PREPARATION AND SHELF- LIFE EVALUATION OF FLAXSEED INCORPORATED BISCUITS

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Preparation And Shelf- Life Evaluation of Flaxseed Incorporated Biscuits.

A dissertation submitted to the Department of Nutrition and Dietetics, Central Campus of Technology, Tribhuvan University in the partial fulfillment of the requirements for the degree of B.Sc. in Nutrition and Dietetics

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Approval Letter

This *dissertation* entitled *Preparation And Shelf- Life Evaluation of Flaxseed Incorporated Biscuits* presented by Vatsala Rai has been accepted as the partial fulfillment of the requirement for the degree of B.Sc. in Nutrition and Dietetics.

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Abstract

Flaxseed incorporated biscuit is a small baked unleavened cake, typically crisp, flat and sweet by using flaxseed flour as the major ingredient. The aim of this research was to develop the formulation for germinated flaxseed flour incorporated biscuit and to study its storage stability. D-optimal design was used for the formulation of recipe and for this, DOE (Design Expert) v13 software was used. The obtained five formulations with 0, 12.5, 25, 37.5 and 50 parts flaxseed flour with wheat flour were prepared in laboratory. The samples were subjected to sensory evaluation for consumer acceptability. The obtained data were statistically analysed using one way ANOVA (no blocking) at 5% level of significance.

From the mean sensory scores, 25 parts flaxseed flour incorporation was selected as the best formulation and subjected for further proximate analysis. Flaxseed incorporated biscuit and wheat biscuit were subjected to calcium and iron content evaluation. At 5% level of significance, control and optimized product were significantly similar to each other. The product was further analysed for prediction of shelf life based on acid value, peroxide value and moisture content and the values were found to be 0.21 mg KOH/g oil, 1.08 meq O_2/kg fat and 4.88% respectively at the end of 2 months which were all within the acceptable limits. These findings suggest that flaxseed flour can be successfully incorporated in refined wheat flour up to the concentration of 25 parts without any adverse effect on sensory attributes. The calcium, iron, protein, ash, fibre and fat content were found to be higher in flaxseed flour incorporated biscuit in comparison to normal wheat flour biscuit.

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List of Abbreviations

Abbreviation	Full form
ANF	Anti Nutritional Factors
ALA	Alpha Linolenic Acid
AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of Variance
BHA	Butylated Hydoxy Anisole
BHT	Butylated Hydroxy Toluene
FF	Flaxseed Flour
FSSAI	Foods Standard and Safety Authority of India
FTQCO	Food Technology and Quality Control Office
HCN	Hydrocyanic Acid
HDPE	High Density Polyethylene
LDL	Low Density Lipoprotein
LSD	Least Significant Difference
NBS	Nepal Bureau of Standards
NGA	Nordihydro Guaiaretic Acid
PG	Propyl Gallate
TIA	Trysin Inhibitor Activity
WF	Wheat Flour
WHO	World Health Organization

Part I

Introduction

1.1 General introduction

The baking industry is one of the leading systematized processed food industry. Bakery products are popular due to their convenience, low price and ready-to-eat nature, easy transportation, availability in numerous tastes and textural profiles (Tiwari and Mishra, 2019). Biscuits are ready to eat, convenient and inexpensive food products containing digestive and dietary principles of vital importance (Agarwal, 1990). The foremost benefit of bakery products is docility for fortification with cereals, millets or other ingredients. Therefore, these products are an effective medium for delivering functional ingredients to consumers (Tiwari and Mishra, 2019).

Because of competition in the market and increased demand for healthy, natural and functional products, attempts are being made to improve the nutritive value of biscuits and functionality by modifying their nutritional composition. Such effects are very often achieved by increasing the ratio of raw materials other than wheat or different types of dietary fibers in basic recipes with the attempt to increase the biscuit’s protein and mineral content for quality and availability or increase dietary fiber content and improve pre biotic characteristics of the final product. They are widely accepted and consumed by almost all profiles of consumers from many countries as they serve as an important source of nutrients. However, several studies indicated that the nutritional, physical and sensory characteristics of biscuit depends on both physiochemical properties of the flour and processing method employed for flour preparation(Asif-Ul-Alam *et al.*, 2014). It therefore offers a valuable supplementation vehicle for nutritional improvement (Cardello and Sawyer, 1992).

Flax (*Linum usitatissimum*) belonging to the family Lineaceae, is a blue flowering annual herb that produces small flat seeds varying from golden yellow to reddish brown color. Flaxseed possesses crispy texture and nutty taste (Rubilar *et al.*, 2010). Flaxseed is also known as linseed and these terms are used interchangeably. Flaxseed is often used to describe flax when consumed by humans while linseed denotes when it is used specifically for industrial applications (Morris, 2007). Flax is grown both for fiber and for oil, with fiber (for linen) derived from the stem of fiber varieties and oil from the seed of linseed varieties (Diederichsen and Richards, 2003).

Flax is considered a functional food or source of functional ingredients because it contains α-linolenic acid (Bozan and Temelli, 2008), lignans and polysaccharides (other than starch), all of which have positive effects in disease prevention.

Beside nutritional and medicinal values of flaxseed, anti-nutritional factors such as cyanate, oxalate, phytic acid and tannin are naturally present in it. These anti-nutritional compounds interfere with digestion, absorption and proper utilization of nutrients (Goyal *et al.*, 2014). It needs to be processed and preserved in such a way, that its therapeutic and nutritional properties are retained the most and should have reduced anti-nutritional factors. Germination is considered one of the most effective processing methods for improving the nutritional quality of pulses and legumes, enhancing the digestibility of nutrients as protein and carbohydrates (Kumar *et al.*, 2021).

Flaxseed is considered as one of the key source of phytochemicals and using of flaxseed in food products will be beneficial to protecting against cancer and lowering cholesterol level, diabetes and heart disease (Gutte *et al.*, 2015). Flaxseed as a nutritional additive for preparation of baked products, ready to eat cereals and fiber bars having good health impacts has been widely recognized in all parts of the world (Rendón-Villalobos *et al.*, 2009).

Shelf- life is an important property of any food and is of interest to everyone in food chain from producer to consumer. Well planned and conducted consumer acceptability tests in the form of appropriate sensory analysis, is an important part of the shelf- life evaluation of any product (Man and Jones, 1994). Moisture and water vapor transfer act as key influencing factor for shelf life (Steele, 2004). Off- flavors, off- odors and loss of crispiness in packaged food are the major cause of consumer rejection. These might be caused by oxidative rancidity of packaged food, permeation, migration and reactions between packaging components with the food components (Ackermann *et al.*, 1995). In modern age, food packaging has become very important to protect the product from contamination by macro and micro- organisms and their filth, prevention from loss or gain of moisture, shielding the product from oxygen and to facilitate handling (Ball, 1960).

1.2 Statement of problem

Snacks constitute major food bulk consumed by a human every day. Hence, like lunch, dinner and breakfast, snacks also should be rich in nutritional value. Biscuits have become a famous snack in developing country like ours due to its low price and ready to eat instant characteristics. Hence, such food must be nutritionally as rich as possible. Many researches have been carried out in context of the nutritional value of normal wheat flour biscuits. Conclusions drawn from those researches point towards a major short coming in such biscuits to be lacking mainly in mineral and protein content. Though they contain adequate amounts of the other essential amino acids, at least for adults, wheat proteins are deficient in the essential amino acid, lysine (Shewry and Hey, 2015).

Refined wheat flour is commonly used for biscuit making owing to its high gluten content but is nutritionally very poor (Agrahar-Murugkar *et al.*, 2015); hence, the low nutritive value of biscuits is an issue of great concern because biscuits are popular among all age groups especially in children. Celiac disease is defined as the abnormal response of the immune system to wheat gluten and related prolamins of rye and barley (Jnawali *et al.*, 2016).

Germination is a natural process occurred during growth period of seeds in which they meet the minimum condition for growth and development. During this period, reserve materials are degraded, commonly used for respiration and synthesis of new cells prior to developing embryo. Several studies on the effect of germination on legumes found that germination can improve digestibility of protein content and dietary fiber, reduce tannin and phytic acid content and increase mineral bioavailability. Germination also was reported to be associated with bioavailability of trace elements and minerals and also germination improved calcium, copper, manganese, zinc, riboflavin, niacin and ascorbic acid contents (Megat Rusydi and Azrina, 2012).

Nepalese diet usually consists of cereals containing high proportion of carbohydrates but not enough protein, dietary fiber and other micro nutrients (Devkota *et al.*, 2015). Though flaxseed is great source of plant based protein, fiber and minerals, it is considered to be edible only from farmers' perspective particularly among the poor in Nepal. Thus, preparation of biscuits with the incorporation of flaxseed can be a better approach towards utilizing the locally available and nutritionally rich plant seeds and to prepare a convenient and healthy snack for consumers rich in protein, fiber and minerals (Subedi and Upadhyaya, 2019).

1.3 Objectives

1.3.1 General objective

General objective of this work is to incorporate germinated flaxseed flour in the wheat flour to prepare flaxseed flour incorporated biscuits and study their storage stability.

1.3.2 Specific objectives

The specific objectives of the study are:

- To determine anti- nutritional factors of flaxseed flour at different germinated time.
- To optimize flaxseed flour for preparation of flaxseed flour incorporated biscuits and assess the sensory attributes.
- To evaluate the physico- chemical properties of the prepared biscuits.
- To determine the shelf- life of the prepared biscuits packed in HDPE (52 μm) at ambient condition.

1.4 Significance of the study

In the present world, interest has been increased in search of functional foods to decrease the risk of the evolution of diseases. Flaxseed is emerging as an important functional food ingredient because it provides oil rich in ω-3, high quality protein and soluble fiber and phenolic compounds.

Beyond its oilseed crop ability, proximate composition of flaxseed makes it more promising for its utilization in different food products. Flaxseed is one of the richest vegetarian sources of α-linolenic acid (ω-3 fatty acid) and soluble mucilage. Flaxseed contains protein (23.4%), lipids (45.2%) and mineral (3.5%) which are nutritionally very important (Mueller *et al.,* 2010). Lipid flaxseed composition makes it an important source of ω-3 fatty acids, especially ALA which constitute up to 52% of the total fatty acid. Hence, it considered as a functional food or source of functional ingredients (Bozan and Temelli, 2008). Flax protein is relatively rich in arginine, aspartic acid and glutamic acid, and the limiting amino acids are lysine, methionine and cysteine. Flaxseed contain total fibre - around (25-28) % and major fibre fractions are cellulose, mucilage gums, and lignin (Coşkuner and Karababa, 2007). These may prevent or reduce the risk of various diseases, such as diabetes, lupus nephritis, arteriosclerosis and hormone-dependent types of cancer (Bilek and Turhan, 2009). In present era, consumer's trend towards functional food has increased significantly as health awareness rose (Ganorkar and Jain, 2013).

Flaxseed, besides its traditional oleochemical uses, is now gaining recognition as a functional food ingredient for human nutrition (Lei *et al.*, 2003; Oomah, 2001). Consumption of flaxseed has been demonstrated to have multitude positive health benefits including decreasing rate of tumor growth, reducing serum cholesterol level and decreasing incidence of breast, prostrate, and colon cancers (Choo *et al.*, 2007; Hemmings *et al.*, 2004; Hosseinian *et al.*, 2006; Muir and Westcott, 2003). The health benefits of flaxseed are mainly attributed to biologically active components such as α-linolenic acid, lignans, unique proteins, phenolic acids and flavonoids (Hosseinian *et al.*, 2006; Kitts *et al.*, 1999; Tarpila *et al.*, 2005; Westcott and Paton, 2001).

Biscuits have significant attributes such as relatively long shelf-life, convenience and good eating quality and suitability for different nutritional/functional novelty trials, which also makes them good alternatives for gluten-free trials (Baljeet *et al.*, 2010). Therefore, in this study, flaxseed (*Linum usitatissimum*) is used to enhance both nutritional and technological drawbacks of commercial wheat biscuits.

1.5 Limitations and delimitations

- Only iron and calcium content of the product was studied.
- Microbial analysis of the product like total plate count and yeast and mould count was not carried out.
- The shelf life of the product could only be studied for 2 months due to time constraint.

Part II

Literature review

2.1 Classification of biscuits

Biscuits are generally classified as hard dough and soft dough type of biscuits as per the protein composition of the flour used. In case of the hard dough biscuits, the flour used should be the weakest possible to obtain and vice versa in case of soft dough biscuit (Smith, 1972). The soft dough group comprises all the sweet biscuits having many factors in common whereas the hard dough biscuits fall naturally into three sections: fermented dough, puff dough and the semi-sweet dough (Whitely, 1971).

2.1.1 Soft dough biscuits

The soft dough group includes all the sweet biscuits, whether they are plain biscuits, shells, or flow type such as gingernuts. Soft or weak wheat is used in the production of flour suitable for the manufacturer of sweet biscuits. The flour should have gluten content in the (7.0-9.0)% range, but in certain cases it may be necessary to weaken the flour with corn flour, or boost the structure-forming gluten content by the addition of a proportion of strong flour. An average quality sweet biscuit has a fat content of approximately 30.0% of the flour content, rising to 35.0% for rich biscuits such as shortcake, and even as high as 45.0% for shortbread. The average quality sweet biscuit will have a combined sugar solids content slightly higher than the fat content, being approximately equal at the 35.0% level, and the sugar content remaining at 35.0%, or even falling to 30.0%, as the fat content increases. At the other end of the scale, as the fat content falls towards 20.0%, the sugar solids content may increase to 45.0% (Whitely, 1971).

Soft doughs do not have a formed gluten structure, because of their high levels of shortening and sugar, and are generally mealy or sandy in texture. They are usually formed by compressing into dies (rotary molded or by extruding and cutting, but some types can be sheeted, then cut. Dough pieces formed from soft doughs tend to retain their shape until baking, but then they spread or flow, becoming thinner (Caballero *et al.*, 2003).

2.1.2 Hard dough biscuits

Hard dough biscuits have relatively high amount of water and low amount of fat and sugar by dough composition. Generally, these biscuits are produced through laminating, dusting, sheeting and cutting processes. Typical hard dough biscuits, which are unsweetened, fermented, aerated, thin and crisp to eat, are highly susceptible for the changes in physical, chemical and organoleptic properties during the accelerated storage life due to their open-flaky texture (Senarathna and Navaratne, 2017).

2.1.2.1 Semi sweet biscuits

Soft flours are used in the production of semi-sweet, hard dough biscuits, and frequently the flour is weakened by the addition of corn flour, arrowroot, or potato flour. The fat content is relatively low and rarely exceeds 22.0% of the flour weight. The sugar content is normally about 2.0% higher than the fat. The flavour is usually rather bland and is dependent upon milk, syrup, and vanillin, or other added background-type flavours (Whitely, 1971).

As the fat content is relatively low and no fermentation is employed to modify the gluten, it might be expected that the resulting biscuits would be hard and tough. In actual fact they are inclined to be tender and brittle. This is achieved by the special mixing technique employed. The method of mixing is an all-in method, whereby the dissolved salt and aerating chemicals are added to the flour and remaining ingredients, and then mixed until the dough becomes developed as for a fermented dough. Mixing still continues, however, until the gluten becomes softened by the mechanical development and eventually loses its elasticity and is completely extensible. When this stage is reached, threads of dough can be peeled off, with no signs of springing back, but with in fact, a stretch like chewing gum. After mixing, the dough is allowed to stand for a period of approximately 1 hr (Whitely, 1971).

Biscuits are baked to low moisture contents, around (1.5-2.0)%. Mixing time on a typical high speed mixer will be (20-25) min. The dough should reach (40-42)°C. At this temperature it should be well kneaded and of correct consistency for machining. Higher dough temperatures result in unstable doughs. The dough may be laminated, but doughs made with sodium metabisulphite are usually sheeted without lamination. Direct Gas Fired and Indirect Radiant ovens are both suitable for baking semi-sweet biscuits either as individual ovens or as a Direct Gas Fired / Indirect Radiant combination oven. Convection zones may be used in the middle and final zones of the oven for drying and coloring the biscuits (Davidson, 2017). Most semi-sweet biscuits are now produced from a warm dough with sodium metabisulphite used to modify the gluten chemically (Manley, 2011).

2.1.2.2 Fermented dough biscuits

This type of biscuits includes two groups: the cream crackers and the soda crackers. Although these two types have variations within them in case of composition and process of manufacture, both of them have basic mode of production as fermentation. Studies show that the manufacture process of salt crackers is standardized whereas a lot of variations might be seen in the manufacture process of cream crackers. Ingredients commonly include flour of medium strength, protein (9.0-9.5)%, shortenings 12% for cream crackers to 14% for salt crackers, sugar basically is used as yeast food only, salt (2-3)%, malt for rapid fermentation due to its diastase activating effect (Smith, 1972).

2.1.2.3 Puff dough biscuits

This hard dough biscuit is leavened with well layered fat between the dough sheets. The dough and the fat should possess nearly the same flow properties and care must be taken that the fat doesn't become the part of the homogenous dough phase as it will not contribute to layering but instead reduce the elasticity of the dough and might give undesirable outcome.

In order to ensure proper thickness of each layer and proper retention of each layer in this final dough piece, the following conditions need to be maintained:

- The gluten must be developed to a point which will properly stretch during reduction.

- The roll in, though plastic and semi-solid, must gradually stretch along with the dough in order to break the continuity of its ultimate shape.

After mixing (15 min) and relaxation (30 min) 60% of the puff dough margarine is applied and sheeted. The sheet is laid off for 15 minutes and rest of the fat is applied (George, 1981).

2.2 Chemical composition of biscuits

Chemical composition of biscuit varies within the biscuit types due to their difference in the raw material composition, method of preparation, end purpose of the biscuit and various other factors. The major and most common difference between the biscuit types namely hard dough, soft dough and fermented dough biscuit is presented in Table 2.1.

Table 2.1 Chemical composition of biscuits

Type	Protein %	Fat %	Total sugar %	Other carbohydrates %	Moisture %	Salt and chemicals %
Soft dough	6.00	20.80	25.88	44.73	1.25	1.34
Hard dough	7.18	12.26	19.15	59.40	0.90	0.56
Fermented dough	7.20	15.00	7.20	67.00	1.50	2.10

Source: (Rao *et al.*, 1991)

2.3 Raw materials for biscuit making

Mostly the common raw materials for biscuit making includes wheat flour, water, emulsifiers, sugar, salt. Apart from these various other raw materials are used for biscuit making in industries. Choice of raw materials is generally done as per the quality and organoleptic requirement of the final product.

Raw materials can be divided into major and minor ingredients, those raw materials which are used in bulk and are a must for biscuit making are considered as major ingredients. For example, Flour, water, sugar and fat are used in bulk in biscuit making procedure.

