sample was accurately weighed into clean dried 50 ml beaker to which 8 ml of hot water was added followed by 3 ml of ammonia solution and the mixture was swirled gently to completely dissolve the spread. The mixture was cooled and 10 ml of ethyl alcohol was added. The contents of the beaker were transferred to mojonnier extraction tube and 25 ml of diethyl ether and 25 ml of petroleum ether (40-60^0C) were added through the beaker used for weighing the sample. The mixture was mixed thoroughly after each addition of the solvents for 90 seconds with intermittent release of gas by removing the stopper. The tube was then

allowed to stand undisturbed for 30 minutes and there after the clear supernatant was decanted into pre-dried and weighed beaker having 2 glass beads.

Second extraction: Fifteen millilitres of each of diethyl ether and petroleum ether were added in order. After each addition of solvent the tube contents were mixed by shaking for 30 seconds. The solvent and water phase were allowed to separate and supernatant was carefully transferred to the same previous beaker.

Third extraction: Five millilitres of each of diethyl ether and petroleum ether were added in same manner. After each addition of solvent the tube contents was mixed by shaking for 30 seconds. The solvent and water phase were allowed to separate and supernatant was carefully transferred to the same previous beaker.

The solvent mixture was evaporated over boiling water and the final traces of water was removed using hot plate and was dried for 1 hour in hot air oven at 100°C to remove traces of water and solvent. The beaker was cooled in desiccators and weighed accurately.

$$\% \text{ Fat} = \frac{\text{Weight of fat residue}}{\text{Sample weight}} \times 100$$

4.3.5 Protein content

Reagents

1. Mixed indicator- mixture of 0.1% bromocresol green and 0.1 percent methyl red indicator (5:1)
2. Two percent boric acid- 10 g of boric acid crystals in 500 ml of boiling distilled water
3. Forty percent NaOH
4. Catalyst for digestion- Potassium sulphate and copper sulphate (5:1)
5. Sulphuric acid of 0.01 N normality
6. Concentrated H_2SO_4

Procedure

The percent protein in flavoured drink was determined by standard Micro Kjeldhal method described in AOAC (2005). The procedure in brief is as follows:

Approximately 5 g flavoured drink sample was weighed accurately and transferred carefully to 300 ml Kjeldhal flask. Five grams of digestion mixture (K_2SO_4 and $CuSO_4$) and 12.5 ml of concentrated sulphuric acid (AR) were added to the flask. The

contents were digested in a digestion assembly until clear and colourless residue was obtained. After cooling, the Kjeldhal flask was washed with10-15 ml of distilled water and added to the Kjeldhal distillation tube. 30 ml of 40% NaOH solution was added to make the solution alkaline. The contents were steam distilled and the liberated ammonia was collected in 25 ml of saturated boric acid solution containing 2-3 drops of the mixed indicator (methyl red and methylene blue). The distillation was continued until about 65-75 ml of distillate was collected. The distillate was titrated against 0.01N H_2SO_4 until purple colour end point appeared. A blank test was carried out simultaneously using all the reagents except the test material and the percent protein was calculated as follows:

$$\%\text{Protein} = \frac{F \times 1.4 \times (\text{Sample reading} - \text{Blank reading}) \times \text{Normality of } H_2SO_4}{\text{Weight of the sample in (g)}}$$

Where, F = factor for conversion of % nitrogen into % protein (6.38).

4.3.6 Moisture (Gravimetric method)

Moisture content in flavoured drink sample was determined according to BIS (1981). About 10 g of milk sample was weighed accurately in to a previously dried and tarred aluminium dish, mixed thoroughly the dish was then transferred to oven maintained at 102 ± 1°C for 4 hours. The dish was taken out from the oven and immediately transferred to desiccators. After cooling for about 30 min the dish was weighed. The process of heating, cooling and weighing was repeated until the loss of weight between two successive weighing was less than 1 mg.

Calculation of moisture content

$$\text{Moisture (\%w/w)} = 100 \times \frac{(W_1 - W_2)}{(W_1 - W)}$$

Where,

W = Weight in g of the empty dish

W1 = Weight in g of the dish with sample before drying

W2 = Weight in g of the dish with sample after drying.

4.3.7 Total ash content

Ash content in flavoured drink was determined as per the method described in AOAC (2005). About 3 g of flavoured drink sample was accurately weighed into dried crucible and charred for 1 hour over hot plate till the smoke stopped coming from the

sample. The crucible containing the charred sample was then transferred to a muffle furnace maintained at 550°C±10°C and kept there for 5-6 hours, cooled and weighed.

$$\% \text{ Ash} = \frac{\text{Weight after ashing} - \text{Weight of crucible}}{\text{Sample weight}} \times 100$$

4.3.8 Total Carbohydrate

Total carbohydrate in the developed flavoured drink was determined by difference method by the formulae given below:

Total Carbohydrate (%) = 100- (% Moisture+% Fat+%Protein+%Ash)

4.3.9 Evaluation of functional flavoured drink for sensory attributes

The organoleptic qualities of flavoured drink were evaluated by an expert panel drawn from the Institute. The 9 point hedonic scale score card was used for sensory evaluation. The evaluation was carried out under proper lighting. The judging parameters were: colour and appearance, body & texture, flavour, sweetness and overall acceptability.

4.3.10 Fatty acid profile analysis

Fatty acid profile of flavoured drink samples were analysed by Gas Chromatography technique after converting to their respective methyl esters.

4.3.10.1 Preparation of Fatty Acid Methyl Esters (FAME)

Fatty acid methyl esters were prepared as described by the method of (Mech *et al*., 2015). About 1.5 ml (1.57g) flavoured drink sample was weighed and taken in a screw capped pyrex culture tube. 1.0 ml internal standard (Tri decanoic acid) followed by 0.7 ml of 10N KOH and then 5.3 ml of methanol were added to the tube then placed in a water bath maintained at 55°C. Tube was incubated in a water bath for 1.5 h with intermittent vigorous shaking for 5 s in every 20 minutes. Tube was then taken out after 1.5 h and was cooled under tap water. After cooling, 0.58 ml of 24 N H_2SO_4 was added. Tube was inverted for proper mixing. After mixing, tube was incubated in a 55°C water bath for 1.5 hour with intermittent vigorous shaking at every 20 minutes interval and was cooled.

Exactly 3 ml hexane was added to the tube and contents were vortex mixed in the tube and then centrifuged for 5 minutes at 1000xg at room temperature in a table top centrifuge. Upper hexane layer containing fatty acid methyl esters was recovered with the help of micropipette and was placed in glass ampoule which was heat sealed with a butane gas burner. Sealed ampoules were kept at -20°C until GC analysis.

4.3.10.2 GC analysis of FAME

Quantification of FAME in samples was performed using gas chromatograph (Model: 7890AGC, Agilent Technologies, United States) equipped with flame ionization detector (FID) and computer assisted programmable data processing and printer. The operating parameters were:

GC analysis condition

Chromatographic system

Model : 7890AGC (Agilent);

Inlet: Split/splitless (Split mode);

Detector: Flame ionization detector (FID);

Column: DB-WAX, 30 m x 0.25mm i.d.x0.25m;

Experimental conditions

Inlet temperature: 250°C;

Injection volume: 1µl;

Split ratio: 50:1;

Carrier gas: Hydrogen;

Head pressure: 53 kPa constant pressure (36 cm/s at 50°C);

Oven temperature: 50°C, 1 min, 25°C/min to 230°C, 18 min;

Detector: 280°C;

Detector gases: Hydrogen: 40 ml/min; Air: 450 ml/min;

Nitrogen make-up gas: 30ml/min

The fatty acids were identified by comparing the retention times with fatty acid methyl esters of standard mixture and quantified by area percent method through the integrated software package. Peak areas were evaluated to estimate proportion of various fatty acids. Fatty acid as percent of total lipid weight of its respective samples are reported. Unknown concentration of analyte 'A' with a known concentration of analyte 'B' (internal standard) was calculated by the below given formula:

$$\text{Concentration of A} = \frac{\text{Peak area A}}{\text{Peak area B}} \times \frac{1}{\text{RRF}} \times \text{Concentration B} \times \frac{1}{\text{Dilution factor}}$$

4.3.11 Microbial Analysis

4.3.11.1 Preparation of saline solution:

The saline solution was used for preparing serial dilutions of the sample for microbiological analysis. Sodium chloride (8.5g) was dissolved in 100 ml distilled water and pH was adjusted to 7 ± 0.1 and filled in the test tubes at the rate of 9 ml in each test tube, plugged with cotton plugs and autoclaved at 121°C for 15 minute.

4.3.11.2 Preparation of Nutrient agar and enumeration of Total Bacterial Count

The dehydrated nutrient agar medium was used for enumeration of total bacteria in the samples. The composition of the nutrient agar is given in table 4.1.

Table.4.1 Composition of the nutrient agar

Ingredients	Gms/litre
Hi veg peptone	5.00
Hi veg extract no.1	3.00
Agar	15.00
Final pH (at 25°C)	6.8±0.2

Six grams of dehydrated agar medium was dissolved in two hundred and fifty millilitre of distilled water and boiled to dissolve the agar. After complete melting of agar, the medium was dispensed into conical flasks of suitable volume, plugged with cotton plug and autoclaved at 121^0C for 15 min. the agar medium was then cooled to room temperature before it was used for plating.

The control and experimental samples were diluted to desired dilution by using 9 mL saline blanks and 1 ml of the diluted sample was pipetted in to sterile petri plate. The nutrient agar was then added and incubated at 37^0C for 24 hours. The plating was done in duplicate.

At the end of incubation period, the bacterial colonies were counted and recorded as cfu/ml of sample.

4.3.11.3 Preparation of Mac Conkey's agar and enumeration of coliform count

The dehydrated MacConkey's agar was used for enumeration of coliform content in the drink samples. The composition of the medium is given below table 4.2.

Table.4.2 Composition of Mac Conkey's agar

Ingredients	Gms/litre
Peptic animal tissue	20.00
Lactose	10.00
Sodium taurocholate	5.00
Sodium chloride	5.00
Bromo cresol purple	0.02
Final pH (at 25°C)	7.2±0.2

Ten grams of the dehydrated medium was added to two hundred and fifty millilitre of distilled water and boiled to dissolve the agar. After complete dissolution of the agar, the medium was transferred to conical flasks and autoclaved at 121^0C for 15 min, then media was cooled to room temperature before it was used for plating.

Coliform count in flavoured drink was enumerated by the pour plate method by plating 1 mL of selected dilution of the product employing MacConkey's agar. Duplicate plates were incubated at 37°C for 24 hrs. Colonies with visual growth were counted and expressed as CFU/ ml of the product.

4.4 STORAGE STUDIES

The flavoured drink samples prepared by using optimized formulation were packed in LDPE pouches and it was stored at refrigeration temperature then shelf life of product was observed on the basis of sensory evaluation (9 point hedonic scale) the samples were drawn at 2 days interval and analysed for sensory, physico-chemical and bacteriological parameters.

4.4.1 Sensory Evaluation

The flavoured milk samples were analysed for sensory parameters on 9 point hedonic scale as described in 4.3.9.

4.4.2 Physico-chemical analysis

The flavoured milk samples were analysed for Viscosity, pH, and Acidity to study the changes those were taking place during storage period.

4.4.3 Microbial analysis

The flavoured milk samples were analysed changes in total bacterial count and coliform count during storage period as described in 4.3.11

4.5 STATISTICAL ANALYSIS

The levels of canola oil, flaxseed oil and their combination (1:1), Inulin, MSNF, sugar optimized for development of functional flavoured drink by using one way analysis of variance (ANOVA). Significance was tested by employing analysis of variance (ANOVA). The storage data were also analysed with SPSS software, version 16.0.

CHAPTER 5

RESULTS AND DISCUSSION

5. RESULTS AND DISCUSSION

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With increase in awareness about the health, the consumers are looking for the nutritional food with functional attributes. Understanding the importance of omega 3 fatty acids and soluble fibres in human nutrition, attempts were made to replace the milk fat with canola oil and flaxseed oil as sources of omega 3 fatty acids and inulin as a source of soluble fibre in milk based flavoured drink. The findings are presented in following paragraphs:

5.1EFFECT OF INCORPORATION OF CANOLA OIL ON SENSORY QUALITY OF FLAVOURED DRINK

Table 5.1 Effect of canola oil on sensory quality of flavoured drink

Sensory attributes	control	Canola oil **:** Milk fat			
		25:75	50:50	75:25	100:0
Colour and Appearance	8.00 ± 0.14^{a}	7.97 ± 0.11^{a}	8.00 ± 0.17^{a}	7.91 ± 0.17^{a}	7.95 ± 0.21^{a}
Body & texture	8.00 ± 0.13^{d}	7.92 ± 0.17^{cd}	7.84 ± 0.13^{c}	7.09 ± 0.12^{b}	6.70 ± 0.11^{a}
Flavour	7.91 ± 0.18^{d}	7.85 ± 0.13^{cd}	7.76 ± 0.13^{c}	6.71 ± 0.11^{b}	6.60 ± 0.12^{a}
Sweetness	7.78 ± 0.20^{a}	7.77 ± 0.19^{a}	7.76 ± 0.21^{a}	7.7 ± 0.24^{a}	7.64 ± 0.23^{a}
Overall Acceptability	8.00 ± 0.16^{d}	7.96 ± 0.13^{cd}	7.87 ± 0.12^{c}	6.81 ± 0.09^{b}	6.70 ± 0.14^{a}

Note: *Fat and MSNF content in all the samples maintained at 3.0% and 9.5% respectively, sugar added @ 8% in all the samples*.
Values are average of three trials. Mean values in same row not sharing common superscript are significantly different ($p<0.05$)

The flavoured drink prepared with different proportions of milk fat and canola oil subjected to sensory evaluation and the scores are presented in table 5.1.

The sensory scores showed the colour and appearance score for control sample was 8.0 and that for experimental sample varied between 7.91±0.17 and 8.00±0.14.

No significant variation between the control and experimental sample were observed. Since lemon yellow colour was added, all the samples had almost similar scores.

The body & texture score revealed that the control drink scored the score of 8.00. The score marginally reduced to 7.92 for the sample containing 25:75 ratio of canola oil, milk fat was used and to 7.84 for sample containing 50:50 ratio of canola oil, milk fat. No significant difference between 25:75 and 50:50 samples was observed. The score significantly reduced to 7.09 for 75:25sample and further reduced to 6.70 for 100% canola oil used sample. It was observed that the consistency of the drink reduced with increased use of canola oil (Gunstone, 2011). The higher levels of unsaturated fatty acids present in canola oil could be the reason for thinner consistency and poor mouth feel. It was also observed that the apparent viscosity of canola oil was less than that of milk fat (ghee).

The control flavoured drink scored a flavour score of 7.91±0.18. The score insignificantly reduced to 7.85±0.13 for 25:75 sample. The score further reduced to 7.76±0.13 for 50:50 and no significant difference was observed between 25:75 and 50:50 samples. When the oil ratio was increased to 75:25, the typical canola oil was felt in the drink and the score significantly reduced to 6.71±0.11. When the milk fat was totally replaced (100:0 sample), the oil flavour became more dominant and the flavour score significantly reduced to 6.60±0.12.

