EFFECT OF FLAXSEED ON THE PHYSIOCHEMICAL AND SENSORY QUALITY OF MUFFIN AS EGG REPLACER

by

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2022

Effect of Flaxseed on the Physiochemical and Sensory Properties of Muffin as Egg Replacer

A dissertation submitted to the Department of Food Technology, Central Campus of Technology, Tribhuwan University, in partial fulfillment of the requirements for the degree of B.Tech, in Food Technology

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November, 2022

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Approval Letter

This ***dissertation*** entitled ***Effect of Flaxseed on the Physiochemical and Sensory Properties of Muffin as Egg Replacer*** presented by **Rupshana Sunwar** has been accepted as the partial fulfillment of the requirement for the B. Tech. degree in Food Technology.

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November 10, 2022

Acknowledge

First and foremost, I would like to express my sincere gratitude my research advisor, Asst. Prof. Mahalaxmi Pradhananga for advice, motivation, assistance, patience and immense knowledge. This paper would have never been accomplished without her continuous support and guidelines. I am very grateful to be associated with such person and is very thankful for your dedication and understanding over my thesis period.

I am thankful to Assoc. Prof. Dr. Dil Kumar Limbu, Campus Chief, Central Campus of Technology and Asst. Prof. Navin Gautam, Head of Department of Food Technology, for providing available facilities to carry out the best in this dissertation work.

I would like to thank my fellow classmates Nisha Marattha, Simron Shrestha, Sangita Rijal, Ranjan Shrestha, Rabu Thapa Magar, Ghanshyam Aryal and Muskan Saru Magar for all the help and support. Special thanks to my juniors Subita Marattha, Kabita Poudel, Monika Shrestha, Supriya Pradhan and Aaradhana Gautam for their support. I am grateful to all those whose perception, observations amd advice have helped me directly and indirectly.

I also take this opportunity to express gratitude to the entire department faculty member. I would like to thank our Lab head Prajwal Bhandari and all staffs of library and laboratory for their support.

Most importantly, I owe my dissertation to my parents whose unconditional love, support and blessings made the whole work inspiring journey.

November 10, 2022

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(Rupshana Sunwar)

Abstract

The present work was carried out to prepare muffin substituted with flaxseed as the egg replacer and evaluate its physicochemical and sensory properties. The flaxseed egg replacer was prepared as 1:3 proportion [ground flaxseed: water]. This egg replacer was substituted in the muffin at the level of 0%, 25%, 50%, 75%, and 100% with the egg and named as sample A, B, C, D and E. Proximate analysis of flaxseed was carried out and superior product obtained through sensory evaluation were determined. The samples were analyzed for physico-chemical properties and acceptability period.

Analysis of variance conducted on the sensory characteristics and overall acceptability indicated a statistically significant effect when replacing oil or eggs for color, flavor, sponginess and overall acceptability ($P \leq 0.05$). Product B was selected as the best product. Statistical analysis for the proximate composition of muffin samples showed that the substitution of flaxseed egg replacer significantly improved the crude fiber, total ash, carbohydrate, calcium and iron content however the protein content and fat content was decreased compared to egg muffin. The phytic acid content and cynogenic glycosides of the flaxseed decreases after baking. The antioxidant activity and total polyphenol content increases in the flaxseed muffin then the control. The calorific value was found to be decreased which resulted in low calorie product. The product kept in LDPE packaging was further analyzed for prediction of shelf life which was up to 4 days based on acid value, peroxide value, TPC, yeast, mold and coliform. The cost analysis showed that the muffin with flaxseed egg replacer was less costly than with egg.

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List of abbreviations

Abbreviation	Full form
ANOVA	Analysis of variance
AOAC	Association of American Analytical Chemist
cm	Centimeter
db	Dry Basis
DE	Design Expert
d.f.	Degree of freedom
DPPH	2,2-diphenyl-1-picrylhydrazyl
et al.	et alibi, and others
etc	Etcetera
Fig	Figure
g	Grams
GAE	Gallic acid equivalent
LSD	Least Significant Difference
meq	Milli equivalent
µg	micro gram
mm	Milli meter
RSA	Radical scavenging activity
SD	Standard Deviation
°C	Degree Centigrade
TFC	Total flavonoids content

Part I

Introduction

1.1 Background

Muffin is small cup-shaped quick bread that is generally dominated by sweet taste and can be served with meal or consumed as a snack (Baixuli *et al.*, 2008). Muffins are described as a quick bread because “quick- acting” chemical leavening agents are used. The characteristic of a good quality muffin is as of symmetrical shape, with golden brown color, rounded top, uniform cells in the crumb, tender and slightly moist in texture, could be easily broken apart, sweet taste, and with pleasant aroma and aftertaste (Hui *et al.*, 2007).

Muffin, a sweet baked product, is highly appreciated by the consumers as it has soft texture and characteristic taste. Flour, egg, sugar, and fat, the principle ingredients of muffins, play important role in structure, appearance, and eating quality of the final product (Karaoğlu and Kotancilar, 2008). Egg, one of the main ingredient in most of the baked goods, provides functional properties such as coagulations , flavoring, tenderizing, emulsification, foaming, leavening, glazing, binding ingredients and also possess high nutritional value (Yang and Baldwin, 1995). However, motivation factors such as less and free from cholesterol foods, low- calorie content, vegan, cheap raw materials, diminished allergens, food safety and far less of microbial concerns led researchers to investigate egg replacers (Lin *et al.*, 2006). However, health risks associated with consumption of eggs and consumer preference for vegan diet led researchers to investigate egg replacers (Murughar *et al.*, 2016).

Flax (*Linum usitassimum)* is a blue flowering annual herb belonging to family Lineaceae. It produces small seeds varying from golden yellow to reddish brown color. Flaxseed possesses crispy texture and nutty taste. 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 rich in fat, protein and dietary fiber. An analysis of brown Canadian flax averaged 41% fat, 20% protein, 28% total dietary fiber, 7.7% moisture and 3.4% ash, which is the mineral-rich residue left after samples are burned (Morris, 2007). Edible flaxseed products include the whole flaxseed, ground meal and extracted oil or mucilage (Singh *et al.*, 2012).

It is a leading source of the omega 3 fatty acid, α-linolenic acid (ALA) (52% of the total fatty acids), and of phenolic compounds commonly known as lignans (>500 μg/g, as is basis), in addition to containing hydro colloidal gum, also referred to as mucilage(about 8% of seed weight), and good quality protein (Oomah, 2001). The functional properties are mostly associated with the protein and carbohydrate fractions of the seed, which will be concentrated in flaxseed meal (Teh *et al.*, 2014). Flaxseed gum is a newly potential source of mucilage that can be applied in bakery products such as fat replacer or egg replacer. It has similar properties with others gums which includes good water holding capacity, water binding ability and also rheological properties (Fedeniuk and Biliaderis, 1994). Since flaxseed mucilage has weak gel properties, it can be used to replace most of the non-gelling gums for food and non-food applications (Chen *et al.*, 2006)..

Flaxseed contains different phytochemicals such as phenols, lignans, tocopherols, flavonoids and some water-soluble vitamin. Its unlimited potential in preventing and/or reducing the risk of several major diseases, including diabetes, coronary heart diseases, lupus nephritis, atherosclerosis, and hormonally dependent cancers undoubtedly make it the nutraceutical food of the twenty-first century (Thompson and Cunnane, 2003)

1.2 Statement of problem

In recent decades, the food industry has been faced with new challenges, and it has had to develop new types of the diets and produce new types of foods that can slow down the spread of chronic diseases. In bakery products such as muffins, egg is the key ingredient. However, eggs are considered the most costly ingredients in some types of cakes. Increasing the amount of eggs in some types of cake, could result in increasing the amount of cholesterol content. According to Savage *et al.* (2007), 1% to 2% of all children are egg allergies and these situations are very common. Eggs are considered a top allergen (Bakerpedia, 2022). Therefore, the use of vegetable proteins for partial or total substitution of eggs in cake formulations appears to be an interesting objective, and especially so for the people with specific dietary needs or restrictions such as vegans, vegetarians, high cholesterol people (Hussain and Oulabi, 2009).

1.3 Objectives

The objectives of the research was divided into two parts:

1.3.1 General objective

The main objective of this work was preparation and the product quality evaluation of the muffin using flaxseed as egg replacer.

1.3.2 Specific objectives

1. To perform physiochemical and nutritional analysis of the flaxseed.
2. To assess anti-nutritional factors of flaxseed like cyanogenic glycosides and phytic acid; and its antioxidant activity and total phenol content in flaxseed.
3. To prepare muffins using different composition of flaxseed egg replacer.
4. To determine the acceptability and the selection of best formulation through sensory analysis.
5. To study physical properties, physiochemical properties and anti-nutritional factors of the best product.
6. To estimate the shelf life of muffin.

1.4 Significance of the study

Flaxseed is a functional food with high nutritional value (Bozan and Temelli, 2008). Flaxseed has emerged as a potential functional food being good source of omega fatty acids (mainly ALA), lignans, high quality protein, soluble fiber i.e., hydro colloidal gums and phenolic compounds. Driven by the health benefits, flaxseed has been incorporated into foods such as bread, muffins, cereals, crackers, energy bars, baking mixes, snacks, soups, and waffles by researchers, and food manufacturers (Daun *et al.*, 2003). The price of the flaxseeds are comparatively lower than the eggs in many part of world. The flaxseed can also be utilized as the vegan alternative of eggs due to different functional properties of its components which can help in long term utilization of it for preparation of baked products. Thus, the present work is solely concerned with the effect of the soaked ground flaxseeds/flaxseed mucilage on the quality evaluation of the muffins due to its nutritional and functional properties and increase the potential use of flaxseed as food ingredient.

1.5 Limitation of the study

1. Only one variety of flaxseed was used.
2. Instrumental textural analysis was not carried

Part II

Literature review

2.1 Flaxseed

Flaxseed is the seed from the flax plants which have a crisp and chewy texture and a pleasant, nutty taste (Carter, 1994). It is rich in fat, protein and dietary fiber. An analysis of brown Canadian flax averaged 41% fat, 20% protein, 28% total dietary fiber, 7.7% moisture and 3.4% ash, which is the mineral-rich residue left after samples are burned. Brown and yellow (Omega) varieties of flaxseed are virtually identical in their nutrient content (Morris, 2007). The composition of flaxseed can vary with genetics, growing environment, seed processing and method of analysis (Cunnane *et al.*, 1994). Seed coat color is determined by the amount of pigment present, a feature that can be changed through normal plant breeding practices (Ganorkar and Jain, 2013)

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Fig.2.1 Flax

Source: Anon. (2022)

3.2.1 Taxonomic classification of Flaxseed.

Flax (*Linum usitatissimum*), also known as common flax or linseed**,** is a member of the genus Linum in the family Linaceae. Several other species in the genus Linum are similar in appearance to L. usitatissimum, cultivated flax, including some that have similar blue flowers, and others with white, yellow, or red flowers. Some of these are perennial plants, unlike L. usitatissimum, which is an annual plant.

Kingdom: Plantae-plants

Subkingdom: Tracheobionta -vascular plants

Superdivision: Spermatophyta-seed plants

Division: Magnoliophyta -flowering plants

Class: Magnoliopsida-dicotyledons

Subclass: Rosidae

Order: Linales

Family: Linaceae-flax family

Genus: Linum

Species: L. usitatissimum

Fig. 2.2 Taxonomic classification of flaxseed

2.1.2 Area production and productivity of flaxseed

The production of flax (*Linum usitatissimum*) and other oilseed crops peak in the temperate climates of the middle mountain and hill farming regions in Nepal. Flax matures in approximately 90 to 125 days and develops most rapidly under the cool, short season of growing. The middle hill region of the Lamjung district (the epicenter of the earthquake devastating Nepal in April 2015) exemplifies an ideal climate for flax production experiencing consistently cool temperatures for most of the year (Schroeder, 1985). The shallow rooting system makes the plant especially susceptible to drought and excess moisture in the soil but easier come time to harvest. Oilseed production in Nepal was largely replaced by grain crops which contain a higher caloric value but requires higher labor and overall decrease in nutritional quality for the Nepalese. Flax production is seen to be increasing as the cool and temperate climate of the mid-hill regions in Nepal present great potential for farmers to maximize their linseed yields and the yield of proceeding cash-crops through disease and pest control (Booker *et al.*, 2006).

The data set "Flaxseed, production quantity (tons)" for Nepal contains data from the year 2003 until 2016 shown in table 2.1 and from 1961 until 2015 shown in figure 2.1.

Table 2.1 Flaxseed, production quantity (tons) for Nepal contains data from the year 2003 until 2020

Year	Value (tons)	Year	Value (tons)
2020	11,237	2010	3,611
2016	7,672	2009	4,917
2015	10,402	2008	5,431
2014	9,136	2007	6,291
2013	7,672	2006	6,400
2012	7,500	2005	6,574
2011	3,361	2004	6,100

Source: (Knoema, 2022)

Flax is harvested for fiber production after about 100 days, or a month after the plants flower and two weeks after the seed capsules form. The bases of the plants begin to turn yellow. If the plants are still green, the seed will not be useful, and the fiber will be underdeveloped. The fiber degrades once the plants turn brown.