Salt, skim milk powder (SMP), ammonium bi-carbonate, sodium bi-carbonate, coloring agents, flavoring agents, emulsifiers, fortifying agents, improvers etc. are used in small amounts and aren't a must for all sort of biscuits. These ingredients are used for developing the taste, texture, flavor and aesthetic value of the product. Therefore, these minor ingredients are also known as the product improvers (Shrestha, 1995). All these ingredients are individually important to obtain more palatable and satisfactory products. The raw materials are found in the form of solid, liquid and paste (Shrestha, 1995).

2.3.1 The major ingredients

2.3.1.1 Flour

Flour is the basic raw material for biscuit making responsible for the major bulk of biscuit (Whitely, 1971). The flour used in biscuit and cracker vary in strength and baking characteristics (Bohn, 1956).

a. Wheat flour

Wheat grain is the only grain naturally capable of producing flour capable of being made into a low density baked product. Wheat is botanically named as *Triticum vulgare*. Wheat flour for biscuit making is obtained from the endosperm in the form of particle size enough to pass through a flour sieve usually 100 mesh per linear inch (Kent and Amos, 1983). Wheat flour is unique among all the cereal flours in that it forms an elastic mass when mixed with correct proportion of water. This unique property is due to the presence of insoluble proteins, collectively called gluten. The gluten forming proteins (Glutenin and gliadin) constitute about (75-80)% of the total flour proteins (Mukhopadhyay, 1990).

Glutenin gives solidity to the product whilst gliadin is the binding agent imparting the soft sticky character to the gluten. Gliadin is soluble in 70% alcohol and may be extracted from flour whereas glutenin is soluble in alcohol and water (Gorinstein *et al.*, 2002). Gluten is elastic, cohesive and rubbery and holds together and holds together the various ingredients of the dough. It has the property of holding the gases given off during fermentation and during baking. It sets in oven to form the firm, porous, open texture during baking which are necessary in the production of biscuits and crackers. Thus gluten is the necessary framework, forming the sustaining wall of the whole structure of baked products (Bohn, 1956).

Wheat flour used for making biscuit should be the product obtained by milling cleaned hard or soft wheat or a combination of both types. Flour strength is usually defined by the percentage of protein present in the flour. Weak flour is casually accepted as the flour with low percentage of protein. Usually, this protein is inferred to be gluten, which when the flour is made into a dough with water, will become very extensible under stress, yet when the stress is removed it will not fully return to its original dimensions. Further, the amount of stress required to facture the dough piece is less than that required under identical conditions when strong flour is used (Smith, 1972).

The flour should be free flowing, dry to touch, should be creamy in color and free from any visible bran particles. It should also have a characteristic taste and should be free from musty flavor and rancid taste. The characteristics as required in flour is given in Table 2.2.

Table 2.2 Requirements for flour characteristics

S. No.	Characteristics	Requirements
1	Moisture content	13.0% max
2	Gluten content on dry basis	7.5% min.
3	Total ash on dry basis	0.5% max.
4	Acid insoluble ash on dry basis	0.05% max.
5	Protein (N × 7.5) on dry basis	9.0%
6	Alcohol acidity as H_2SO_4 in 90% alcohol	0.1%
7	Water absorption	55%
8	Sedimentation value	22%
9	Uric acid (mg/100 gm)	10% max.
10	Granularity	To satisfy the taste

Source: (Arora, 1980)

b. Corn flour

Corn flour and maize starch are prepared from the cereal *Zea mays*. Maize is Indian corn. The two chief varieties are known respectively as flint maize and dent maize (Sanchez *et al.*, 2002). Corn flour is milled from the endosperm of maize and is very nearly pure starch, because of its high starch content; it can be used to weaken flour which is too strong.

c. Rice flour

It is prepared from the cereal *Oryza sativa*. Rice contains a larger proportion of starch than any other cereals. Although rice is deficient in minerals, fat and protein its use in biscuit making is due to its very easily digestible carbohydrate (Corrêa *et al.*, 2007). Apart from nutritional value its used in biscuit making is done as dusting agent, when dough release from a rotary moulder die is not effective, dies are lightly dusted with rice cones before they receive the dough (Smith, 1972).

d. Oat flour

The use of oat products in biscuit making is due to its high nutritive value owing to high proportion of protein and fats. Generally, the oat meal contains higher level of fat (about 6%). The major drawback of its use is due to the off-flavor development during baking and during long storage and baking. However stabilizers may be used to inhibit this problem (Worgan, 1960).

e. Soy flour

Soya flour is used in dough due to its emulsifying property and higher level of protein content. The emulsifying action is due to its higher level of lecithin content (Whitely, 1971).

f. Arrowroot flour

Another tropical plant, a herbaceous perennial, *Maranta arundinacea*, has swollen roots from which the starch is extracted. This was used in a particular variety of British semi-sweet biscuits to weaken the flour and give an improved smoothness to the palate. The combination of a world crop failure in the 1960s and the availability of cheaper starches with almost identical properties has effectively precluded its use in biscuits now (Manley, 2011).

g. Flaxseed flour

Flax (*Linum usitatissimum*) belonging to family Lineaceae, is a blue flowering annual herb that produces small flat seeds varying from golden yellow to reddish brown color. Flaxseed also has potential health benefits due to its anti-diabetic, antioxidant, anti-inflammatory functions which in turn reduce risk of cancer, cardiovascular, renal and bone disorders (Katare *et al.*, 2012). Flaxseed flour contains high concentration of calcium, which is a key component to mineralize the bone matrix (Theobald, 2005). Since, flaxseed flour has several benefits, incorporating flaxseed flour containing lignin-rich dietary fiber to deliver a product seems to be beneficial nutritionally (Kaur *et al.*, 2019).

2.3.1.2 Fat or shortening

Fat in a biscuit can be denoted in several ways: it can be seen on the label as butter, animal fat (although this is less common now), vegetable fat or vegetable oil (including the named types such as palm oil, sunflower oil, etc). It may also be presented in a generic form such as 'shortening' which is a term used to describe one of the functions of fat in biscuits which is to 'shorten' the dough that is, to give it that typical 'melt in the mouth', crumbly texture which is characteristic of biscuits (Manley, 2011). Recipes with high fat contents require little water for producing a cohesive dough and produce soft, short doughs. During mixing, the fat coats the flour particles and this inhibits hydration and interrupts the formation of the gluten. Fats also tend to inhibit the leavening action of the carbon dioxide diffusion in the dough during baking, and this produces a softer, finer texture (Davidson, 2016). Shortening also aids dough aeration during the creaming step. The overall effect improves palatability, extends shelf-life, improves flavor and, of course, adds caloric energy (Caballero *et al.*, 2003)

2.3.1.3 Sweetening agents

White and brown sucrose, glucose, glucose syrup, golden syrup, invert sugar syrup, and highfructose corn syrup are all used in as sweetening agents (Wrigley *et al.*, 2015). Also malt syrup, malt extract, maple syrup, honey, etc are also used (Whitely, 1971)

The sugar crystals which have a coating of syrup are known as raw sugar or brown sugar. The colour of the syrup varies so the brown sugar may be golden brown or quite dark. Brown sugars are extensively used in baking for the distinctive flavour they give (Manley, 2011). Golden syrup is made by the refiner from low grade sugars and uncrystallised syrups. It is a mixture of sucrose and invert sugar in solution with a small proportion of gums, acid, and mineral salts. Glucose is a clear, thick, viscous fluid, only about half as sweet as sucrose. Glucose is readily fermented by yeast. During baking it readily caramelises, giving a good colour to the face of the biscuit, and it also assists in soft dough formation. Malt extract is very poor as a sweetening agent, but consists of approximately 50% malt sugar (maltose), the rest being water, dextrins, and a small proportion of protein. Its main use is for flavour, but can also be used in fermented doughs to assist gluten modification and as a yeast food. Whereas malt extract is generally prepared from malted barley, malt syrup is produced from barley and maize starch and is consequently sweeter. It is used for flavour and as a yeast food (Whitely, 1971).

Maple syrup is obtained from the sap of certain maple trees. The sap contains about 3% sucrose and has a distinctive flavour. This sap is concentrated to about 70–75% sucrose. Maple syrup is used primarily as a flavouring ingredient and is relatively expensive (Manley, 2011).

Sugars affect product dimensions, color, hardness, structure, surface finish, and sweetness. At low levels, the sugar goes into solution during heating and forms a glass-like structure when cooled. This results in an open texture when no gluten has been developed. The hard-eating properties of crunch biscuits are due to their high sugar content. Sugar inhibits gluten development by competing with the flour for the recipe water. In short dough biscuits, which have high sugar and low recipe water levels, some of the sugar 20 dissolves in the water to form a saturated sugar solution, leaving no free water to hydrate the flour protein to form gluten (Wrigley *et al.*, 2015).

Many biscuit manufacturers mill crystalline sugar to change the particle size distribution. When a crunchy, coarse-eating product is desirable, a proportion of granulated sugar is added. Particle size affects the rate of solution of sucrose during mixing and in short-dough biscuits affects biscuit dimensions and texture. Larger-particle-sized sugar produces doughs that flow less during baking, meaning that the biscuits produced are shorter, wider, and thicker. Large sugar particles increase in biscuit hardness (Wrigley *et al.*, 2015).

Large sugar particles, which have not dissolved during mixing, melt to form a glass on the surface during the early stages of baking. As the dough expands, the glass cracks, resulting in the typical cracked surface appearance. Sugar contributes to color via Maillard browning reactions, which require the presence of both reducing sugars and amino acids. Sucrose is not a reducing sugar but is partially hydrolyzed to glucose and fructose (reducing sugars) during baking. Some of the compounds produced by Maillard reactions contribute to biscuit flavor. Brown sugars contribute to biscuit flavor and color both via Maillard reactions and due to small quantities of aromatic compounds from the molasses (Wrigley *et al.*, 2015). Dissolved sugar tends to inhibit starch gelatinisation and gluten formation and creates a biscuit with a more tender texture. Undissolved sugar crystals give a crunchy, crisp texture (Davidson, 2016). Texture is a very important characteristic which makes a significant contribution to the overall acceptance of food products (Kulthe *et al.*, 2017).

2.3.2 The minor ingredients

2.3.2.1 Emulsifying agents

Emulsifiers are chemicals, which stabilize mixture of immiscible liquids like fat and water. They also modify and stabilize fat crystallization, change dough consistency, stickiness and starch gelling as well as lubricate low fat doughs. It acts by reducing the surface tension. Also certain emulsifiers complex with amylose fraction of starch. Emulsifiers like Soya lecithin, Glycerol Mono Stearate (GMS) Polyoxy ethylenes, Sorbitan monostearates, polyglycerol esters, Diacetyl tartaric acid esters of mono glyceroids, Stearoyllactylates, Sucrose esters and many more.

The emulsifiers are used at 0.5 to 1.0% level by dissolving in fat and using at creaming stage. It helps in the uniform distribution of fat throughout the mass. Apart from lecithin, GMS, a white flaky substance is used in biscuits.

The use of emulsifiers / surfactants improves the texture of biscuits. It also reduces stickiness in low fat dough by complexing with starch and proteins.

2.3.2.2 Leavening agents

Leavening are the gassing agents which causes the dough to spring off or puff up to give a porous open texture to the final product. Ammonium and sodium bicarbonate are the major chemical leaveners, while yeasts are the biological leaveners. Similarly, mechanical leavening can be done by incorporating the air within the dough matrix by mechanical agitation. Reaction of two or more chemicals also leads to production and incorporation of gas, mainly the reaction takes place between bicarbonates of ammonia as well as sodium with acidulants. To discuss about the major and most common leavening agent the baking powder, it should possess the following properties (Smith, 1972) :

a. Maximum gas strength-greatest volume of gas for least weight of the product.
b. Proper balance of ingredients to prevent any impairment of the taste or appearance of the biscuit.
c. Innocuous ingredients and residues.
d. Optimum velocity of reaction to be susceptible to control.
e. Keeping quality under diverse and extreme conditions to remain unimpaired over reasonable periods of time.
f. Minimum cost of production, economical in use.

The chemical reaction during use of chemical leaveners and acidulants is as given below:

$$NH_4HCO_3 \xrightarrow{heat} NH_3 + CO_2 + H_2O$$

Ammonium bicarbonate Ammonia Carbon dioxide Water

$$(NH_4)_2CO_3 \xrightarrow{heat} 2NH_3 + CO_2 + H_2O$$

Ammonium carbonate Ammonia Carbon dioxide Water

The chemical equations for the reaction of soda and the commonly used acidulants are as below:

$$NaHCO_3 + HX \longrightarrow NaX + CO_2 + H_2O$$

Sodium bicarbonate Acid Carbon dioxide Water

$$NaHCO_3 + C_4H_5O_6K \longrightarrow C_4H_4O_6NaK + CO_2 + H_2O$$

Cream of tartar Sod. Pot. Tartarate

Both sodium and ammonium bicarbonate react with acidic ingredients if any, in the dough. Use of an excess ammonium bicarbonate makes the dough more alkaline and thereby may affect the protein structure of the dough. Use of an excess sodium bicarbonate will affect the crumb and crust color often with an accompanying unpleasant or soapy taste, unless any acidic material is used to neutralise the residual sodium carbonate (Bohn, 1956).

2.3.2.3 Milk solids

Milk and milk solids are considered to be the ingredients of value addition during biscuit making. Apart from increasing the nutritive value of the biscuit, milk and milk solids help in retention of flavors. Usually, use of milk in biscuit making is done in SMP and full cream form due to its higher stability and easy storage facility. Milk solid when used in biscuit making have proved to enhance crust bloom and color, tenderness and texture without altering the symmetry and crumb color. The coloration may be due to the fact that the lactose in milk solid remains as lactose in the biscuit because it is not fermentable by yeast. Lactose helps in the formation of melanoids, the principle crust coloring substances, formed by the reaction of sugars and amino acids from the proteins under the influence of heat. Probably this reaction takes place in all biscuit dough baking (Smith, 1972).

Higher milk flavor can be obtained by the use of condensed milk during biscuit making. Similarly, among all the milk products, butter is the potent product for better flavor development but due to economical aspect their use have nearly completely been replaced by butter flavors. Other milk products that are also sometimes used are cheese, whey, butter milk etc (Shrestha, 1995).

2.3.2.4 Salt (Sodium Chloride)

Salt is used for flavour and flavour enhancement. Mostly used at (1-1.5)%. It toughens gluten and thus reduces stickiness. Salt should be free flowing with particle size suitable especially for sprinkling on the surface (Egyankosh).

2.3.2.5 Flavoring and coloring agents

Flavor is the quality of the thing that affects the sense of taste and smell. The majority of the flavors used in biscuit making are derived from natural sources and these are in many ways most satisfactory. To get good distribution in a dough, the flavor should be creamed with the sugar and shortening at the beginning of mixing. Except from the added flavors, flavor can also be obtained from the various ingredients such as nuts, fruits etc. Most commonly used flavoring agents are common salt, yeast, extracts, spices and essences (Whitely, 1971).

Coloring agents are mostly not added externally during biscuit making. Other ingredients like sugar, invert syrup, milk solids etc. provide color to the product mainly due to caramelisation. Coloring agents not only include the synthetic as well as natural colors only but various ingredients used during biscuit making also serve to provide appealing color to the product (George, 1981).

2.3.2.6 Water

Water affects textural properties of baked products. Water acts as a plasticizer, and the amount of water used is adjusted to produce a batter or dough of acceptable consistency for processing. Water is needed for hydrating the proteins, gelatinizing the starch, making leavening agent function, activating the enzymes, dissolving sugar and salt, as well as acting as major heat transfer mechanism during baking through evaporation and condensation (Shiksha).

2.3.2.7 Anti-oxidants

These are chemical components, which prevent spoilage of fat in biscuit during storage. Some of the permitted antioxidants are:

- Ethyl/Propyl gallate or mixture (at 0.01%).
- Octyl / Dodecyl gallate (at 0.01%).
- Ascorbyl palmitate.
- Tertiary butyl hydroxy quinone (TB HQ) (at 0.02%).
- Citric / Tartaric acid (synergists) (at 0.02%).

Propyl gallate and TB HQ along with citric acid are commonly used.

Source: (Egyankosh)

2.4 General specifications of biscuits as published by NBS

Biscuit should be properly baked, crisp and uniform in texture and appearance. They should not possess rancid flavor, fungal infection, off odour and any insect infestation. For filled biscuits any of the fillers like jam, jellies, marshmallow, cream, caramel, figs, raisins etc can be used. The biscuits may be coated with caramel, cocoa or chocolates. Use of antioxidant as well as permitted preservative can be done not exceeding the maximum dosages. The general specifications of biscuits as described by Nepal Bureau of Standards (NBS) is given in Table 2.4.

Table 2.3 General specification for biscuits as published by NBS

S. No.	Characteristics	Requirements
1	Moisture	6% max
2	Acid-insoluble ash (on dry basis)	0.05% max
3	Acidity of extracted fat (as oleic acid)	1.00% max

Source: (NBS, 2040)

Processed edible oil used in biscuit making means any edible vegetable oil neutralized with alkali, refined, deodorized and bleached.

2.5 Nutritive value of biscuits

Biscuit is a ready to eat good source of nutrient as it contains carbohydrates, fats, proteins, minerals and vitamins. Proteins are nutrients for growth and repair of tissues while carbohydrate and fat provide heat and energy. Similarly, minerals provide nutrient for bone growth. Vitamins are responsible for normal metabolic activities and maintaining normal vitality of the body. Nutritive value of biscuit is given in Table 2.4.

Table 2.4 Nutritive value of biscuit (per 100 gm)

Weight per serving	100 gm
Calories (kcal)	450
Protein (gm)	6.4
Fat (gm)	15.2
Carbohydrate (gm)	71.9
Calcium (gm)	-
Phosphorous (gm)	-
Iron (mg)	-
Vitamin A value (I.U.)	-
Thiamine (mg)	-
Riboflavin (mg)	-
Niacin (mg)	-

Source: (Anonymous, 2017)

(Sarwar, 2010) reported proximate values of wheat flour as 13%, 0.60%, 0.90%, 0.30%, 11.3%, and 87% respectively and (Poudel, 2021) reported proximate values of wheat flour (db) as 12.35%, 0.57%, 1.55%, 0.61%, 10.47% and 86.8% respectively.

2.6 Technology involved during biscuit making

Technology is the factor which enables easy doing of something by significantly reducing the labour, time, expenditure and increasing the quality. Technology is always beneficial until it is under control. Hence the skill to handle the available technology is the fore most need during an operation. Not only the technology controller or his department is responsible with the machinery control but he is also with the total control from ingredient purchase to sales (Rao *et al.*, 1991). The technology of biscuit production is shown in Fig. 2.1.