The sweetness scores shows that the control sample scored as score of 7.78±0.20 and the scores for experimental sample varied between 7.77±0.19and, 7.64±0.23 and no significant differences between the control and experimental samples was observed. Since canola oil was bland in taste, it did not contribute to the sweetness of the drink.

The overall acceptability scores followed the trend of body& texture and flavour scores. The control flavoured drink scored a highest overall acceptability score of 8.00±0.16 and the score insignificantly reduced to 7.96±0.13 for 25:75sample. But the scores reduced to 7.87±0.12 for 50:50sample and no significant difference was observed between 25:75 and 50:50 sample. The score significantly reduced to 6.81±0.09 for 75:25 and 6.70±0.14 for 100:0 samples, mainly due to thinner consistency and detection of oil flavour in these samples.

5.2EFFECT OF INCORPORATION OF FLAXSEED OIL ON SENSORY QUALITY OF FLAVOURED DRINK

Table.5.2 Effect of flaxseed oil on sensory quality of flavoured drink

Sensory attributes	Control	Flaxseed oil : Milk fat			
		25:75	50:50	75:25	100:0
Colour and Appearance	8.00 ± 0.15^{a}	8.02 ± 0.11^{a}	7.98 ± 0.15^{a}	7.95 ± 0.14^{a}	7.91 ± 0.23^{a}
Body & texture	7.86 ± 0.16^{c}	7.77 ± 0.15^{bc}	7.71 ± 0.12^{b}	6.86 ± 0.16^{a}	6.78 ± 0.15^{a}
Flavour	7.84 ± 0.13^{d}	7.77 ± 0.11^{cd}	7.69 ± 0.15^{c}	6.66 ± 0.10^{b}	6.44 ± 0.13^{a}
Sweetness	7.8 ± 0.19^{a}	7.75 ± 0.18^{a}	7.69 ± 0.19^{a}	7.68 ± 0.20^{a}	7.67 ± 0.19^{a}
Overall Acceptability	7.93 ± 0.14^{d}	7.87 ± 0.13^{cd}	7.80 ± 0.14^{c}	6.74 ± 0.11^{b}	6.55 ± 0.10^{a}

Note: *Fat and MSNF content in all the samples maintained at 3.0% and 9.5% respectively, sugar added @ 8% in all the samples.*
Values are average of three trials. Mean values in same row not sharing common superscript are significantly different ($p<0.05$)

The milk fat was replaced by flaxseed oil at different proportions in the flavoured drink to study the effect of flax seed oil on the sensory scores of the drink. The results are presented in table 5.2.

The result showed that the control flavoured drink scored a colour and appearance score of 8.00±0.13, while that for experimental samples prepared with different levels of flaxseed oil varied between 7.91 and 8.02. The statistical analysis showed that no significant difference was observed between control and experimental samples.

The body and texture scores showed that the control sample scored a higher score of 7.86±0.16 and the score marginally reduced to 7.77±0.15 for 25:75sample. Statistically there was no significant difference in scores of 25:75 and 50:50 samples. When the ratio of flaxseed oil and milk fat was increased to 75:25, the score significantly reduced to 6.86±0.16 to 6.78±0.15 for 100% flaxseed oil used flavoured drink. It was observed that with increase in flaxseed oil proportions, the consistency of the flavoured drink reduced which resulted in lowering scores.

Similarly the flavour score shows that control drink sample scored a higher score of 7.84±0.13 and the scores reduced with increased proportion of flaxseed oil. However the flavour score for 25:75 blended drink was 7.77±0.11 and the score was not significantly different from that of control sample. Similarly the score for 50:50drink was 7.69±0.15 which was not significantly different from that for 25:75 drink, but the flaxseed oil flavour was observed in 75:25drink and the flavour was prominent when the milk fat was completely replaced with flaxseed oil (100:0).

The sweetness scores for control and experimental samples varied between 7.80±0.19 and 7.67±0.17 and no significant differences were observed between the control and experimental samples. This indicates that the flaxseed oil did not interfere with the sweetness of the product.

Generally the body and texture and flavour have significant effect on the overall acceptability of a food. In the present study the overall acceptability of the drink followed the trend of body & texture and flavour scores. The control sample scored a higher overall acceptability score of 7.93±0.14 and the score reduced with proportion of flaxseed oil in the total fat. The score for 25:75 drink was 7.87±0.13 which was not significant from the score of the control sample. Similarly the score for 50:50 drink was 7.80±0.14 and it did not significantly differed from that for 25:75 drink sample. The 75:25 and 100:0 samples scored 6.74±0.11 and 6.55±0.10 and these scores significantly differed from the 50:50 sample. Hall and Schwarz , (2002) reported that ice cream prepared by replacing milk fat by using 25% flax seed oil exhibited an oil like mouth feel; however, the presence of the oil in product could not be detected by 60% of the panellists using an informal sensory evaluation.

5.3 EFFECT OF INCORPORATION OF CO & FSO MIXTURE (1:1) ON SENSORY QUALITY OF FLAVOURED DRINK

The canola oil and flax seed oil were mixed in 1:1 ratio and was incorporated at different proportions to study the effect of mixture of two oils on the sensory attributes of flavoured drink. The scores are tabulated in table 5.3.

The colour and appearance scores for control sample was 8.00±0.13 and that for experimental samples varied between 7.91±0.23 and 7.99±0.15. No significant differences were observed of scores of control and experimental samples.

Table 5.3 Effect of CO& FSO mixture (1:1) on sensory quality of flavoured drink

Sensory attributes	Control	*Oil mixture **:** Milk fat			
		25:75	50:50	75:25	100:0
Colour & Appearance	8.00 ± 0.13^{a}	7.97 ± 0.11^{a}	7.95 ± 0.13^{a}	7.99 ± 0.15^{a}	7.91 ± 0.23^{a}
Body & texture	7.85 ± 0.12^{d}	7.76 ± 0.16^{cd}	7.70 ± 0.20^{c}	6.81 ± 0.10^{b}	6.69 ± 0.11^{a}
Flavour	7.88 ± 0.15^{d}	7.80 ± 0.17^{cd}	7.75 ± 0.17^{c}	6.73 ± 0.12^{b}	6.52 ± 0.15^{a}
Sweetness	7.98 ± 0.17^{a}	7.96 ± 0.08^{a}	7.93 ± 0.21^{a}	7.94 ± 0.16^{a}	7.89 ± 0.14^{a}
Overall Acceptability	7.90 ± 0.11^{d}	7.82 ± 0.14^{cd}	7.75 ± 0.15^{c}	6.79 ± 0.11^{b}	6.66 ± 0.11^{a}

Note:*Canola and flaxseed oil mixture (1:1), Fat and MSNF content in all the samples maintained at 3.0% and 9.5% respectively, sugar added @ 8% in all the samples.*

Values are average of three trials. Mean values in same row not sharing common superscript are significantly different ($p<0.05$)

The body& texture scores revealed that control drink scored a score of 7.85±0.12, while the sample containing 25 parts of oil mixture and 75 parts of milk fat (25:75) in total fat scored a marginally lower score of 7.76±0.16 which was not significantly different from that of control sample. When the oil mixture and milk fat ratio was increased to 50:50, the score further reduced to 7.70±0.20. Though this score was significantly different from that for control sample, the differences in the scores of 25:75 and 50:50sample was not significant. However when the oil mixture content increased to 75:25, the score significantly reduced to 6.81±0.10, and further reduced to 6.69±0.11 when milk fat was completely replaced by the oil mixture (100:0). The canola oil and flaxseed oil are rich in PUFA (%) which contributes to thinner consistency when compared to that of milk fat which has about 65% saturated fatty acids (Gunstone, 2011).

The flavour scores shows that the control drink scored a score of 7.88±0.15 and the 25:75 sample scored a lower score of 7.80±0.17. But no significant difference was observed between the two scores. When the oil mixture proportions was increased to 50% (50:50) the score further reduced to 7.75±0.17 with slight perception of oil flavour in the drink. Due to this the score significantly differed from the score of control sample. But it did not differed from the score for 25:75 sample. When the oil mixture proportion was

increased to 75% (75:25) the score significantly reduced to 6.73±0.12 due to objectionable oily flavour. The intensity of oily flavour increased when the milk fat was totally replaced by the oil mixture, and score significantly reduced to 6.52±0.15.
The sweetness score for control sample was 7.98±0.17 and those for experimental samples varied between 7.89±0.14 and 7.96±0.08. No significant differences were observed between control and experimental sample scores, indicating that the oil mixture did not interfere with the sweetness of the product.

The overall acceptability scores followed the trend of body& texture and flavour scores. The control sample scored a higher score of 7.90±0.11and the score reduced insignificantly to 7.82±0.14 for the 25:75 sample. When 50% of milk fat is replaced by mixture of flaxseed oil and canola oil (50:50) the score insignificantly reduced to 7.75±0.15 when compared to the score for 25:75 sample. However significant difference was observed between the scores of control and 50:50 sample. The score significantly reduced to 6.79±0.11 and 6.66±0.11 for 75:25 and 100:0 samples respectively due to perception of oily flavour in the product. In 100:0 sample, the oily flavour was predominant.

Based on the sensory attributes it was observed that an acceptable quality milk based flavoured drink can be prepared by replacing milk fat up to 50% by the mixture of canola oil and flaxseed oil (mixed in 1:1 ratio), which can be comparable to control sample. Though the drink prepared by using 50:50 ratio of oil mixture and milk fat had slight oily flavour, this level was selected to mask the flavour with inulin. Hence this combination fat & oil was selected for further trials.

5.4 EFFECT OF INCORPORATION OF INULIN ON QUALITY OF FLAVOURED DRINK

5.4.1 Effect of incorporation of inulin on sensory quality of flavoured drink

The inulin was incorporated at different levels in flavoured milk prepared by using selected level of oil mixture and milk fat, to study the effect of inulin on sensory quality and viscosity of the drink.

The inulin was incorporated at different levels in to the flavoured drink prepared by incorporating 50% of canola oil and flaxseed oil mixture (mixed in 1:1 ratio) and 50%

milk fat to study the effect of inulin on the sensory quality of flavoured drink. The sensory scores are tabulated in table 5.4.

Table.5.4. Effect of incorporation of inulin on sensory quality of flavoured drink

Sensory attributes	Control	Experimental sample				
		Inulin (%)				
		2	3	4	5	6
Colour and Appearance	7.92±0.12^{a}	7.95±0.11^{a}	7.94±0.12^{a}	7.90±0.13^{a}	7.91±0.15^{a}	7.94±0.14^{a}
Body& texture	7.70±0.09^{b}	7.78±0.10^{b}	7.8±0.08^{b}	8.02±0.12^{c}	8.07±0.14^{c}	7.50±0.10^{a}
Flavour	8.03±0.11^{c}	7.73±0.13^{a}	7.78±0.12^{a}	7.83±0.12^{b}	7.89±0.09^{bc}	7.91±0.11^{bc}
Sweetness	8.01±0.14^{e}	7.92±0.12^{de}	7.85±0.10^{cd}	7.73±0.14^{bc}	7.63±0.15^{ab}	7.57±0.11^{a}
Overall Acceptability	7.97±0.12^{c}	7.74±0.10^{b}	7.80±0.10^{b}	7.85±0.13^{bc}	7.89±0.12^{c}	7.60±0.09^{a}

Note: *Control sample contains 3% fat, 9.5% SNF and Experimental sample contains 50% milk fat: 50% oil (canola oil and flaxseed oil mixture 1:1)), 9.5% MSNF, all the samples contains 8% sugar.*

Values are average of three trials. Mean values in same row not sharing common superscript are significantly different ($p<0.05$)

The colour and appearance scores revealed that scores for control was 7.92±0.12 and that for experimental samples varied between 7.91±0.13 and 7.95±0.11. The inulin formed a colourless solution in water. Hence the addition of inulin even at 6% did not affect the colour and appearance of the product.

The addition of inulin has showed the positive effect on the body& texture of the drink. The control drink scored a body and texture score of 7.70±0.09. When 2% and 3% inulin was incorporated, though the score increased to 7.78±0.10 and 7.80±0.14respectively did not vary significantly from that of control sample. When 4% inulin incorporated in the drink the score significantly increased to 8.02±0.17, and further increased to 8.07±0.16 for 5% inulin incorporated sample. The addition of inulin resulted in increased viscosity and thus the mouth feel of the drink. On the other hand when inulin

level was increased to 6%, the viscosity of the drink increased to beyond the acceptable level resulting in significantly lowering of body& texture score to 7.50±0.10.

The flavour scores presented in the table shows that, control sample scored a score of 8.03±0.11 and the score significantly reduced to 7.73±0.13 and 7.78±0.12 for 2 and 3% inulin incorporated drinks respectively. The scores significantly increased to 7.83±0.12, and 7.89±0.09 and 7.91±0.11 for 5% and 6% inulin incorporated samples over the scores for 2 and 3% , inulin. No significant when compared to control. This shows that the slight oily flavour which was observed in 2 and 3% inulin incorporated samples could be masked when higher level of inulin was used in the drink preparation.

The sweetness scores showed that the control sample scored a score of 8.01±0.13 and the scores reduced with increased incorporation of inulin. The reduction in the scores was mainly due to increased sweetness contributed by inulin %. When 2% inulin was used in the drink, the sweetness score insignificantly reduced to 7.92±0.12 when compared to control. But use of higher level of inulin significantly reduced the scores when compared to control sample. The drink with 6% inulin scored least score of 7.57±0.11 due to the higher sweetness than the desired level.

The over acceptability scores revealed that the control sample scored a higher score of 7.97±0.12, while the sample with 2 and 3% inulin scored significantly lower overall acceptability scores of 7.74±0.10 and 7.80±0.10 respectively due to higher sweetness and poorer flavour . When the inulin incorporation was increased to 4 and 5% the scores increased to 7.85±0.13 and 7.89±0.12 respectively and no significant differences were observed between these scores and that of control sample. On the other hand when the inulin content was increased to 6.0% the scores significantly reduced to 7.60±0.09 due to the higher sweetness and higher viscosity than the desired in the product. Hence based on the sensory evaluation use of 5% inulin was selected for further studies.

5.4.2Effect of inulin incorporation on the viscosity of flavoured drink

The results presented in fig.5.1 showed that the control sample recorded the viscosity of 2.08cP. The cow milk generally shows the viscosity of 2.00cP (Mathur *et al.*, 1999). The increased viscosity of control flavoured milk over normal milk could be due to added sugar which has increased the total solids in the control drink sample. When 2% inulin was added, the viscosity increased to 2.18cP and the values increased with

increased level of inulin in the product. When 5% inulin was used in the drink, the viscosity value was 2.46cP the body and texture scores presented in table 5.4 also shows improved score up to 5% inulin incorporation in the drink due to better mouth feel over that in control sample. When 6% inulin was incorporated, the viscosity value increased to 2.58cP and which had negative effect on the body and texture due to abnormally higher viscosity than to a desired level.