Flax grown for seed is allowed to mature until the seed capsules are yellow and just starting to split; it is then harvested in various ways. A combine harvester may either cut only the heads of the plants, or the whole plant. These are then dried to extract the seed. The number of weeds in the straw affects its marketability, and this, coupled with market prices, determines whether the farmer chooses to harvest the flax straw. If the flax straw is not harvested, typically, it is burned, since the stalks are quite tough and decompose slowly (i.e., not in a single season). Formed into windrows from the harvesting process, the straw often clogs up tillage and planting equipment. Flax straw that is not of sufficient quality for fiber uses can be baled to build shelters for farm animals, or sold as biofuel, or removed from the field in the spring.

2.1.3 Physical properties of flaxseed

The spherical fruit capsules contain two seeds in each of five compartments. The seed itself is flat and oval with a pointed tip. It is a little larger than a sesame seed and a smooth-glossy surface. Flaxseeds range in color from medium, reddish-brown to a light yellow. Seed color is determined by the amount of pigment in the outer seed coat the more pigment, the darker the seed. The dimensions of the seed vary approximately 3.0–6.4 mm in length, 1.8–3.4 mm in width and 0.5–1.6 mm in thickness (Freeman, 1995).

2.1.4 Functional component of flaxseed

- **Protein**

The protein fraction of flax has not yet attracted as much interest as the other seed macro components. This may partly be attributed to the use of whole seeds in foods without recognizing them as a source of vegetable protein. Proteins in flaxseed were found to be made up of about 20% albumins (1.6S and 2S) and 80% legumin-like proteins (11S and 12S) (Daun *et al.*, 2003).Flaxseed proteins were found to be structurally more lipophilic than soybean proteins. Other specific proteins are oleosins, cadmium binding proteins and antifungal proteins (Wanasundara and Shahidi, 2003). The commercial utilization of flaxseed proteins in food products depend on its functional properties before its incorporation in various food products (Oomah and Mazza, 1993). The gum in flaxseed has been implicated in enhancing the viscosity and the water-binding, emulsifying, and foaming properties of linseed-protein products (Mazza and Biliaderis, 1989)

- **Mucilage**

The flaxseed coat, together with the endosperm, forms six layers. Mucilage or gum comes from the secondary wall material in the outermost layer. Mucilage makes up approximately 8% of the seed weight (Daun *et al.*, 2003).. It is easily extracted from the seed coat by soaking in water. When hydrated, the mucilage cells swell, and their content exude on the surface of the seeds. Flax mucilage contains between 50-80% carbohydrates and 4- 20% proteins and ash. The major constituent of flax mucilage consists of two polysaccharide components, neutral and acidic. The neutral fraction contains L-arabinose, D-xylose and D-galactose in a mole ratio of 3.5:6.2:1 and the acidic fraction contains L-rhamnose, L-fucose, L-galactose, and Dgalacturonic acid in a mole ratio of 2.6:1:1.4:1.7 (Oomah et al., 1995). Crude fat content from flaxseed gum were found in range of 0.44 %-0.39% in different varieties of flaxseed mucilage (Mehtre *et al.*, 2017).

Flaxseed mucilage is a water-soluble hydrocolloid. Flaxseed mucilage has potential to be used as a food gum as a result of its thickening and emulsifying properties. For 1% (w/v) solutions, flax seed mucilage gave foam values about 75% of those of ovalbumin and had similar time-dependent stability (Mazza and Biliaderis, 1989).

Table 2.2 Functional properties of flaxseed constituents

Functional ingredient	**Applications**
Mucilage	Emulsifier & stabilizer in sauces, sausages, meat emulsions, salad dressings
	Anti-staling agent in baked products
	Improves cooking quality of noodles
	Functional food ingredient (interaction of mucilage and protein regulate blood glucose level)
Protein	Stabilizer & emulsifier in ice cream, sauces and meat emulsions
	Antifungal property
	Viscoelastic texture to extruded pastes for breakfast cereals and snacks
	Enhances nutrition in gluten free meal
	Egg and gelatin substitute in baked goods and ice cream
	Functional food ingredient

Source: Kajla *et al.* (2015)

Table2.3 Functional properties of flaxseed flour

Parameter	Content
Bulk density	0.78 g/ml
Water absorption	1.48 g/g
Fat absorption	1.20 g/g
Foam capacity	14.60 ml
Foam stability	8.80 ml

Source: Hussain *et al.* (2008)

2.1.5 Nutritional composition of flaxseed

Flaxseed contains lipids, polysaccharides, and proteins as the major components. It is also an excellent source of ω-3 fatty acids (FA), particularly linolenic acid, which is beneficial to both humans and animals (Treviño *et al.*, 2000). In a previous work Heimbach (2009), there were no significant nutritional or safety-related differences between flaxseeds of different colors. Flaxseed content varied from 38 to 45 % oil and FA distribution depending on location, cultivation, and environmental condition (Mazza and Oomah, 1997).

All carbohydrate in flaxseed are dietary fibers and are considered as a source of soluble and insoluble fractions. About one-third of the fiber in flaxseed is soluble and it may help to lower cholesterol and to regulate levels of blood sugar. The remaining two thirds of the fiber is insoluble which aids digestion by increasing bulk and preventing constipation (Anon., 2002)

Canadian flaxseed has been found to contain about 23% crude protein (or 20% true protein or 18% true protein corrected for non-protein nitrogen. Flax protein is relatively rich in arginine, aspartic acid and glutamic acid, and the limiting amino acids are lysine, methionine and cysteine (Coşkuner and Karababa, 2007). Potassium and phosphorus were the major mineral components of flaxseed. Flaxseed also contained significant quantities of iron, zinc, and manganese. Flaxseed contains water soluble vitamins. Flaxseed also contains significant quantities of complex phenolics known as lignans (Daun *et al.*, 2003).

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. The nutritional composition of flaxseed is shown in table 2.4.

Table 2.4 Nutritional composition of flaxseed

Parameter	Quantity	Parameter	Quantity
Energy	534 kcal	Vitamin	
Carbohydrate	28.88 g	Thiamin (B1)	1.644 mg
Sugar	1.55 g	Riboflavin (B2)	0.161 mg
Dietary fiber	27.33 g	Niacin (B3)	3.08 mg
Fat	42.16 g	Pantothenic acid (B5)	0.985 mg
Saturated	3.663 g	Vitamin (B6)	0.437 mg
Monounsaturated	7.527 g	Folate (B9)	0.1 mg
Polyunsaturated	28.730 g	Vitamin c	0.6 mg
Omega-3	22.8 g	Minerals	
Omega-6	5.9 g	Calcium	225 mg
Protein	21.76 g	Iron	5.73 mg

Source: Anon. (2020)

2.1.6 Antioxidant property and total polyphenols

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate 10 degenerative diseases. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydro peroxide and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease (Miller *et al.*, 1986).

2.1.7 Anti-nutritional factors

Flaxseeds contain anti-nutrients that may have adverse influence on the health and well-being of human population. Antinutrients or antinutritional 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 (Akande *et al.*, 2010).

Carraro *et al*., (2012) reported that flaxseed contains cyanogenic compounds of 264– 354 mg per 100 g. 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 (2003), 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 (Anon, 2010). Wolever *et al*., (1995) reported that cyanogenic glycoside measured as HCN/100 g flaxseeds, decreased from 20.8 to 1.0 mg/100 g after roasting.

Phytic acid, another anti-nutrient present in flaxseed, ranges from 23 to 33 g/kg of the flaxseed meal (Mazza *et al*., 1996a). Phytic acid interferes with the absorption of calcium, zinc, magnesium, copper and iron. It is a strong chelator, forming protein and mineral-phytic acid complexes and thus reducing their bioavailability (Akande *et al*., 2010). Preeti and Chimmad, (2010) analyzed flaxseed and recorded phytic acid of 969 mg/100 g.

2.7 Health benefits of flaxseed

Flaxseed contains n-3 fatty acids, soluble fibers, vitamin E, lignans, and other phenolic and peptide compounds which are found to exert potential diverse actions thought to benefit health, e.g., anti-inflammation, vessel relaxation, antioxidant, hypocholesterolemic, anticarcinogenic, and attenuation of the postprandial insulin response (Carraro *et al*., 2012). Ground flaxseed of 50g/day consumed over 4 weeks increased the average daily ALA plasma levels by about 10 times in healthy adults (Cunnane *et al*., 1995). All the omega-3 fatty acids regulates the cholesterol, triglycerides and blood pressure, whereas alpha linolenic acid especially helps in proper growth of infants in reducing the risk of cardiovascular diseases (Horrobin and Manku, 1990).

Consumption of 50 g of ground flaxseed daily for four weeks lead to reductions in serum total cholesterol level up to 6-9% and low density lipoprotein-cholesterol (LDL-C) up to 9-18% in healthy young adult men and women with moderately high levels of blood cholesterol and postmenopausal of 20 women (Bierenbaum *et al*., 1993). The efficacy of partly ground and defatted flaxseed (*Linum usitatissimum*) on constipation patients, predominant irritable bowel syndrome was documented by Tarpila *et. al,* (2003).

2.2 Muffin

Muffin is small cup-shaped quick bread that is generally dominated by sweet taste and can be served with meal or consumed as a snack. Muffin is characterized by a typical porous structure and high volume. To obtain such a structure, a stable batter lodging many tiny air bubbles is required (Baixauli *et al.*, 2008).

Muffins are sweet baked products highly appreciated by consumers due to their good taste and soft texture, perfect for breakfast, brunch and snacks. Muffin composition is a fat in water emulsion obtained from an egg-sugar-water-fat mixture as a continuous phase, and air bubbles represent a discontinuous phase where the flour is dispersed. Muffins are generally associated with a high porous spongy texture (Matos *et al.*, 2014). Traditionally, a muffin recipe is composed of wheat flour, vegetable oil, eggs and milk (Sanz *et al.*, 2009).

2.2.1 Varieties of muffin

A muffin is an individual-sized, baked product. It can refer to two distinct items, a part-raised flatbread that is baked and then cooked on a griddle (typically unsweetened) and a cup muffin-like quick bread (often sweetened) that is chemically leavened and then baked in a mold. While quick bread muffins are often sweetened, there are savory varieties made with ingredients such as corn and cheese. The flatbread is of British or European derivation, and dates from at least the early 18th century, while the quick bread originated in North America during the 19th century. Both are common worldwide today (Baixauli *et al.*, 2008) .

Muffins made by large commercial bakeries are more cakelike, and those made in the home or small institutions tend to be more breadlike. The differences between cakelike and breadlike muffins are that cakelike muffins are higher in fat and sugar and use soft wheat flours. A common problem encountered in bread-type muffins is tunnel formation resulting from overdevelopment of gluten. However, this problem is avoided in cake-type muffins, because sugar, fat, and soft wheat flours interfere with gluten development and prevent tunnel formation. Bread-type muffins contain 12% of both fat and sugar compared with 18–40% fat and 50–70% sugar in cake-type muffin (Cross, 2007).

2.2.2 Muffin mixing method

There are two primary methods for mixing muffins – the cake method and the muffin method. The cake method involves creaming sugar and shortening together, followed by addition of liquid ingredients and the final addition of dry ingredients. The muffin method of mixing involves two to three steps. First, dry ingredients are mixed together; second, shortening or oil and other liquids are mixed together; and third, the liquids are added to the dry ingredients and mixed until the dry ingredients are moistened (Cross, 2007).

2.2.3 Objective of mixing

The primary objective in mixing is to achieve a homogenous mixture; generally, this means, attaining a nearly uniform distribution of the ingredient. A distinction may be drawn between batch and continuous process. Overall, the concentration of the ingredient should uniformly distributed in the output stream, should not vary with time and the processing of each part of the mixture should be same (Cullen, 2009).

2.2.4 Ingredients and their effects in Muffin characteristics

2.2.4.1 Flour

Flour is the primary ingredient in baked products. Wheat is the only grain, which could yield flour capable of being made into low-density baked products. Most muffin formulas contain either a blend of cake or pastry flour and a higher protein flour such as bread flour, or all bread flour (11.0-12.0% protein) (Willyard, 2000). The protein in flour is needed to provide structure in quick breads made with limited amounts of sugar. Flour contains starch and the proteins glutenin and gliadin, which hold other ingredients together to provide structure to the final baked product. Hydration and heat promote gelatinization of starch, a process that breaks hydrogen bonds, resulting in swelling of the starch granule, which gives the batter a more rigid structure (McWilliams, 2016a).

Table 2.5 Requirements for flour characteristics

Characteristics	Requirements
Moisture content	13.0% max
Total ash (dry basis)	0.5% max
Gluten content (dry basis)	7.5% max
Protein (dry basis)	9.0% min
Acid insoluble (dry basis)	0.05% max
Alcohol acidity as H_2SO_4 in 90% alcohol	0.1%
Water absorption	55% min
Sedimentation value	22%
Uric acid (mg/100g)	10% max
Granularity	To satisfy the taste

Source: Arora (1980)

- **Nutritive value of wheat flour**

The nutritive value of wheat flour is the same as that of wheat flours of lower extraction rate viz. white flour and whole flour as milled, differ from wheat I nutritive value because of removal of varying amounts of bran, germ and other endosperm in which the concentration of protein, mineral and vitamins is higher than in inner endosperm (Kent, 1983).