Preparation of ingredients

↓ (Weighing, recipe calculation and fermentation of dough in case of fermented biscuits.)

Creaming and mixing

↓ (Performing is done for hard dough and fermented dough biscuits.)

Laying the dough

↓

Dough forming

↓ (Moulding in case of soft dough biscuit, sheeting for other types)

Baking

↓ (Temp. 176˚C to 204˚C for 4 to 6 minutes)

Cooling

↓ (For 6 to 7 minutes)

Wrapping and packaging

↓

Labeling and storage (RH 40 to 59% at room temp.)

Source: (Smith, 1972)

Fig 2.1: Flow sheet of manufacturing process of biscuit

2.6.1 Dough mixing

Mixing is the major step during biscuit making. Properly mixed dough has a great influence in the final quality of the product. Mixing of the dough can be done in various ways as per requirement. Mixing in industries is carried out by use of electrical mixers, most commonly used mixers are two speed mixers. In top speed, the creaming up time is (3-5) minutes in two speed mixer types, while the flour should be mixed for 10 minutes on slow speed (Whitely, 1971).

There are two basic methods of mixing the dough but each may have several variations designed to achieve the best result under the particular circumstances prevailing and depending upon the type of equipments used.

a. Creaming up method

This may be done in either two stages or three stages, but the principle is the same.

(i) Shortening, sugar, sugar syrup, reconstituted milk and other ingredients are mixed together to form a smooth cream. Then water is added and mixed well.

(ii) Suspension of salt, alkaline ingredients colour and flavour in the remaining water is made and added to the cream and mixed well.

(iii) The acidic ingredients and flour are added to get a desired dough consistency.

This type of mixing procedure encourages the formation of dough emulsion of shortening, sugar and water and hence strong gluten development is retarded.

Source: (Egyankosh)

b. All in one method

Salt, leavening chemicals, colour, flavour etc., are dispersed in part of water. All the ingredients, including salt and leavening chemicals suspension are mixed together till a satisfactory dough results. Creaming method is generally followed for soft doughs and the dough prepared is either processed in rotary moulder or wise-cut machine. All-in-one method is quite satisfactory to produce hard doughs. The mixed dough should be sufficiently developed to form sheet when forced between sheeting rollers and subsequent reduction through gauge rollers. It should withstand cutting action without farming cracks and hang together as a network when lifted as scrap from the cut-out biscuits. In case offermented dough any of the two methods given below are followed:

(i) All-in-one mixing and fermentation.

All ingredients including yeast suspension are mixed together till the flour is wetted. The dough is then allowed for 3 to 8 hrs fermentation and then taken for further processmg.

(ii) Two stage mixing and fermentation.

a) Sponge

70% of the total flour, sugar, yeast, suspension, part of shortening and whole of water are mixed for 2-3 min and fermented for 19 hrs.

b) Dough

When the sponge is ready, rest of the ingredients are mixed together and allowed for a shorter fermentation and taken for further processing.

Source: (Egyankosh)

2.6.2 Laying the dough

Lay time refers to the halt of the dough between mixing and machining. Lay time for various products is variable. Lay time for fermented dough is highly necessary and is long while in case of sulphited doughs, it is avoided. A minimum of 15 minutes of lay off time should be given to the dough for the achievement of good surface gloss, color, and weight. It also makes the dough easily machinable (Smith, 1972).

2.6.3 Forming and preforming

The shaping or forming of the dough into various shapes and to the required thickness before baking is one of the major steps in biscuit making. In case of hard dough and fermented dough forming is done by using sheeters and laminators, which reduces the thickness of the dough to convert it into a thin sheet. Occluded air is eliminated from the dough. It also helps in proper spread of fat, salts which helps in producing a product with short eating and layered appearance.

Soft dough is directly fed into moulding or embossment disc which cuts them into required size, shape and appearance (Shrestha, 1995).

2.6.4 Baking

Baking is the major step of biscuit production without which the product loses its eating quality. During baking, the product is cooked, flavor and color is developed and the raw dough is converted into an edible snack named biscuit. The main objective of baking is to remove the moisture present in the dough pieces by gradual heating. The dough contains more than 25% moisture, a part of which is bound water present in the flour and other ingredients while other part is the free water added externally for dough making and easy machinability (Bloksma, 1990).

Every baking process depends upon the heat transfer from a hot source to the product being baked. Method of heat transfer during baking is mainly by three methods namely, conduction, convection and radiation. During baking a major part of heat transfer to the dough pieces is by radiation while the heat transfer by convection is very low as long as the air velocity in the tunnel is not higher than 5 feet per second, after which the heat transfer by convection tends to be higher. Apart from these three modes of heat transfer, high frequency heating is also used which has a higher rate of moisture removal (Smith, 1972).

Every oven used till date consists of four basic parts:

a) A heat source.
b) A base (sole or hearth), capable of being heated, on which the dough piece is placed.
c) A cover over the base, making up a chamber in which to retain the heat.
d) A closable opening through which the dough piece can be put into and taken from the baking chamber.

During baking the dough undergoes gradual changes physically as well as chemically. Physical changes include:

a) Formation of a film crust on the dough.
b) Melting of the fat in the dough.
c) Gas release and volume expansion.
d) Conversion of water into steam.
e) Escape of carbon dioxide, other gases and steam.

Chemical changes include:

a) Gas formation.
b) Starch gelatinization.
c) Protein changes.
d) Caramelisation of sugar.
e) Dextrinization.

Temperature in the baking oven has different effect on the raw dough which is shown in Table 2.5.

Table 2.5 Temperature related changes in biscuit during baking

Temperature (°F)	Changes occured
90-100	Top crust skin formation (Evaporation of surface moisture).
90-120	Evolution of CO_2 Within crumb (Less solubility of CO_2).
90-150	Increase in volume due to CO_2.
90-210	Gas expansion (CO_2 and steam).
125-210	Starch gelatinisation (Biscuit structure).
170-190	Evaporation of alcohol, yeast action ceases.
170-250	Evaporation of alcohol, yeast action ceases.
350-400	Coagulation of protein (Irreversible).
370-400	Dextrinization (surface gloss).

Source: (Mukhopadhyay, 1990)

During baking it is necessary to have more steam in the oven than that derived from the moisture from the dough and the combustion of the fuel. Introducing steam into the baking chamber, either immediately at the entry of the dough pieces or at a point very early in their passage through the oven, helps to create a shiny crust formation, prevention of cracked crusts, increased volume and to some degree agitation of the oven atmosphere. The need of steam injection can be removed by using fast moving fans recirculating air at speeds of 2000 cu ft per minute. The dampers present at the ovens play a vital role in releasing the high positive pressure within the oven created due to high heat evaporation, similarly if high moisture cookies or biscuits are desired than the dampers at the last zone must be closed (Smith, 1972).

2.6.5 Cooling

During cooling, the flexible structure becomes rigid as sugar and fat solidify, Cooling should be extended as long period as possible and the humidity of the cooling atmosphere should be controlled. There are two systems of cooling followed.

Atmospheric multi-tier conveyor

In the atmospheric multi-tier conveyor, the biscuits from the oven band travel on to a canvas web having single, double or three tiers. The biscuits on these are cooled by surrounding atmosphere.

The forced draft cooling conveyor

Where space does not permit gradual and atmospheric cooling, forced draft cooling is used. Filtered air is blown against the direction of travel of biscuits coming out of the oven on the cooling conveyor thereby cooling the biscuits earlier then the normal time.

Source: (Egyankosh)

2.6.6 Packaging and storage

Biscuits are low moisture content food. Their mandatory standards state them to be of low moisture content, mainly below 6%. The relative humidity of freshly baked biscuit is very low so in order to prevent rapid uptake of moisture from the atmosphere, the biscuits must be packed in a water vapour resistant material. Up take of moisture by biscuit make them prone to microbial attack similarly open access to the atmosphere make them prone to oxidative rancidity as fat is a major ingredient used during biscuit making.

Packaging materials are those materials which contain the product within them providing necessary conditions and protection to the product inside to keep them safe and consumable over a long period of time. In case of biscuit a good packaging material must be:

a) Resistant to water vapour.
b) Non- tainting material with good grease resistance.
c) Should be strong enough to protect against any possible mechanical injury.
d) Should be opaque.
e) Easily printable.

Packaging in case of biscuits must be close up together in order to provide a mutual reinforcing effect which prevents them from breakage. Packaging of biscuits at commercial level is generally done in triple laminates consisting of polyethylene, aluminium foil and paper (Paine and Paine, 1983).

The characteristics of the packaging materials are described in Table 2.6.

Table 2.6 Some characteristics of packaging materials

Polymer materials	Abbreviations	Associated properties
Low density polyethylene	LDPE	Sealability, formability, moisture barrier, low cost.
Linear low density polyethylene	LLDPE	Sealability, abuse resistance, moisture barrier, formability.
Polypropylene	PP	Moisture barrier, thermal resistance, dimensional rigidity.
Ethylene vinyl acetate copolymer	EVOH	Sealabilty, improved abuse resistance over LDPE, clarity.
Polyesters	PET	Mechanical resistance, heat resistance, medium O_2 barrier.
Ethylene vinyl alcohol	EVA	High O_2 barrier, good co- extrusion, processability, clarity.
Polyamides	PA	Mechanical strength, O_2 barrier (moisture sensitive), formability.
Polyvinylidene chloride	PVDC	High O_2 barrier; (moisture stable), grease and fat barrier.
High density polyethylene	HDPE	More gas impermeable than LDPE, low cost, strong, reduced clarity.
Polyvinyl chloride	PVC	Versatile, shrink properties, sparkling clear, low cost.
Polystyrene	PS	Excellent clarity, low cost, readily thermoformed and injection moulded.
Ionomer	-	Heat sealability, produce films of unusual toughness and clarity.
Polycarbonate	PC	High clarity, strong, imact resistance, dimensional rigidity.

Source: (Kerry *et al.*, 2002)

2.7 Sensory perception of biscuits

Before launching the biscuit in the market, a panel of experts evaluate the overall acceptability of the final product which is known as sensory perception or evaluation. Many basis of evaluation include appearance, crispiness, color, flavor and finally the overall acceptability.

- **Appearance**

  Appearance is related to the geometry and the structure of the biscuits, and the outer ends of appearance, from an irregular biscuit with an irregular surface produced by evaporation bubbles, to a very compact biscuit with a smooth surface (San José *et al.*, 2018).

- **Crispiness**

  Crispiness is a factor that influences the perception of freshness. The crispiness of a product can be characterized by the sound during breaking of the product (Anonymous).

- **Color**

  Color is defined as the degree of toasting (San José *et al.*, 2018).

- **Flavor**

  Flavor is the the combined effect of taste sensations, aromatics, and chemical feeling factors evoked by a substance in the oral cavity (Astm, 2009).

- **Overall acceptability**

  Overall acceptability is a mixture of all of the sensory attributes and therefore the outer ends of the scale go from: very little like the commercial standard biscuit, with a very light color, little flavor and taste of biscuit, a very crumbly and soft biscuit, to a very dark biscuit, with a strong taste of toasted, a very hard and compact biscuit, at the upper end (San José *et al.*, 2018).

The evaluation is marked on the score cards which is later on discussed in order to find out the best product. Consumer research, case history research is also some of the factors that need to be included which drawing conclusions from the analysis (Smith, 1972).

2.8 9-point hedonic rating scale

The hedonic scale method has proven to be a very useful tool in food research. There are only limited number of basic psychometric methods, and the hedonic scale is no more than a special application of the most generally useful one- the rating scale. It represents a direct approach to the measurement of psychological states.

In the hedonic scale method, the stimuli (actual samples of food) are presented singly and are rated on a scale where the 9 categories range from "dislike extremely" to "like extremely." History, methods of use in laboratory and field, analysis of the data, reliability, essential characteristics, applications, special effects requiring control, and interpretation of results are discussed and evidence is cited for the method's validity for predicting food behaviour. Major advantages of this method are:

- Subjects can respond meaningfully without prior experience.
- It is suitable for use with a wide range of populations.
- The data can be handled by the statistics of variables.
- Results are meaningful for indicating general levels of preference.

Source: (Peryam and Pilgrim, 1957)

2.9 Flaxseed (Linum usitatissimum)

Flax or linseed is among the oldest crop plants cultivated for the purpose of oil and fiber. It belongs to the genus *Linum* and family Linaceae. The botanical name, *Linum usitatissimum* was given by Linnaeus in his book “Species Plantarum” (Linnaeus, 1857). It is an annual herbaceous plant with shallow root system. The common names flax and linseed are used in North America and Asia, respectively, for *L. usitatissimum*. Oilseed varieties and fiber varieties are specialized development of this species (Millam *et al.*, 2005). The cultivars grown primarily for seed/oil purpose are relatively short in height and possess more secondary branches and seed bolls (seed capsule). The cultivars grown for fiber purpose are tall growing with straight culms and have fewer secondary branches.

Flax, while a minor crop, is grown in a wide range of countries, climates and for many different products. Because of its adaptability and product diversity, it is being considered as a platform for the development of novel bioproducts. Research on use of flax for bioproduct production is being conducted in Australia, North America, Europe and Asia. Flax for fiber purpose is grown primarily in China, Russia, Egypt, and near the northwestern European coast for the production of high quality linen and several other products (Vromans, 2006).

2.9.1 Classification and nomenclature

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

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

Source : (USDA, 2018)

2.9.2 Physical and functional properties

It is essential to study the physical properties of kernels, grains and seeds which are necessary for the design of equipment to handle, transport, process and storage. Commercial utilization of flaxseed proteins in food products depend on its functional properties before its incorporation in various food products. Study of functional properties is necessary where functional properties have the role in food formulations. Bulk density is one of the functional properties where higher the bulk density is desirable as this property to reduce the paste thickness. This is an important factor in infant formulas where bulk is of concern (Anwar *et al.*, 2005).

In the study of Janaki, Surabhi and Nagarkot, varieties flaxseed (*Linum usitatissimum*), it was reported that flaxseeds are in oval shape and brown in color with smooth glossy appearance. Thousand seeds weight ranged from (5.01-6.05) g and seed length ranged between (4.50-5.45) mm per seed in three varieties. The seeds were reported to be flat and pointed and the width ranged between (0.90-1.45) mm (S. Arora and Modgil, 2006). Some physical properties of flaxseed (*Linum usitatissimum* L.) were analyzed by (Coşkuner and Karababa, 2007) in which the value of bulk density was found to be 0.66 g/cm^3. On the same study it was reported that the bulk density values of (555.6-726.6) kg/m^3 for the moisture range of (6.09–16.81)% (d.b.), whereas on other study it was observed that the bulk density of (545.0-690.5) kg/m^3 for the moisture range of (6.09–16.81)% (d.b.) for the commercial variety of linseed. The decrease in bulk density of flaxseed may be due to the increase in seed size with moisture content which gives rise to decrease in quantity of seeds occupying the same bulk volume (Khan and Saini, 2016).

Similarly. another study was carried out to analyze the functional properties of full fat roasted and non-roasted flax seed. There was no significant difference between full fat roasted and full fat unroasted as bulk density was 0.83 and 0.78 g/ml, water absorption capacity was 1.83 and 1.48 g/g and fat absorption capacity of 1.31 and1.20 g/g respectively. This study showed the higher fat absorption in flax flour of full fat roasted (Hussain *et al.*, 2008). On the observation of functional properties of flaxseed protein concentrate found that it has better foam stability and emulsifying capacity was 83.3% and 84.76 ml/g and emulsifying activity was 88.37% (Flores *et al.*, 2006). The improvement in functional properties may be achieved either by genetic modification, physical treatment or chemical processing Water absorption is the ability of protein to bind water imparts moisture to many food products. The difference in the water absorption capacity between the varieties may be due to the differences in the proportion of hydrophilic groups (Oomah and Mazza, 1993). The study reported that higher values for water absorption capacity, may be due to reduction of polyphenols and phytic acid, which might have made more protein available for holding the water (Pawar *et al.*, 2001).

2.9.3 Nutritional composition of flaxseed

Various edible forms of flax are available in the food market—whole flaxseeds, milled flax, roasted flax and flax oil. According to its physicochemical composition, flaxseed is a multicomponent system with bio-active plant substances such as oil, protein, dietary fiber, soluble polysaccharides, lignans, phenolic compounds, vitamins (A, C and E) and mineral (P, Mg, K, Na, Fe, Cu, Mn and Zn) (Goyal *et al.*, 2014). According to food composition table by DFTQC the nutritional composition of flaxseed is 28.3g carbohydrate, 37.7 g fat, 20.3 g protein 2.4 g minerals and 4.8 g fiber per 100gm.

Table 2.7 Nutritional composition of flaxseed

Component (%)	Golden flax	Brown flax
Moisture	6.73±0.03	6.52±0.04
Ash	2.84±0.01	2.63±0.01
Total lipids	37.57±0.71	38.13±1.39
Crude protein	23.24±0.06	24.42±0.11
Carbohydrates	29.61±0.76	28.29±1.45

Source: (Sargi *et al.*, 2013)

2.9.4 Health benefits of flaxseed

Cardiovascular health benefits

As the building block for other messaging molecules that help in prevention of excessive inflammation, ALA can help protect the blood vessels from inflammatory damage. Numerous studies have shown the ability of dietary flaxseeds to increase blood levels of ALA, even when those flaxseeds have been ground and incorporated into baked goods like breads or muffins (Dodin *et al.*, 2008). When flaxseeds are consumed, two other ω-3 fatty acids have also been shown to increase in the bloodstream, namely, eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA). Increase in EPA and DPA also helps in the inflammatory protection (Dodin *et al.*, 2008).

Diabetes prevention

Low glycemic index foods containing soluble fiber not only prevent certain metabolic ramifications of insulin resistance, but also reduce insulin resistance (Reaven *et al.*, 1993). Soluble fiber and other components of flaxseed fractions could potentially affect insulin secretion and its mechanisms of action in maintaining plasma glucose homeostasis. Flaxseed was shown to reduce the postprandial blood glucose response in humans (Cunnane *et al.*, 1993; Jenkins *et al.*, 1999). A consumption of 50 g/day ground flaxseed by young females over a 4-week period caused a reduction in blood glucose levels (Cunnane *et al.*, 1993). Similar findings were observed in post-menopausal women who were fed 40 g/day flaxseed fortification diet (Lemay *et al.*, 2002). Bread containing 25% flaxseed gave a glycemic response that was 28% lower than the control (no flaxseed) bread (Jenkins *et al.*, 1999).

Post- menopausal benefits

Flax is currently being researched as a supplement for women entering into menopause (Dodin *et al.*, 2008). The high fibre of flax coupled with the health benefits of the essential fatty acids have shown to have a positive impact on hormonal modulation in clinical trials of women over the age of fifty. Women who enjoy high fibre diets experience fewer problems and side effects due to menopause (Hallund *et al.*, 2008).