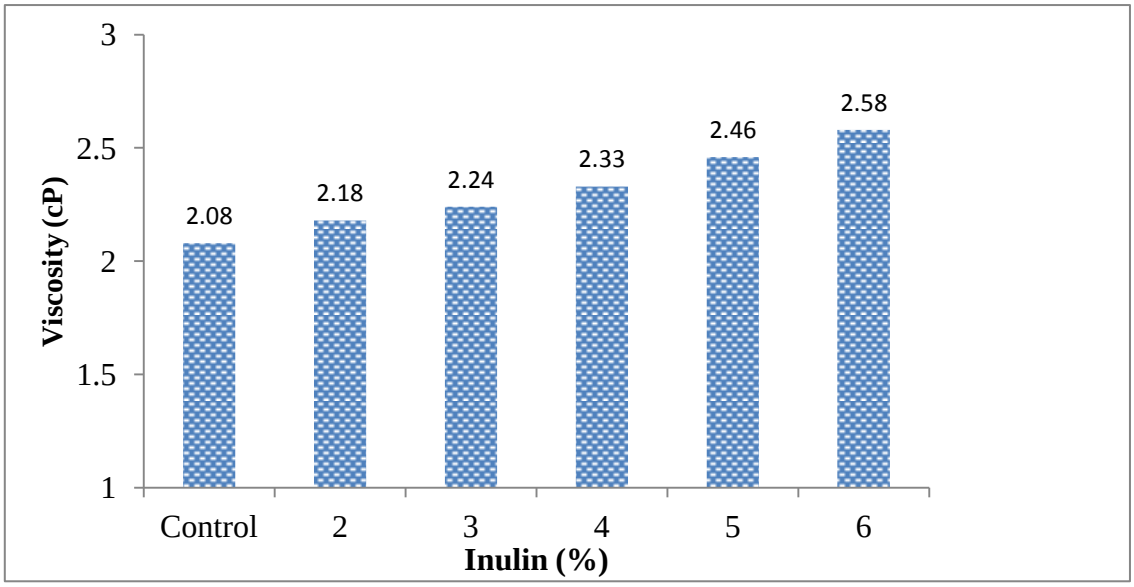


Fig. 5.1 Effect of inulin incorporation on the viscosity of flavoured drink

Note: *Readings were taken at 40°C*

5.5 EFFECT OF DIFFERENT LEVELS OF MSNF ON QUALITY OF FLAVOURED DRINK

The milk SNF was adjusted to different levels of 8.5, 9.0 and 9.5% in the inulin incorporated flavoured milk to study the variation in MSNF on the quality of flavoured milk and the MSNF in control was maintained at 9.5%. Findings are presented in following paragraphs.

5.5.1 Effect of different levels of MSNF on sensory quality of flavoured drink

The flavoured drink prepared with different levels of MSNF was subjected to sensory evaluation and the scores are tabulated in table 5.5.

The milk SNF of the experimental flavoured drink with 5% inulin were adjusted to three different levels of 8.5, 9.0, 9.5% to study the variation in MSNF on the sensory qualities of the flavoured drink. The MSNF in control drink was maintained at 9.5%.

Table 5.5 Effect of different levels of MSNF on sensory quality of flavoured drink

Sensory attributes	Control	Experimental sample		
		MSNF (%)		
		8.5	9	9.5
Colour and Appearance	7.96±0.11[a]	7.92±0.15[a]	7.91±0.16[a]	7.94±0.12[a]
Body& texture	7.72±0.13[a]	7.85±0.11[b]	7.98±0.11[c]	8.01±0.16[c]
Flavour	7.95±0.14[b]	7.60±0.11[a]	7.88±0.13[b]	7.90±0.15[b]
Sweetness	7.96±0.12[b]	7.70±0.13[a]	7.68±0.15[a]	7.61±0.16[a]
Overall Acceptability	7.85±0.11[b]	7.66±0.10[a]	7.81±0.14[b]	7.78±0.15[b]

Note: *Control contains 3% fat: 9.5% SNF and experimental sample contains milk fat, oil (50:50) with 5% inulin and all the samples contains sugar @ 8%.*
Values are average of three trials. Mean values in same row not sharing common superscript are significantly different ($p<0.05$)

The colour and appearance score for the control drink was 7.96±0.11 and that of experimental samples with different level of MSNF varied between 7.91±0.16 and 7.94±0.12. The scores of the experimental samples did not vary significantly from that of the control drink.

The body& texture scores showed that the control sample which had 9.5% MSNF scored a score of 7.72±0.13 whereas the experimental sample with 8.5% MSNF scores significantly higher score of 7.85±0.11. The increase in the score could be due to presence of 5% inulin, which has contributed for the viscosity in the product. When MSNF was increased to 9.0% the score further significantly increased to 7.98±0.11, but the score insignificantly increased to 8.01±0.16, when MSNF was increased to 9.5%. This indicates that 9% MSNF could give optimum body& texture to the drink containing 5% inulin.

The flavour scores revealed that the control sample scored a score of 7.95±0.14. The score significantly reduced to 7.60±0.11 for the sample with 8.5% MSNF and 5% inulin. The sample exhibited slightly oily flavour. When MSNF increased to 9.0%, the score increased to 7.88 and the sample was comparable to that of control sample. Further increase of MSNF to 9.5% resulted in marginal increase in the score to 7.90±0.15 as

shown in table 5.5. This study shows that MSNF is one of the factors which could mask the oil flavour in the drink.

The sweetness scores indicate that the scores of experimental samplesvaried between 7.61±0.16 and 7.70±0.13 and no significant variation observed between the experimental samples. On the other hand the control samples scored significantly higher score of 7.96±0.12 when compared to experimental sample. The higher sweetness in the experimental samples contributed by the inulin resulted in lower scores and the variation in MSNF did not show any influence on the sweetness scores.

The control sample scored an overall acceptability score of 7.85±0.11 and the sample with 8.5% MSNF scored a significantly lower score of 7.66±0.10.When the MSNF content in experimental sample was increased to 9.0. The score significantly increased to 7.81±0.14 it was observed that no significant differences were observed between control sample with 9.5% MSNF and experimental samples with 9.0% MSNF. The scores marginally but insignificantly reduced to 7.78±0.15, when the MSNF level was increased to 9.5%.

The study shows that a minimum of MSNF of 9.0% MSNF is to be maintained in the drink for the production of acceptable quality flavoured drink with 5% inulin.

5.5.2 Effect of MSNF on the viscosity of flavoured drink

The viscosity of the flavoured milk prepared with different levels of MSNF was measured and the values are depicted in fig 5.2.

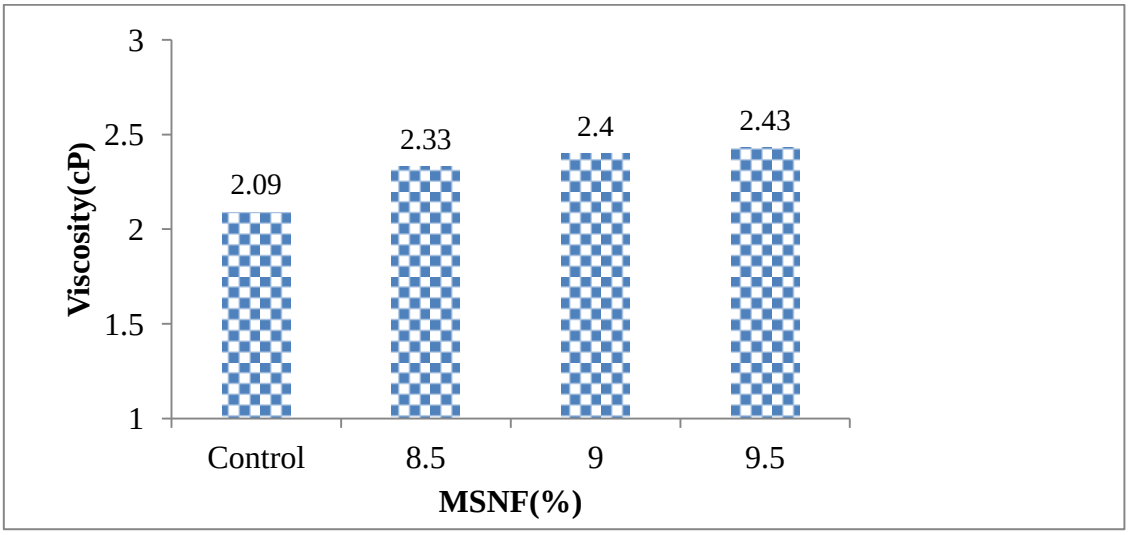


Fig.5.2 Effect of MSNF level variation on the viscosity of flavoured drink

Note: *Readings were taken at 40°C*

The figure shows that the control sample recorded the viscosity of 2.09cP, whereas the experimental sample containing 5% inulin and 8.5% MSNF showed higher viscosity of 2.33cP when compared to control. This increase in viscosity was mainly due to presence of inulin. When MSNF was increased to 9.0% and maintaining the inulin level at 5%, the viscosity marginally increased from 2.40cP to 2.43cP for the drink containing 9.5% MSNF and 5% inulin due to increased MSNF. This study shows that the MSNF has positive effect on viscosity of the milk drink.

5.6 EFFECT OF VARIATION IN SUGAR LEVEL ON THE QUALITY OF FLAVOURED DRINK

The sugar was added in three different levels of 6, 7 and 8% in the flavoured milk prepared by using optimized level of inulin and MSNF, to study the effect of sugar on the sensory quality and viscosity of flavoured milk. The results are presented in following paragraphs.

5.6.1 Effect of sugar level variation on the sensory quality of flavoured milk

When the flavoured milk prepared by different levels of sugar subjected to sensory evaluation and the scores are tabulated in table 5.6.

Table.5.6. Effect of sugar level variation on the sensory quality of flavoured drink

Sensory attributes	Control	Experimental sample		
		Sugar (%)		
		6	7	8
Colour and Appearance	7.90 ± 0.15^{a}	7.89 ± 0.15^{a}	7.88 ± 0.15^{a}	7.86 ± 0.15^{a}
Body and texture	7.54 ± 0.10^{a}	7.76 ± 0.10^{b}	7.89 ± 0.15^{c}	7.94 ± 0.12^{c}
Flavour	7.90 ± 0.16^{c}	7.28 ± 0.11^{a}	7.50 ± 0.10^{b}	7.82 ± 0.13^{c}
Sweetness	7.89 ± 0.13^{b}	7.71 ± 0.14^{a}	7.84 ± 0.10^{b}	7.68 ± 0.13^{a}
Overall Acceptability	7.88 ± 0.15^{c}	7.36 ± 0.09^{a}	7.58 ± 0.11^{b}	7.82 ± 0.15^{c}

Note: *Control contains 3% fat: 9.5% SNF and experimental sample contains milk fat, oil (50:50), 5% inulin and 9.0% MSNF.*

Values are average of three trials. Mean values in same row not sharing common superscript are significantly different ($p<0.05$)

Since sugar does not contribute to the colour and appearance of a food product, no significant difference between the scores of control and experimental drink were observed.

The body and texture scores shows that the control drink scored a score of 7.54±0.10 and the scores significantly increased to 7.76±0.10. This could be due to presence of inulin in the experimental sample. The scores further increased significantly to 7.89±0.15 when 7% sugar was used which could be due to increased total solids in the drink. But when the sugar was increased to 8.0%, the scores marginally increased to 7.94±0.12 shown in table 5.6.

The sugar had shown the significant effect on the flavour of the drink. The control sample which had 8% sugar had scored a score of 7.90±0.16 and the scores significantly decreased to 7.28±0.11 and 7.50±0.10 respectively further drinks prepared by using 6 and 7% sugar. The oily flavour was felt in these samples. The flavour score significantly increased to 7.82±0.13 where 8% sugar was used in the flavoured drink. Though the flavoured drink had higher sweetness, the score did not significantly vary from that of control sample due to masking of the oily flavour.

The sweetness score for control sample which had 8% sugar scored the score of 7.89±0.13. The score significantly reduced to 7.71±0.14 when 6% sugar was used in the experimental sample due to perception of less sweetness. When sugar level was increased to 7%, the score significantly increased to 7.84±0.10. No significant difference was observed between this score and the score for control sample. When the sugar level was increased to 8% the score significantly reduced to 7.68%±0.13 due to higher sweetness in the product. This shows that the sweetness of drink with 7% sugar incorporation and 5% inulin could be compared to 8.0% sugar in control sample without inulin.

The overall acceptability scores indicate that the control sample scored a score of 7.88±0.15. The score significantly reduced to 7.36±0.09when 6% sugar was used, this was mainly due to less sweetness and presence of oily flavour. Though the body and texture and sweetness scores improved with addition of 7% sugar, still the overall acceptability score was 7.58±0.11 which was significantly lower than that of control sample which was mainly due to presence of oily flavour. When the sugar level was increased to 8%, though the sweetness score reduced to 7.68±0.13 due to higher sweetness, the body and texture and flavour scores were significantly higher than that of

control sample. This has resulted in higher overall acceptability with a score of 7.82±0.15.

5.6.2Effect of sugar on viscosity of flavoured drink

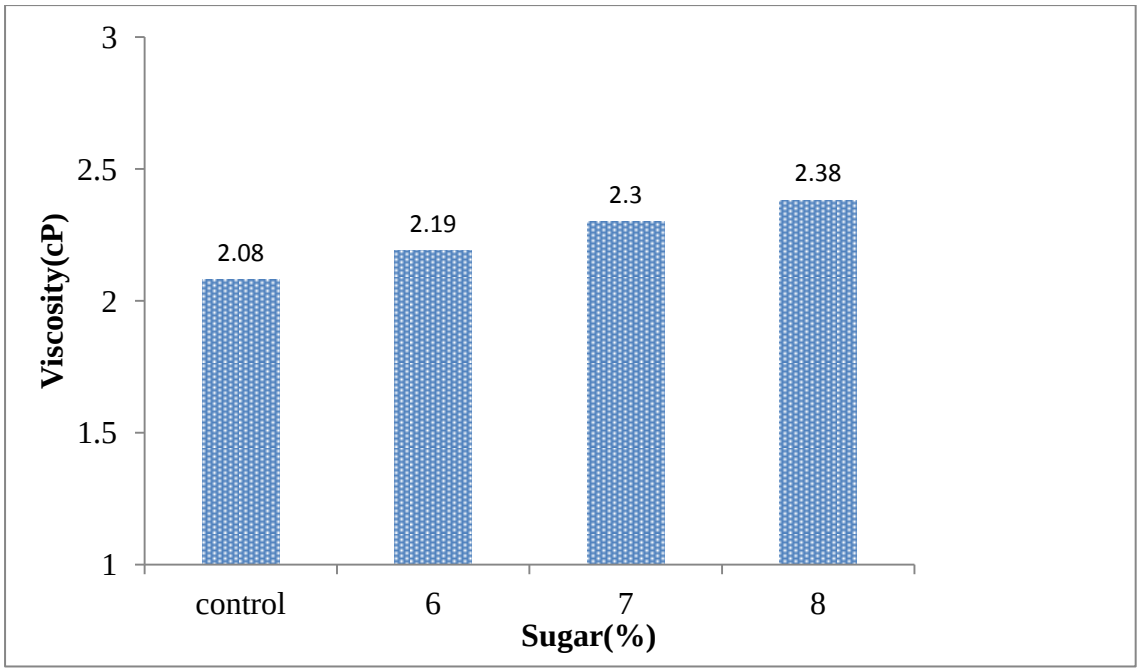


Fig.5.3. Effect of sugar level variation on viscosity of flavoured drink

Note: *Readings were taken at 40°C*

The variation of sugar level on viscosity of the flavoured drink was studied and the results are presented in fig.5.3.