Table 2.6 The chemical composition of wheat flour

Types of wheat	Moisture	Protein	Fat	Crude fiber	Ash	Carbohydrate
Soft	13.6	11.2	1.5	2	0.55	71.15
Hard	12.3	12.5	2	2.5	0.57	70.13

Table 2.7 Vitamins and minerals content (mg/100 g) in wheat flour

	Thiamine	Riboflavin	Niacin	Iron	calcium
Wheat	3.4	1.1	4.5	4.6	0.48

Source: Kent (1983)

2.2.4.2 Egg (whole egg)

Eggs are used in baked foods for several important functional properties, as an ingredient, such as binding, leavening, tenderizing, volume, texture, stabilization(for firmness and elasticity), emulsification (for texture and consistency), foaming(for formation of air cells in batters),coagulation(heat setting and structure forming) flavor, color and food/nutritional value (Geera *et al.*, 2011). Eggs provide natural yellow hue, and contribution to Maillard browning reaction(Grizio and Specht, 2016). Egg acts as a moistening agent which and is important for consistency and texture (Feeney, 1964; Julianti *et al.*, 2016) Some, if not all, of these functional attributes of egg originate from its unique composition. As a natural biological product, egg possesses a unique composition of proteins, lipids and other nutrients that are not only important in providing functionalities in food processing, but also make it nutritionally “wholesome” (Seuss-baum, 2007).

Upon baking, the protein in egg white coagulates to provide structure. Adding egg whites to muffin batter provides structure to the finished product and produces a muffin that is easily broken without excessive crumbling. Substituting egg whites for whole eggs, however, will result in a dry, tough muffin unless the formula is adjusted to increase the amount of fat. Fat in the yolk acts as an emulsifier and contributes to mouth-feel and keeping qualities (Cross, 2007).

Table 2.8 Nutritional composition of fresh chicken egg (per 100 g)*

Parameter	whole egg	yolk	white
energy (kcal)	149	358	50
water (g)	75.33	48.81	87.81
protein (g)	12.49	16.76	10.52
fat (g)	10.02	30.87	0
cholesterol (mg)	425	1,281	_
carbohydrate (g)	1.22	1.78	1.03
vitamin A (IU)	635	1,945	_
riboflavin (mg)	0.508	0.639	0.452
calcium (mg)	49	137	6
phosphorus (mg)	178	488	13

*100 g is approximately equal to two large whole eggs

Source: Agriculture (1985)

2.2.4.3 Fat

Fat is one of the most important ingredients. It is the primary enriching agents used in the flour confectionary and provides the most concentrated source of energy of any food stuff. Fat increases the palatability and also modifies the dough characteristics. One of the most important function of fat is to shorten baked goods which otherwise might be solid masses firmly held together by strand of gluten. Being insoluble in water, fat prevents cohesion of gluten strands during mixing. Fat must be digestible nature and for this reason only vegetable and animal fat are suitable. (Renzyaeva, 2013)

The important functional properties of fat from baking point of view are as follows:

a. Shortening: it prevents the formation of tough gluten structure, producing the quality known as shortness.

b. Creaming: The creaming ability of fat, that is, its ability to entrap air, is a very important factor in the production of good volume and texture in the muffin.

c. Layering: The typical structure of muffin and similar baked products is dependent on the formation of layers of dough separated by layers of fat. The fat for this purpose must have good plasticity over fair temperature range so that there is no tendency for it to be absorbed by the dough causing shortness, or to have such a high melting point as to be difficult to and unpleasant to the palate.

d. Emulsifying: Muffin butter is an emulsion of a fatty phase and a phase composed of the remaining ingredients. The emulsifying powder of a fat determines how much liquid can be incorporated in a batter without curdling taking place. The more liquid which can be added to a muffin batter, the more sugar will be able to hold dissolved in the liquid. (Renzyaeva, 2013)

2.2.4.4 Sugar

Cane sugar is used for sweetening and characteristics on dough. During muffin making various forms of sugar namely crystalline, pulverized, liquid, brown or soft sugar are used as per product requirement. Generally most commonly used form of sugar in muffin making is 18ulverized sugar. Sugar contributes tenderness, crust color, and moisture retention in addition to a sweet taste. Sucrose promotes tenderness by inhibiting hydration of flour proteins and starch gelatinization. Sugar is hygroscopic (attracts water) and maintains freshness (Cross, 2007). Chemical changes in sugars during baking contribute characteristic flavors and browning. Caramelization of sugar is responsible for the brown crust of muffins. Caramelization involves dehydration and polymerization (condensation) of sucrose. (McWilliams, 2016b)

2.2.4.5 Leavening agent

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).

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

The chemical reaction during use of chemical leaveners and acidulants is as given in this section.

$$NH_4HCO_3 \xrightarrow{\text{Heat}} NH_3 + CO_2 + H_2O$$

Ammonium bicarbonate | Ammonia | Carbon dioxide | Water

$$(NH_4)2CO_3 \longrightarrow 2NH_3 + CO_2 + H_2O$$

Ammonium carbonate

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

$$2NaHCO_3 + C_4H_5O_6K \longrightarrow C_4H_4O_6NaK + 2CO_2 + 2H_2O$$

Baking soda | Tartaric acid | Sodium tartarate

Sodium carbonate is a product of an incomplete reaction in formulas with excess sodium bicarbonate. Excess sodium carbonate results in a muffin with a soapy, bitter flavor and a yellow color because of the effect of an alkaline medium on the anthoxanthin pigments of flour. Also, formulas with too much baking powder or soda result in a muffin with a coarse texture and low volume because of an overexpansion of gas, which causes the cell structure to weaken and collapse during baking. Inadequate amounts of baking powder will result in a compact muffin with low volume (McWilliams, 2016b).

2.2.4.6 Milk powder

Milk powder is added to dry ingredients, and water or fruit juice is used for liquid in muffin formulas. Milk powder binds flour protein to provide strength, body, and resilience – qualities that are helpful in reducing damage during packing and shipping. In addition, milk powder adds flavor and retains moisture. The aldehyde group in lactose in milk combines with the amino group in protein upon heating, contributing to Maillard browning (Willyard, 2000).

2.2.5 Baking profile

Baking is the major step of muffin 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 muffin. The main objective of baking is to remove the excess moisture present in the dough by gradual heating (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.

1. A heat source

2. A base (sole or hearth), capable of being heated, on which the dough piece is placed.

3. A cover over the base, making up a chamber in which to retain the heat.

Muffin baking is considered a simultaneous heat and mass transfer process, characterized by a rapid increase of core temperature and the development of a dry surface crust. Also, the increase of internal temperature is associated to several chemical reactions and physical phenomena, which are responsible for the transformation of the cake batter into crumb and the product volume expansion (Ureta *et al.*, 2013)

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

1. Formation of a film crust on the dough.

2. Melting of the fat in the dough.

3. Gas release and volume expansion.

4. Conversion of water into steam.

5. Escape of carbon dioxide, other gases and steam.

6. Chemical changes include:

7. Gas formation

8. Starch gelatinization

9. Protein changes

10. Caramelization of sugar

11. Dextrinization

2.3 Egg replacement

Egg replacement involves eliminating eggs partially or totally from a food formula and replacing them with ingredients that can offer similar performance (Feeney, 1964). Enhancing the functionality of egg replacers can be applied in some situations especially where this egg alternatives give better functionality than eggs. For instance, some egg replacers giving better in foaming abilities, binding properties, and enhancing the flavor. Meanwhile, as for nutrition and health, plant based ingredients can contribute or replace in some components that is healthier for example this egg replacers can replace the unhealthy component that egg provide such as cholesterol. Egg replacers also helps in sustainability which, when eggs were laid, more manure is produced that ammonia within it will lead to some pollution such as water pollution (Grizio and Specht, 2016).

2.3.1 Egg replacer

Many food and bakery systems can use egg replacement ingredients such as cakes, cookies, muffins, pie fillings, icings, etc. Commercially-available egg replacers are classified into three main categories: proteins, polysaccharides and emulsifiers (Feeney, 1964):

Table 2.9 Categorization of egg replacers

Component	Functionality	Challenges
Proteins (whey, soy, wheat, pea, chickpea)	• Foam structure • Elasticity • Firmness • Water binding	• Potential allergenicity • Flavor issues (bitterness) • Volume reduction • Emulsifier required
Polysaccharides /gums (xanthan, guar)	• Structure • Thickening	• Development of very high viscosity at low shear rates. • Tendency to aggregate and fall out of solution/dispersion • Good thermostability
Emulsifiers (lecithin, sucrose esters)	• Emulsification and binding	• Potential allergenicity • Not enough functionality

Source: Feeney (1964)

New plant based alternatives or their combinations have been used to effectively replace eggs in certain baked goods including (Grizio and Specht, 2016):

- Chia seed gel: Chia seed gel, when used at 25% level, provides similar emulsifying capacities to eggs (Borneo *et al.*, 2010).
- Natural colors: Lycopene, annatto, turmeric and paprika extracts can be used to substitute the yellow hue provided by egg yolk.
- Flaxseed gel: provides humectancy and binding capacity. Typically 1 egg is substituted by 1 tablespoon of flaxseed meal with 3 tablespoons of water. Uhlman and Schumacher (2014) found that acceptable pumpkin bars can be achieved by the use of ground flaxseed.
- Fruit purees: Banana puree and applesauce provide some biding and humectancy capacity.
- Vegetable oils: used to replace egg wash to provide glow to baked goods.
- Algal flours: provide emulsifying and humectancy capacity due to the presence of phospholipids and starch.

Consideration when using egg substitutes (Grizio and Specht, 2016):

- A combination of several substitutes yield better results than one single ingredient to perform all of the egg's functions.
- Full egg replacement should be implemented when producing baked goods for egg-allergic consumers.
- Replacement level should be carefully designed when using substitutes with strong flavor that may change baked goods flavor profile.

Part III

Materials and methods

3.1 Materials

3.2.1 Raw materials

Wheat flour in the form of maida used for muffin making was obtained from local market of Dharan. Flaxseed grown in Lahan, Nepal was collected from the local market of Dharan. The other raw materials such as sugar, butter, milk powder, egg, baking powder was purchased from supermarket of Dharan.

3.1.3 Glassware, equipment and chemicals

All glassware, equipment and chemicals were used from the laboratory of Central Campus of Technology, Hattisar, Dharan.

3.2 Method

3.2.1 Analysis of flaxseed

Flaxseed

↓

Winnowing and handpicking

↓

Clean flaxseed

↓

Physical, proximate and nutritional analysis of each sample

Methanolic extraction for each sample(50 ml)

↓

Estimation of total phenol, antioxidant activity, Phytic acid and cyanogenic glycosides

Fig 3.1 Steps in analysis of flaxseed

Source: El and Karakaya (2004)

3.2.1.1 Physical characteristics of flaxseed varieties

The parameter studied under physical characteristics includes seed color, length, breadth, thickness, porosity, true density, bulk density, sphericity and thousand kernel weight were analyzed in triplication.

Seed Color

Seed color was observed by using horticultural color charts through visual observation.

Seed Length (mm) and breadth (mm)

Determined by using Vernier calipers holding the single grain length wise and breadth wise respectively.

Sphericity

It was determined by measuring length (a), breadth (b) and thickness(c) and calculation was done as:

Sphericity= $(abc)^{1/3} \div a$

1000 kernel weight

Thousand seeds in three replications was weighed in electronic weighing balance; the mean weight of 1000 seeds will be expressed in grams.

3.2.1.2 Functional properties

Bulk Density (g/ml)

The bulk density of the sample was calculated and the results were expressed as g/ml.

True density (g/ml)

True density obtained by measuring the displaced volume of water by known weight of grain sample.

True density = (weight of sample/ volume of water displaced)

Water and Oil Absorption Capacity (g/g)

The determination of water and oil absorption capacities were carried out according to method described by Sosulski *et al.* (1976). After mixing 10 ml distilled water or oil with 1g flaxseed flour, the contents were allowed to rest at 30±2°C for 30 min and then centrifuged at 200g for 30 minutes and finally the water and oil absorption capacities of the flour were expressed as grams of water or oil absorbed by 1g of flaxseed flour.

Determination of porosity of seed

Porosity, (%) indicates the amount of pores in the bulk material and was calculated as per (Mohsenin, 1980). The porosity of the seed was calculated from the average values of bulk density and true density using the relationship.

Porosity (%) = (1-Bulk density/True density) ×100%

3.2.2 Preparation of flaxseed egg replacer

The flaxseed egg replacer was prepared by grinding the clean whole flaxseed in fine particle in grinder, sieved in sieve of mesh size 60 and then soaked in room temperature water for 25 min in ratio 1:3 (Uhlman and Schumacher, 2014).