Flaxseed in cancer

Studies on the activity of lignans on breast, colon, prostate and thyroid cancer has generally shown beneficial effects although there are some studies with no conclusive or negative effect. Flaxseed has been shown to reduce the early risk markers for and incidence of mammary and colonic carcinogenesis in animal models (Serraino and Thompson, 1992). Flaxseeds significantly increased urinary excretion of lignans without changing the serum hormone concentration of premenopausal women suggesting that the chemoprotective effects reported for flaxseed may have resulted from mechanism other than a hormonal effect (Frische *et al.*, 2003).

Flaxseed in bone health

Alpha linolenic acid, the ω-3 fat found in flaxseed promotes bone health by helping to prevent excessive bone turnover-when consumption of foods rich in these ω-3 fat results in a lower ratio of ω-6 to ω-3 fats in the diet (Griel *et al.*, 2007). When the women who had been having 14 hot flashes per week for at least a month weren't taking estrogen to relieve their menopausal symptoms, they were fed 2 tablespoons of crushed flaxseed twice daily for six weeks and the women halved their number of daily hot flashes while taking flaxseed. In addition, the intensity of the women's hot flashes dropped by 57%. Side effects included abdominal bloating (14 women) and mild diarrhoea (8 women) (Pruthi *et al.*, 2007).

2.9.5 Dietary fibre

Flaxseed meal is reach in crude, acid detergent, neutral detergent and total fibers (cellulose, lignin and hemicellulose). Flaxseed contains soluble and insoluble dietary fibers in a proportion that varies between 20:80 and 40:60. The major insoluble fiber fraction consists of cellulose and lignin and the soluble fiber fractions are the mucilage gums (Cui *et al.*, 1996; Qian *et al.*, 2012).

Dietary fibers from flaxseed were found to have a direct relation to health in particular in body weight regulation through both hunger suppression and diminished nutrient absorption (Kristensen *et al.*, 2012) generally, soluble fiber forms a gel when mixed with water. This gel slows down the emptying of the stomach, potentially lowering blood glucose levels. Cholesterol is also lowered as it is surrounded by the gel, which inhibits its absorption and leads to more cholesterol being excreted. (Ibrügger *et al.*, 2012) conducted a crossover acute study about the influence of flax drink and flax tablet on hunger suppression. Sensation of satiety and fullness were similar for flax tablets and flax drink as they did not differ by more than (1-4) % (Ibrügger *et al.*, 2012).

Flaxseed protein demonstrated the reduction of the fat absorption by fecal excretion in animal and human. Addition of a flax dietary fiber extract rich in viscous dietary fibers significantly increased fat excretion and lowered total and LDL cholesterol, with no effect on appetite (Kristensen *et al.*, 2012).

Metabolism

Dietary fiber of flaxseed reaches the large intestine and is fermented by colonic micro flora with production of short chain fatty acids (SCFA), hydrogen, carbon dioxide, methane and biomass and exhibit laxative effects (Kritchevsky, 1979).

In the large intestine, both soluble and insoluble fibers have their bulking effect resulting in increasing both dry and wet weight of the colon contents and feces. Soluble fiber increases water binding, initially by the binding capacity of its macromolecules, later by increasing the mass of microbial cells. The contribution of soluble fiber to fecal weight was insignificant compared to insoluble fiber. Recent studies, however, have shown that it is of the same magnitude (Malkki, 2004).

2.9.6 Anti-nutritional factors

Anti-nutrients or anti-nutritional factors are those substances generated in natural feed stuffs by the normal metabolism of species and by different mechanisms which exerts contrary to optimum nutrition. Anti-nutritional factors such as tannin, trypsin inhibitors, oxalates etc., are found in food grains (Hiremath, 2013).

2.9.6.1 Phytic acid

Phytic acid, another anti-nutrient present in flaxseed, ranges from (23-33) g/kg of the flaxseed meal. Phytic acid interferes with the absorption of calcium, zinc, magnesium, copper and iron. It is a strong chelator, forming protein and mineral-phytic acid complexes and thus reducing their bioavailability (Oomah and Mazza, 1993). Phytic acid (also known as Inositol hexakisphosphate) (InsP6) is the salt form of phytic acid, are found in plants, animals and soil. It is primarily present as a salt of the mono- and divalent K^{+}, Mg^{++}, and Ca^{++} and accumulates in the seeds during the ripening period. It serves as a storage of phosphorous and minerals and accounts for (60-90)% of the phosphorous in the plant. Besides phytic acid, other inositol phosphates are present in the seeds, however to a much lower extent. In addition, phytic acid has been suggested to serve as a store of cations, of high energy phosphoryl groups, and, by chelating free iron, as a potent natural anti-oxidant (Mueller-Harvey, 2001).

In cereals, phytic acid is located up to 80% in the aleurone layer, but is also found in the germ, while the endosperm is almost free of phytic acid. During the germination of seeds, phytic acid is hydrolysed, and phosphorous along with minerals such as calcium, magnesium and iron are liberated, becoming available for germination and development of the seedlings (Frolich *et al.*, 2011).

2.9.6.2 Tannins

Tannins are very important commercial products. However, their chemistry is very complex and diverse. They can be classified into two groups, the proanthocyanidins (or condensed tannins) and the polyesters of gallic acid and (or) hexahydroxydiphenic acid (hydrolysable tannins, respectively, gallo- and ellagitannins) (Özacar and Şengil, 2002). Condensed tannins are derivatives of flavanols and hydrolysable tannins are esters of a sugar, usually glucose (Adamczyk *et al.*, 2017). The co-occurrence of both kinds of tannins in the same plant or plant tissue is often observed. Tannins are found in the leaves of trees (Özacar and Şengil, 2002).

Trypsin inhibitors are anti-nutritional factors which form an indigestible complex with trypsin. This prevents trypsin activity and thus decreases trypsin concentration in the small intestine. The pancreas of the animal compensates for this by secreting more trypsin and thus increases the protein requirements of the animal for these increased secretions. Trypsin inhibitors are present in flaxseed meal; their activity level appears to be much lower than the trypsin inhibitors found in both soybean meal and canola meal. The same researcher reported the trypsin inhibitor activity (Ogbonna *et al.*) level for soybean meal is 1650 units of TIA, canola meal is 99 units of TIA and for flaxseed meal it ranges from 42 to 51 units of TIA depending on cultivar and processing (Bhatty, 1993).

Anti-nutritional factors in flaxseed were analyzed. 325mg/100g tannins and phytic acid of 969mg/100g were reported by (Preethi and Chimmad, 2010). Trypsin inhibitors are reported in flaxseed, though activity is insignificant as compared to soybean and canola seeds (Bhatty, 1993).

2.9.6.3 Hydrocyanic acid

Flaxseed contains cyanogenic compounds of (264–354) mg per 100 g. Cyanate are naturally present in the plants and on hydrolysis convert into hydrogen cyanide. Cyanide if present in high amount per low dose repeated exposure will be toxic to human as it inhibits cytochrome Coxidase system involved in respiratory chains (Enneking and Wink, 2000).

These cyanogenic compounds are toxic to humans. It was found that ingestion of 100 mg/day may be lethal to adult individuals. However, these compounds present in seeds are instable when subjected to thermal and mechanical processes, including cooking in microwaves, autoclaving and boiling. Average tolerance of ingestion of cyanogenic compounds without adverse effects, as established by the World Health Organization, is 0.11 mg/kg weight in the form of cyanogen chloride, it means that an individual weighing 60 kg may consume up to 0.66 mg of cyanogen chloride (WHO, 2003). Food Standards and Safety Authority of India (FSSAI) mentioned maximum permissible limit of hydrogen cyanide in food grains as 37.5 mg/kg (FSSAI Act, 2006). Considering the concentration of cyanogenic compounds in flaxseed as reported in literature, the daily ingestion of flaxseed indicated (30 g) may contain on average 106 mg of cyanogenic compounds, which is above the tolerable level. However, it gets reduced when subjected to thermal and mechanical processes. Same study reported that cyanogenic glycoside measured as HCN/100 g flaxseeds, decreased from 20.8 to 1.0 mg/100 g after roasting (Hiremath, 2013).

Flaxseed contains cyanogenic glycosides and linamarin (acetone– cyanohydrinbeta–glucoside $C_{10}H_{17}O_6N$) in small amounts (Hall III *et al.*, 2005). Whole flaxseed contains (250–550) mg/100 g cyanogenic glycosides (Mazza, 2008), of which linustatin and neolinustatin are the major components. (Park *et al.*, 2005) reported 207 and 174 mg/100 g seed of linustatin and contribute to releasing the poisonous hydrogen cyanide (HCN). However, adequate processing of foodstuffs containing cyanogenic glycosides helps in reducing the potential risks associated with poisoning. Flaxseed meal also contains 10 mg/100 g Linatine (gammaglutamyl- 1- amino-D-proline) which induces vitamin B_6 deficiency (Mazza, 2008). The linatine (Vitamin B_6 antagonist) in flaxseed did not affect vitamin B_6 levels or metabolism in people fed up to 50 g of ground flaxseed per day. It has been reported that flaxseed depressed vitamin E levels in rats only when fed at very high levels (Ratnayake *et al.*, 1992). The cyanogenic glycosides in flaxseed raise thiocyanate levels in the blood very briefly, after which the levels drop, but even these levels are less than those of persons smoking tobacco (Kajla *et al.*, 2015).

2.9.6.4 Oxalic acid

The anti-nutritional factor that is of primary concern is oxalic acid. Oxalic acid (ethanedioic acid, $H_2C_2O_4$) is a strongly oxidized and corrosive compound with good chelating activity, synthesized by a broad range of animals, plants and microorganisms (Stewart *et al.*, 2004). Oxalic acid and its salts are extensively spread in numerous plant tissues as the end products of metabolism. Oxalic acid content in foodstuffs has long been a concern in human diets, due to the negative health effects connected to a high intake of oxalic acid. Incidences of kidney stones, hypocalcemia and hyposideremi (low plasma levels of calcium and iron) that correspond strongly with the intake of oxalic acid that perform as an absorption inhibitor are common (Palaniswamy *et al.*, 2002).

High oxalate content in urine and blood causes several diseases such as hyperoxaluria and vitamin deficiencies. Small dose of oxalate in the body may result in pain, headaches, and twitching in muscles and cramps. Larger doses can result in a drop in blood pressure, weak, irregular heartbeat and signs of heart failure. Large doses of oxalate may rapidly put a person in a shock-like state, causing convulsions (because of low plasma calcium), coma, and even death. The mean fatal dose for an adult is about (15-30) g, but the lowest reported fatal dose is merely 5 g (or about 70 mg/kg) (Tsai *et al.*, 2005). Consumption of foods high in oxalic acid in the long term can be troublesome. Healthy persons can securely consume such foods moderately, but those with gout, rheumatoid arthritis, kidney disorders, or certain forms of chronic vulvar pain (vulvodynia) are normally advised to stay away from foods high in oxalates or oxalic acid (Shimi and Haron, 2014). On flaxseed powder the oxalate content was found to be (2-10) mg per serving (oxalate content) while in roasted flaxseed it was found to be less than 2 mg per serving (oxalate content). The oxalate concentration was in the range of (6.43-19.40) mg/100 g for whole cooked samples, (9.03-11) mg/100 g for raw soy products, and (4.36-7.99) mg/100 g for cooked ones (Shimi and Haron, 2014).

In a study, it was found that oxalate content in raw flaxseed range from (2-10) mg/kg (Hiremath, 2013). (Hui, 1992) stated that intake of 5g or more oxalic acid would be fatal to humans but the negative effect can be seen at even low values. In a study, the roasted pistachio and chestnuts contained very low level than that of raw samples (Ritter and Savage, 2007).

2.9.7 Processing methods to reduce Anti-nutritional factors (ANFs)

The abundance of anti-nutritional factors and toxic influences in plants used as human foods certainly calls for concern. Therefore, ways and means of eliminating or reducing their levels to the barest minimum should be discovered (Soetan and Oyewole, 2009).

2.9.7.1 Heat treatment

Heat treatment is a usual process in food processing. It is an effective means of inactivating the thermo-labile ANF. This improves protein quality by inactivating anti-physiological factors, particularly trypsin inhibitor and hemagglutinins and by unfolding the protein structure, thus making them more susceptible to attack by digestive enzymes (Alegbejo, 2013). Also, dry heat was less effective than cooking (moist heat) for the improvement of growth promoting action in soybeans but the degree of inactivation is governed by temperature, duration of heating and particle size.

Popping

Popping involves heating of the grain in a hot pot. Popping is achieved by rapid, intense heating of grain; it makes water expand all at once; thereby expanding the grain. As expansion takes place, some of the granules are gelatinized resulting in the grain being much more available to digestive enzymes (Njoki *et al.*, 2014).

Blanching

(Noonan and Savage, 1999) suggested mild boiling (75–95)°C is sufficient to inactivate endogenous enzymes and avoid cooking; however, the heat is minimal to eliminate oxalic acid. Typically blanching is carried out by treating the vegetables and seeds with steam or hot water for (1-10) min at (75-95)°C. The time/ temperature combination depend upon the types of seeds and vegetables (Cano, 1996).

Extrusion

It is a form of high temperature short time (HTST) processing involving a combination of high temperature, pressure, and shear processing and is responsible for reducing the ANF content in food. Extruded amaranth grain exhibited better nutritional value than raw amaranth and the product required no additional cooking prior to consumption (Mendoza and Bressani, 1987).

2.9.7.2 Soaking

This process implies exposure to water and salt solutions with or without additive to encourage ANF loss. Oxalates and tannins may be removed from food by cooking in water, although this is not the most effective method. Soaking followed by wet cooking may reduce oxalates more rapidly when compared with just wet cooking (Hotz and Gibson, 2001).

2.9.7.3 Germination

Germination is also considered as a highly suitable method for reducing the anti-nutrient components of plant-based foods (Nkhata *et al.*, 2018). Germination of seeds generally activates the enzyme phytase, which degrades phytate and leads to decreased phytic acid concentration in the samples. Germination commonly changes the nutritional level, biochemical property and physical features of the foods. Germinated cereals showed enhanced activity of phytase-degrading enzyme while in non-germinated cereals the endogenous activity of phytase enzyme was observed in diminished amounts (Vashishth *et al.*, 2017). Reduction of anti-nutrients like tannin and phytic acid in germinated cereals increase the bioavailability of several minerals, which led to increased nutritional value of the food products (Ogbonna *et al.*, 2012; Oghbaei and Prakash, 2016).

2.9.8 Effect of different method of processing

2.9.8.1 Germination

The cyanogenic content varied from (421.20-559.15) mg/kg in raw flaxseed varieties. (Mandokhot and Singh, 1983) also reported similar values for cyanogenic glycosides in flaxseeds. A recorded reduction was observed in cyanogenic glycosides content in germinated flaxseed varieties. Similar results were also quoted by (Wanasundara *et al.*, 1999) and reported about 70% reduction in cyanogenic glycosides in germinated flaxseeds.

According to (Selmar *et al.*, 1988), linamarin is first converted to the diglycoside linustatin which is the translocatory form of cyanogenic glycosides that may not be broken down by linamarase during transportation. During the germination phase of leaf expansion, linustatin is transported out to the growing parts of seedlings, where the enzyme diglucosidase splits gentiobiose. The HCN produced by dissociation of the resulting acetone cyanohydrins is immediately fixed by β- cyanoalanine synthase to â-cyanoalanine. The β-cyanoalanine, so produced, may be hydrolysed to afford asparagine. This pathway of degradation of linustatin suggests that cyanogenic glycosides are not solely stored as secondary metabolites and may function as a source of nitrogen when required, such as in certain developmental stages. From a nutritional point of view, disappearance of cyanogenic glycosides during germination reduces the risk of HCN production, thus improving the nutritional quality of sprouts.

Germination is mainly a catabolic process that supplies important nutrients to the growing plant through hydrolysis of reserve nutrients. Phytic acid content of flaxseed varieties reported by (Kajla *et al.*, 2017) showed significant variations from 21.5 g/kg (JL-27) to 25.8 g/kg (JLS-6). Phytic acid decreased significantly during germination. Germinated flaxseed varieties JL-27 (10.6 g/kg) and JL-23 (10.5 g/kg) showed an appreciable decrease in phytic acid.

Reduction in phytic acid in germinated flaxseed varieties might be attributed to increase in phytase activity. Similar results were reported by different workers (S. Arora and Modgil, 2006; Hooda and Jood, 2003). However, phytic acid may be one of the factors responsible for reducing mineral availability and its loss during germination may enhance nutritional bioavailability of flaxseeds.

2.9.8.2 Soaking

This process implies exposure to water and salt solutions with or without additive to encourage ANF loss. Generally, cereals and legumes are soaked in water overnight; phytic acid is water-soluble, so a considerable amount of phytic acid is removed into water. In addition, this process also enhances the action of naturally occurring phytic acid in cereals and legumes (Greiner and Konietzny, 1999).

(Noonan and Savage, 1999) suggested that oxalate also can be reduced by leaching in soaking solutions. Oxalates and tannins may be removed from food by cooking in water, although this is not the most effective method. Soaking followed by wet cooking may reduce oxalates more rapidly when compared with just wet cooking. As HCN and cyanogenic glycosidase was found significantly lower because of its extreme solubility in water (FAO/WHO, 1965). In a study of chickpea, soaking the seeds at room temperature (for 22 hr) resulted in a smaller decrease in polyphenols content, total flavonoids contents than soaking at 60˚C (for 2 hr) (Aharon *et al.*, 2011).

2.10 Shelf life of biscuits

The shelf-life of the product in the package under specified conditions can be confirmed by several methods, viz., (1) weight gain or loss method, (2) method based on testing the performance of the product, (3) chemical (acid value, peroxide value) changes during storage. All these tests are related to water vapor and oxygen permeability of the packaging material, which in turn indicates the increase of acid value and peroxide value of the product overtime. All shelf-life assessment methods use accelerated and controlled conditions so that an accurate prediction of shelf-life can be possible within a short time (Kumar, 2001).

Peroxide value and free fatty acid tests are analytical methods typically used for fats and oils. If the fat must first be extracted from food products in order to run these tests, the following statements must be true to ensure valid results (Sewald. and DeVries, 2000).

a. The fat extracted must be representative of the fat in the food.
b. No non-fat compounds, which would interfere with the test, should be extracted with the fat.
c. No active fat compounds can be either produced or destroyed during the extraction process.
d. The solvents used must be free of any active substances.

Storage temperature conditions should then be chosen which fit the product and give reliable results in a reasonable amount of time. Common temperatures used would be 20, 30, 40, and 55°C (68, 86, 104, and 131°F). A control, stored at 0°F, can also be used. The frequency of the analytical testing is the next important decision. The higher the storage temperature, the more frequent should be the testing. Weekly tests are common for most products (Sewald. and DeVries, 2000).