The results are showed that the control drink containing 8% sugar had the viscosity of 2.08cP while that in experimental drink having 6% sugar showed higher viscosity of 2.19cP, mainly contributed by inulin in the sample. The viscosity values further increased to 2.30cP and 2.38cP respectively for the samples containing 7 and 8% sugar. This study shows that, in addition to inulin, the sugar also contributes to the viscosity of the flavoured drink.

Based on the studies it was observed that acceptable quality milk based flavoured drink can be prepared by blending milk fat and mixture of canola oil and flaxseed oil (blended in 1:1 ratio) in 50:50 ratio, maintaining 9.0% MSNF and 8% sugar with 5% inulin.

The optimized flavoured drink was further studied for estimation of proximate physical composition and its shelf life.

5.7 EFFECT OF FLAVOURS ON ACCEPTABILITY OF THE FLAVOURED DRINK

In the studies, banana flavour and corresponding lemon yellow colour were used as flavouring and colouring agents in the flavoured drink. In order to understand the compatibility of the flavours in the developed drink having vegetable oils and inulin, the banana, mango and chocolate flavours were used as flavouring agents, the sensory evaluation showed that though the flavours are of individual liking, the banana and mango flavoured drinks were more acceptable over chocolate flavoured drink. Since the chocolate flavour had slightly bitter flavour.

5.8STUDY OFSTABILITY OF FLAVOURED MILK TO STERILIZATION CONDITIONS

The flavoured drink prepared by using the optimized formulation was subjected to sterilization condition of 121°C for 15 min. It was observed that the developed flavoured drink was stable to sterilization conditions and was comparable to control sample.

Based on the studies it was observed that acceptable quality milk based flavoured drink can be prepared by blending milk fat and mixture of canola oil and flaxseed oil (blended in 1:1 ratio) in 50:50 ratio, maintaining 9.0% MSNF and 8% sugar with 5% inulin.

5.9 ANALYSIS OF DEVELOPED FLAVOURED DRINK

The optimized flavoured drink was further studied for estimation of proximate physical composition and its shelf life.

5.9.1 Proximate composition of developed flavoured drink

Table 5.7 Physico-chemical composition of the developed flavoured drink

Parameters	Control	Developed drink
Fat (%)	3.00±0.1	2.9±0.1
Protein (%)	3.47±0.02	3.35±0.03
Carbohydrates (%)	13.55±0.10	18.63±0.17
Ash (%)	0.78±0.01	0.72±0.02
Moisture (%)	79.20±0.20	74.40±0.20
pH	6.56±0.02	6.55±0.01
Acidity (%) L.A.	0.166±0.0	0.17±0.0
Viscosity (cP) @ 40°C	2.08±0.02	2.40±0.02

The milk based flavoured drink prepared by using optimized formulation was analysed for its physicochemical composition and the results are presented in table 5.7. The fat content in developed drink was 2.9±0.1% and that in control sample was 3.0±0.1%. Since the fat content in both samples was standardized to 3.0% fat, no much variation in the fat content was observed. The protein content in control sample was 3.47±0.02%, and the value decreased marginally to 3.35±0.03%. Similarly the ash content in control sample was 0.78±0.01% and corresponding value for developed drink was 0.72±0.02%. The reduced protein and ash content in the developed drink due to dilution of these constituents by inulin, which is a pure carbohydrate and contains no protein and ash. On the other hand the experimental sample had a total carbohydrate content of 18.63±0.17 when compared to 13.11±0.10 in control sample. The added inulin has contributed solely to the increased carbohydrate in the sample. According the moisture content in the developed sample was 74.40±0.20 due to higher T.S. content while that in control sample was 79.20±0.20%.

The acidity of control and experimental samples were 0.166% and 0.17% L.A indicating the added ingredients in the experimental sample did not contribute much to the acidity in the product. The corresponding pH values are 6.56±0.02 and 6.55±0.01. The viscosity of the control drink was 2.08±0.02 and that for experimental sample was 2.40±0.02cP, mainly due to the incorporation of inulin.

5.9.2. Analysis of fatty acid profile of developed flavoured drink

Table.5.8. Fatty acids composition of control and final product in grams per 100gm fat

Sample	Fatty acids (%)		
	C18:1	C18:2	C18:3
Control	21.3	1.8	ND
Developed drink	23.6	7.8	13.3

The fatty acid profile of the fat present in the developed flavoured drink which had the mixture of canola oil, flaxseed oil (mixed in 1:1 ratio) and milk fat was analysed and results are presented in table 5.8.

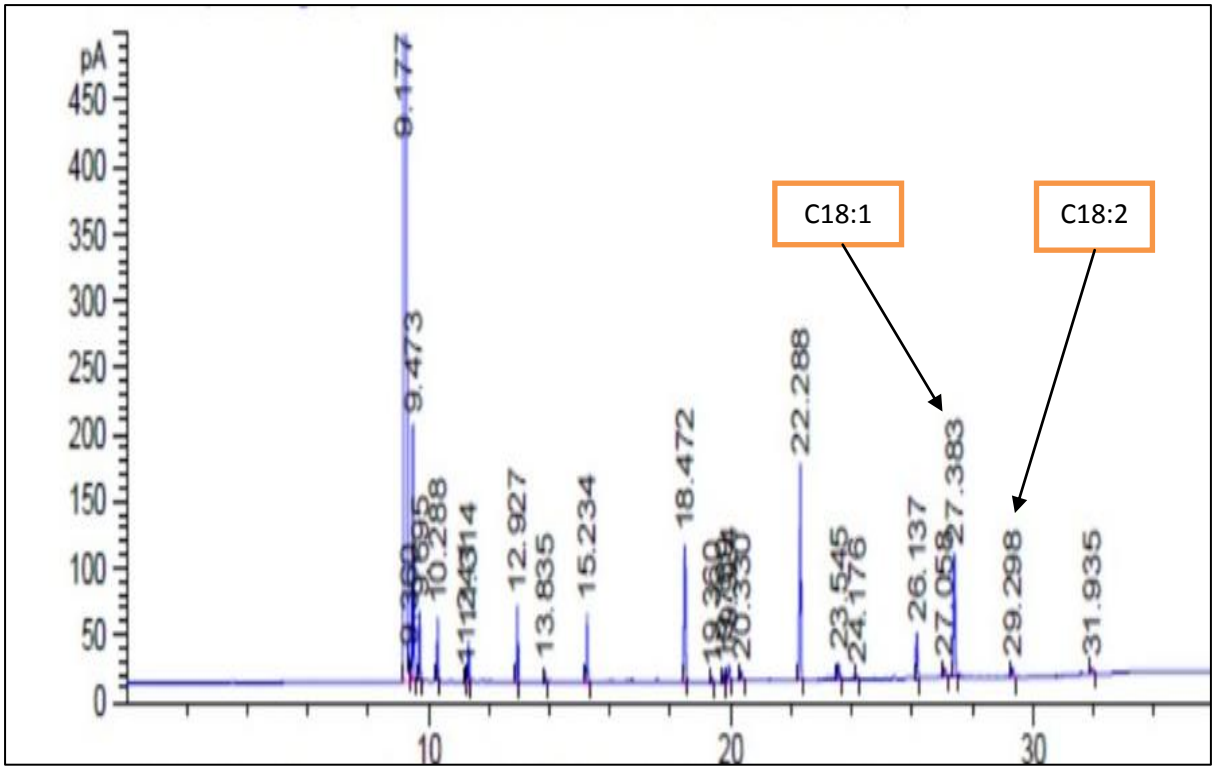


Fig.5.4. Fatty acid profile of fat present in control sample

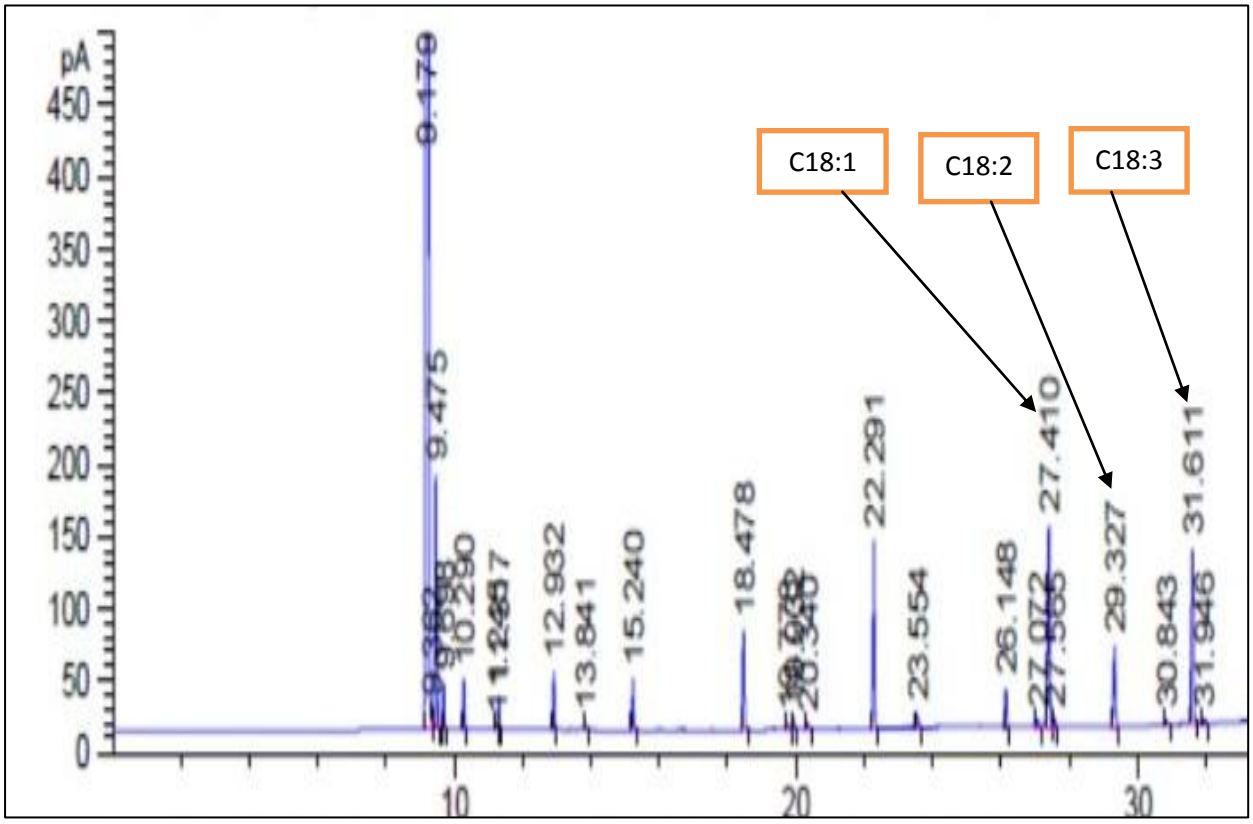
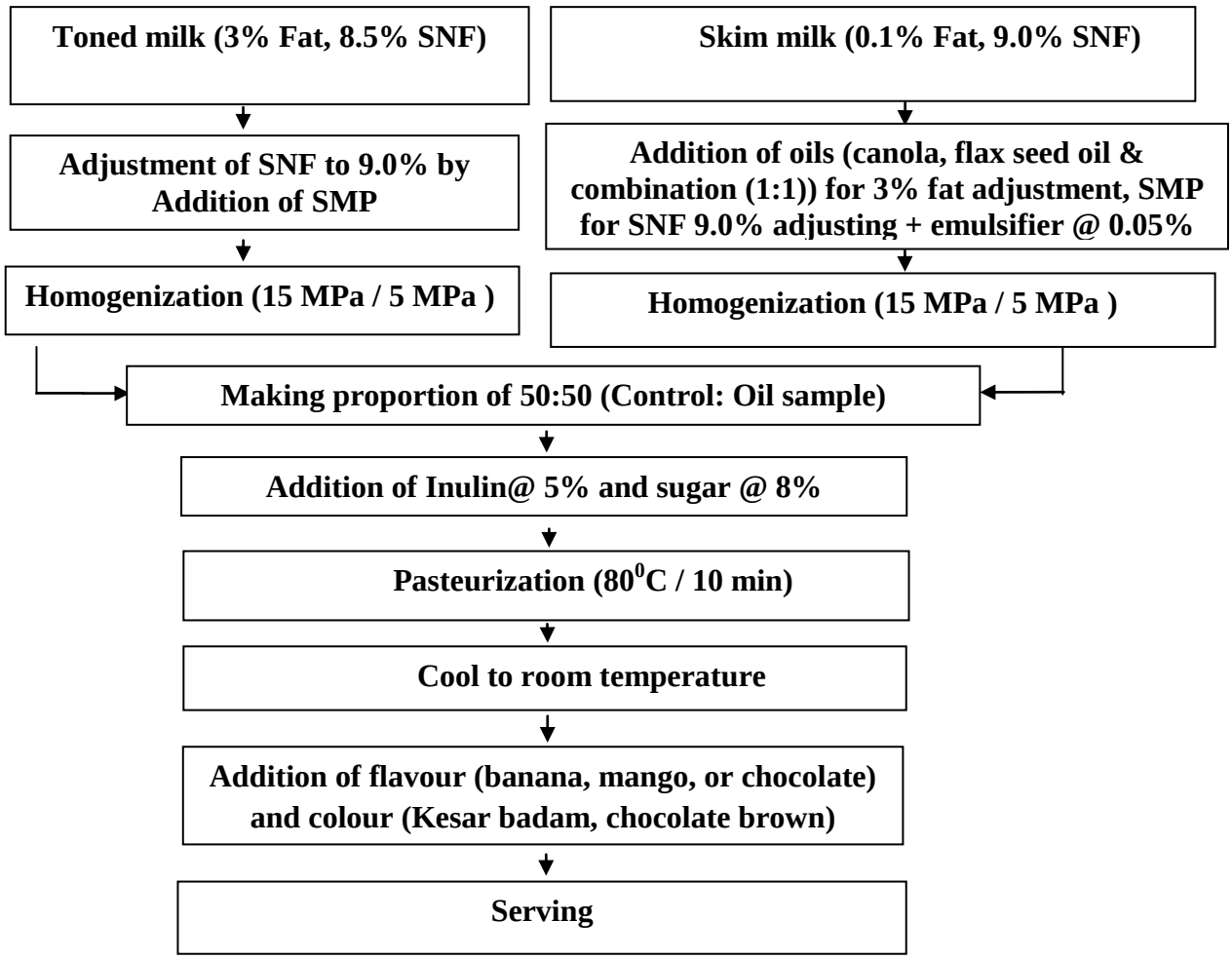


Fig.5.5. Fatty acid profile of fat present in developed flavoured drink

X axis and Y axis in fig. 5.4 and 5.5 represents retention time (min) and peak area respectively. The results presented in fig. 5.4, 5.5 and table 5.8 showed that the oleic acid (C18:1) content in control sample was 21.3%, while that in experimental sample was 23.6%. The higher oleic acid content of canola oil of low erucic acid resulted in the higher values in the experimental drink. Gunstone (2011) reported that presence of 56% oleic acid and 26% linoleic acid in low erucic acid canola oil. The linoleic acid (C18:2) content in control sample was 1.8% while that in experimental sample significantly increased to 7.8% which is contributed by flaxseed oil and canola oil. The omega 3 fatty acid i.e. linolenic acid (C18:3) which was not found to the level of 13.3% in experimental sample. Daun *et al*. (2003) reported that flaxseed oil is a rich source of linolenic acid which contains about 50 to 62% and 10% linoleic acid. Souci *et al*. (1989) also reported that flaxseed oil contains 54.2% alpha linolenic acid.