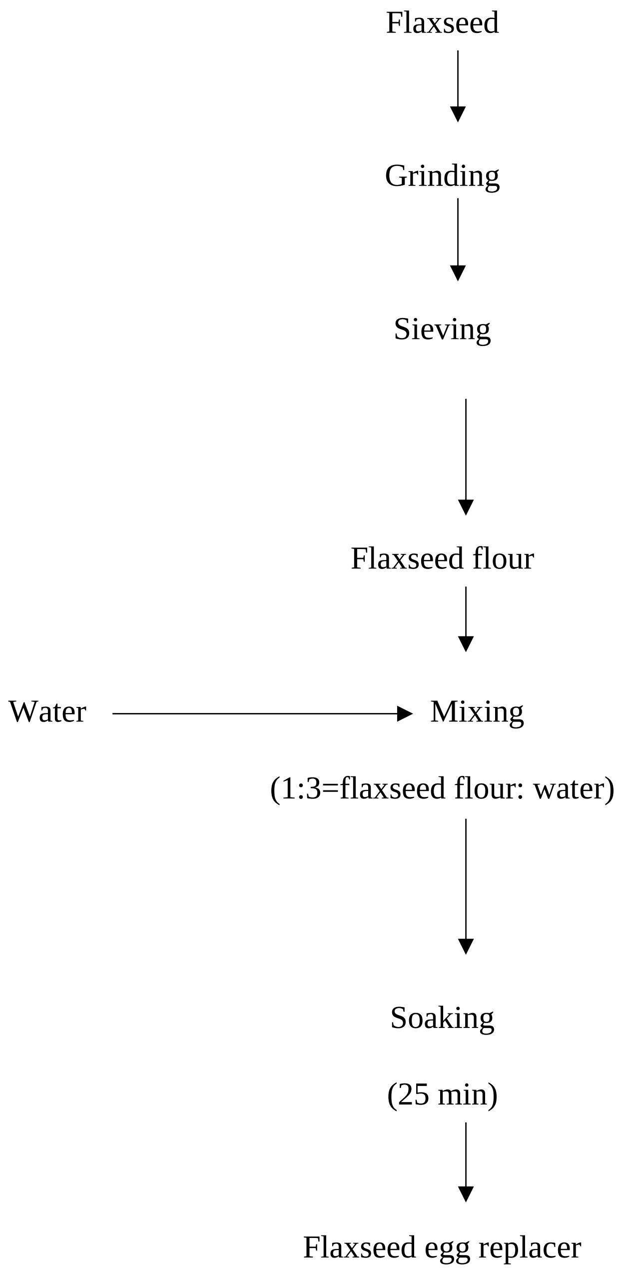

Fig 3.2 Steps in preparation of flaxseed egg replacer

Source: Uhlman and Schumacher (2014)

3.2.3 Preparation of Muffin

Muffin was prepared by the single stage mixing method as described by Mishra, (1991). The ingredient used was as follows:

Flour blend: 100 g

Fat: 105 g

Sugar: 82.5 g

Egg: 112.5 g

Milk powder: 37.5 g

Baking powder: 1.875 g

By keeping all ingredients constant, egg proportion was varied according to design of experiment version 13 (DOE) shown in table 3.1.

Table 3.1 Proportion of flaxseed egg incorporated with egg

Sample	Egg (%)	Flaxseed egg replacer (%)
A	100	0
B	75	25
C	50	50
D	25	75
E	0	100

3.2.4 Method for preparation of Muffin

There are two primary methods for mixing muffins – the cake method and the muffin method. However, the cake method was used in the study. The cake method involves creaming sugar and shortening together, followed by addition of liquid ingredients(milk and eggs) and the final addition of dry ingredients (flour, salt, baking soda) (Cross, 2007). For the flaxseed muffin, the flaxseed was cleaned, grinded by electric grinder and then soaked

25 min prior to mixing. The batter was filled in paper muffin cup and baked at 215ºC in oven for 20±3 min (Khouryieh *et al.*, 2005)

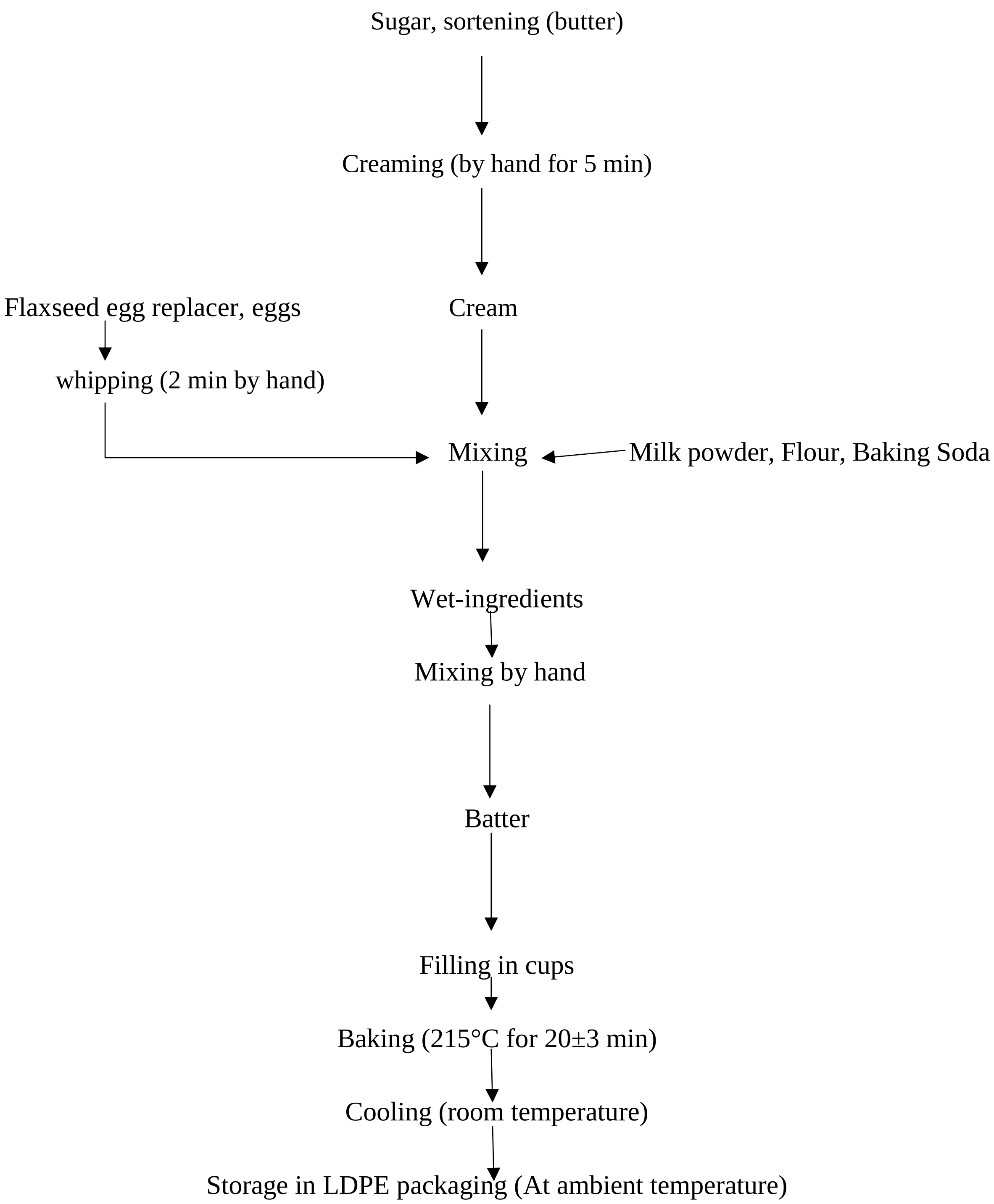

Fig 3.3 Flow-chart for the preparation of the muffin with egg and egg replacer (flaxseed)

Source: Cross (2007)

Mixing

The main objective of mixing is to blend all ingredients together to form a homogenous mass. Therefore, mixing is the most important unit operation of baking industry. Proper mixing equipment and mixing method play a vital role in good quality production.

Baking

The main objective of baking is to cook and remove the extra moisture present in the batter by heating to optimum degree. Within the oven various physical and chemical changes occur. Physical changes-:

1. Crust formation on top
2. Melting of fat in dough
3. Conversion of water to steam
4. Escape of CO_2 and other gases.

Chemical changes-:

1. Gas formation
2. Starch gelatinization
3. Protein changes
4. Caramelization of sugar

Cooling

Muffin leaving the oven is cooled to room temperature (21ºC) before packing. On cooling, sugar in the muffin imparts strength and stiffness to the product.

De-panning

The cooled muffin was de-panned and packed for further analysis.

3.2.5 Analysis of Muffin

3.2.5.1 Physical properties

Muffin Height

The muffin product was taken out from the paper baking case, and the muffin height will be measured as the vertical distance from the bottom to the top(highest point) of the muffin center using vernier calipers (Lee *et al.*, 2020).

Muffin Volume

The volume of the muffins was measured by the rapeseed displacement method. Each muffin was placed in a plastic beaker of known volume (total volume, Vt), the remaining space in the plastic beaker will be then filled with rapeseed; the volume of the rapeseed required (Vs) will be then determined by graduated cylinder. Muffin volume was calculated as the difference between the total volume and volume of rapeseed, the muffin volume = Vt – Vs (Lin *et al.*, 2006).

Muffin density

It was calculated as the ratio of weight of muffin to volume of muffin (Hera *et al.*, 2012).

Baking loss

Baking loss is the weight loss (WL%) during baking was calculated by using following equation (Rodríguez García *et al.*, 2013):

$$WL(\%) = (W_{batter} – W_{muffin}/W_{batter}) \times 100$$

Where, W denotes weight in g.

3.2.5.2 Physiochemical analysis of muffin

Moisture content

Moisture content was determined by using a hot air oven as per described by Ranganna (1986). It was calculated using following formula:

$$\%\ \text{Moisture content} = \frac{\text{loss in weight}}{\text{weight in sample}} \times 100$$

Crude fat

Crude fat was determined by soxhlet extraction method as per described by Ranganna (1986). The percent of crude fat was expressed as follows:

$$\%\text{Crude Fat} = \frac{\text{weight of oil}}{\text{weight of sample}} \times 100$$

Crude protein

Crude protein was determined by micro Kjeldhal method. The procedure was followed as described by Ranganna (1986). The percentage of nitrogen and protein was calculated by the following equation:

$$\%\ \text{Nitrogen} = \frac{\text{TS-TB} \times \text{Normality of acid} \times 0.014 \times \text{Dilution factor}}{\text{Aliquot taken} \times \text{weight of sample}} \times 100$$

Where, TS= Titre volume of the sample(ml), TB= Titre volume of blank(ml), 0.014=M.eq. of N

% Protein= Nitrogen × 6.25

Crude fiber

Crude fiber was determined by using chemical process, sample was treated with boiling dilute sulphuric acid, boiling sodium hydroxide and then with alcohol as standard method described in Ranganna (1986).

Carbohydrate

Total carbohydrate content was determined as total carbohydrate by difference, calculated by substracting the measured protein, fat, ash, fiber in dry basis as per Ranganna (1986).

Ash content

Ash content was determined by following the method given by Ranganna (1986) using muffle furnace. Drying the sample at 100°C and churned over an electric heater. It was then ashed in muffle furnance at 550°C for 5 hrs. It was calculated using the following formula:

$$\% \text{ Ash content} = \frac{\text{Wt. of ash}}{\text{Initial wt. of sample}} \times 100$$

Calcium

Calcium content was determined by following the method given by Ranganna (1986). Calcium was precipated as calcium oxalate with ammonium oxalate. The precipate was washed with ammonia to remove the chloride ions. The washed precipate was then made to react with 1N sulphuric acid. The liberated oxalic acid was now estimated by titrating against standardised potassium permanganate. The amount of oxalic acid liberated s proportional to the amount of calcium.

Iron

Iron content was determined by following the method given by Ranganna (1986). The iron was determined by spectrophotometer at 580nm.

Energy value

Energy computed as followed for all the samples.

Energy (kcal) = [Protein (g) x 4] + [Carbohydrate (g) x 4] + [Fat (g) x 9]

3.2.1.4 Phytochemicals/anti-nutrients in flaxseed and muffin

Anti-nutrients like cyanogenic glycosides and phytic acid was analyzed along with Antioxidant property and polyphenol content was estimated.

Preperation of methanolic extract of the samples

1 g of each sample of flaxseed was ground with 30 ml of methanol in mortar and pestle for homogenization. After recovery of the homogenate, 15 ml methanol was used to wash the mortar and pestle and then pooled with the first homogenate. The mixture was refrigerated for half an hour and allowed to centrifuge at 4,500 rpm for 15 min at room temperature (27°C). Supernatants obtained by filtered using Whatman filter paper was made volume up to 50 ml with methanol (El and Karakaya, 2004).

Phytic acid

Phytic acid was determined by following the method given by Sadasivam and Manickam, 1991).

Cyanogenic glycosides

5 g of weighed and prepared sample was taken in clean mortar and pestle. Then extraction was carried out in 50 ml distilled water by grinding it homogenously for 1 to 2 min. The extract was filtered through a moderately retentive filter paper Whatman No. 42. The filtrate was taken and 5 ml of this aliquot was transferred to a clean test tube followed by the addition of 10 ml of alkaline picrate and 5 ml of distilled water. This was sample solution. Similarly, blank was made with each set of samples containing 0 ml of sample filtrate, 10 ml of alkaline picrate and 5 ml of distilled water. The test tubes containing sample and color reagents were incubated for 15 min in water bath at 37°C. Then before reading were added 15 μl of sulfuric acid to stop the reaction and increase the stability of reading. All the sample and blank was shaken well and the absorbance was immediately read at 540 nm (Brito *et al*., 1998).

HCN content (ppm or mg/kg) = (Y×50×1000)/ (aliquot taken×sample weight taken)

Where, Y = 0.2476X + 0.009 (R2 = 0.9536)

X = absorbance

Total Polyphenols

Total polyphenols was determined by following the method given by Sadasivam and Manickam, 1991).

Antioxidant Activity by DPPH Method

Antioxidant Activity was measured by DPPH free radical scavenging method. The DPPH radical absorbs at 517 nm and the antioxidant activity can be determined by monitoring the decrease in this absorbance (Singh *et al*., 2008a).

Reagent preparation: 0.1 mM DPPH solution was prepared by dissolving 4 mg of DPPH in 100ml of ethanol.