Part III

Materials and methods

3.1 Raw materials

Refined wheat flour used for biscuit making was obtained from local market of Dharan. Flaxseed was collected from Birtamode, Jhapa. The other raw materials such as sugar, salt, fat in the form of butter (Amul), skimmed milk powder, baking powder were collected from local market of Dharan. Lecithin was collected from Asian Thai Foods, Sonapur. Sodium bicarbonate, ammonium bicarbonate and flavor (vanilla) were obtained from the laboratory of Central Campus of Technology, Dharan.

3.2 Chemicals required

The required chemicals obtained from the laboratory of Central Campus of Technology is mentioned in appendix B.

3.3 Apparatus required

The required apparatus obtained from the laboratory of Central Campus of Technology is mentioned in appendix B.

3.4 Preparation of flaxseed flour

3.4.1 Cleaning

Flaxseed grains were first winnowed with woven bamboo trays (nanglo). In this step; husk, immature grains and light particles were winnowed away and heavier particles such as specks and stones were separated by gravity during winnowing.

3.4.2 Steeping

Cleaned seeds were transferred to the stainless steel vessel and water was added 1.5 times that of flaxseed. Light materials present in the sample were skimmed off. Agitation was done to clean the seed. The grains were steeped for 24 h at room temperature 21°C and drained to remove the excess water.

3.4.3 Germination

The steeped grain was first collected in a muslin cloth and swirled in order to drain excess water. The grains were placed in humidity chamber and kept for germination in incubator at (23±3)°C and RH 90% for 7 days.

3.4.4 Grinding and packaging

All the samples were powdered in grinder and sieved and packed in high density polythene bags (52 µm) separately. Thus, seven different samples were prepared for analysis.

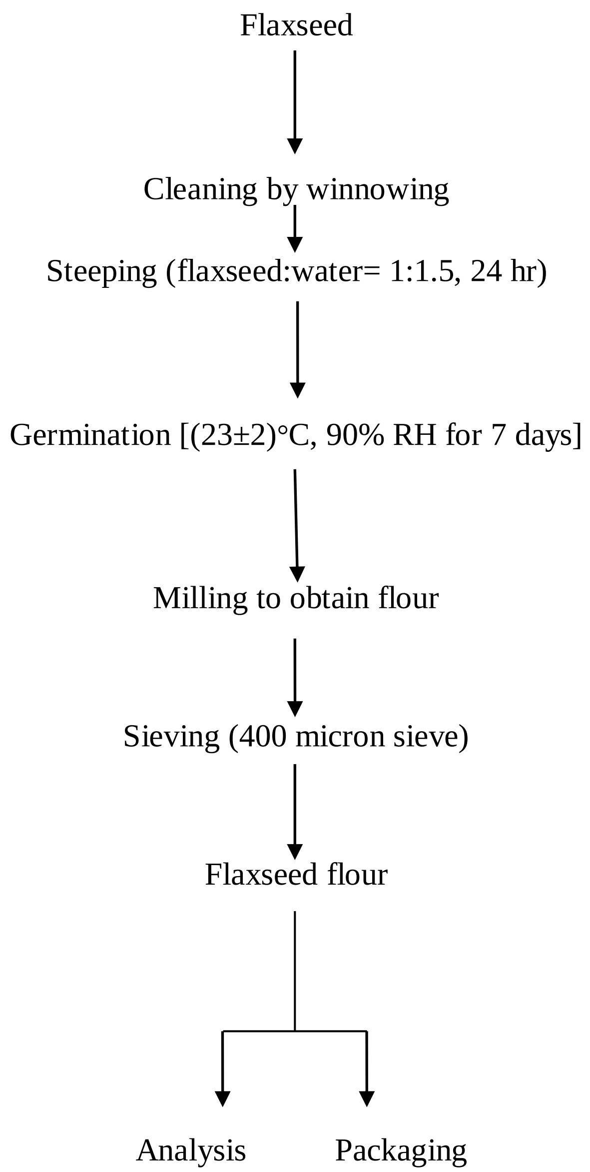

Fig 3.1 Preparation of flaxseed flour

Source: (Anonymous)

3.5 Determination of threshold of flaxseed flour

The independent variable of the experiment is flaxseed flour. The determination of threshold for flaxseed flour was carried out by the help of trial experiment. The result of the experiment concluded that above 50%, the biscuit was not acceptable. Therefore, the threshold for flaxseed flour is set between (0-50) %.

3.6 Methods

3.6.1 Experimental design

Design Expert v13 software was used to create the recipe. D- optimal design was used to formulate the recipe.

Run	Component 1 A: Flaxseed flour	Component 2 B: Wheat flour
1	50	50
2	0	100
3	25	25
4	37.5	62.5
5	12.5	87.5
6	50	50
7	0	100
8	25	75

Formulation of recipe

The recipe formulation for the flaxseed flour incorporated biscuit was carried out as given in Table 3.1.

Table 3.1 Recipe formulation of flaxseed flour incorporated biscuit

Ingredients	A	B	C	D	E
Wheat flour	100	87.5	75	62.5	50
Flaxseed flour	0	12.5	25	37.5	50
Fat	35	35	35	35	35
Pulverized sugar	35	35	35	35	35
Salt	0.3	0.3	0.3	0.3	0.3
Skimmed milk powder	6	6	6	6	6
Baking powder	1.5	1.5	1.5	1.5	1.5
Ammonium bicarbonate	1.5	1.5	1.5	1.5	1.5
Sodium bicarbonate	0.75	0.75	0.75	0.75	0.75
Soya lecithin	1.5	1.5	1.5	1.5	1.5
Flavor (Vanilla)	0.1	0.1	0.1	0.1	0.1
Water (ml)	10	10	10	10	10

3.7 Formulation of biscuit recipe with incorporation of malted flaxseed flour

Fat, sugar powder and soya lecithin were firstly creamed. Salt was dissolved in water and added to the prepared cream mixture. Other raw materials like skimmed milk powder and chemicals like ammonium bicarbonate were then added to the cream mixture for final creaming. As creaming process was continued, all flour and baking powder were added and stirred well together. The full prepared dough was sheeted (5-6) mm thick and was cut to form required circle shape. The formed biscuits were baked at 200°C for 15 min. After cooling to about 35°C, the biscuits were packed in HDPE packages (52 µm). The process for preparing flaxseed flour incorporated biscuit is shown in Fig. 3.2.

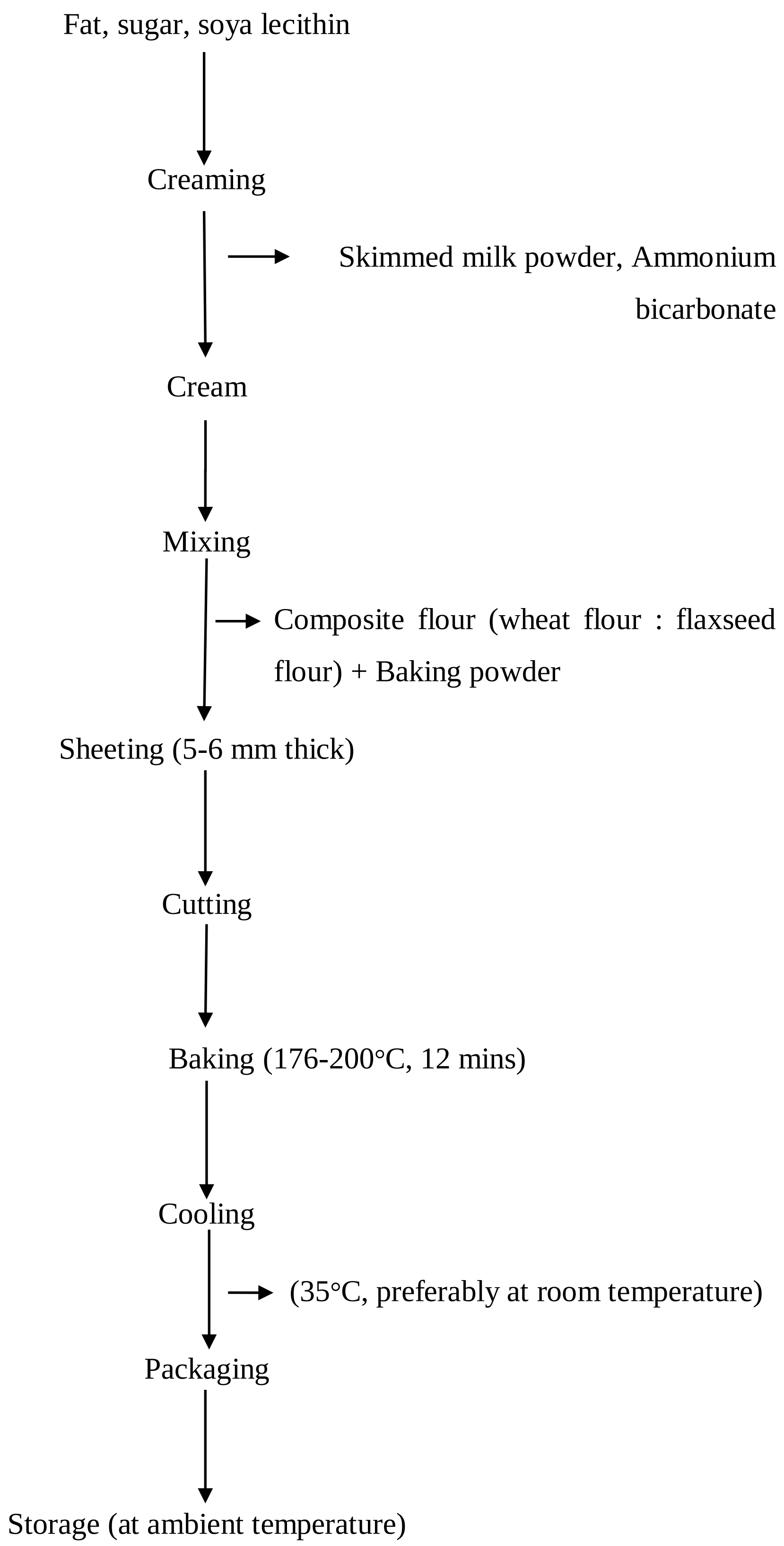

Fig 3.2 Flow chart for the preparation of flaxseed flour incorporated biscuit

Source: (Smith, 1972)

3.8 Analysis of the product

3.8.1 Physical parameter analysis

3.8.1.1 Spread ratio

The spread ratio of the biscuit was determined by using the formula as per (AOAC, 2005).

$$\text{Spread ratio}= \frac{\text{Diameter (mm)}}{\text{Thickness (mm)}}$$

Where, diameter was measured in millimeter (mm) by vernier calliper and thickness was measured in mm by screw gauge.

3.8.1.2 Volume

Volume of the biscuit was determined by the area of biscuit multiplied by thickness as per (AOAC, 2005).

$$\text{Volume (cm}^3\text{)} = \frac{\pi d^2 t}{4}$$

Where, t = Average thickness of biscuit (mm)

d = Diameter of biscuit (mm)

3.8.1.3 Density

Density of biscuit was obtained by the ratio of mass to the volume of the biscuit as per (AOAC, 2005)

$$\text{Density(g/cm}^3\text{)} = \frac{\text{Mass (g)}}{\text{Volume (cm}^3\text{)}}$$

3.8.2 Physicochemical analysis

3.8.2.1 Moisture content

Moisture content of the flour and biscuit samples were determined by weight loss during heating in a thermostatically controlled oven at 105°C by hot air oven method (KC and Rai, 2007).

3.8.2.2 Ash content

Flour and biscuit samples were ignited at (550-600) °C in a Muffle furnace until it resulted a white grey ash (KC and Rai, 2007).

3.8.2.3 Crude fat

Crude fat in flour and biscuit samples were determined by Soxhlet extraction method using soxhlet apparatus using petroleum ether. Fat was extracted by repeated syphoning (KC and Rai, 2007).

3.8.2.4 Crude fiber

The crude fiber content of flour and biscuit samples were determined by using Buchner's filtration assembly (KC and Rai, 2007).

3.8.2.5 Crude protein

Protein content of flour and biscuit samples were estimated by Kjeldhal method using automatic digestion and distillation set. The protein value was calculated from nitrogen content (N_2). The factor 6.25 was to used for flour and biscuit samples (KC and Rai, 2007).

3.8.2.6 Carbohydrate

Percentage of carbohydrate was determined by difference between 100 and the sum of moisture, protein, crude fat, fiber and total ash content.

3.8.2.7 Iron content

Flour and biscuit samples were first digested with acid and aliquots were used for determination of iron by spectrophotometer in 480nm wavelength (KC and Rai, 2007).

3.8.2.8 Calcium content

Flour and biscuit samples were first digested with acid and aliquots were used for determination of calcium by titration (KC and Rai, 2007).

3.8.2.9 Energy value

The calorific value of the control sample and the biscuit sample was determined as 4 × protein + 4 × CHO +9 × fat = energy (kcal).

3.8.2.10 Determination of phytic acid

The phytic acid was extracted with trichloroacetic acid and was precipitated as ferric salt. The iron content of the precipitate was determined colorimetrically and phytate phosphorus content was calculated from that value assuming a constant 4Fe:6P molecular ratio in the precipitate (Sadasivam and Manickam, 2016). Result was presented as phytic acid mg per 100 g sample.

$$\text{Phytic acid (mg/100 g) sample} = \frac{\mu\text{gFe} \times 15}{\text{wt of sample (g)}}$$

3.8.2.11 Determination of tannin

The tannins were determined by Folin-Dennis method. Tannin like compounds reduce phosphotungstomolybdic acid in alkaline solution to produce a highly coloured blue solution, the intensity of that was proportional to the amount of tannins. The intensity was measured in a spectrophotometer at 760nm (Sadasivam and Manickam, 2016).

3.9 Sensory analysis

The sensory evaluation for overall quality was carried out with 12 semi-trained panelists (teachers and student of Central Campus of Technology). The parameters for sensory evaluation are color, flavor, crispiness, texture and overall acceptability. Sensory evaluation was performed by hedonic rating test (Peryam and Pilgrim, 1957).

3.10 Statistical analysis

Data were statistically analyzed by statistical software Gene stat version 12.1.0.3338. Means of the data were compared by using Fisher's Protected LSD method at 5% level of significance.

3.11 Packaging and storage of the biscuit

High density polyethylene bag (52 μm) was used for the packaging of the biscuits and was stored at ambient temperature.

3.12 Determination of shelf life

Acid value, peroxide value and moisture content of the biscuit were evaluated for determination of shelf- life of biscuits. The analysis was carried out on a monthly basis up to two months.

PART IV

Results and discussion

The wheat flour and the flaxseed flour were collected to formulate flaxseed flour incorporated biscuit of 0 part, 12.5 parts, 25 parts, 37.5 parts and 50 parts of flaxseed flour incorporation. Proximate composition of the flour as well as biscuit was carried out. The best product among the five variations was determined by carrying out sensory evaluation and the detailed nutritional value of the best product was analyzed.

4.1 Proximate composition

Wheat flour and flaxseed flour were analysed for proximate components. The results of analysis of wheat flour and flaxseed flour in dry basis are tabulated in Table 4.1.

Table 4.1 Proximate composition of wheat flour and flaxseed flour

Parameters	Wheat flour	Flaxseed flour
Moisture (% wb)	11.67±0.01	4.8±0.1
Ash (% db)	0.33±0.01	3.99±0.02
Fat (% db)	1.58±0.03	27.52±0.02
Fiber (% db)	0.75±0.01	4.9±0.1
Protein (% db)	10.32±0.02	13.28±0.01
Carbohydrates (% db)	87.02±0.02	50.31±0.01

Values presented are the average of triplicates determination ± standard deviation.

The p- values for the proximate comonents for both wheat flour and flaxseed flour were found to be less than the signicance level of 0.05. It means that the null hypothesis is rejected which further means that all the parameters for both flours are significantly different at 5% level of significance.

The moisture content, ash, fat, fiber, protein and carbohydrate of wheat flour was found to be 11.67%, 0.33%, 1.58%, 0.75%, 10.32% and 87.02% respectively. (Sarwar, 2010) reported proximate values (db) as 13%, 0.60%, 0.90%, 0.30%, 11.3%, and 87% respectively and (Poudel, 2021) reported proximate values (db) as 12.35%, 0.57%, 1.55%, 0.61%, 10.47% and 86.8% respectively. The moisture content in wheat flour 11.67% was lower than that obtained by (Sarwar, 2010) and (Poudel, 2021). The ash and protein content was found to be lower whereas the fat, carbohydrate and crude fiber content was found to be higher than the value obtained by (Sarwar, 2010) and (Poudel, 2021). The reason for this difference could be attributed to species and drying conditions (temperature, relative humidity and time).

The moisture content, ash, fat, crude fiber, protein and carbohydrate of flaxseed flour was found to be 4.8%, 3.99%, 27.52%, 4.9%, 13.28% and 50.31% respectively. (Masoodi and Bashir, 2012) reported proximate values as 8.66%, 3.59%, 41.63%, 9.97%, 16.07% and 30.13% respectively and (Maghaydah *et al.*, 2022) reported proximate values as 6.4%, 3.86%, 34.60%, 17.91%, 16.53% and 20.70% respectively.

The moisture content, protein and fat was found to be lower whereas the ash and carbohydrate content was found to be higher than the values reported by (Masoodi and Bashir, 2012) and (Maghaydah *et al.*, 2022).

The crude fiber of the flaxseed flour was found to be 4.9% which is comparatively greater than that of wheat flour 0.67%. A half ounce of dry whole flax seed provides between (20-25) % of our daily fiber needs. Flax contains polysaccharides (other than starch) which, due to their anti-hypercholesterolemic, anti-carcinogenic and glucose metabolism controlling effects, may prevent or reduce the risk of various important diseases, such as diabetes, lupus nephritis, arteriosclerosis and hormone-dependent types of cancer (Bilek and Turhan, 2009; Williams *et al.*, 2007).

4.2 Anti-nutrients in flaxseed flour

The mean values of different anti- nutrients determined are presented in the Figure 4.1.