5.10. OPTIMIZED FORMULATION FOR PRODUCTION OF FLAVOURED DRINK Fig.5.6. Flow chart for the development of milk based functional flavoured drink with optimized levels of ingredients



5.11 STORAGE STUDY OF DEVELOPED FLAVOURED DRINK

The flavoured drink prepared by the optimized formulation was packed in LDPE pouches and stored at refrigerated temperature. The changes in sensory, physicochemical and bacteriological qualities of the drink were analysed during storage period at regular intervals and the results are presented in following paragraphs.

5.11.1 Changes in sensory quality of flavoured drink during storage

The changes in the sensory quality of the stored drink samples were studied during the storage period and analysed presented in annexure II and fig. 5.7, 5.8, 5.9 and 5.10.

The flavoured milk stored at refrigerated temperature was analysed for changes in sensory qualities during storage and the results are tabulated in following figures.

5.11.1.1 Colour and appearance

The colour and appearance scores presented in fig. 5.7 shows that fresh control scored a score of 8.00 and the developed drink showed almost similar score of 7.99. At the end of storage period of 6 days, the scores marginally but insignificantly reduced to 7.90 and 7.88 respectively.

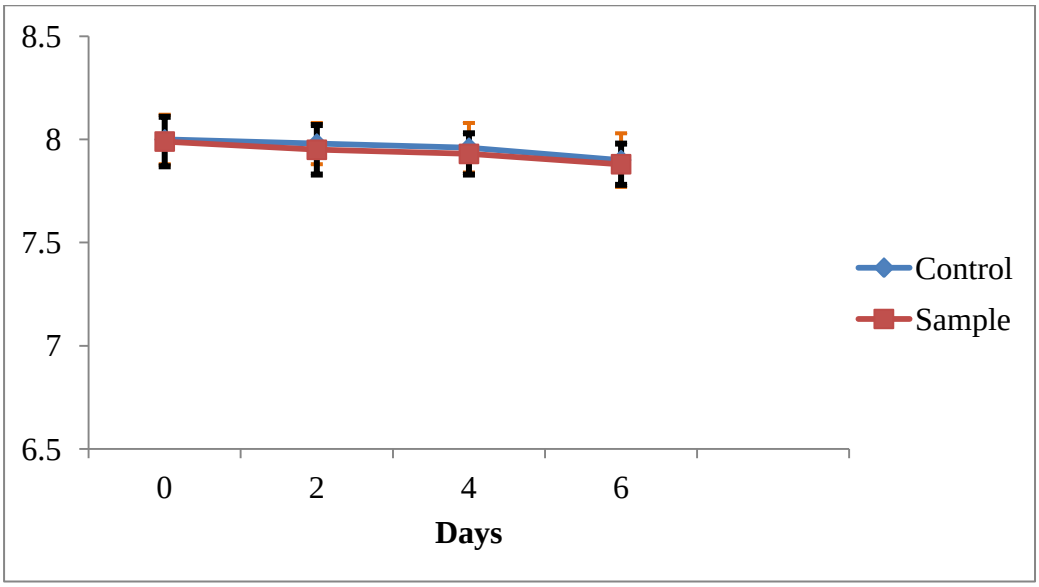


Fig.5.7. Changes in colour and appearance of developed flavoured drink during storage

5.11.1.2 Changes in body& texture scores of flavoured drink during storage

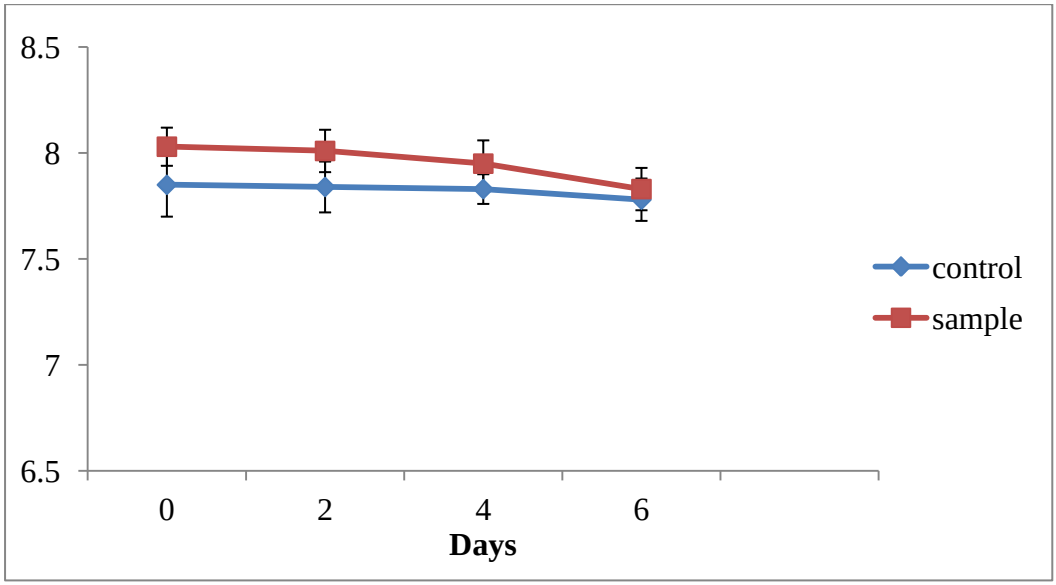


Fig.5.8. Changes in body& texture scores of flavoured drink during storage

The changes in the body and texture scores presented in fig.5.8 shows that fresh developed drink scored significantly higher score of 8.03 when compared to 7.85 for control sample due to better mouth feel contributed by the incorporated inulin. During 6 days of storage the score for control sample gradually reduced to 7.78 but no significant differences in reduction was observed. The score for developed drink also reduced gradually during the storage. The scores up to 4 days of storage did not vary significantly from that of fresh sample. The scores were significantly higher than that for control sample during corresponding storage period of days. But at the end of 6 days of storage the score significantly reduced to 7.83 and the score did not vary significantly when compared to that of control sample.

5.11.1.3 Changes in flavour score of flavoured drink during storage

The changes in the flavour score of flavoured drink samples presented in fig.5.9 shows that the fresh control sample scored higher score of 7.98 when compared to that in fresh developed drink which scored a score of 7.88. At the end of 4 days of storage scores of both the samples reduced to 7.91 and 7.80 respectively and no significant difference between the samples and the storage period were observed. At the end of 6 day storage, the scores for both the sample reduced to 7.76 and 7.68 respectively and the scores were significantly different from corresponding scores for fresh samples.

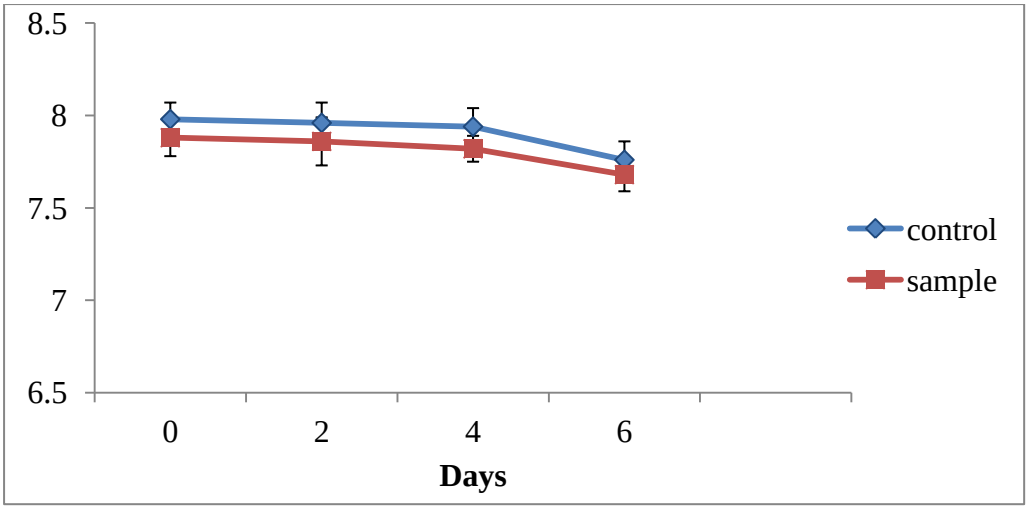


Fig.5.9. Changes in flavour score of flavoured drink during storage

5.11.1.4 Changes in sweetness of flavoured drink during storage

The changes in sweetness scores of flavoured drink samples during storage period presented in fig.5.10 showed that the similar trend of flavour scores. The fresh control and developed drink scored the score of 8.03 and 7.85 respectively.

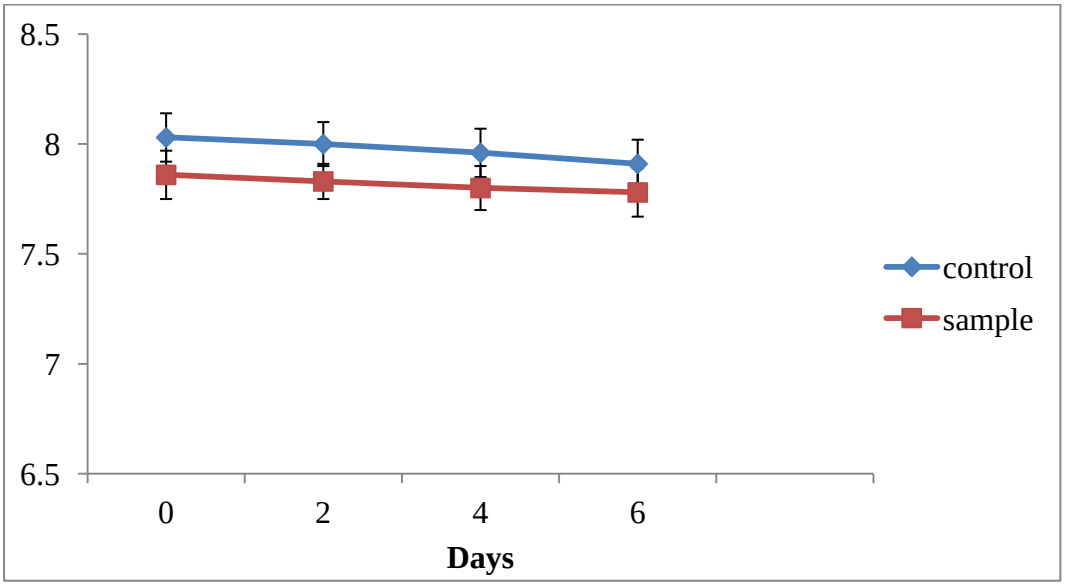


Fig.5.10. Changes in sweetness of flavoured drink during storage

The lower score for fresh developed drink was due to higher sweetness than the desired level which was contributed by the incorporated inulin in the sample. Though the sweetness values reduced to 7.90 for control and 7.77 for developed drink at the end of six days of storage, no significant differences were observed when compared to corresponding fresh samples. However as in the fresh samples, the sweetness of

developed flavoured drink was significantly differed from that of control drink was observed during storage due to inulin presence which contributes to higher sweetness of developed drink.

5.11.1.5 Overall acceptability

The overall acceptability scores presented in fig 5.11 revealed that the fresh control and developed drink scored a score of 7.96 and 7.89 respectively and no significant difference was observed between the samples. During storage period the scores of all the sensory parameters decreased. Accordingly the decreased trend in overall acceptability scores was observed during 6 days of storage period. However no significant differences between the two samples during storage periods were observed.

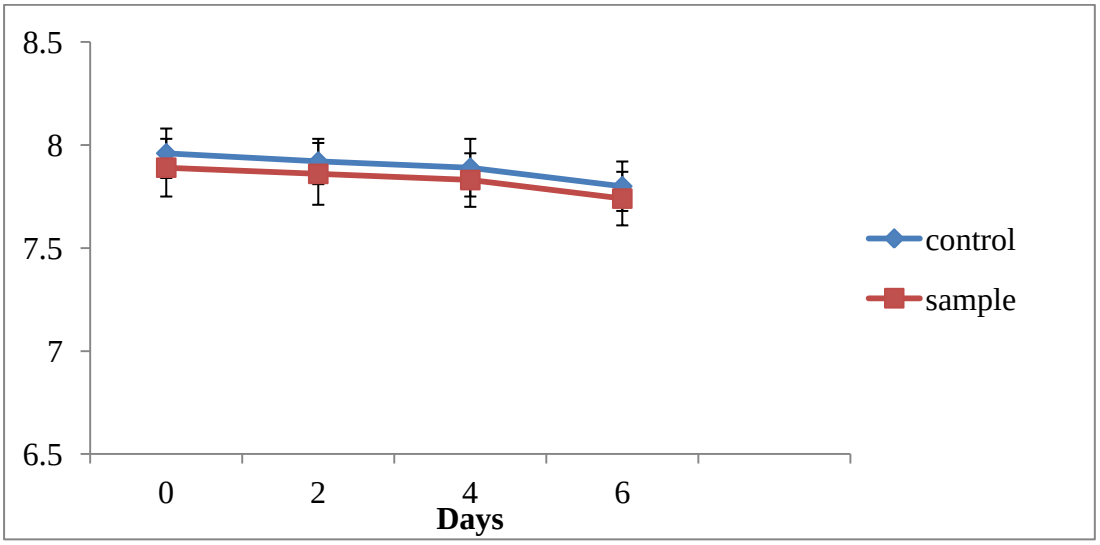


Fig. 5.11. Changes in overall acceptability of flavoured drink during storage

5.11.2 Physico-chemical changes in flavoured drink during storage

Changes in physicochemical properties of flavoured drink during storage were analysed and presented in annexure III.

The developed flavoured drink was packed in LDPE pouches and stored at refrigerated temperature to study the physicochemical changes during the storage. The findings are presented in annexure III and fig. 5.12, 5.13 and 5.14.