The sample was reacted with the stable DPPH radical in an ethanol solution. Freshly prepared sample of 0.5 ml, 4ml of ethanolic solution of DPPH was added. After incubation in the dark for 30 min, the OD of the solution was read spectrophotometrically at 517nm. The OD of DPPH solution without sample addition was read. The difference in OD of DPPH solution and DPPH solution + sample was calculated. The decrease in OD with sample addition was used for calculation of the antioxidant activity. Finally, percentage scavenging activity was determined using following equation:

[DPPH scavenging activity (%) = (Ac-At)/Ac×100], where Ac is the absorbance of control sample, and at is the absorbance of test sample (Singh *et al.*, 2008a).

3.2.6 Determination of peroxide value

Peroxide value was determined based on the method described by KC and Rai (2007). 5 g of extract incorporated ground meat sample was weighed accurately (by difference) in the Iodine flask. 25 ml of solvent was added. 1 ml of KI solution was added and allowed and to stand for 1 min (with gentle shaking). 35 ml of distilled water was added and few drops of starch indicator was added. Appearance of blue color on addition of starch indicates presence of free iodine. Liberated iodine was titrated with 0.01N or 0.1N sodium thiosulphate until the blue color vanished. Blank determination was carried out simultaneously. Peroxide value was calculated using the following equation,

$$\text{PV (meq/kg)} = \frac{\text{N}\times(\text{Vs-Vb})\times1000}{\text{Wt. of sample (g)}}$$

Where,

N = normality of sod-thiosulfate

V_S = sod-thiosulfate consumed by sample (ml)

V_B= sodium-thiosulfate consumed by blank (ml).

3.2.7 Determination of acid value

0.1 -0.3 g of fat sample was 100 ml Erlenmeyer flask. 10 ml of n-Hexane and 1-2 drops of indicator was added. The solution titrated was against 0.02N KOH solution. The end point was reached when pink (phenolphthalein) or blue (thymolphthalein) color persists for 30 seconds. A blank test was carried out without using fat sample. The acid value was determined using following formula (Kim, 2022):

Acid value (mg/g) = 56.11 x 0.02 x (Vs — Vb) x F W

Where,

Vs = titration volume of sample (ml)

Vb = titration volume of blank (ml);

W = weight of fat in the volume of extract usd (g);

F = factor of 0.02 KOH solution,

Where,

F = 5 Vf: Vf is the volume of 0.02N KOH required to neutralize 5 ml of the 0.02N H_2SO_4 solution.

56.11 = Molecular weight of KOH

0.02 = Concentration of KOH

3.2.6 Sensory evaluation

The laboratory prepared samples were evaluated for aroma, taste, texture, color sponginess and overall acceptability on a 9-point hedonic rating scale by semi-trained panelist (include teachers and research students) of Central Campus of Technology, Dharan. The sample was given to the panel member with the evaluation card containing value from 1 to 9 where 9 indicates the like extremely whereas 1 indicate the dislike extremely. They were told to give the score from 9 to 1 according to their acceptance of the product based on color, flavor, taste, texture, sponginess and overall acceptability.

3.2.7 Statistical analysis

For all chemical analysis three replicates of the same sample was used for the determination of each constituent. Mean values with standard deviations was computed. The raw data were subjected to analysis of variance and read at 0.95 confidence level using statistical software

GenStat (12th edition) developed by VSN International Limited. Fisher's least significance differences (LSD) test was used to define differences between means at the 5% significance level ($p<0.05$).

3.2.8 Microbiological analysis

Total Plate Count (TPC) was determined by pour plate technique on Plate Count Agar (PCA) medium (incubated at 30°C/48 h). Coliform count was determined by pour plate technique on MacConkey medium (incubated at 37°C/48 hr) (AOAC, 2005)

3.2.9 Storage stability of muffin

Acceptability period of the product was determined by acid value, peroxide value of the extracted fat and moisture content of the muffin. The analysis was carried out for 6 days.

3.2.10 Cost calculation of muffin

The total cost associated with the best product was calculated including overhead cost (processing and labor cost) and profit of 10%.

Part IV

Results and discussions

Flaxseed grown in the Lahan, Nepal, was collected for analysis of nutritional components and anti-nutritional factors. This flaxseed was then grinded and soaked for 10 min which was then substituted in the muffin at different ratios according to the recipe of Design expert version 13. The five products each with substitution of 0% (A), 25% (B), 50% (C), 75% (D) and 100% (E) were obtained. Finally, the effect and changes occurred in sensory analysis, physical characteristics and physiochemical properties of muffin were studied. The acceptability period of best product was calculated.

4.1 Physical and Functional properties of flaxseed varieties

Physical and functional properties of flaxseed were analyzed. Under physical properties seed length, seed breadth, seed size (length/breadth ratio) and 1000 seeds weight and under functional properties bulk density, true density, porosity, water and oil absorption capacities were analyzed. The results are presented in the Table 4.1.

Table 4.1 Physical and functional properties of flaxseed

Parameters	Flaxseed
Length (mm)	4.5±0.01
Breadth (mm)	3.4±0.02
Thickness (mm)	1.7±0.1
Sphericity (%)	64.84±0.01
Bulk density g/cm3	0.77±0.01
True density g/cm3	1.24±0.24
Porosity (%)	18.54±0.11
1000 kernel weight (g)	7.1±0.2
Oil absorption capacity (g/g)	1.2±0.3
Water absorption capacity (g/g)	1.51 ±0.25

[The values in the table are the means of triplicates. Figures in the parentheses are the standard deviation.]

4.1.1 Seed color

The seed color of selected variety was found to be dark brown 166A RHS.

4.2 Chemical composition of Flaxseed

The chemical composition of flaxseed was analyzed and the data are as shown in table 4.2

The moisture content of raw flaxseed was determined by weight loss during heating in hot air oven and was found to be 6.23±0.12% (Table 4.2). In a study, the moisture content of flaxseed was found to be 6.5% (Morris, 2007). The protein content of flaxseed was determined to be 21.08±0.06% by Kjeldahl method. In the study, the protein content of the raw flaxseed was found to be 17.96% (Kajla *et al.*, 2015). The fat content was determined to be 40.85±0.15% by using Soxhlet extraction method. In the study, the fat content of raw flaxseed was found to be 40.3%.It is higher than then value of fat content of flaxseed give in Food composition table given by DFTQC i.e., 37.7%. Hussain and Oulabi (2009) reported 38.76g fat per 100g of flaxseed. The ash content was determined to be 2.7±0.08% in raw flaxseed. It is similar to ash content 2.4% given in the Food Composition Table for Nepal by DFTQC. In the present study, the crude fiber content in raw flaxseed was found to be 8.1±0.1%, which is lower than the fiber content 11.09 % in a study by (Kajla *et al.*, 2015). Carbohydrate and energy were computed using the formula, in this present study Carbohydrate and energy value for raw flaxseed 27.27 and 557.9 Cal/100gm.

In present study, the calcium content of the raw flaxseed was found to be 232.5±0.4 mg/ 100 g. The obtained calcium content is much higher than the one given in the Food Composition Table for Nepal given by DFTQC, in which the calcium content is 170 mg/100 g. In a similar study of nutritional composition of three selected varieties of flaxseeds, the calcium content ranged from 223 to 240 mg/100 g (Hiremath, 2013). The iron content in this study was found to be 7.21±0.08 mg per 100 g in raw sample of flaxseed. In a study, the iron content of raw sample of flaxseed was found to be 6.10 mg/ 100 g. In a study, the iron content of raw sample of flaxseed was found to be 6.10 mg/ 100 g.

Table 4.2 Chemical composition of Flaxseed

Parameter (%) (db)	Raw flaxseed
Moisture content	6.23±0.12
Protein	21.08±0.06
Crude fat	40.85±0.15
Ash	2.7±0.08
Crude fiber	8.1±0.1
CHO	27.27±0.07
Energy value(Cal/100gm)	536.11±0.09
Calcium(mg/100gm)	232.5±0.4
Iron (mg/100gm)	7.21±0.08

* The values in the table are the means of triplicates. Figures in the parentheses are the standard deviation

4.3 Phytochemicals / anti-nutrients in Flaxseed

4.3.1 Phytic acid

The data in table 4.3 shows that the phytic acid was found to be 26.2±0.66. The contents of phytic acid were significantly different among cultivars. AC Linora has a lowest phytic acid content of 2280 mg/100 g and low ALA yellow-seeded cultivar Linola 947 has the highest content (3250 mg/100 g seed) among the eight cultivars reported (Mazza and Oomah, 1997). Phytic acid interferes with the absorption of minerals and act as strong chelator, forming protein and mineral-phytic acid complexes and thus reducing their bioavailability (Akande *et al.*, 2010).

Table 4.3 Phytochemicals / anti-nutrients in flaxseed

Parameter (db)	Flaxseed
Phytic acid (g/kg)	26.2±0.66
Cyanogenic glycosides (mg/Kg)	880±1.83
TPC (mg GAE/100 gm)	536.22±0.82
Antioxidant activity (%)	46

* The values in the table are the means of triplicates. Figures in the parentheses are the standard deviation

4.3.2 Cyanogenic glycoside

Hydrocyanic acid content in flaxseed powder of raw was found to be 880±1.83 mg/kg as shown in table 4.3. In a study, whole flaxseed contains 250–550 mg/100 g cyanogenic glycoside. In a study, cyanogenic glycosides are the major anti-nutrients and are fractionated into linustatin (213–352 mg/100 g), neolinustatin (91–203 mg/100 g), linmarin (32 mg/100 g). The content of these three glycosides depend upon cultivar, location etc (Mazza and Oomah, 1997)

4.3.3 Total polyphenol

This study shows that the total phenol content was found to be 536.22±0.82 (Table 4.3). In a study, it was found that the total polyphenol content in selected flaxseed varieties ranged from 440.00 to 536.33 mg GAE/100 g (Hiremath, 2013).

4.3.4 Antioxidants

The present study found that the antioxidant activity of raw flaxseed was found to be 46%(Table 4.3).On the study of raw flax seed Linum usitatissimum was 80.32 ± 0.12 in JL-27 variety (Kajla *et al.*, 2015)

4.4 Physical analysis of muffin

The physical analysis investigated on the formulated muffins are muffin volume, muffin height, baking loss and muffin density as shown in Table 4.4. These analyses are of great importance since they determine the quality of baked products prepared.

4.4.1 Muffin volume and density

The increasing substitution of flaxseed egg replacer in muffin decreases the muffin volume, which ranged from 24.67±0.47cm^3 to 15±0.81 cm^3 in A and E muffin. The density however being inversely proportional, E muffin had greatest density. This means that product is less aerated and dense.

4.4.2 Muffin height

Muffin height of A was highest which decreases with the increase of proportion of flaxseed egg replacer in the formulation. The height of A was 22.79±0.021mm and ranges to 20.12±0.02mm in E. In a similar study, the muffin height decreased from 4.133±0.25cm to1.300±010 cm in a complete substitution of eggs by soaked ground flaxseed (Kostor *et al.*, 2022).

4.4.3 Baking loss

In this study, the baking loss decreases with the partial substitution of flaxseed egg replacer which is due to the water binding capacity of the flaxseed gum as shown in table 4.

Table 4.4 Physical characteristics of flaxseed egg replacer substituted muffin

Parameters	Muffin volume(cm^3)	Muffin density(g/cm^3)	Muffin height(mm)	Baking loss(%)
A	24.67 ± 0.47^a	0.42 ± 0.003^d	22.79 ± 0.021^a	13.43 ± 0.04^a
B	23.33 ± 0.47^a	0.44 ± 0.004^d	22.5 ± 0.045^b	13.39 ± 0.01^a
C	18.7 ± 0.47^b	0.55 ± 0.004^c	21.68 ± 0.02^c	13.12 ± 0.07^b
D	17.33 ± 0.94^b	0.61 ± 0.004^b	20.91 ± 0.086^d	12.84 ± 0.03^c
E	15 ± 0.81^c	0.71 ± 0.008^a	20.12 ± 0.02^e	12.41 ± 0.06^d

* The values in the table are the means of triplicates. Figures in the parentheses are the standard deviation

4.5 Sensory evaluation

The muffin prepared from using different proportions of flaxseed egg replacer and egg was subjected to sensory evaluation. The different muffin with different proportions were coded as A, B, C, D and E. The coded samples were provided to 10 semi trained panelists. They were asked to score the experimental muffin for appearance, taste, texture, color and overall acceptability as in the score sheet given in appendix B.1. Best muffin was selected statistically at 5% level of significance.

4.5.1 Color

The mean sensory score for color were found to be 7.8±0.4, 8.4±0.48, 7.1±0.3, 6 and 5.2±0.4 on 9-point hedonic rating scale for the muffin formulation A, B, C, D and E respectively. ANOVA at 5% level of significance showed that the partial substitution of egg with flaxseed egg replacer had significant effect ($p\leq0.05$) on the color of the different muffin formulations which are represented in figure 4.1.