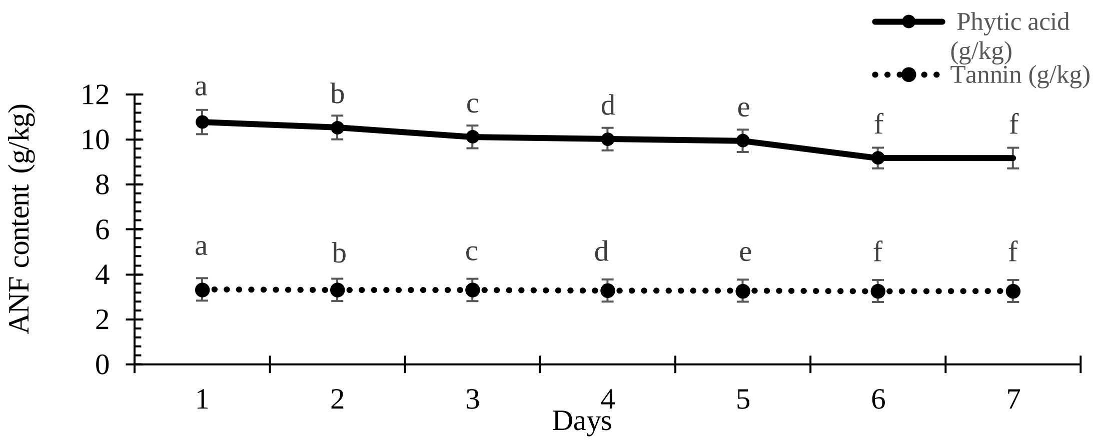

Fig 4.1 Line diagram showing the content of ANF at different germinated time

Values presented are the average of triplicates determination ± standard deviation. All values are expressed on dry basis.

The flaxseed was soaked and germinated at a temperature of (23±3)°C and 90% RH for 7 days since there was no significant difference in phytic acid and tannin content on the 6^{th} and 7^{th} day. 500 gm of flaxseed was taken out each day, dried in cabinet drier at (50±3)°C for 24 hours and grinded into flour and seven different samples were prepared accordingly to analyze the anti-nutritional factors. The anti-nutritional factors of seven different flaxseed flour samples were analyzed.

4.2.1 Phytic acid

Phytic acid is an anti-nutritional factor present in legumes which reduces the bioavailability of nutrients from them. The flaxseed was germinated for seven days. The change in phytic acid content was analyzed in each day of germinated sample. Phytic acid content reduced on the progressive days of germination. The phytic acid content (g/kg) was found to be 10.78±0.03, 10.54±0.05, 10.12±0.07, 10.02±0.04, 9.94±0.01, 9.18±0.06 and 9.18±0.08 respectively. This study shows a decreasing trend in the content of phytic acid as days of germination progressed which is similar to the finding reported by (Kajla *et al.*, 2017) who reported a decrease in the content of phytic acid of flaxseed from 25.8 g/kg to 21.5 g/kg during germination.

The analysis of variance (Appendix C) showed that there was significant difference in phytic acid content in the different days of germinated samples ($p < 0.05$) except the sixth and seventh day of germination.

Reduction in phytic acid in germinated flaxseed varieties might be attributed to increase in phytase activity (S. Arora and Modgil, 2006; Hooda and Jood, 2003).

4.2.2 Tannin

Tannin is a potential antinutrient present in legumes. The flaxseed was germinated for seven days. The change in tannin content was analyzed in each day of germinated sample. Tannin content reduced on the progressive days of germination. The tannin content (g/kg) was found to be 3.34±0.09, 3.32±0.07, 3.31±0.14, 3.29±0.01, 3.28±0.08, 3.27±0.11 and 3.27±0.23 respectively. This study shows a decreasing trend in the content of tannin as days of germination progressed which is in accordance with the results obtained by (Khandelwal *et al.*, 2010) who in their study, mentioned that it is expected that total phenolics and tannin content changed differently in different legumes during germination. (Khandelwal *et al.*, 2010) also reported that total phenolics and tannin content was reduced significantly in germinated green gram compared to bengal gram, red gram and lentil.

The analysis of variance (Appendix C) showed that there was significant difference in tannin content in the different days of germinated samples ($p < 0.05$) except the sixth and seventh day of germination.

Reduction in tannin content after germination may be attributed to the leaching out effect during hydration which was reported by (Kataria *et al.*, 1989).

Since phytic acid and tannin content values were recorded least on Day 7, sample of day 7 was used to prepare the final product.

4.3 Influence of flaxseed flour on physical parameters of biscuits

Physical characteristics of biscuits such as thickness, diameter, spread ratio, weight, volume and density were affected by the substitution increment of the level of flaxseed flour which is presented in Table 4.2. The results indicated that diameter and thickness of biscuit were slightly increased with increasing substitution percentage of flaxseed flour. 50 parts flaxseed flour incorporated biscuit revealed the maximum diameter and thickness (65.09 and 7.485 mm). The results agree with work done by (Hussain *et al.*, 2006) who found that diameter and thickness of cookies showed gradual increment as the level of flour substitution increased.

Moreover, the results of spread ratio of biscuit revealed a reduction in spread ratio from 14.27 to 8.947. It is clear that, as the flaxseed flour level increased, spread ratio for different treated biscuits gradually decreased. These results are on the line with the findings of (Ganorkar and Jain, 2014) who reported that the reduction in spread ratio might be due to increase in dietary fibre and protein percentage with increasing level of flaxseed flour because dietary fibre and protein has more water binding power. Also, when more water is present, more sugar is dissolved during dough mixing (Ganorkar and Jain, 2014).

One way ANOVA at 5% level of significance shows that the thickness of samples C, D and E are significantly similar to each other but significantly different than samples A and B. Diameter of samples D and E are significantly similar to each other and almost significantly similar to sample C and significantly different than samples A and B. Weight, spread ratio and volume of all samples are significantly different than each other. Density of samples B, C D and E are significantly similar to each other but significantly different than sample A.

Table 4.2 Physical parameters of flaxseed flour incorporated biscuits

Samples	Thickness (mm)	Diameter (mm)	Weight (gm)	Spread ratio	Volume (cm^3)	Density (g/cm^3)
A	4.4±0.16[a]	63.01±0.004[a]	7.983±0.004[a]	14.27±0.29[a]	13.76±0.15[a]	0.58±0.016[a]
B	5.4±0.24[b]	63.32±0.02[ab]	8.153±0.004[b]	14.11±0.62[b]	14.123±0.18[b]	0.57±0.048[b]
C	7.275±0.05[c]	64.34±0.82[bc]	10.003±0.009[c]	11.914±0.16[c]	17.55±0.23[c]	0.56±0.040[b]
D	7.415±0.08[c]	64.4±0.47[c]	11.583±0.004[d]	10.0625±0.05[d]	20.84±0.15[d]	0.55±0.065[b]
E	7.485±0.09[c]	65.09±0.43[c]	12.043±0.004[e]	8.947±0.02[e]	24.207±0.61[e]	0.49±0.057[b]

Values are the means of three determinations ± standard deviations.

4.4 Sensory analysis of flaxseed flour incorporated biscuit

Statistical analysis of the sensory scores were obtained from 12 semi-trained panelists using 9- point hedonic rating scale (9=like extremely, 1= dislike extremely) for composite biscuit formulations. Sensory analysis was performed with the aid of different panelists evaluating texture, crispiness, color, flavor and overall acceptability of flaxseed flour incorporated biscuit.

4.4.1 Color

The mean sensory score for color of sample C was found to be 6.83 and was highest of all other biscuit formulations. The lowest score was 5.25 for sample E. Statistical analysis showed that the partial substitution of wheat flour with flaxseed flour had significant effect ($p < 0.05$) on the color. One way ANOVA at 5% level of significance shows that samples A & C; B & C were almost significantly similar to each other. Whereas, samples D & E were significantly similar to each other but significantly different than sample C. Fig. 4.2 represents the mean sensory scores for color of flaxseed flour incorporated biscuit.

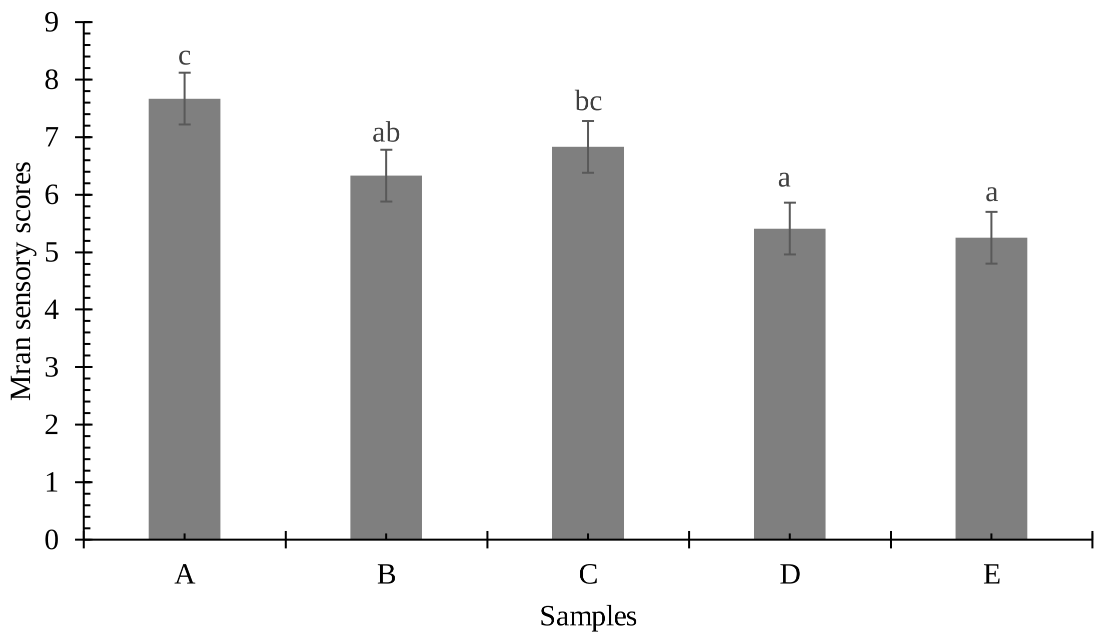

Fig 4.2 Mean sensory scores for colour of flaxseed flour biscuit

[A, B, C, D, E denotes 0 part, 12.5 parts, 25 parts, 37.5 parts, 50 parts. Vertical errors bar represent ± standard deviation of scores given by 12 panelists.]

The sample C got the highest score which may be due to the appropriate amount of flaxseed flour incorporation (25 parts) and brown colour of the product which might have been attractive to panelists. The effect of higher amount of incorporation of the flaxseed flour may be the cause of dark brown color that is observed in the case of sample E which could be the cause of lower acceptance of the color. The result is in accordance with (Masoodi and Bashir, 2012) who found that the color of the fortified biscuits attained more dark color as the supplementation was increased.

4.4.2 Crispiness

Statistical analysis showed that partial substitution of wheat flour with flaxseed flour had significant effect ($p < 0.05$) on the crispiness. The sample C got highest score of 7.58 while sample E got the lowest score of 6.16 because incorporation of high level of flaxseed flour depresses the water holding capacity. Samples B & D were significantly similar to each other, almost significantly similar to samples C & E significantly different than sample A. Fig. 4.3 represents the mean sensory scores for crispiness of flaxseed flour incorporated biscuit.

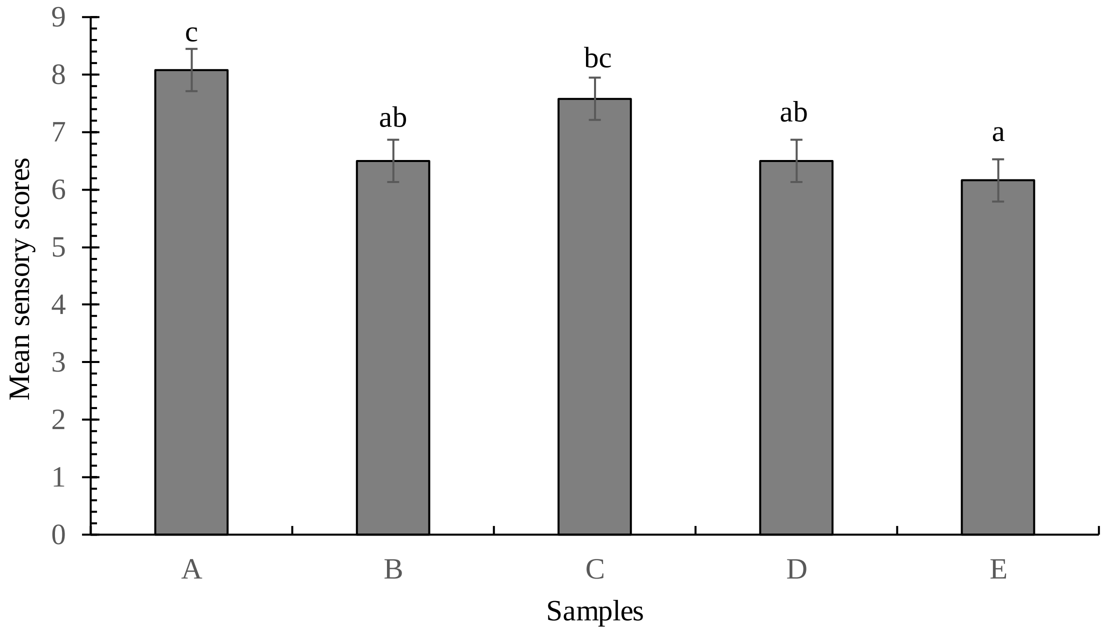

Fig. 4.3 Mean sensory scores for crispiness of flaxseed flour biscuit

[A, B, C, D, E denotes 0 part, 12.5 parts, 25 parts, 37.5 parts, 50 parts. Vertical errors bar represent ± standard deviation of scores given by 12 panelists.]

The reduced water content increases the glass transition temperature of the crust and contributes to the development of the crust crispiness. A glassy state of the solids would provide brittleness to the product, but the porous structure of the product and the solid, thin pore membranes may significantly contribute to the sensory properties of the product as stated by (Roos, 1995).

4.4.3 Flavor

The mean sensory score for flavor of sample C was 7.67 and was the highest score scored among the different formulations. The lowest mean sensory score was of sample E of 5. Statistical analysis showed that the partial substitution of wheat flour with flaxseed flour had significant effect ($p < 0.05$) on the flavor. Samples A & C were significantly similar to each other, almost significantly similar to sample D and significantly different than sample B & E. Fig. 4.4 represents the mean sensory scores for flavor of flaxseed flour incorporated biscuit.

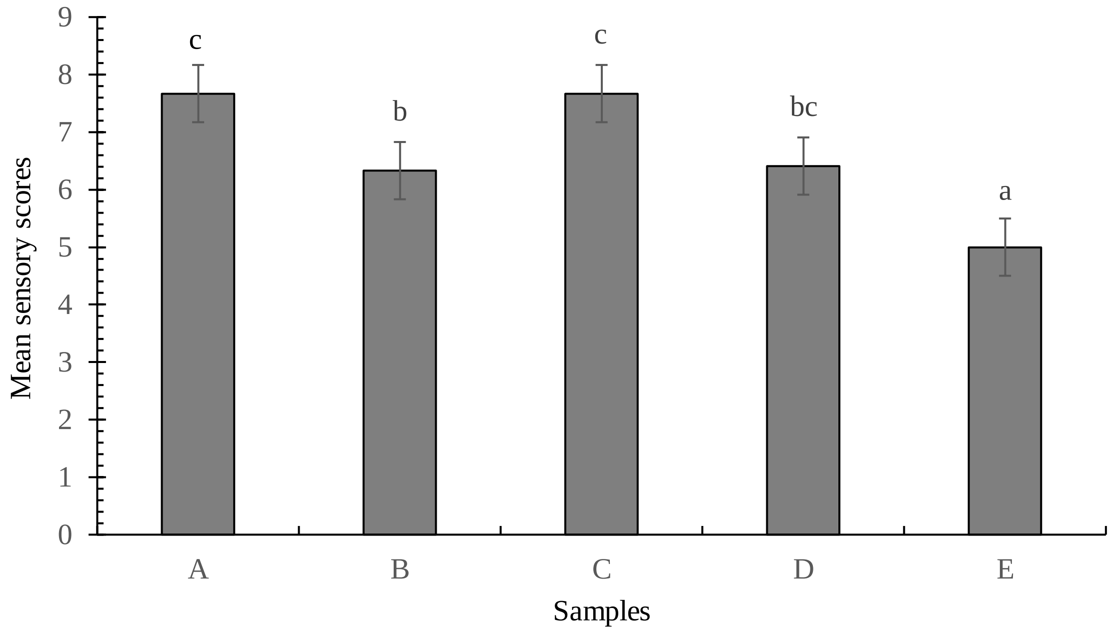

Fig. 4.4 Mean sensory scores for flavour of flaxseed flour biscuit

[A, B, C, D, E denotes 0 part, 12.5 parts, 25 parts, 37.5 parts, 50 parts. Vertical errors bar represent ± standard deviation of scores given by 12 panelists.]

The biscuit with the highest amount of flaxseed flour incorporation such as sample E had the lowest score of 5 and the reason behind this could be attributed to intensified flaxseed nutty flavor, un-pleasure after taste, dark brownish color, rough surface, less crisp and gritty mouth feel, making them to score low in sensory evaluation (Eisa, 2006). The flavor of sample C was found to be of balanced flavor which was preferable to other product formulations and got the highest score of 7.67.

4.4.4 Texture

The mean sensory score for texture of sample C was found to be 7.16 which was the highest score of all the biscuit formulations The lowest mean sensory score was of sample E of 6.33. Statistical analysis showed that the partial substitution of wheat flour with flaxseed flour had no significant effect ($p > 0.05$) on the texture. Samples B, D & E were significantly similar to each other, almost significantly similar to sample C and significantly different than sample A. Fig. 4.5 represents the mean sensory scores for texture of flaxseed flour incorporated biscuit.

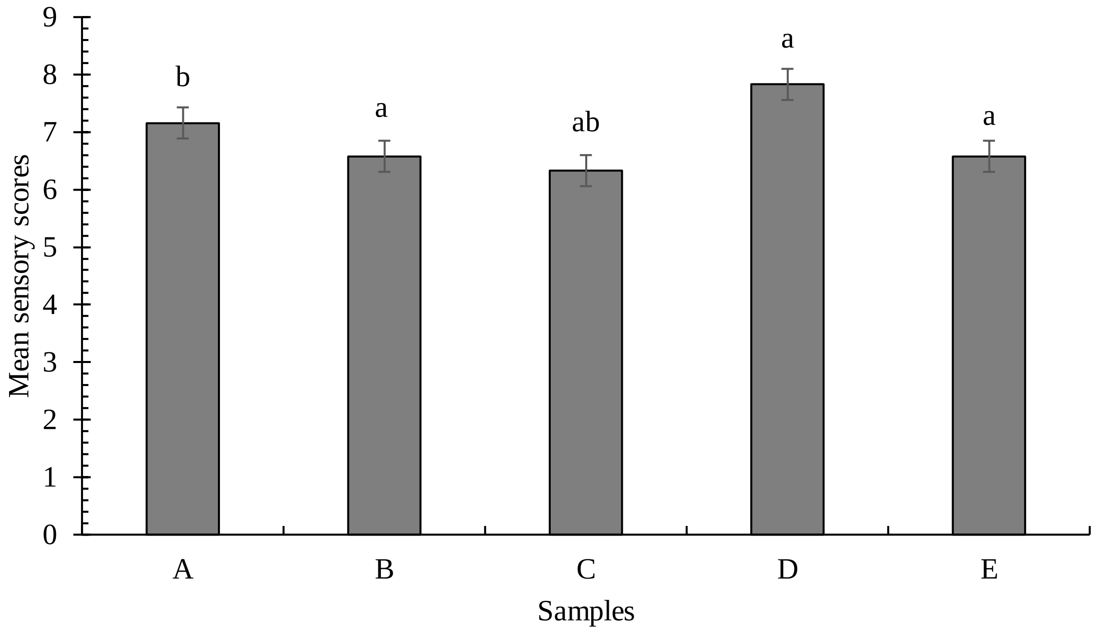

Fig. 4.5 Mean sensory scores for texture of flaxseed flour biscuit

[A, B, C, D, E denotes 0 part, 12.5 parts, 25 parts, 37.5 parts, 50 parts. Vertical errors bar represent ± standard deviation of scores given by 12 panelists.]