5.11.2.1 Changes in acidity values of flavoured drink during storage

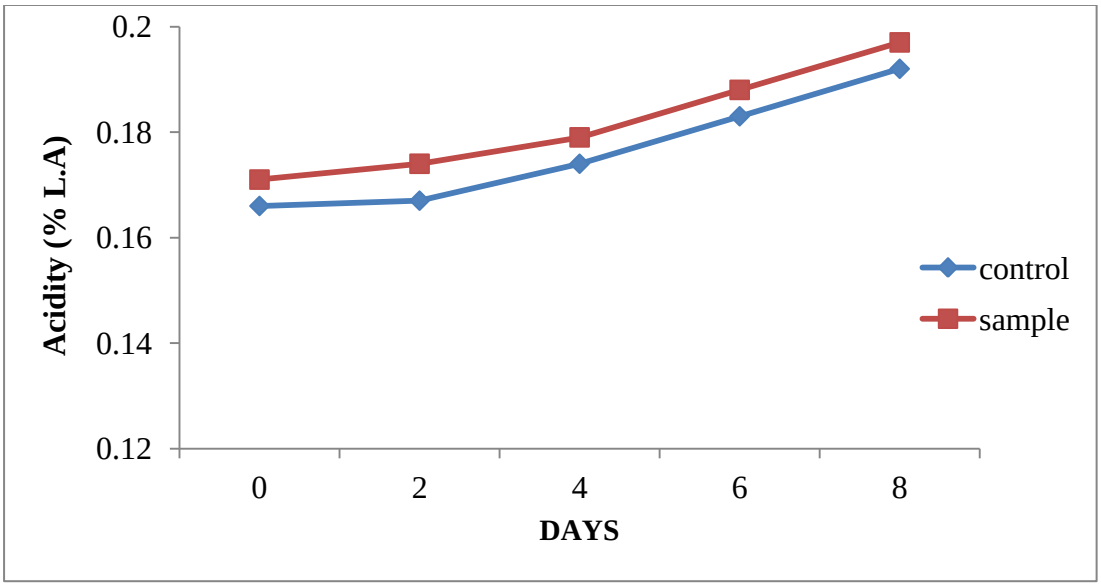


Fig.5.12. changes in acidity values of flavoured drink during storage

As shown in the fig.5.12 the initial acidity of fresh control sample was 0.166% LA and the corresponding value for developed drink was 0.171% LA. During the storage period the gradual increase in acidity in both the samples, increased and at the end of 6 day of storage the corresponding acidity values were 0.183 and 0.188% LA. At the end of 8 day of storage, the acidity values were 0.192, 0.197% and both the samples answered positive for COB test due to developed acidity.

5.11.2.2 Changes in pH values of flavoured drink during storage

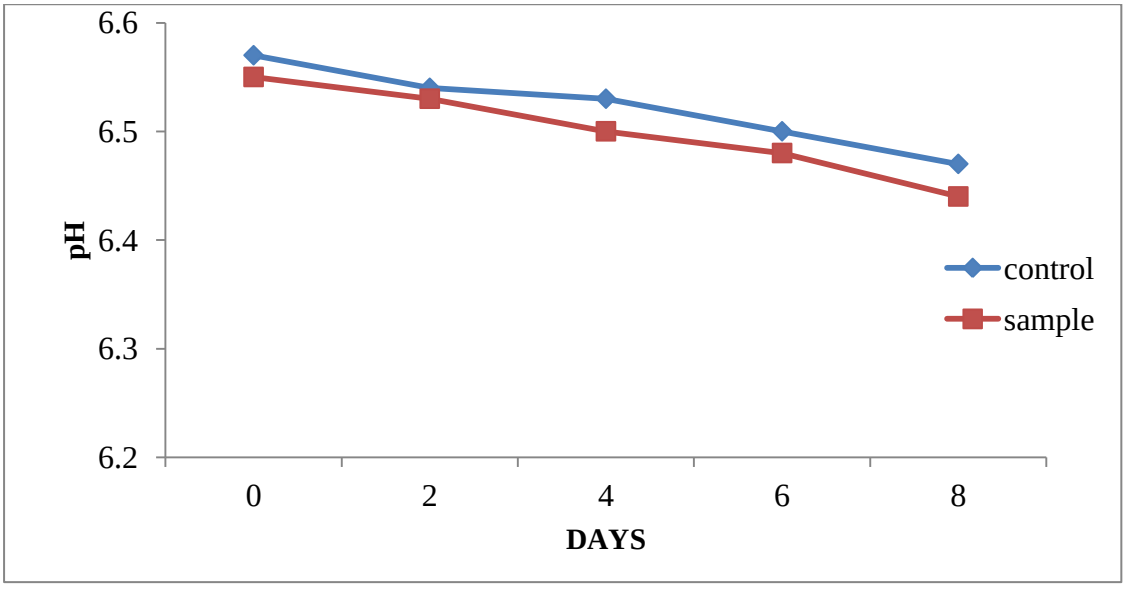


Fig. 5.13 Changes in pH values of flavoured drink during storage

The pH value of fresh control and developed drink were 6.57 and 6.55 respectively and the values decreased during storage period as shown in the fig. 5.13. At the end of 8 days of storage the corresponding values were 6.47 and 6.44. The values correlated with the increased acidity of samples during storage.

5.11.2.3 Changes in viscosity values of flavoured drink during storage

The results presented in the fig.5.14 shows that viscosity values for fresh control and developed drink were 2.07±0.01 and 2.40±0.02 respectively. The inulin incorporation in the developed drink was mainly attributed to increased viscosity. The values of both the samples gradually increased during storage period and at the end of 6 days of storage, the corresponding values were 2.11±0.01 and 2.49±0.01. At the end of 8 days of storage the values were 2.13±0.02 and 2.55±0.02. The increase in viscosity can be directly related to the increased acidity which had influenced the changes in protein structure.

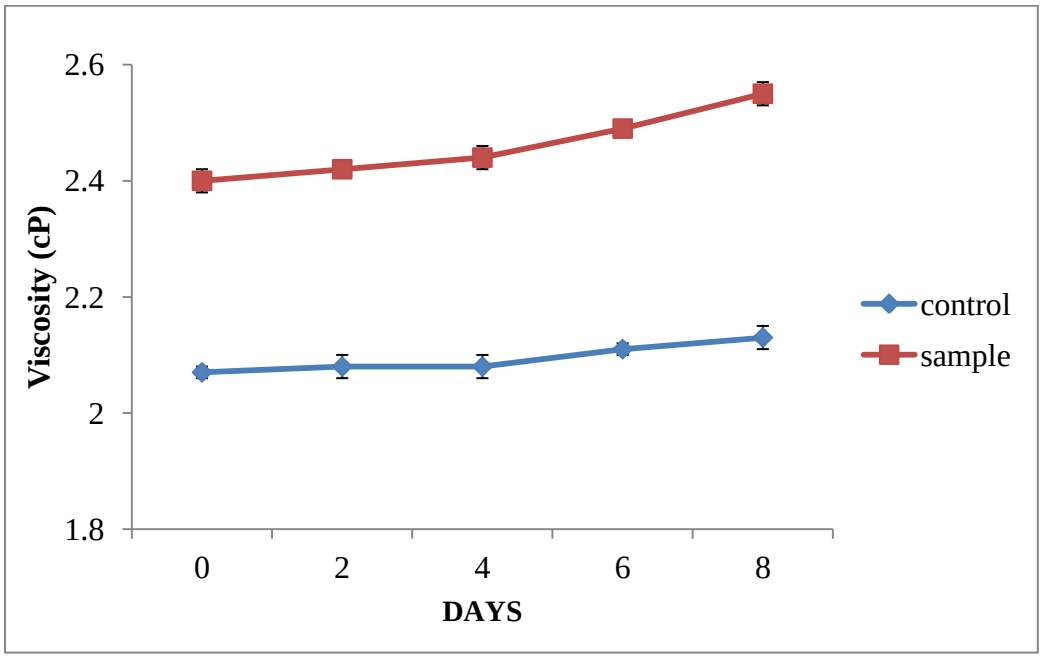


Fig.5.14 Changes in viscosity values of flavoured drink during storage

Note: *Readings were taken at 40°C*

5.11.3 Bacteriological changes during storage of flavoured drink

5.11.3.1 Total bacteria count change during storage of flavoured drink

The total bacterial count presented in table 5.9 shows that the fresh control sample and experimental drinks had the count of 80×10^2 and 95×10^2cfu/ml respectively. During the storage period under refrigerated conditions, the counts increased gradually and at the

end of 8 days of storage the corresponding counts were 26×10^3 cfu/ml and 27×10^3 cfu/ml. The results indicate that the use of inulin or the vegetable oils in the experimental drink did not influence the bacterial activity during storage period.

Table 5.9 Total bacteria count change during storage of flavoured drink

Storage period (days)	Total bacteria count (CFU/ml)	
	Control	Final product
0	80×10^2	95×10^2
2	115×10^2	135×10^2
4	14×10^3	16×10^3
6	17×10^3	20×10^3
8	26×10^3	27×10^3

5.11.3.2 Coliform count changes during storage of flavoured drink

Table 5.10 Coliform count changes during storage of flavoured drink

Storage period (days)	Coliform count (CFU/ml)	
	Control	Final product
0	<10	<10
2	<10	<10
4	<10	<10
6	<10	<10
8	<10	<10

The coliform counts presented in table 5.10 shown that, the coliform counts in both control and developed product was <10 cfu/ml and even at the end of the storage period of 8 days, the counts were <10 cfu/ml. This shows that proper hygiene practices were followed during the preparation of the drinks.

The study showed that an acceptable quality milk based flavoured drink could be prepared by incorporation of mixture of canola oil and flaxseed oil (mixed in 1:1ratio) and 5% inulin with improved fatty acid profile. The developed drink had a shelf life of 6 days at refrigerated temperature which was comparable to control sample.

CHAPTER 6

SUMMARY AND CONCLUSION

6. SUMMARY AND CONCLUSION

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With the increase in consumer awareness about the nutritional facts, the demand for food products which are rich in functional ingredients such as soluble fibre and omega 3 fatty acids are increasing in the present investigation attempts were made to develop milk based drink prepared by incorporating selected vegetable oils, inulin and results are summarized in the following paragraphs.

6.1 The sensory evaluation of the flavoured drink prepared by replacing milk fat with canola oil at different proportions showed that the canola oil can be replace the milk fat up to 50% without affecting the sensory qualities of the flavoured drink.

6.2 The sensory evaluation of the flavoured drink prepared by replacing milk fat with flaxseed oil at different proportions indicated that the flaxseed oil can replace the milk fat up to 50% for the preparation of flavoured drink without affecting the sensory parameters.

6.3 Attempts were made to replace the milk fat with the combination of canola oil and flaxseed oil (mixed in 1:1 ratio) to study its effect on the sensory qualities of flavoured drink, the sensory score revealed that the mixture of these oils can replace the milk fat up to 50% without affecting the sensory qualities. The oily flavour was perceived in the flavoured drink in which milk fat is replaced by 75% and above.

6.4 To study the effect of inulin on the sensory qualities of flavoured drink, the inulin was incorporated at different levels in the flavoured drink and the sensory scores showed that inulin cab be incorporated up to 5% in the flavoured drink. The incorporation of inulin was found to increase the viscosity of the drink. The viscosity of control sample was 2.08cP while that of the flavoured drink with inulin had the viscosity of 2.40cP. Use of 6% inulin resulted in higher viscosity than the desired in the flavoured drink.

6.5 The sensory evaluation study on optimization of milk SNF level in the flavoured drink revealed that use of 9.0% MSNF in milk with 5% inulin was comparable to the control flavoured milk with 9.5% MSNF.

6.6 It was observed that the viscosity of the drink increased with increase in MSNF level in the drink. The viscosity of the drink with 8.5 and 9.0% were 2.33, 2.40cP and which was increased to 2.43cP when MSNF was increased to 9.5%.

6.7 Inulin contribution to sweetness to the product. In the study on optimization of sugar level in the drink, it was observed that use of 7% sugar got optimum sweetness in the drink which was comparable to 8% sugar in the control sample. However, at 7% level, slight oil flavour was observed in the drink which could be masked when 8% sugar was used in the drink preparation. It was observed that the viscosity of the drink increased with increase in sugar level 6, 7 and 8% the corresponding values were 2.19, 2.3 and 2.38cP respectively.

6.8 The analysis of proximate composition of drink shows that the fat and protein content in developed flavoured drink were 2.9 and 3.35% respectively and corresponding values for control sample were 3.0 and 3.47%. The total carbohydrate content in the developed drink was 18.63 due to addition of inulin and that in control sample was 13.55%. The viscosity of the developed flavoured drink was 2.40 while that of control sample was 2.08. No significant variation in acidity and pH were observed between control and developed drink.

6.9 The fatty acid profile analysis of the drink samples indicate that the control flavoured drink had oleic acid (C18:1) and linoleic acid (C18:2) content of 21.3 and 1.8% on total fat basis while the corresponding values of developed flavoured drink were higher at 23.6 and 7.8%. The linolenic acid (C18:3) which was not detectable in the control samples where it was 13.3% in developed flavoured drink.

6.10 Among the different flavours tried, the sensory evaluation showed that though the flavours are of individual liking, the banana and mango flavoured drinks were more acceptable over chocolate flavoured drink.

6.11 The flavoured drink prepared with selected level of canola oil and flax seed oil, and inulin was found to be stable at sterilization conditions.

6.12 The sensory evaluation of the developed drink during storage indicated that the drink had a shelf life of 6 days when stored under refrigeration temperature and was comparable to control sample.

6.13 The physico-chemical changes in the developed product when stored under refrigerated temperature showed the marginal increase in the acidity and correspondingly lower pH till the end of 6 days of storage. At the end of 8 days of storage both control and developed drinks answered positive for COB test. The marginal increase in viscosity in both the control and developed samples were observed.

6.14 The bacteriological analysis of the drink samples during storage showed that the fresh control and developed drinks had the total bacteria count of 80×10^2 and 95×10^2 cfu/ml respectively and at the end of 6 days of storage the corresponding counts were $26\times10^3$and 27×10^3 cfu/ml.

6.15 The coliform counts were less than 10 cfu/ml in fresh samples of control and developed drink. Even at the end of 8 days of storage period, the counts in both the sample were less than10 cfu/ml.

It can be concluded that the canola oil and flaxseed can individually or in combination (mixed in 1:1 ratio) could be replace the milk fat up to 50% in the milk based flavoured drink containing a total fat of 3.0%. Maintenance of 9.0% MSNF with incorporation of 5.0% inulin and 8% sugar was found to give optimum sensory attributes to the drink. The developed functional flavoured drink had higher levels of oleic acid, linoleic acid and linolenic acids compared to those in control sample. The drink had a shelf life of 6 days when stored under refrigeration temperature.

BIBLIOGRAPHY

BIBLIOGRAPHY

AACC (American Association of Cereal Chemists). (2001). The definition of dietary fiber. Report of the Dietary Fiber Definition Committee to the Board of Directors of the American Association of Cereal Chemists. *Cereal Foods World*, **46:** 112–126.

Abou-zeid, N. A. (2016)**.** Nutraceutical Ingredient "Poly-Unsaturated Fatty Acids" (PUFAs) Fortification in Milk and Dairy Products: A Review. *International Journal of Advanced Research in Science, Engineering and Technology*, **3** (2), ISSN**:** 2350-0328.

Akin, M. B., Akin, M. S., & Kirmaci, Z. (2007). Effects of inulin and sugar levels on theviability of yogurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice cream. Food Chemistry, **104:** 93–99.

Alles, M.S., Hautvest, J. G. A, Nagengast, F.M, Hartermink, R, Van Laere K. M. J, and Jansen J. B. M. (1996). Fate of fructo-oligosaccharides in the human intestine. *British Journal of Nutrition*, **76**: 211-221.

Andrew, L., Strong, M. L., & Strong, M. J. (1996). *U.S. Patent No. 5,580,600*. Washington, DC: U.S. Patent and Trademark Office.

AOAC. (2005). *Association of official analytical chemists, official method of analysis*. 18th edn., Washington D.C., USA.

Aqilah, M. A. (2010). Enhancement of probiotics survival by microencapsulation with alginate and prebiotics MMG 445. *Basic Biotechnology*, **6:** 13–18.

Arora, K. J. and Kalra, K.K. (1984). Lactometric determination of total solid of flavoured milk. *Indian journal of Dairy sci*., **38**(1)**:** 18-24.