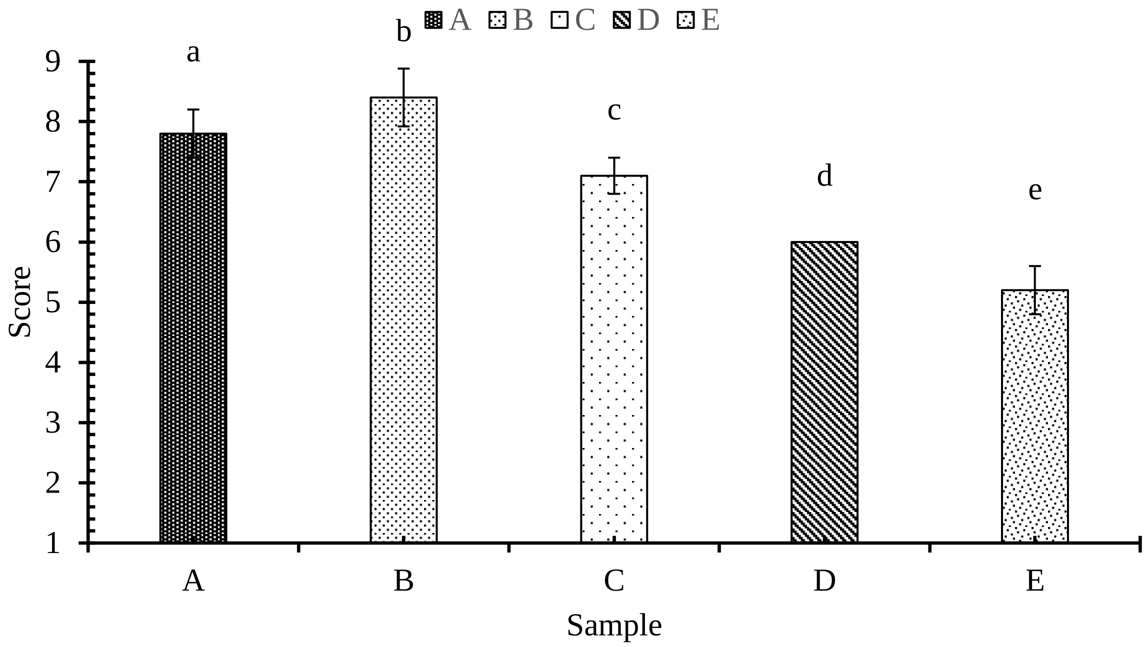

Fig 4.1 Mean sensory scores for color of flaxseed egg replacer substituted muffins of different formulations. Bars with similar alphabets at the top are not significantly different.

Sample E got lowest score which was significantly different with sample A, B, C and D. The decrease in the score with the incorporation of the flaxseed can be seen which may be due to the darker color of muffin samples. Color in baked goods comes from two sources: intrinsic color imparted by individual ingredients and developed color resulting from interaction of ingredients (Acosta and Cavender, 2011). Millard browning results from interactions of free amino groups with reducing sugars, and when compared with amylose, amylopectin has more reducing ends (Zanoni *et al.*, 1995).The cause of dark color may be due to the dark brown color of the flaxseed. The result is in accordance with the (Ahmad *et al.*, 2021).

4.5.2 Texture

The mean sensory score plus minus for texture were found to be 8.1±0.3, 8.1±0.3, 7.9±0.3 ,7.7±0.45 and 7.7±0.44 on a 9-point hedonic rating scale for the muffin formulation A, B, C, D and respectively which are shown in appendix table C.1.

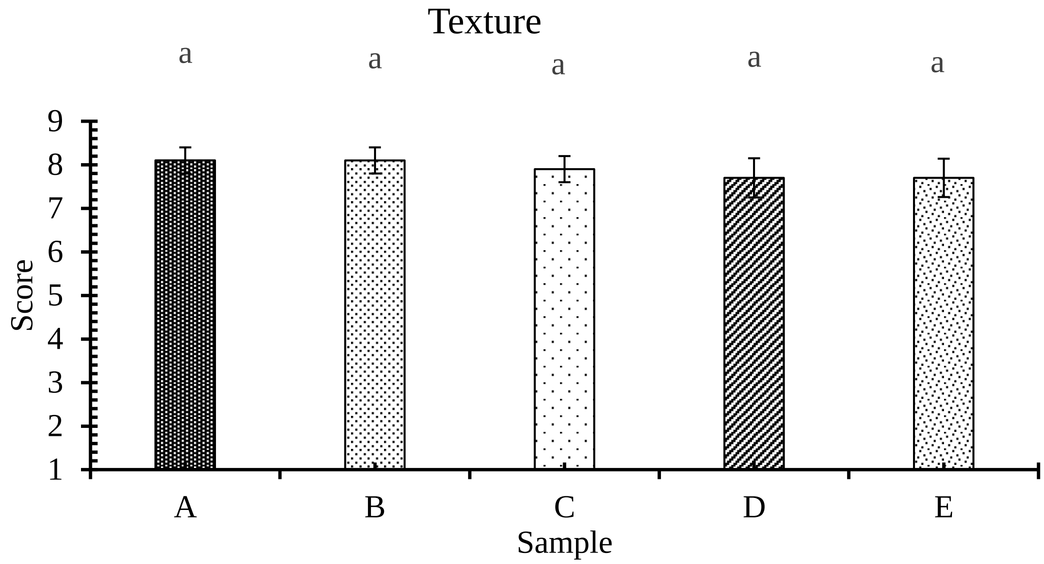

Fig 4.2 Mean sensory scores for texture of flaxseed substituted muffins of different formulations. Bars with similar alphabets at the top are not significantly different.

ANOVA at 5% level of significance showed that the partial substitution of the flaxseed in muffin had no significant effect ($p>0.05$) on texture of different muffin formulation as shown in fig 4.2. Similar result were obtain in the study where texture was found to be similar. This may be due to the functional characteristics of the flaxseed mucilage which gives texture to the muffin as egg. In a similar study, the texture of muffins were not significantly different for egg and flaxseed muffin(Ahmad *et al.*, 2021).

4.5.3 Sponginess

The mean sensory score for sponginess were found to be 8.1±0.3, 8.6±0.8, 8±0.15, 7.8±0.4 and 7.4±0.8 on a 9-point hedonic rating scale for the muffin formulation A, B, C, D and E respectively. ANOVA at 5% level of significance showed that the partial substitution of flaxseed had significant effect ($p\leq0.05$) on aroma of different muffin formulation. Product A and B were not significantly different as shown in figure 4.3. Product C and D were not significantly different and were related to product A, B and E. However, product E were significantly different to all other product. The sponginess of A (25% flaxseed egg replacer) and B (100% egg) were found to be significantly superior. Kostor *et al.* (2022) found that flaxseed muffin had lower springiness as compared to control muffin which may be due to lower no of air bubbles incorporated into muffins.

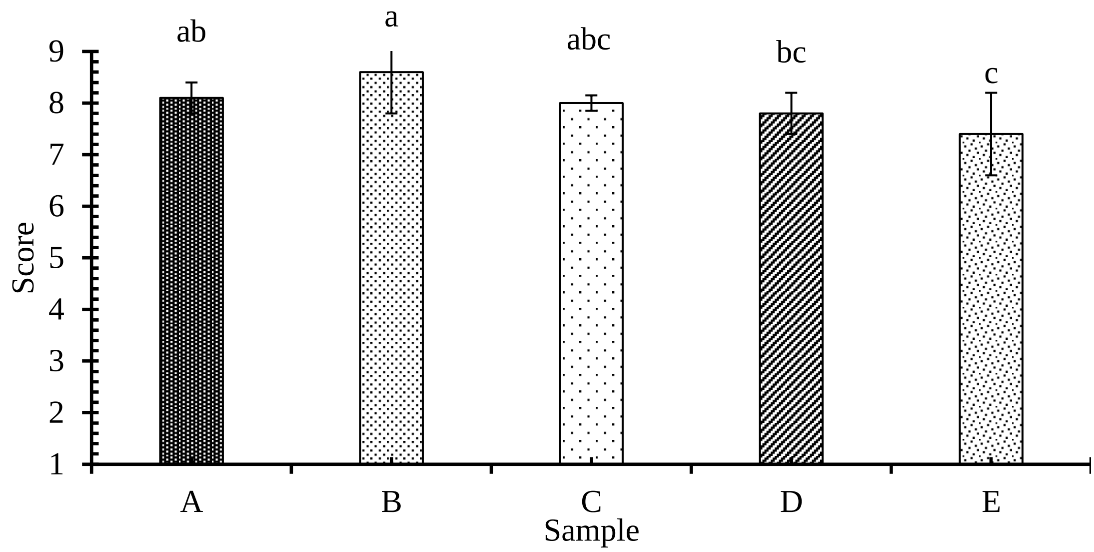

Fig 4.3 Mean sensory scores for sponginess of flaxseed substituted muffins of different formulations. Bars with similar alphabets at the top are not significantly different.

4.5.4 Taste

The mean sensory score for taste were found to be 8.1±0.3, 8.1±0.3, 7.9±0.3, 7.8±0.4 and 7.7±0.45 on a 9-point hedonic rating scale for the muffin formulation A, B, C, D and E respectively and is plotted in figure 4.4. Statistical analysis showed that the partial substitution of flaxseed had no significant effect ($p>0.05$) on the taste of the different muffin formulations. None of the sample was significantly different from each other as sweetener, shortening agent and leavening agent used were same for all formulation and taste from these ingredient overcome the taste of flaxseed and egg.

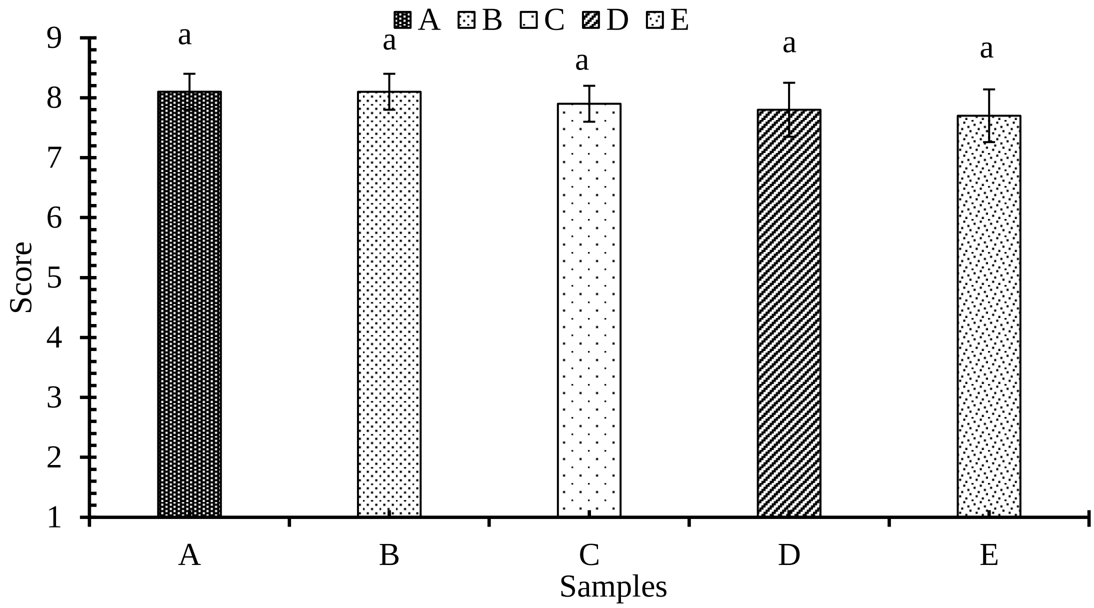

Fig 4.4 Mean sensory scores for taste of flaxseed substituted muffins of different formulations. Bars with similar alphabets at the top are not significantly different.

4.5.5 Flavor

The mean sensory score for flavor were found to be 7.7±0.4, 8.5±0.4, 7.2, 7.2 and 7±0.44 for muffin formulation A, B, C, D and E respectively and is plotted in figure 4.5. ANOVA at 5% level of significance showed that the partial substitution of flaxseed had significant effect ($p \leq 0.05$) on the flavor of the different muffin formulations. The product A and B were not statistically different statistically and got highest score. The product C, D and E were not statistically different but statistically different that other and have lower scores, which is shown in fig 4.5. The difference in the scores may be due to the replacement of egg flavor with the nutty flavor of the flaxseed.

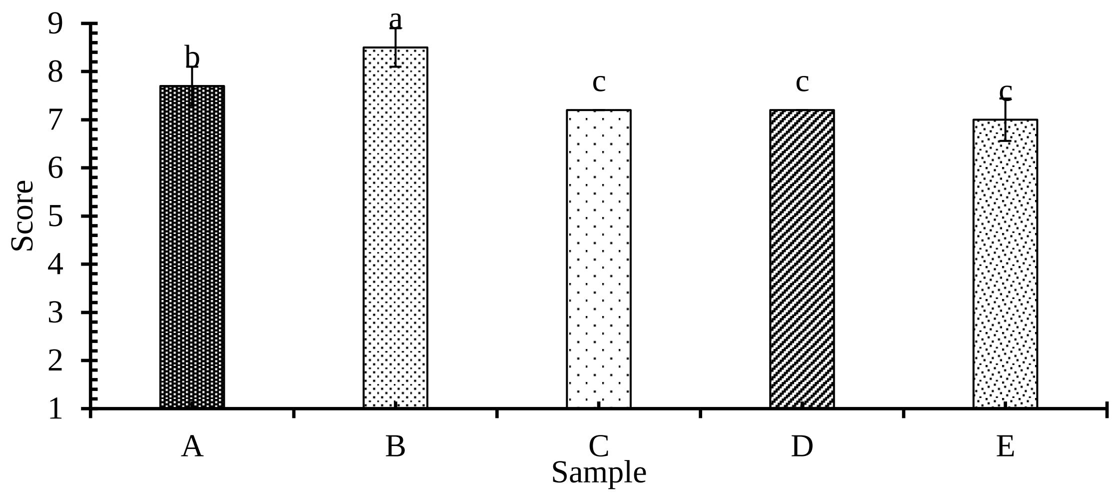

Fig 4.5 Mean sensory scores for flavor of flaxseed substituted muffins of different formulations. Bars with similar alphabets at the top are not significantly different

4.5.6 Overall acceptability

The mean sensory score for overall acceptability were found 8.3±0.45, 8.4±0.48, 7.7±0.4, 7.6±0.48 and 7±0.534 on 9-point hedonic rating scale for the muffin formulation A, B, C, D and E respectively. ANOVA at 5% level of significance showed that the partial substitution of egg with flaxseed had significant effect ($p \leq 0.05$) on the overall acceptability of the different muffin formulations as shown in figure 4.6.