The probable cause for higher score for control than flaxseed flour incorporated biscuit may be due to the least gluten development as higher amount of flaxseed flour is used which is gluten-free affecting the formation of gluten development in wheat flour resulting in the tougher texture and cracks on the crust. The result is in accordance with (Sudha *et al.*, 2007) who found similar result with the increase in the level of substituted flour in biscuit. Sample E was found to have the lowest mean score of 6.33 due to the least gluten development as higher amount of flaxseed flour is used which is gluten less affecting the formation of gluten development in wheat flour. As proportion of flaxseed flour increased, texture score decreased which may be due to tougher texture and cracks on the crust. Sample A showed firm texture and no cracks, which might be due to adequate amount of gluten development. Texture is an important factor of comparing the biscuit as it greatly affects consumer acceptance of the product (Eisa, 2006).

4.4.5 Overall acceptability

Sample C scored highest in overall acceptability of the sensory conducted among the panelists with score of 7.58 and sample E was scored the lowest with a score of 5.5. Statistical analysis from the experimental data showed that the partial substitution of flaxseed flour in samples showed significant difference ($p < 0.05$) in overall acceptability of samples. Samples A & C were significantly similar to each other, almost significantly similar to sample B and significantly different than samples D & E. Fig. 4.6 represents the mean sensory scores for overall acceptability of flaxseed flour incorporated biscuit.

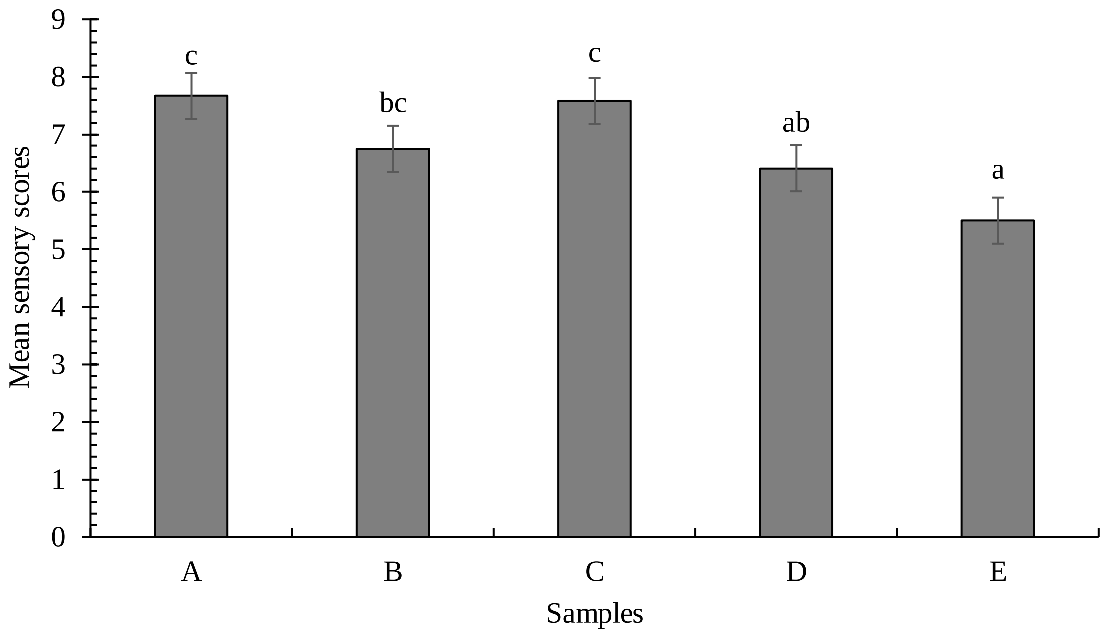

Fig. 4.6 Mean sensory scores for overall acceptability of flaxseed flour biscuit

[A, B, C, D, E denotes 0 part, 12.5 parts, 25 parts, 37.5 parts, 50 parts. Vertical errors bar represent ± standard deviation of scores given by 12 ppanelists.]

Furthermore, biscuits containing 25% flaxseed flour showed maximum sensory scores compared to other samples and non-significant difference with control biscuit. The results were similar with the results obtained by (Moraes *et al.*, 2010) who found that acceptance of flaxseed, as a dietary ingredient of functional food in cakes, revealed consumer acceptance up to 30% supplementation level.

Since samples A (control) & c (25 parts flaxseed flour) are significantly similar to each other, both samples A & C were subjected to further analysis.

4.5 Proximate and ultimate composition of the control sample and optimized sample

The composition of the optimized product and the control sample (wheat biscuit) from chemical analysis was carried out. The result of the analysis is given in the Table 4.3.

Table 4.3 Proximate and ultimate composition of control sample and optimized sample

Parameters	Wheat biscuit	Flaxseed biscuit
Moisture (% wb)	2.6±0.39	3.14±0.02
Ash (% db)	1.84±0.02	2.47±0.04
Fat (% db)	16.33±0.04	18.27±0.40
Fiber (% db)	0.68±0.08	3.78±0.13
Protein (% db)	7.84±0.14	13.12±0.05
Carbohydrates (% db)	73.31±0.02	62.36±0.01
Iron (db)	1.94±0.02	4.25±0.07
Calcium (db)	20.59±0.03	168.83±0.08
Energy value (kcal/ 100 gm)	471.57	466.35

Values are the means of three determinations ± standard deviations.

The p- values for the moisture content for both wheat biscuit and flaxseed biscuit were found to be more than the signicance level of 0.05. It means that the null hypothesis is accepted which further means that the moisture content for both the products are significantly similar at 5% level of significance. Whereas, the p- values for ash, fat, fiber, protein, carbohydrates, iron, calcium content and energy value were found to be less than the standard significance level of 0.05. It means that the null hypothesis is rejected which further means that the ash, fat, fiber, protein, carbohydrates, iron, calcium content and energy value for both the products are significantly different at 5% level of significance.

The composition of flaxseed flour incorporated biscuit is shown in Table 4.3. The ash content of biscuits increased in flaxseed flour incorporated biscuit. The increase in ash content may be due to the high mineral content in the flaxseed flour incorporated biscuit i.e. calcium and iron. The high ash content in the flaxseed flour supplemented food would be of nutritional importance in most developing countries like Nepal.

The moisture content ranged from 2.6% in wheat biscuit to 3.14% in flaxseed flour incorporated biscuit. This might be due to the presence of high fibre (gum mucilage) in flaxseeds which retain maximum moisture content (Cui and Mazza, 1996). The fiber content ranged from 0.68% in wheat biscuit to 3.67% in flaxseed flour incorporated biscuit. Flaxseed contains (35–45) % of fibre and two-third is insoluble and one third is soluble fiber. Insoluble fiber consists of cellulose, hemicellulose and lignin (Morris, 2007; Oomah and Mazza, 1993). Therefore, flaxseed flour incorporated biscuit showed an increase in the fiber content.

The fat and protein content ranged from 16.33% and 7.84% respectively in wheat biscuits to 18.27% and 13.12% in flaxseed flour incorporated biscuits whereas the carbohydrate content ranged from 73.31% in wheat biscuits to 62.36% in flaxseed flour incorporated biscuits. The results agree with work done by (El-Demery *et al.*, 2015; Khorshid *et al.*, 2011) who reported that the addition of flaxseed to cookies significantly increased all-chemical composition except the total carbohydrates.

There is increase in iron content from 1.94 mg/100 gm in control to 4.25 mg/100 gm in optimized product. Similarly, there is vast increase in calcium content which ranges from 20.59 mg/100gm in control to 168.83 mg/100 gm in optimized product. The iron content in the optimized product was lower than that reported by (Gupta *et al.*, 2017) as 10.01 mg/100 gm whereas the calcium content in optimized product was lower than that reported by (Verma *et al.*, 2017) as 170 mg/100gm and higher than the value reported by (Mekebo and Chandravanshi, 2014) as (54-74) mg/100 gm.

This new formulated functional biscuit had lower calorie count (466.35 kcal/100g) than the control product (471.57 kcal/100g) and can be included in the diet of every age group which will increase the sensory characteristics and nutrient intake by maintaining the good health and promoting immunity against infections.

4.6 Shelf- life evaluation of the biscuit

The shelf- life of the biscuit was studied for 2 months with triplicate samples. The product was packed in high density polyethylene bags (52 µm) and stored in ambient temperature. The acid value, peroxide value of the extracted fat and the moisture content of the product was evaluated from the date of manufacture upto 2 months.

4.6.1 Change in acid value

In general, acid value is the indication of free fatty acid content in the product. The increment in the fatty acid of the product was found increased with storage time and also depends on packaging material.

The acid value (AV) of the product was observed to be 0.13 at initial which reached 0.21 within 60 days. The analysis was almost significantly similar between 0 days and 30 days; 30 days and 60 days while a significant difference in the acid value of the extracted fat was observed in case of 0 day and 60 days analysis. This study shows an increasing trend in the acid value content as days of storage progressed which is in accordance with the findings of (Singh *et al.*, 2000) who reported that free fatty acids of soy- fortified biscuits increased with increased storage period and (Rajiv *et al.*, 2012) who also reported an increase in acid value of flaxseed incorporated cookies from 1.24 to 3.43 mg KOH/ gm oil during (0-90) days of storage.

The data obtained in this study was below the unacceptability level of maximum 0.3 mg KOH/ gm oil as described by (Mukhopadhyay, 1990) till the last date of analysis. The rate of increase in AV shows a gradual increase which suggests that it will be self-preserved till 6 months as described by (Smith, 1972). The change in acid value of the product is shown in Fig. 4.7.

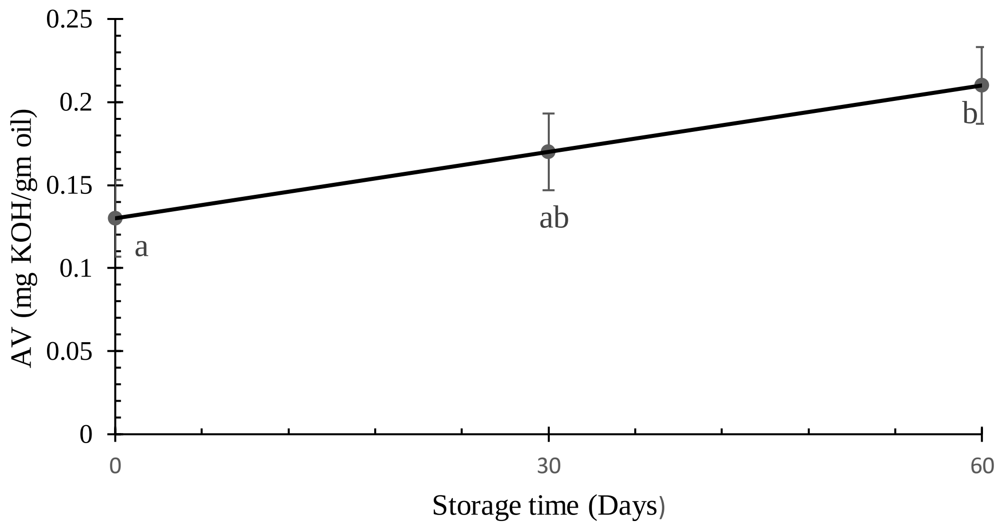

Fig 4.7 Change in acid value during storage

4.6.2 Change in peroxide value

The peroxide value (PV) of the product was observed to be 1.2 at initial which reached 1.8 within 60 days. Significant difference in the peroxide value of the extracted fat was observed in case of 0 day, 30 days and 60 days analysis. This study shows an increasing trend in the peroxide value content as days of storage progressed which is in accordance with the findings of (Smitha, 2011) who reported that peroxide value increased for biscuits prepared from blends of partially deoiled groundnut meal, cow milk coprecipitate and cereal products respectively during storage for 120 days at room temperature and (Rajiv *et al.*, 2012) who reported that peroxide value of flaxseed incorporated cookies increased significantly from 1.44 to 5.11 $MeqO_2$/kg fat, during (0–90) days of storage.

The values obtained in this study was far below the unacceptable level of maximum 3 meqv O_2/kg fat as described by (Mukhopadhyay, 1990). The peroxide value of the product is shown in Fig. 4.8.

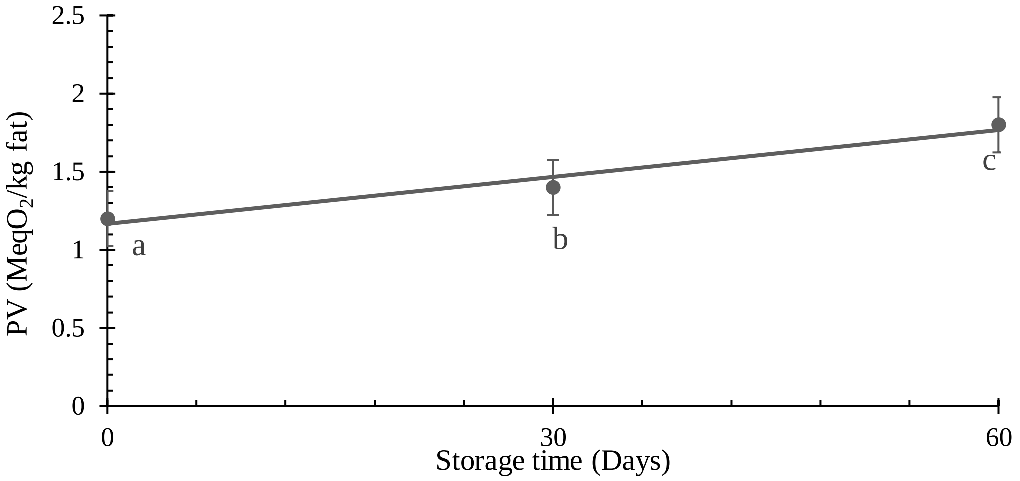

Fig 4.8 Change in peroxide value during storage

4.6.3 Change in moisture content

The moisture content of the product was observed to be 3.14 at initial stage which reached 4.88 within 60 days of analysis. Moisture content was significantly different within 0 day and 30 days; 0 day and 60 days while a significant similarity in the moisture content of the extracted fat was observed in case of 30 days and 60 days analysis. This study shows an increasing trend in the moisture content as days of storage progressed which is in accordance with the results reported by (Rajiv *et al.*, 2012) who also noted an increase in moisture content of flaxseed incorporated cookies during 90 days of storage.

The value was below the acceptable level of maximum 6% (Mukhopadhyay, 1990). Hence, the biscuit could be considered safe for consumption till date. The change in moisture content of the product is shown Fig. 4.9.

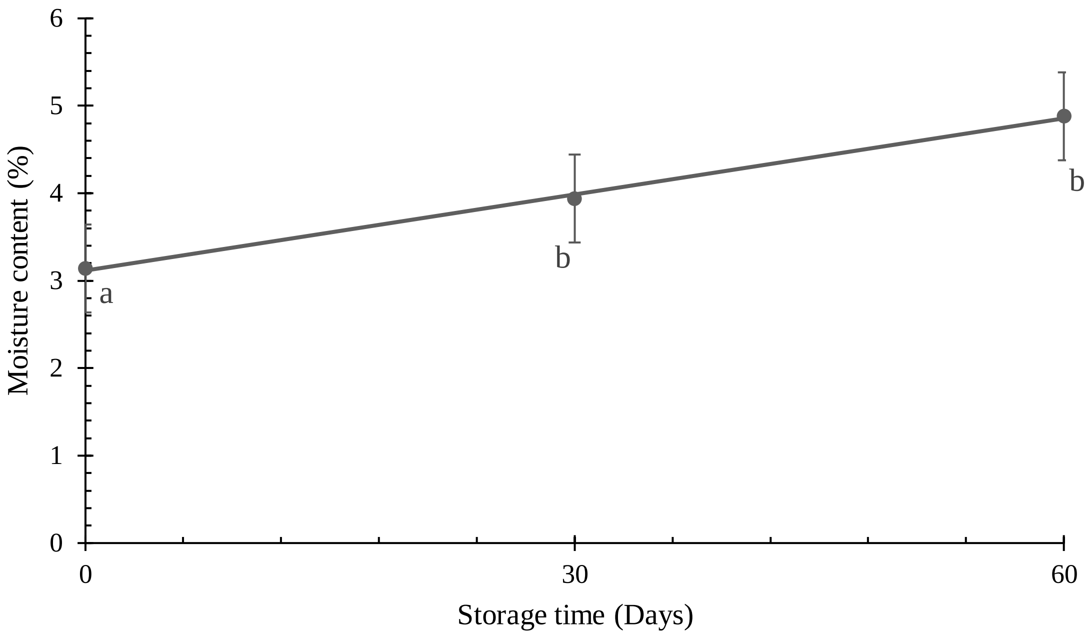

Fig 4.9 Change in moisture content during storage

Hence, the shelf- life of the product was evaluated and all the parameters determining the shelf life of biscuit were found to be within the standard limit. The rate of increase in AV, PV and moisture content signified that the product would be safe for consumption till standard best before time of six months. Packaging in laminate packets would have further increased the stability of the biscuit.

4.6.4 Comparison of control and optimized product

The total cost associated with the optimized product (flaxseed flour incorporated biscuits) was calculated and the cost per 100 g was found to be NRs. 57.517 including 20% overhead cost. The cost of control product (refined wheat flour biscuits) was found to be NRs. 52.56 per 100 g including 20% overhead cost. The optimized product (flaxseed flour incorporated biscuits) were found to be NRs. 4.957 (per 100 gm) more expensive than the control product (refined wheat flour biscuits).

From the cost calculation given in appendix F, it can be seen that due to the high cost of flaxseed to prepare flaxseed flour incorporated biscuits, the cost of biscuit has been increased.