Ashes, J. R., S. K. Gulati, L. J. Cook, T. W. Scott, and J. B. Donnelly. (1992). Assessing the biological effectiveness of protected lipid supplements for ruminants. *J. Am. OilChem. Soc*.**56:** 552.

Babu, G., & Nithyalakshmi, V. (2011). Influence of prebiotic composition on probio-tic survivability in calcium alginate coated symbiotic microcapsules at thermalincubation. *Agricultural Journal*, **6:** 231–236.

Barclay, T., Ginic-Markovic, M., Cooper, P. and Petrovsky, N. (2010). Inulin-a versatile polysaccharide with multiple pharmaceutical and food chemical uses. *Journal of Excipients & Food Chemicals*, **1**(3).

Bhardwaj, P. K., Kapoor, C.M., Beniwal, B. S and Dabur, R. S. (2002). Development of low calorie flavoured milk. *International conference on traditional dairy foods*. 2007: 76.

Bhatty, R. S. (1995). Nutrient composition of whole flaxseed and flaxseed meal. *Flaxseed in human nutrition*. AOAC Press. Champain, IL: 56-58.

BIS. (1981) IS: SP:18, Part-XI. Hand book of food analysis. Dairy products, *Bureau of Indian Standards*, New Delhi.

Bourre, J. M. (2004). Cited by Joseph, J. Collin, (2010). Omega-3 Essential fatty acids. *Nutri news*. Doughlas Laboratories. Pittsburg, **9**(2**):** 1-8.

Brenna, J. T., Salem, N., Sinclair, A. J., & Cunnane, S. C. (2009). α-Linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. *Prostaglandins, leukotrienes and essential fatty acids*, **80**(2), 85-91.

Burdge, G.C., Jones ,A.E. and Wootton,S.A. (2002). Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men. *Br J Nutr*, **88**(4):355-364.

Burdge, M. and Wootton, S.A. (2002). Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. *Br J Nutr*; **88**(4)**:** 411-420.

Carmichael, H. E., Swinburn, B. A. and Wilson, M. R. (1998). Lower fat intake as a predictor of initial and sustained weight loss in obese subjects consuming an otherwise ad libitum diet. *Journal of American Diet Association*, **98:** 35–39.

Carrapsio, M. R; Aguayo, C and Carrapsio, A.I. (2004). Effect of fat content on the sensory characteristics of vanilla flavoured milk. *Ciencia-Y-Technological Alimentaria*, **4**(4)**:** 246-250.

Carter, J. F. (1993). Potential of flaxseed and flaxseed oil in baked goods and other products in human nutrition. *Cereal Foods World*. **38**(10)**:** 753–759.

Chavan R. S., Nalawade and Kumar, A. (2015). Studies on the Development of Whey Based Mango Beverage. *Journal of Food and Dairy Technology*, **3**(2)**:** pp-ISSN**:** 2347-2359.

Chawla, R., & Patil, G. R. (2010). Soluble dietary fiber. *Comprehensive Reviews in Food Science and Food Safety*, **9**(2), 178-196.

Chimmad, B.V. (2010). Flax: A minor oil seed for major health benefits. Proceedings of winter school on *Nutraceuticals*: Challenges & opportunities in 21st century. Held at University of Agricultural Science, GKVK, Bangalore in Nov 29th to Dec 19th, 2010.

Chourasia, s. (2010). *Development of novel sterilized herbal flavoured milk* (doctoral dissertation, indira gandhi krishi vishwavidyalaya, raipur (cg).

Connor, W. E. (2007). Cited by Joseph, J. Collin, (2010). Omega-3 Essential fatty Acids. *Nutri news*. Doughlas Laboratories. Pittsburg, **9**(2)**:** 1-8.

Coskuner, Y. and Karababa, E. (2007). Some physical properties of flaxseed (Linum usitatissimum). *J. Food Eng*, **78:** 1067–1073.

Cunnane, S. C., Ganguli, S., Menard, C., Liede, A.C., Hamadeh, M.J., Zhen, Y.C., Wolever, T.M.S. and Jenkins, D.J.A. (1993). High alpha-linolenic acid flaxseed (Linum usitatissimum): some nutritional properties in humans. *Brit. J. Nutr*. **69**(2):443-453.

Cunnane, S.C. (2003). Dietary sources and metabolism of α-linolenic acid. In: Thompson L, Cunnane SC, editors. *Flaxseed in Human Nutrition*. 2nd ed. Champaign, IL: AOCS Press. pp**:** 63-91.

Cunnane, S.C., Hamadeh, M.J., Liede, A.C., Thompson, L.U., Wolever, T .M. and Jenkins, D.J. (1995). Nutritional attributes of traditional flaxseed in healthy young adults. *American Journal of Clinical Nutrition*, **61:** 62-68.

Daun, J. K., Barthet, J.V., Chornick, T.L. and Duguid, S. (2003). Structure, composition, and variety development of flaxseed. In ‘‘*Flaxseed in Human Nutrition’’* (S.C. Cunnane and L.U. Thompson, eds), 2nd Ed., pp. 1–40. AOCS Press, Champaign, IL.

Daun, J. K., Mazur, P. B., and Marek, C. J. (1983). Use of gas liquid chromatography for monitoring the fatty acid composition of Canadian rapeseed. *Journal of the American Oil Chemists' Society*, **60**(10)**:** 1751-1754.

Dayane de C. Morais., Erica A. Moraes., Maria Ines de S. Dantas., Julia C. C. Carraro., Cassiano O. da Silva., Paulo R. Cecon., Hercia S. Duarte Martino. and Sonia M. Rocha Ribeiro. (2011). Heat Treatment and Thirty-Day Storage Period Do Not Affect the Stability of Omega-3 Fatty Acid in Brown Flaxseed (Linum Usitatissimum) Whole Flour. *Food and Nutrition Sciences,* **2:**281-286.

De, S. (1980). Special milks In: “Outlines of dairy technology”, 1stedn, pp: 98-99.

Dehkordi, N., Rao, D. R., Warren, A. P., and Chawan, C. B. (1995). Lactose malabsorption as influenced by chocolate milk, skim milk, sucrose, whole milk, and lactic cultures. *Journal of the American Dietetic Association*, **95**(4)**:** 484-486.

Delzenne, N. M. (2003). Oligosaccharides: state of the art. *Proceedings of the nutrition Society*, **62**(1)**:** 177-182.

Donkor, O. N., Nilmini, S. L. I., Stolic, P., Vasiljevic, T. and Shah, N. P. (2007). Survival and activity of selected probiotic organisms in set-type yoghurt during cold storage. *International Dairy Journal*, **17:** 657–665.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., and Attia, H. (2011). Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food chemistry*, **124**(2)**:** 411-421.

Eskin, N.A.M., McDonald, B.E., Przybylski, R., Malcolnson, L.J., Scarth, R., Mag, T., Ward, K. and Adolph, D. (1996). In: *Bailey's industrial oil and fat products*, Hui, Y.H. (Eds), Wiley, Newyork, pp.1.

Estrada, J. D., Boeneke, C., Bechtel, P., & Sathivel, S. (2011). Developing a strawberry yogurt fortified with marine fish oil. *Journal of dairy science*, **94**(12), 5760-5769.

Franck, A. (2002). Technological functionality of inulin and oligofructose. *British journal of Nutrition*, **87**(S2): S287-S291.

Frankel, E. N. (1993). In search of better methods to evaluate natural antioxidants and oxidative stability in food lipids. *Trends Food Sci.Tech.,* **4**: 220-225.

FSSAI, A. (2006) and rules (2011). *Akalank's food safety and standards Act, rules and regulation, Akalank publication,* pp- 293.

Garden, J. (1993). Flaxseed gum: Extraction, characterization, and functionality. Doctoral Dissertation of North Dakota State University, Fargo, ND, 157.

Ghoddusi, H. B., Grandison, M. A., Grandison, A. S. and Tuohy, K. M. (2007). In vitro study on gas generation and prebiotic effects of some carbohydrates and their mixtures. *Anaerobe*, **13**(5)**:** 193-199.

Ghosh, P., Chakraborty, M., Sorenson, B., Berzonsky, W.A. and Hall, C. (2004). Effects of ground flaxseed on the processing and quality parameters of wheat flour tortilla. Presented at III AACC Annual Meeting Conference, San Diego, CA.

Gibson, G. R. (1999). Dietary modulation of the human gut microflora using the prebiotics oligofructose and inulin. *J Nutr* , **129**:1438S–41S.

Gibson, G. R. and Roberforid, MB. (2008). Handbook of Prebiotics, CRC Press, Boca Raton, FL. probiotic bacteria. *International Dairy Journal*, **11:** 935–942.

Gibson, G. R., and Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *The Journal of nutrition*, **125**(6): 1401.

Giltay, E.J., Gooren, L.J., Toorians, A.W., Katan, M.B. and Zock, P.L. (2004). Docosahexaenoic acid concentrations are higher in women than in men because of estrogenic effects. *Am J Clin Nutr*, **80**(5)**:**1167-1174.

Goyal, A., Sharma, V, Upadhyay, N., Gill, S., and Sihag, M. (2014). Flax and flaxseed oil: an ancient medicine & modern functional food review, *Journal of Food Science and Technology*, **51:** 1633–1653.

Goyal, A., Sharma, V., Sihag, M. K., Singh, A. K., Arora, S., & Sabikhi, L. (2016). Fortification of dahi. *Journal of food science and technology*, ***53***(5), 2422-2433.

Green, A.G. and Dribnenki, J.C.P. (1994). Linola -A new premium poly unsaturated oil. *Lipid Tech*., **6**: 29-33.

Gulati, S.K., May, C., Wynn, P.C and Scott, T.W. (2002). Milk fat enriched in n-3 fatty acids. *Journal of Animal Feed Science and Technology*: 143–152.

Gunstone, F. D. (1996). *Fatty acid and lipid chemistry*. Blackie Academic & Professional.

Gunstone, F. D. (edn.). (2011). Vegetable oils in food technology: composition, properties and uses. Blackwell publishing, UK, pp. 18-52, 98-124.

Gunstone, F.D., Hatwood, J.L. and Padley, F.B. (1994). *The lipid hand book*. Chapman and Hall, London.

Hall, C. and Schwarz, J. (2002). Functionality of flaxseeds in frozen desserts-preliminary report. In ‘‘Proceedings of the 59th Flax Institute of the United States’’, held at Fargo, ND. pp. 21–24.

Hall, C., Niehaus, M., Wolf-Hall, C., Bauer, A., Auch, C. and Stoerzinger, C. (2004). Flaxseed in yoghurt: Lignan stability and effect on processing. Proceedings of the 60th Flax Institute of the United States. Fargo, ND. pp. 1–7.

Hanif, M., Muhammad J., Hussain, B., Ayub M. and Alizi N. A. (1996). Acceptability of cow and buffalo flavoured milk. *Sarhad J. Agric*. **12** (4)**:** 463 - 470.

Homler, B. E. (1984). Aspartame: Implications for the food scientist. *Aspartame: Physiology and Biochemistry*, 247.

Hu, F. B., Manson, J.E. and Willet, W.C. (2001). Types of dietary fat and risk of coronary heart disease: a critical review. *Journal of the American College of nutrition*, **20**(1)**:** 5-19.

Ibrahim, G. A., Mehanna, N. S., El-Rab, D. G., Abd El-Salam, M. H., Kholif, A. M., Abdou, S. M., and El-Shibiny, S. (2004). Preparation and properties of set fermented milk containing inulin and different probiotics. In *Proc. 9th Egyptian Conf. Dairy Sci. & Techn* (Vol. **117**).

ISI. 1981(b). SP 18, Part XI, ISI Hand book of food analysis. Dairy products, *Bureau of Indian Standards*, New Delhi.

ISI. 1981. SP 18, Part XI, ISI Hand book of food analysis. Dairy products, *Bureau of Indian Standards*, New Delhi.

Jason, R., Karp, Jeanne, D., Johnston and Sandra Tecklenburg. (2006). Chocolate milk as a Post Exercise Recovery Aid. *International Journal of sport Nutrition and Exercise Metabolism*. **16:** 78-91.

Jenab, E. (2013). Lipase-catalyzed interesterification between canola oil and fully hydrogenated canola oil in contact with supercritical carbon dioxide. *PhD* dissertation, University of Alberta, Canada.

Jenner, M. R. (1989). Sucralose properties and applications. Food ingredients, Europe, Conference proceedings, pp. 247-248.

Kalyani, N. K., Suman, K. and Thompkinson, D.K. (2010). *Food Reviews International*. **26:** 189-203.

Karimi, R., Hossein Azizi, M., Ghasemlou, M. and Moharam, V. (2015). Application of inulin in cheese as prebiotic, fat replacer and texturizer: A review. *Carbohydrate Polymers*, **119:** 85–100.

Kaufmann, N., and Ulf Andersen and Lars Wiking. (2012). The effect of cooling rate and rapeseed oil addition on the melting behaviour, texture and microstructure of anhydrous milk fat. *International Dairy Journal*, **25:** 73–79.

Kaur, N., and Gupta, A. K. (2002). Applications of inulin and oligofructose in health and nutrition. *Journal of biosciences*, **27**(7)**:** 703-714.

Kocer, D., Hicsasmaz, Z., Bayindirli, A. and Katnas, S. (2007). Bubble and pore formation of the high-ratio cake formulation with polydextrose as a sugar-and fat-replacer. *Journal of Food Engineering*. **78:** 953-964.

Kozlowska, J. (1989). The use of flax seed for food purposes. In ‘‘Flax in Europe Production and Processing. Proceedings of the European Regional Workshop on Flax’’ (R. Kozlowska, ed.), Poznan, Poland.

Kris-Etherton, P. M., Harris, W.S. and Appel, L.J. (2002). Fish consumption, fish oil, omega-3 fatty acids and cardiovascular disease. *Circulation*, **106**(21)**:** 2747-57.

Kumar, M. (2000). Physico-chemical characteristics of low calorie lassi and flavoured dairy drink using fat replacer and artificial sweeteners. M.SC Thesis, NDRI, Karnal, India.

Kumari, A., Choudhary, S., Arora, S., & Sharma, V. (2016). Stability of aspartame and neotame in pasteurized and in-bottle sterilized flavoured milk. *Food chemistry*, *196*, 533-538.

Lakka, H.M., Laaksonen, D.E., Lakka, T. A., Niskanen, L. K., Kumpusalo, E, Tuomilehto J. and Salonen,J.T. (2002).The metabolic syndrome and total and cardiovascular disease mortality in middle and aged men. *J Am Med Assoc*, **288**:2709-2716.

Lau, C. S. (2007). *Formulation and Physical, Chemical and Sensory Analysis of a Novel Flaxseed-enriched Milk-based Beverage to Deliver Omega-3 Fatty Acids* (Doctoral dissertation).

Lee, R. E., Manthey, F.A., and Hall, C.A., III. (2003). Effects of boiling, refrigerating, and microwave heating on cooked quality and stability of lipids in macaroni containing ground flaxseed. *Cereal Chem*, **80:** 570–574.

Lee, R.E., Manthey, F.A. and Hall, C.A, III. (2004). Content and stability of hexane extractable lipid at various steps of producing macaroni containing ground flaxseed. *J. Food Proc. Preser*, **28**: 133–144.