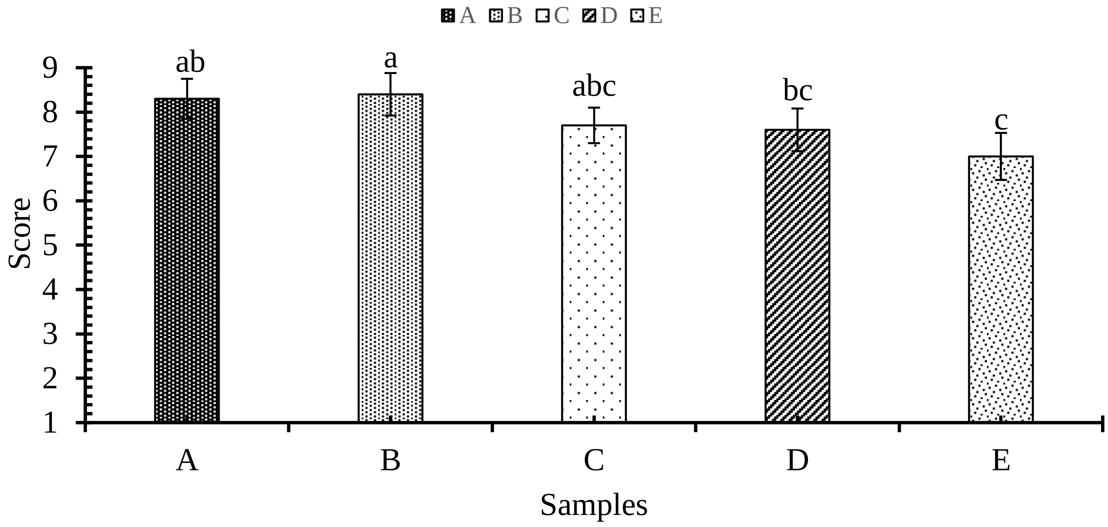

Fig 4.6 Mean sensory scores for overall acceptability of flaxseed substituted muffins of different formulations. Bars with similar alphabets at the top are not significantly different

The highest score for overall acceptability was found to be 8.4±0.48 with the incorporation of 25% flaxseed i.e. product B. The product E (100%) was significantly different than other and has lowest score i.e. 7±0.534, however, it was not disliked by the panelists.

4.6 Chemical composition of muffin

The proximate composition of muffin with 25% and 100% flaxseed incorporation was analyzed and obtained results are given in the table 4.5.

Table 4.5 Chemical composition of muffin

Parameter (db)	Control	Best sample	100% Flaxseed muffin
Moisture content	17.67±0.87[a]	17.84±0.40[a]	18.2±0.84[a]
Crude fat	17.9±0.12 [a]	16.875±0.012 [b]	15.23±0.16[c]
Crude fiber	0.6±0.008[a]	0.7±0.008[b]	1.3±0.081[c]
Total ash	1.283±0.004[a]	1.583±0.062[b]	1.97±0.047[c]
Crude protein	7.41±0.06[a]	7.10±0.01[b]	6.13±0.12[c]
Carbohydrate	72.8±0.009[a]	73.832±0.012[a]	75.28±0.016[b]
Energy	481.94±0.021[a]	475.60±0.06[b]	462.71±0.006[c]
Calcium(mg/100g)	19.38±0.42[a]	28.23±0.16[b]	69.40±0.44[c]
Iron(mg/100g)	1.46±0.063[a]	1.79±0.012[b]	2.22±0.012[c]

[Data are expressed in dry basis and the values are the means of triplicate ± standard deviation. Means bearing different superscripts in a row are significantly different (p<o.o5)].

The moisture content of both the egg muffin and flaxseed muffin were not significantly different. The substitution of egg with flaxseed does not have any effect on the moisture content of the formulated muffins. In a similar study, Kostor *et al.* (2022) found that the moisture content is not affected by the replacement of the egg by flaxseed. The fat and protein content decreased with increase in the ground soaked flaxseed proportion. Egg muffin had the highest protein content and it was due to rich protein in egg (Chepkemoi *et al.*, 2017) whereas the flaxseed mucilage or flax egg is low in protein content (Mehtre *et al.*, 2017)which resulted in lower protein content in muffin. Flax egg muffin had less fat content then egg muffin. Mehtre *et al.* (2017) reported that crude fat content ranged from 0.39-0.44% of flaxseed mucilage which was lower than 2.34% of fat content in egg (Chepkemoi *et al.*, 2017).In a similar study, Ahmad *et al.* (2021) found similar result on the fat content and protein content of the flaxseed muffin.

In present study, the fiber content has significant difference between the product A (0%), B (25%) and E (100%) in increasing pattern. Flaxseed contains about 28% of both soluble and insoluble fiber of which one-third of the fiber is soluble (S. Hussain *et al.*, 2006). The ash content was increased from 1.283% in egg muffin to 1.583% in the flaxseed muffin. Ash content in flaxseed mucilage ranged from 2.85-3.11% (Mehtre *et al.*, 2017), which was higher than in egg (0.86%) (Chepkemoi *et al.*, 2017). However, the carbohydrate content was found to be greater in the flaxseed muffin as compared to the egg muffin. Similar result were found in a study by Ahmad *et al.* (2021).

Calcium content was found to be increased to 28.23mg/100g in product B and 69.40mg/100g in product E flaxseed muffin than the egg muffin which contain 19.38mg/100g calcium. The increase in calcium content in flaxseed muffin is due to ta high calcium content in the flaxseed which contains about 230.5 mg/100g (Fig4.1)

Regarding the iron content, 25% flaxseed substituted muffin contain 1.79mg/100g and 100% substituted muffin contain 2.22 mg/100g, whereas egg muffin contains 1.46 mg/100g. The increase in iron content in flaxseed muffin is due to high iron content in the flaxseed which contain about 6.10 mg/100g (Fig 4.1)

4.7 Anti-nutritional composition of flaxseed and muffin.

4.7.1 Phytic acid

The phytate content of the flaxseed muffin was found to be 0.000089 g/kg which is negligible amount with respect to the flaxseed which contain 0.000185g/kg phytate and is plotted in figure 4.7. This value was obtained from the standard curve of Ferric nitrate which is shown in appendix D. The phytate content decreased when processed into muffin, which is due to further heat treatment during baking.

It was found that the reduction in the phytate content of muffin is due to soaking of ground flaxseed and also due to high baking temperature for long time as phytate is converted into insoluble phytins between phytic acid and some minerals. According to Daneluti and Matos (2013) ,phytic acid undergoes thermal decomposition when heated above 150°C.

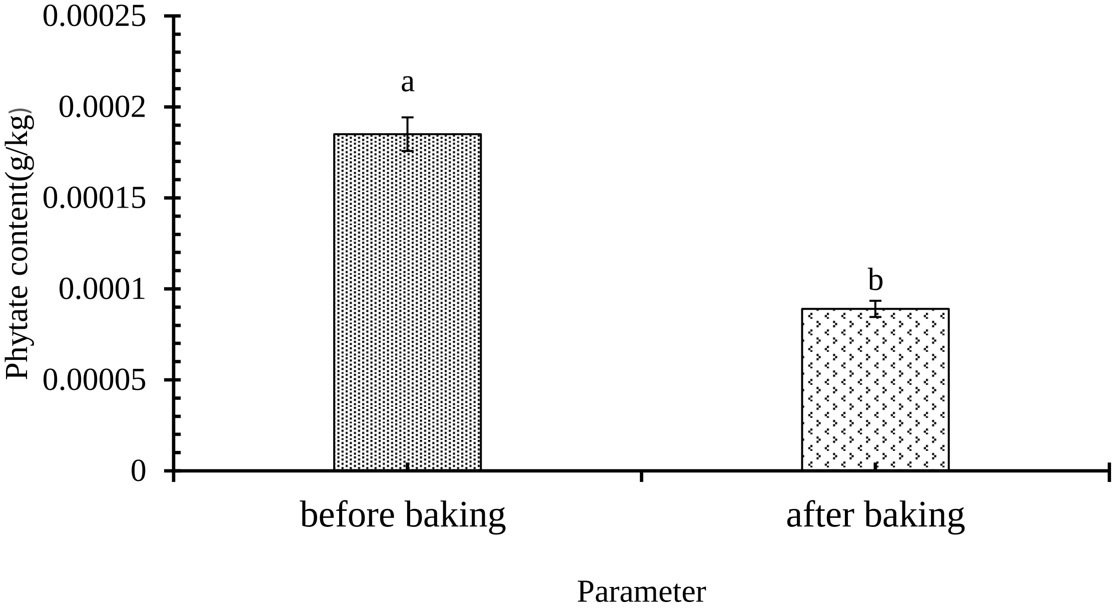

Fig 4.7 Comparisons of phytic acid content in flaxseed and muffin before and after baking.

4.7.2 Cynogenic glycosides

The cynogenic glucosides of the flaxseed muffin after baking were found to be 0.00287 mg/kg and that of ground flaxseed before baking was 0.00623 mg/kg as shown in figure 4.8.

It was found that the higher reduction in the cyanogenic glycosides of muffin is due to the high baking temperature for long time as Hydrocyanic acid content is liable to thermal treatment and easily destroyed by heat processing methods and by certain detoxifying enzymes such as β-glycosidases, releasing hydrogen cyanide which can be evaporated by using steam(Yamashita *et al.*, 2007) and this may be the reason for decrease in cyanogenic glycosides in baked flaxseed muffin.

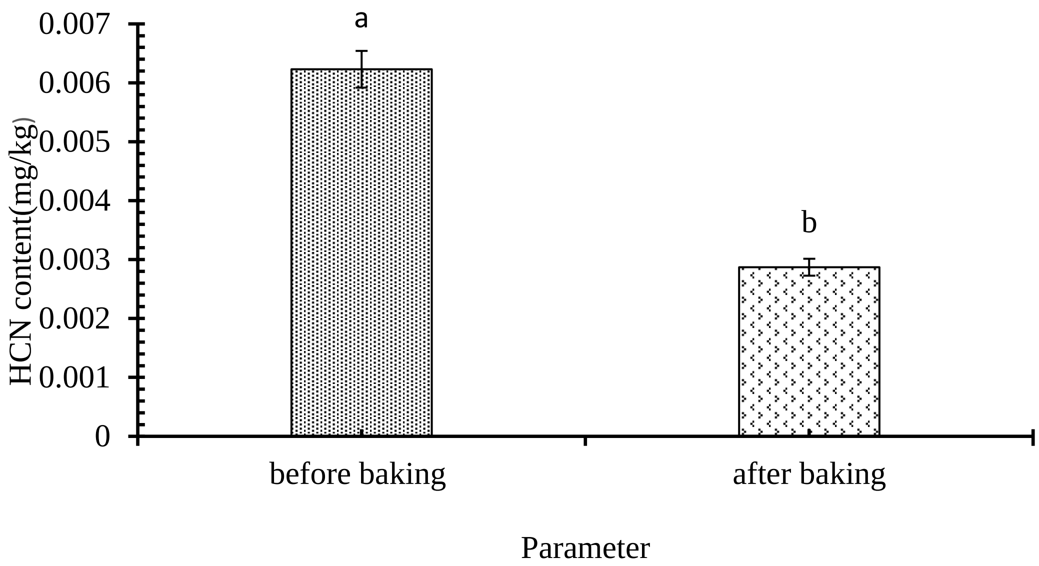

Fig 4.8 Comparisons of cyanogenic glycosides content flaxseed and muffin before and after baking.

4.8 Phytochemicals

4.8.1 Total polyphenol content (TPC)

The TPC of flaxseed muffin was found to be 128.4 mg GAE/100g and TPC of egg muffin was found to be 102.65 mg GAE/100g as shown in figure 4.9.