Part V

Conclusion and recommendations

5.1 Conclusion

On the basis of research, following conclusions can be drawn:

- Biscuit formulation containing 25 parts (w/w) flaxseed flour was found to be appropriate formulation on the basis of sensory score.
- Phytic acid and tannin were found to be significantly lower at the seventh day of germination of flaxseed.
- From 2 months shelf- life study of biscuits, acid value, peroxide value and moisture content were found to be at safe level.
- Cost of biscuit per 100 g was found to be Rs. 57.517 that includes 20% of overhead cost.

5.2 Recommendations

The experiment can be further continued with the following recommendations:

- Entrepreneurs can utilize germinated flaxseed flour upto 25 parts to enrich nutritional value of general biscuits.
- Commercial production of flaxseed flour incorporated biscuits can be done by selecting suitable packaging material.

PART VI

Summary

Biscuits are the low cost, processed food which offers good taste along with nutritional values at affordable price with convenient availability. Biscuits have in general, a good shelf life in comparison to most of the other snack items. Since, biscuit is a kind of dry food having a long shelf life, the problem of deterioration is very low in comparison to other bakery products. Biscuits owing to their shelf life can be beneficial for feeding programmes and other immediate catastrophic conditions. Further value of biscuit can be added by incorporating flaxseed flour. Incorporation of wheat flour with flaxseed flour to make biscuits provides a good opportunity to improve the nutritional quality of the fibre and minerals consumed by many people especially growing children and old people due to which helps to rise the nutritional status of the population.

A study was carried out to know about the effects of incorporation of ANF treated flaxseed flour on biscuit quality. Design expert (D- optimal design) was used to design the experiment for formulating the recipe of biscuit. Flaxseed collected from Jhapa district was used. Grains were steeped for 24 h at 21°C. After steeping, the grains were germinated for 7 days at (23±3)°C and 90% RH. Then, the germinated grains were dried in cabinet dryer at (50±3)°C for 24 h to obtain desired moisture content of 10% for the next 7 days. The dried samples of seven days were grinded and packed in seven different packets and samples of each day was taken for analysis. Analysis of chemical properties of flour and physical and chemical properties of the optimized product prepared from the ANF treated flour was performed.

Five different biscuit formulations, namely A (0 part flaxseed flour), B (12.5 parts flaxseed flour), C (25 parts flaxseed flour), D (37.5 parts flaxseed flour) and E (50 parts flaxseed flour) were prepared by soft dough process. The other ingredients: fat 35 parts, pulverized sugar 35 parts, salt 0.3 parts, SMP 6 parts and baking powder 1.5 parts were taken constant. The biscuit formulated was of soft dough type. The five different biscuits samples were prepared and subjected to sensory evaluation. The sensory analysis was carried out based on texture, crispiness, color, taste and overall acceptance. The data obtained were statistically analysed using one-way ANOVA (no blocking) at 5% level of significance. Sample C (FF:WF = 25:75) got the highest mean sensory score. The proximate analysis for moisture, crude protein, crude fat, crude fibre, total ash and carbohydrate content of flaxseed flour was found to be 4.8%, 13.28%, 27.52%, 4.9%, 3.99% and 50.31% respectively whereas

the proximate analysis for moisture, crude protein, crude fat, crude fibre, total ash and carbohydrate content of the sample C (optimized product) was found to be 3.14%, 13.12%, 18.27%, 3.78%, 2.47% and 62.36% respectively.

Further, flaxseed flour incorporated biscuits were subjected for calcium and iron content evaluation. The calcium and iron content in flaxseed flour incorporated biscuit was 4.25 mg/100 g and 168.83 g/100 g respectively. The energy content of the control sample was found to be 471.57 kcal whereas the energy content of the optimized product was found to be 466.35 kcal. At 5% level of significance the two samples were significantly similar from each other. Thus, prepared biscuits were packed in HDPE (52 μm) and stored at ambient temperature. The product was further analysed for prediction of shelf life based on acid value, peroxide value and moisture content found to be 0.21 mg KOH/g oil, 1.8 meq O2/kg fat and 4.88% respectively at the end of 60 days which were all within the acceptable limits. These findings suggest that flaxseed flour can be successfully incorporated in refined wheat flour up to the concentration of 25 parts without any adverse effect on sensory attributes giving a nutritionally enriched product especially calcium, iron content, ash, fibre and fat content with best acceptability. The cost of the flaxseed flour incorporated biscuits (FFIB) were found to be NRs. 57.517 per 100 g including 20% overhead cost. The cost of refined wheat flour biscuits (RWFB) were found to be NRs. 52.56 per 100 g including 20% overhead cost. The FFIB were found to be slightly expensive than the RFWB. The FFIB were found to be NRs. 4.957 (per 100 gm) more expensive than the RWFB which might be due to the addition of an expensive legume like flaxseed.

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Appendices

Appendix A

Sensory evaluation score sheet for biscuit

Date:

Name of Panelist:

Name of the product: Flaxseed Flour Incorporated Biscuit (FFIB)

Dear panelist, you are provided 5 samples of Flaxseed Flour Incorporated Biscuit (FFIB) on each proportion with variation on flaxseed flour content. Please test the following samples of biscuit and check how much you prefer for each of the samples. Give the points for your degree of preferences for each parameter for each sample as shown below:

Judge the characteristics on the 1-9 scale as below:

Like extremely- 9	Like slightly- 6	Dislike moderately- 3
Like very much- 8	Neither like nor dislike- 5	Dislike very much- 2
Like moderately- 7	Dislike slightly- 4	Dislike extremely- 1

Parameters	Sample Code				
	A	B	C	D	E
Crispiness					
Color					
Flavor					
Texture					
Overall acceptabilty					

Any comments:

Signature:

Appendix B

Chemicals and apparatus requirements

Chemicals required

The following chemicals were obtained from the lab of Central Campus of Technology.

- Acetone
- Methyl orange indicator
- Sodium hydroxide
- Hydrochloric acid
- Sulphuric acid
- Mixed indicator solution
- Boric acid
- Digestion mixture
- Petroleum ether
- Sodium thiosulfate
- Neutral alcohol
- Methyl red indicator
- Saturated ammonium oxalate
- Potassium thiocyanide
- Standard iron solution
- Distilled water
- Phenolphthalein indicator
- Toulene
- Alcohol
- Oxalic acid
- Ammonium hydroxide
- Potassium iodide
- Silver nitrate
- Potassium oxalate
- Starch
- Acetic Acid
- Ammonia
- Potassium permagnate
- Saturated potassium persulfate

Apparatus required

The following apparatus were obtained from the lab of Central Campus of Technology.

- Burette
- Soxhlet assembly
- Conical flask
- Dessicator
- Measuring cylinder
- Heating arrangement
- Beaker
- Buchner filter assembly
- Pipette
- Kjeldhal digestion and distillation set
- Ashless filter paper
- Colorimeter
- Thermometer
- Whatmann filter paper
- Weighing balance
- Muffle furnace
- Vernier calliper
- Silica crucible
- Screw gauge
- Petridish
- Hot air oven
- Conical flask
- Volumetric flask

Appendix C

Independent t-test for physico- chemical analyses of samples, and paired t-test for control and optimized product

Table C.1 Independent t- test comparison of proximate components of wheat and flaxseed flour

		Mean	Variance	s. d.	F probability ratio
Moisture content	WF	11.67	0.0001	0.01	<0.001
	FF	4.8	0.010	0.1	<0.001
Protein content	WF	10.32	0.0004	0.02	<0.001
	FF	13.28	0.0001	0.01	<0.001
Fat content	WF	1.58	0.0009	0.03	<0.001
	FF	27.52	0.0004	0.02	<0.001
Fiber content	WF	0.75	0.0001	0.01	<0.001
	FF	4.9	0.01	0.1	<0.001
Carbohydrate content	WF	87.02	0.0004	0.02	<0.001
	FF	50.31	0.0001	0.01	<0.001
Ash content	WF	0.33	0.0001	0.01	<0.001
	FF	3.99	0.0004	0.02	<0.001

Table C.2 Paired t- test comparison of proximate, ultimate components and energy values of wheat biscuit and flaxseed biscuit

		Mean	Variance	s. d.	F probability ratio
Moisture content	WF	2.6	0.01	0.39	0.016
	FF	3.14	0.01	0.02	0.016
Protein content	WF	7.84	0.003	0.14	<0.001
	FF	13.12	0.003	0.05	<0.001
Fat content	WF	16.33	0.0004	0.04	<0.001
	FF	18.27	0.0004	0.40	<0.001
Fiber content	WF	0.68	0.002	0.08	<0.001
	FF	3.78	0.002	0.13	<0.001
Carbohydrate content	WF	73.31	0.001	0.19	<0.001
	FF	62.36	0.001	0.32	<0.001
Ash content	WF	1.84	0.003	0.02	0.003
	FF	2.47	0.003	0.04	0.003
Iron content	WF	1.94	0.001	0.02	<0.001
	FF	4.25	0.001	0.07	<0.001
Calcium content	WF	20.59	0.001	0.03	<0.001
	FF	168.83	0.001	0.08	<0.001
Energy values	WF	471.57	0.0001	0.01	<0.001
	FF	466.35	0.0001	0.01	<0.001

Appendix D

Table D.1 ANOVA for phytate

Source of variation	Degree of freedom	Sum of square	Mean squares	Variance ratio	F probability ratio
Days	6	6.769E+04	1.128E+04	4.513E+05	<.001
Residual	14	3.500E-01	2.500E-02		
Total	20	6.769E+04			

Since $p < 0.05$, there is a significant difference between the samples in different germination days, so LSD testing is necessary.

Table D.2 LSD of means for phytate

No of days	Mean	Letter used for significant test	l.s.d	d.f.
1*	10.78±0.03	a	0.2769	14
2*	10.54±0.05	b		
3*	10.12±0.07	c		
4*	10.02±0.04	d		
5*	9.94±0.01	e		
6*	9.18±0.06	f		
7*	9.18±0.08	f		

*= Significantly different

Table D.3 ANOVA for tannin

Source of variation	Degree of freedom	Sum of square	Mean squares	Variance ratio	F probability ratio
Days	6	131.924429	21.987405	2629.47	<.001
Residual	14	0.117067	0.008362		
Total	20	132.041495			

Since $p < 0.05$, there is a significant difference between the samples in different germination days, so LSD testing is necessary.

Table D.4 LSD of means for tannin

No of days	Mean	Letter used for significant test	l.s.d.	d.f.
1*	3.34±0.09	a	0.1601	14
2*	3.32±0.07	b		
3*	3.31±0.14	c		
4*	3.29±0.01	d		
5*	3.28±0.08	e		
6*	3.27±0.11	f		
7*	3.27±0.23	f		

*= Significantly different

Appendix E

Tannin calibration curve

Tannic acid Concentration(μg)	Absorbance
20	0.016
40	0.232
60	0.356
80	0.553
100	0.688
120	0.794
140	0.823

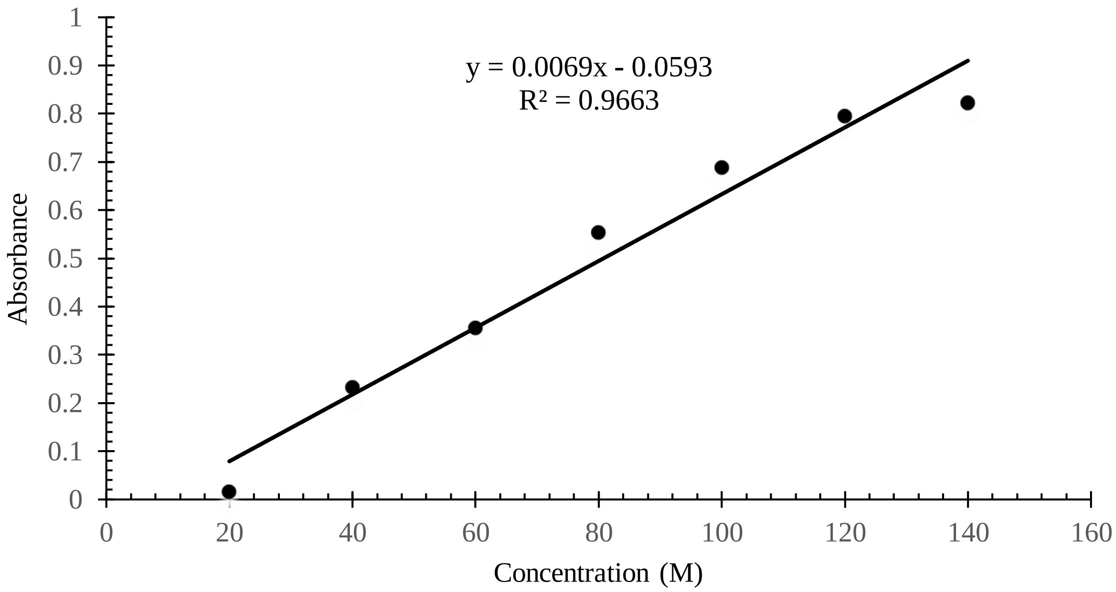

Fig E.1 Standard curve for tannin content

Appendix F

ANOVA for physical parameter analysis of biscuits

Table F.1 One way ANOVA (no blocking) for thickness

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Samples	4	23.91885	5.97971	187.55	<.001
Residual	10	0.31883	0.03188		
Total	14	24.23768			

Table F.2 One way ANOVA (no blocking) for diameter

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Samples	4	8.6952	2.1738	6.62	0.07
Residual	10	3.2849	0.3285		
Total	14	11.9802			

Table F.3 One way ANOVA (no blocking) for weight

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Samples	4	4.245E+01	1.061E+01	1.193E+05	<.001
Residual	10	5.547E-04	5.547E-05		
Total	14	4.245E+01			

Table F.4 One way ANOVA (no blocking) for spread ratio

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Samples	4	67.7702664	16.9425666	1.634E+05	<.001
Residual	10	0.0010370	0.0001037		
Total	14	67.7713034			

Table F.5 One way ANOVA (no blocking) for volume

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Samples	4	239.272794	59.818198	36779.51	<.001
Residual	10	0.016264	0.001626		
Total	14	239.289058			

Table F.6 One way ANOVA (no blocking) for density

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Samples	4	0.0159576	0.0039894	7.63	0.004
Residual	10	0.0052320	0.0005232		
Total	14	0.0211896			

Appendix G

Table G.1 Summary of ANOVA of sensory evaluation of flaxseed flour incorporated biscuit

Sample code	Color	Texture	Crispiness	Flavor	Overall acceptability
A	7.67 ± 0.79^{c}	7.83 ± 1.37^{b}	8.08 ± 1.37^{c}	7.67 ± 0.74^{c}	7.67 ± 0.62^{c}
B	6.33 ± 1.6^{ab}	6.58 ± 1.37^{a}	6.5 ± 1.55^{ab}	6.33 ± 1.97^{b}	6.75 ± 1.42^{bc}
C	6.83 ± 1.32^{bc}	7.16 ± 1.42^{ab}	7.58 ± 1.42^{bc}	7.67 ± 1.02^{c}	7.58 ± 1.03^{c}
D	5.41 ± 1.59^{a}	6.58 ± 1.59^{a}	6.5 ± 1.5^{ab}	6.41 ± 1.6^{bc}	6.41 ± 1.18^{ab}
E	5.25 ± 1.54^{a}	6.33 ± 1.54^{a}	6.16 ± 0.92^{a}	5 ± 1.51^{a}	5.5 ± 1.7^{a}

Appendix H

ANOVA for shelf life of the product

Table H.1 One way ANOVA (no blocking) for acid value

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Samples	2	0.0096000	0.0048000	10.29	0.012
Residual	6	0.0028000	0.0004667		
Total	8	0.0124000			

Table H.2 One way ANOVA (no blocking) for peroxide value

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Samples	2	0.560000	0.280000	254.55	<.001
Residual	6	0.006600	0.001100		
Total	8	0.566600			

Table H.3 One way ANOVA (no blocking) for moisture content

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Samples	2	1.191200	0.595600	186.12	<.001
Residual	6	0.019200	0.003200		
Total	8	1.210400			

Appendix I

ANOVA for physical analysis of samples

Table I.1 One way ANOVA (no blocking) for color

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Formulation	4	48.433	12.108	4.62	0.003
Residual	55	144.167	2.621		
Total	59	192.600			

Table I.2 One way ANOVA (no blocking) for crispiness

Source of variation	Degree of freedom	Sum of squares	Mean sqaures	Variance ratio	F probability ratio
Formulation	4	32.433	8.108	4.39	0.004
Residual	55	101.500	1.845		
Total	5932.433	133.933			

Table I.3 One way ANOVA (no blocking) for flavor

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Formulation	4	59.267	14.187	5.78	<0.001
Residual	55	140.917	2.562		
Total	59	200.183			

Table I.4 One way ANOVA (no blocking) for texture

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Formulation	4	25.567	6.392	3.52	0.012
Residual	55	99.833	1.815		
Total	59	125.400			

Table I.5 One way ANOVA (no blocking) for overall acceptability

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Formulation	4	38.433	9.608	5.64	<.001
Residual	55	93.750	1.705		
Total	59	132.183			

Appendix J

Table J.1 Cost calculation of flaxseed flour incorporated biscuits

Particulars	Cost (NRs/kg)	Weight in a lot (g)	Cost (NRs)
Wheat flour	60	75	4.5
Flaxseed	250	25	6.25
Fat	105/ 100 gm	35	36.75
Pulverized sugar	70	35	2.45
Salt	25	0.3	0.0075
SMP	426/400 gm	6	6.39
Baking powder	60/100 gm	1.5	0.9
Raw material cost			57.2475
20% overhead cost			11.4495
Grand total cost			68.697
Average weight of FFIB		9.953	
Total no of FFIB formed		12	
Total weight of FFIB (g)		119.436	
Total cost of FFIB (NRs/100 g)			57.517

Table J.2 Cost calculation of refined wheat flour biscuit (control sample)

Particulars	Cost (NRs/kg)	Weight in a lot (g)	Cost (NRs)
Wheat flour	60	100	6
Fat	105/100 gm	35	36.75
Pulverised sugar	70	35	2.45
Salt	25	0.3	0.0075
SMP	426/400 gm	6	6.39
Baking powder	60/100 gm	1.5	0.9
Raw material cost			52.4975
20% overhead cost			10.4995
Grand total cost			62.997
Average weight of RFWB		9.987	
Total no of RWFB formed		12	
Total weight of RWFB		119.844	
Total cost of RWFB (NRs/100 g)			52.56

Color plates

Plate 1: Germination bed

Plate 1: Prepared flaxseed flour incorporated biscuits ready for baking

Plate 3: Vacuum packed biscuits for sensory analysis

Plate 4: Crude fiber analysis of the optimized product