Lobato calleros, C., Ramirez, C, Santiago and Osorio, V.J. (2002). Microstructure and texture of manchego cheese like products made with canola oil, lipophilic and hydrophilic emulsifiers. *Journal of Texture Studies*: 165–182.

Luck, H., Grothe, J. (1973). Fruit juice-flavoured milk, a healthy beverage. *S. Afr. J. Dairy Technol*, **5:** 47.

Madhusudan, R. (1992). *Studies on Use of Non-conventional Sweeteners in the Preparation of Flavoured Milk* (Doctoral dissertation, University of Agricultural Sciences, GKVK).

Mag, T. K. (1990). Further processing of canola and rapeseed oils. Canola and rapeseed: Production, chemistry, nutrition and processing technology, edited by Fereidoon Shahidi, 251-76.

Mangsi, A. S., & Barhaml, G. S. (2012). Production and Comparison of Banana and Chikoo Flavoured Milk-based Beverages. *Pakistan Journal of Nutrition*, **11**(6), 600-604.

Manthey, F. A., Lee, R.E. and Kegode, R.K. (2000). Quality of spaghetti containing ground flaxseed. *Proceedings of the 58th Flax Institute of the United States*. Fargo, ND. pp. 92–99.

Manthey, F., Lee, R., & Hall, C. (2002). Stability of alpha-linolenic acid in macaroni containing ground flaxseed. *Proceedings of the 59th Flax Institute of the US*, 14-20.

Mathur, M. P., Datta Roy, D. and Dinakar, P. (1999). Textbook of dairy chemistry. Chapter-1. Indian council of Agricultural Research, New Delhi. pp. 18.

Mech. A., Rao, S.B.N., Soren, N.M. and Naveen, B.D. (2015). Estimation of fatty acids composition of animal feeds by gas chromatography. In *Recent techniques in feed and fodder evaluation for assessing feed quality and safety* (Samanta, Ashish and Bhatta, edn.). 2nd edn, ICAR-NIANP, Bengaluru, India, pp. 47-50.

Menendez, J.A. (2006). Cited by Joseph, J. Collin, (2010). Omega-3 Essential fatty acids. *Nutrinews*. Doughlas Laboratories. Pittsburg, **9**(2)**:**1-8.

Meyer, D., Bayyari, S., Torrega and Costell, E. (2011). Inulin as texture modifier in dairy products. *Food Hydrocolloids*, **25:** 1881–1890.

Miller, G. D., Jarvis J. K., and Me Bean, L. D. (2007). *Handbook of Dairy Foods and Nutrition* Boca Raton, FL: CRC press.

Miocinovic, J., Pu–da, P., Radulovic, Z., Pavlovic, V., Miloradovic, Z., Radovanovic, M. (2011). Development of low fat UF cheese technology.

Mittal, S., & Bajwa, U. (2012). Effect of fat and sugar substitution on the quality characteristics of low calorie milk drinks. *Journal of food science and technology*, **49**(6): 704-712.

Mueller, K., Eisner, P., Yoshie-Stark, Y., Nakada, R. and Kirchhoff, E. (2010). Functional properties and chemical composition of fractionated brown and yellow linseed meal (Linum usitatissimum), *J. Food Eng*, **98**(4): 453-460.

Nataraj, K. B. (2008). Studies on production of sterilized flavoured milk with low calorie sweetners. M.Tech. Thesis, Dept. of Dairy Technology, S. V. V. U, Tirupati, AP, India.

National Research Council. (2005). Dietary Reference Intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids (macronutrients). Washington DC: National Academy Press. pp. 464-471.

NDDB. Annual Report. (2016).

Newsome, R. L. (1986). Sweeteners-nutritive and nonnutritive. *Food Technology*, **40**(8)**:** 195-206.Ogawa, A. *et al*., (2009). Cited by Joseph, J.Collin, (2010) Omega-3 Essential fatty Acids. *Nutri news*. Doughlas Laboratories. Pittsburg, **9**(2)**:** 1-8.

Oomah, B. D., & Mazza, G. (1999). Health benefits of phytochemicals from selected Canadian crops. *Trends in food science & technology*, **10**(6), 193-198.

Oomah, B.D. and Mazza, G. (1998). Flaxseed products for disease prevention. P.91-138.In: Mazza, G. (ed.). Functional Foods: Biochemical and Processing Aspects. Lancaster, PA: Technomic.

Phillips, G. O., and Williams, P. A. (2000). Introduction to food hydrocolloids. In G. O.Phillips, & P. A. Williams (Eds.), Handbook of Hydrocolloids Cambridge, England: Woodhead Publishing Limited, pp. 1–22.

Pradhan, R., Meda, V., Rout, P., Naik, S. and Dalai, A. (2010). Supercritical CO_2extraction of fatty oil from flaxseed and comparison with screw press expression and solvent extraction processes. *J. Food Eng*, **98**(4)**:** 393-397.

Prakash, S., Huppertz, T., Karvchuk, O., & Deeth, H. (2010). Ultra-high-temperature processing of chocolate flavoured milk. *Journal of food engineering*, **96**(2), 179-184.

Przybylski, R., Liu, H., Biliaderis, C. G., and Eskin, N. M. (2011). Physical behaviour and composition of low-and high-melting fractions of sediment in canola oil. *Food chemistry*, **53**(1)**:** 35-41.

Ramasamy, D, Abraham, R. J and Manoharan, A. (2005). Organoleptic study on naturally coloured and flavoured sterilized milk with extracts of plant. *Journal of veterinary public health*, **3**(2)**:** 115-117.

Ravindra, M. R., Rao, K. J., Nath, B. S., & Ram, C. (2014). Extended shelf life flavoured dairy drink using dissolved carbon dioxide. *Journal of food science and technology*, **51**(1), 130-135.

Renner, E. and Zewrmann, K. (1988) studies on the optimum sugar content for flavoured milk drink , *Deutsce Molkezei Zeitung*,**109**(10)**:** 276-279.

Repate, K. C; Kamble, V. J., Hassan Bin Awaz and Thombre, B. M. 2010. Studies on preparation of flavoured milk from cow milk blended with safflower milk. *J. Dairying, Foods & H.S.*, **29** (2)**:** 92 – 96.

Revathy, T., Mythili, S., and Sathiavelu, A. (2011). Assessing the growth of probiotic bacteria in selected prebiotic foods rich in oligosaccharides.

Rezaei, K and Temelli, F. (2000). Lipase-Catalyzed Hydrolysis of Canola Oil in Supercritical Carbon Dioxide. *J. Am. Oil Chem. Soc*,**77:** 8.

Rieter, J. 1993. Flavoured milk's sleeping giants. *Dairy foods*, **94**(1)**:** 29-30.

Rousseau, D., & Marangoni, A. G. (1998). The effects of interesterification on physical and sensory attributes of butterfat and butterfat–canola oil spreads. *Food Research International*, **31**(5), 381-388.

Rubilar, M., Gutierrez, C., Verdugo, M., Shene, C.and Sineiro, J. (2010). Flaxseed as a source of Functional Ingredients.*J. Soil Sci. Plant Nutr*. **10** (3)**:** 373 – 377.

Sales, J. E., Gignac, M. and Hawker, G. (2008). The relationship between disease symptoms, life events, coping and treatment, and depression among older adults with osteo-arthritis. *The Journal of Rheumatology*, **35**(2)**:** 335-342.

Sameen, A., Tariq, M. R., Huma, N., & Khan, M. I. (2013). Effect of stabilizers on the quality of carbonated flavoured whey drink. *African Journal of Agricultural Research*, **8**(5): 445-448.

SanGiovanni, J. P. (2005). Cited by Joseph, J.Collin,(2010.) Omega-3 Essential fatty Acids. *Nutri news*. Doughlas Laboratories. Pittsburg, **9**(2)**:** 1-8.

Satyanarayana, M. A. V. (2008). Technological innovations for production of sterilized herbal flavoured milk. M. Tech. Thesis. Dept. of Dairy Technology, S. V. V. U, Tirupati, AP, India.

Simmons, R. K., Alberti, K.G., Gale, E. A., Colagiuri ,S., Tuomilehto, J., Qiao, Q,.,Ramachandran ,A., Tajima, N., Brajkovich Mirchov ,I., Ben-Nakhi ,A., Reaven, G., Hama Sambo B., Mendis S. and Roglic, G. (2010). The metabolic syndrome: useful concept or clinical tool? Report of a WHO expert consultation. Diabetologia, **53**(4)**:** 600-605.

Simopoulos, A. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother*, **56**(8)**:** 365-79.

Simopoulos, A.P. (1999). Essential fatty acids in health and chronic disease. *Am. J.Clin. Nutr***, 70:** 560S-9S.

Singh, C., Grewal, K. S., & Sharma, H. K. (2005). Preparation and properties of carrot flavoured milk beverage. *J. Dairying Foods Home Sci*, **24**:184-189.

Sivakumar, S. (2014). Development of process optimization for production of functional yoghurt. *Ph.D. Thesis*, NDRI, Karnal, Haryana, India.

Souci, S. W., Fachmann, W., & Kraut, H. (1989). Food composition and nutrition tables 1989/90. In Food composition and nutrition tables 1989/90. Wissenchaftliche Verlagsgesellschft mbH.

Strohman, D. R. (2014). *Flavor changes in stored extended shelf-life flavored milks* (Doctoral dissertation, University of Minnesota).

Takeuchi, H. (2007). Cited by Joseph, J.Collin. (2010). Omega-3 Essential fatty Acids. *Nutri news*. Doughlas Laboratories. Pittsburg, **9**(2)**:** 1-8.

Treen, M. Arch Biochem Biophys. (1992). Cited by Joseph, J. Collin. (2010). Omega-3 Essential fatty Acids. Nutri news.Doughlas Laboratories.Pittsburg **9**(2)**:** 1-8.

Tudorica, C. M., Kuri, V., & Brennan, C. S. (2002). Nutritional and physicochemical characteristics of dietary fiber enriched pasta. *Journal of agricultural and food chemistry*, **50**(2): 347-356.

Tungland, B. C. and Meyer, D. (2002). Nondigestible oligo- and polysaccharides (Dietary Fiber): their physiology and role in human health and food. *Comprehensive reviews in food science and food safety*, **1**(3)**:** 90-109.

Vijayalakshmi, R. and Tamilarasi, M. (2001). Shelf life and microbiological quality of selected dairy products. *J. Food Sci and Technol*, **38:** 385.

Von Schacky C. and Harris, W. S. (2007). Cardiovascular benefits of omega-3 fatty acids. *Cardiovasc Res*, **73**(2)**:** 310-315.

Whelan, J., & Rust, C. (2006). Innovative dietary sources of n-3 fatty acids. *Annu. Rev. Nutr.*, **26:** 75-103.

Woodman, R. J. (2003). Cited by Joseph, J.Collin. (2010). Omega-3 Essential fatty Acids. *Nutri news*.Doughlas Laboratories.Pittsburg **9**(2)**:** 1-8.

Yan cheng. (2007). Development on chocolate flavoured milk. Dairy Science Abstracts, **69** (8): 810.

Yanes, M., Durán, L., and Costell, E. (2002). Effect of hydrocolloid type and concentration on flow behaviour and sensory properties of milk beverages model systems. *Food hydrocolloids*, **16**(6)**:** 605-611.

Yurko-Mauro and Curr, K. Alzheimeres. (2010). Cited by Joseph, J.Collin. (2010). Omega-3 Essential fatty Acids. *Nutri news*.Doughlas Laboratories.Pittsburg **9**(2)**:** 1-8.

Zambon, D., J. Sabate., S. Munoz., B. Campero., E. Casals., M. Merlos., J.C. Laguna and Ros. E. (2000). Substituting walnuts for monounsaturated fat improves the serum lipid profile of hypercholestrolemic men and women: a randomized crossover trial. *Ann. Int. Med*, **132:** 538-546.

ANNEXURES

ANNEXURE- I

Sensory Evaluation of flavoured drink on 9-point Hedonic Scale

Name of the Judge: -
Date:-

Attributes	**A**	**B**	**C**	**D**
Colour & Appearance				
Body & Texture				
Flavour				
Sweetness				
Overall Acceptability				

Comments on **A**:

Comments on **B**:

Comments on **C**:

Comments on **D**:

Signature:-

9-like extremely; 8-like very much; 7-like moderately; 6-like slightly; 5- neither like nor dislike; 4-dislike slightly; 3-dislike moderately; 2-dislike very much; 1-dislike extremely

ANNEXURE- II

Changes in sensory quality of flavoured drink during storage

Sensory attributes	Samples	Days			
		0	2	4	6
Colour& Appearance	Control	8.00 ± 0.12^{aA}	7.98 ± 0.10^{aA}	7.96 ± 0.12^{aA}	7.90 ± 0.13^{aA}
	Experimental drink	7.99 ± 0.12^{aA}	7.95 ± 0.12^{aA}	7.92 ± 0.10^{aA}	7.88 ± 0.10^{aA}
Body& texture	Control	7.85 ± 0.10^{aB}	7.84 ± 0.12^{aB}	7.82 ± 0.07^{aB}	7.78 ± 0.10^{aB}
	Experimental drink	8.03 ± 0.09^{aA}	8.01 ± 0.10^{aA}	7.95 ± 0.11^{aA}	7.83 ± 0.10^{bA}
Flavour	Control	7.98 ± 0.09^{aA}	7.96 ± 0.11^{aA}	7.91 ± 0.10^{abA}	7.76 ± 0.10^{bA}
	Experimental drink	7.88 ± 0.10^{aA}	7.86 ± 0.13^{aA}	7.80 ± 0.07^{abA}	7.68 ± 0.09^{bA}
Sweetness	Control	8.03 ± 0.11^{aA}	8.00 ± 0.10^{aA}	7.95 ± 0.11^{aA}	7.90 ± 0.11^{aA}
	Experimental drink	7.85 ± 0.11^{aB}	7.83 ± 0.08^{aB}	7.80 ± 0.10^{aB}	7.77 ± 0.11^{aB}
Overall Acceptability	Control	7.96 ± 0.12^{aA}	7.92 ± 0.11^{aA}	7.89 ± 0.14^{aA}	7.80 ± 0.12^{aA}
	Experimental drink	7.89 ± 0.14^{aA}	7.86 ± 0.15^{aA}	7.83 ± 0.13^{aA}	7.74 ± 0.13^{aA}

Note: *Values are average of three trials*; *values with different superscripts (A, B, C) and (a, b, c) differ significantly with in the columns and rows at $p<0.05$, # refrigerated temperature*

ANNEXURE- III

Physico-chemical changes in flavoured drink during storage

Samples	Parameters	Days				
		0	2	4	6	8
Control	Acidity (% L.A)	0.166	0.167	0.174	0.183	0.192
	pH	6.57±0.01	6.54±0.01	6.53±0.01	6.5±0.01	6.47±0.01
	Viscosity(cP) @ 40°C	2.07±0.01	2.08±0.02	2.08±0.02	2.11±0.01	2.13±0.02
Experimental drink	Acidity(% L.A)	0.171	0.174	0.179	0.188	0.197
	pH	6.55±0.01	6.53±0.01	6.5±0.01	6.48±0.01	6.44±0.01
	Viscosity (cP)@ 40°C	2.40±0.02	2.42±0.01	2.44±0.02	2.49±0.01	2.55±0.02