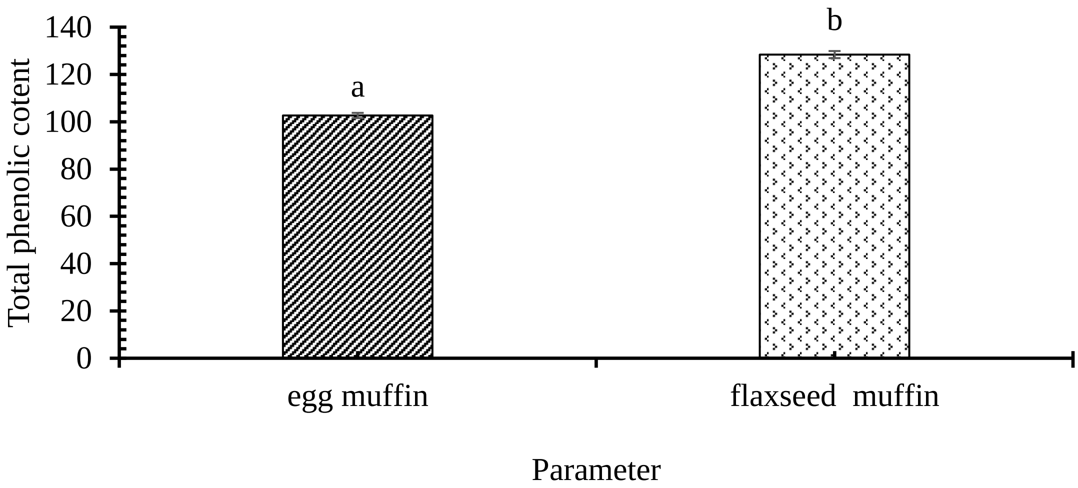

Fig 4.9 Comparisons of Total poly phenol content in egg and flaxseed muffin

This result indicates the TPC content of the increases with the substitution of flaxseed in the muffin. In a study carried out on Chinese steamed bread (CSB) containing flaxseed hull extracts shows that the total phenolic content was increased to 405 mg FAE/100 g bread sample from 170 mg FAE/100 g bread sample (Meili and Trust, 2012). The increase in phenolic content may be due to the high phenolic content in the flaxseed which is 536.22 GAE/100g.

Considering the broad human health benefits of flavonoids, most of which are having the anti-oxidant properties, they have gained significant interest. Baking decreased the TFC value to significant level. Baking at high temperature (180°C) decreased the total flavonoids content (TFC) value, which is due to breakdown of heat labile flavonoid compounds upon exposure to high temperatures. Losses in flavonoid content of different formulations under baking are expected to occur due to breakdown of complex polyphenols into other phenolic and non-phenolic compounds when subjected to high temperature conditions (Baojun *et al.*, 2008).

4.8.2 Antioxidant activity

The DPPH radical scavenging activity (DPPH RSA) of flaxseed muffin was found to be 8.57% and DPPH (RSA) of egg muffin was found to be 6.56%. Similar result of increase of antioxidant activity were found observed in flaxseed flour supplemented muffin. The increase in antioxidant activity of muffin is due to the incorporation of flaxseed containing high antioxidant activity (i.e.46%). The increasing pattern of antioxidant activity is in accordance to previous study carried out on Chinese steamed bread containing flaxseed hull extracts which shows that the antioxidant activity was increased to 12.87% compared to control (i.e.6.75%).

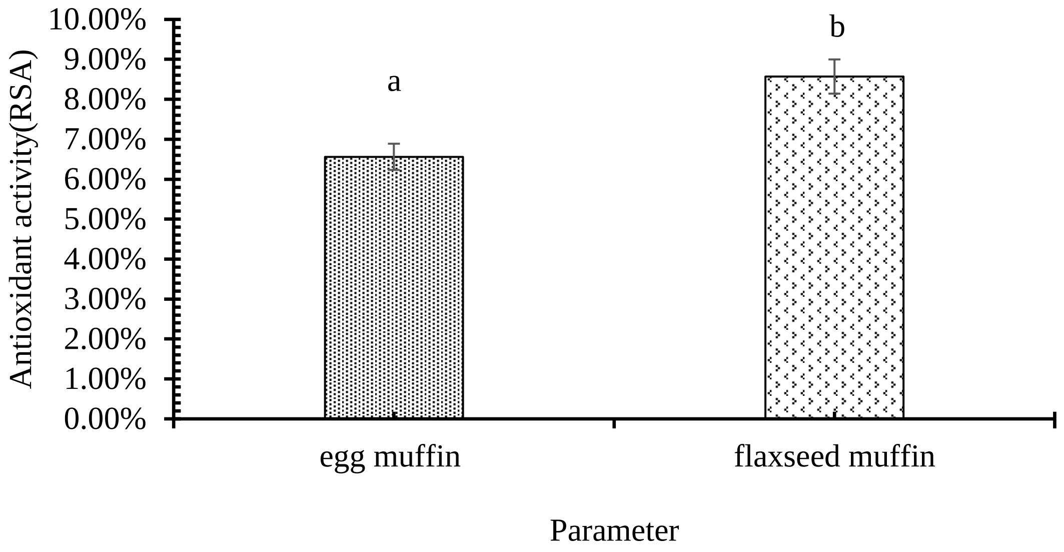

Fig 4.10 Comparisons of antioxidant activity of egg and flaxseed muffin

4.9 Chemical and microbiological analysis of product.

The shelf life of product B which was kept in packaging material i.e. LDPE (50μ) at normal atmospheric condition was studied. The acid value and peroxide value of extracted fat, yeast count, mold count, coliform and total plate count of the product were obtained to evaluate its shelf life.

4.9.1 Acid value

The standard AV value for the muffin should not exceed 0.5 mgKOH/g. After preparation of the muffin the acid value was found to be 0.154 mgKOH/g. After 1 day of storage in the LDPE package at room temperature, acid value was found to be 0.230 mgKOH/g. After 2 days of storage, acid value was found to be 0.341 mgKOH/g. After 3days of storage, acid value was found to be 0.389 mgKOH/g. After 4 days of storage, acid value was found to be 0.468 mgKOH/g. After 5th days of storage, acid value was found to be 0.630 mgKOH/g. From the data, the muffin is not acceptable after 4 days of storage in LDPE packaing material at normal atmospheric condition as shown in figure 4.11.

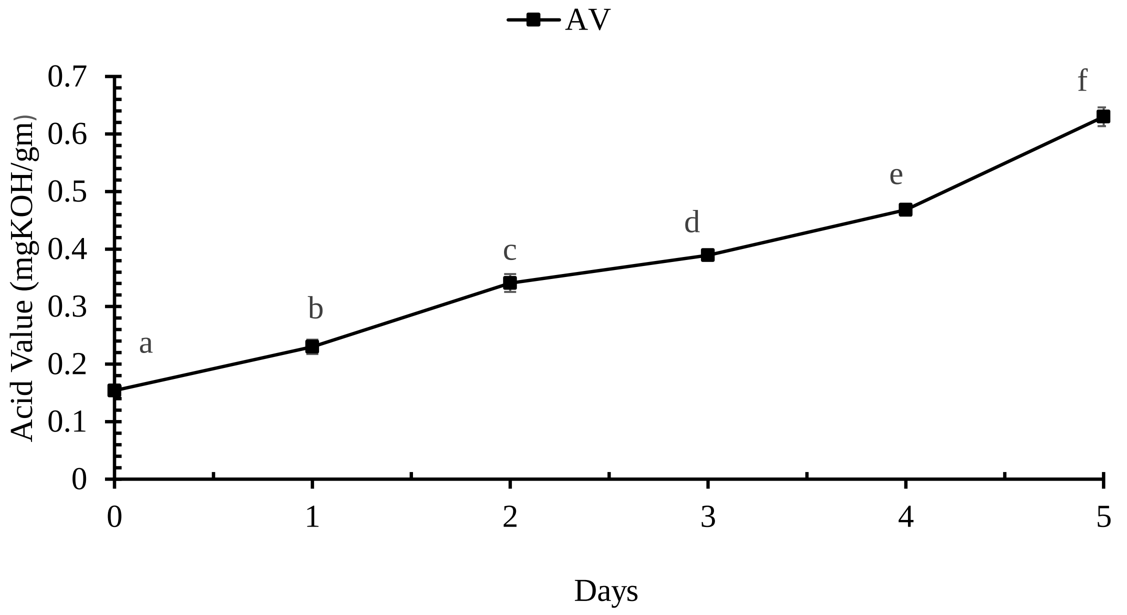

Fig 4.11 Changes in acid value (AV) with respect to no of days of storage

4.9.2 Peroxide value

After preparation of muffin, peroxide value of muffin was found to be 0.732 meq/kg. After 1 day of storage peroxide value of muffin was found to be 1.86 meq/kg. After 2 days of storage, peroxide value was found to be 3.70 meq/kg. After 3 days of storage peroxide value was found to be 5.22 meq/kg. After 4 days of storage peroxide value was found to be 7.46 meq/kg. After 5 days of storage, peroxide value was found to be 11.35 meq/kg. The peroxide value of the muffin should not exceed 10 meq/kg. So it is clear that muffin is not acceptable to eat after 4th day of storage as shown in figure 4.12.

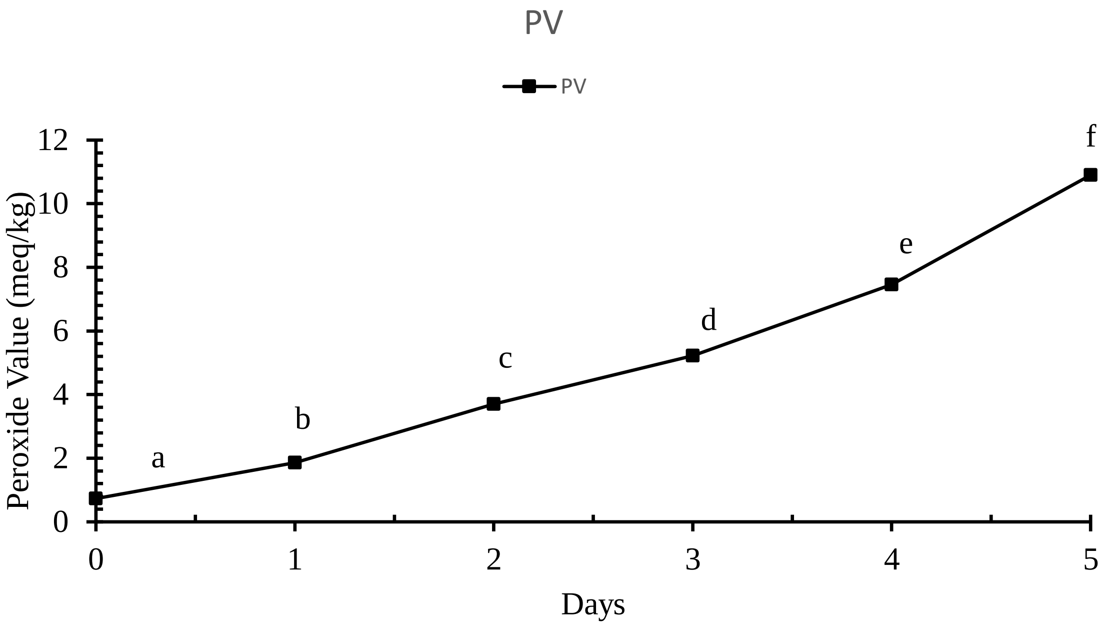

Fig 4.12 Changes in peroxide value (PV) with respect to number of days of storage.

4.9.3 Microbiological analysis

There were no colonies of coliform found. Also, there were no any colony of yeast and mold up to 4 days. The total plate count was also found to be nil till 4th day. They were destroyed during the baking of muffin before packaging further contamination may be restricted by the packaging material i.e. LDPE.

4.10 Cost of the flax-egg incorporated muffin.

The total cost associated with the products was calculated and the cost of flaxseed incorporated muffin were NRs.51 (25%) and NRs.42.53 (100%) which were lower than the price of egg muffin (0%) i.e. NRs. 53.84 including overhead cost and profit of 10%. The amount of flaxseed required to prepare ground soaked flaxseed is in ratio 1:3 due to which the amount and cost of the flaxseed required is low. From cost calculation given in appendix D, it can be seen that due to comparatively low cost of flaxseed from egg, the cost of muffin has been decreased.

Part V

Conclusions and recommendations

5.1 Conclusions

On the basis of results and discussion, the following conclusions were done:

1. Muffin height, muffin volume and baking loss decreases while muffin density increases with the incorporation of flaxseed in the muffin.
2. Muffin formulation containing 25 % flaxseed was found to be best with respect to sensory parameters color, taste, flavor, texture, sponginess and overall acceptance however formulations up to 100% replacement were not disliked.
3. The crude fiber, total ash, carbohydrate, calcium and iron content increased with the substitution of flaxseed.
4. The protein, fat and calorie content of muffin decreased with increase in substitution of flaxseed.
5. The phytic acid and cynogenic glycoside of flaxseed were decreased after baking.
6. Total phenolic content (TPC) and Antioxidant activity were found to be higher in flaxseed muffin.
7. The chemical and microbiological analysis of product showed acceptability of flaxseed muffin up to four days at room temperature in LDPE package without any artificial preservatives.
8. The flaxseed muffin was comparatively cheaper than egg muffin.

5.2 Recommendation

1. The optimization of flaxseed to water ratio should be studied.
2. Texture of the prepared muffin can be analyzed using texture meter.
3. Flaxseed can be used as plant based vegan egg replacer commercially

Summary

Muffin is small cup shaped quick bread which is sweet in taste i.e., baked in appropriate portion and is highly appreciated by the consumers. Flaxseed (*Linum usitassimum)* is small seed produced by annual herb, with high nutritional value and is a functional food. It has several uses, however, is underutilized in commercial way. Egg is the major ingredient of muffin, however, increasing its amount in some types of cake, could result in increasing the amount of cholesterol and are related to cardiovascular disease. Also, it acts as allergen. Therefore, the aim of this study is to investigate the functional properties of seed, physicochemical and sensorial properties of muffin with flaxseed as egg replacer.

For the preparation of flaxseed muffin, the flaxseed was finely grinded and then soaked in water to extract the mucilage. Design expert version 13 software was used in which d-optimal method was used for the formulation of recipe. Five different muffin formulation namely A (0 parts egg replacer), B (25 parts egg replacer), C (50 parts egg replacer), D (75 parts egg replacer) and E (100 parts egg replacer) were prepared by cake method and subjected to sensory evaluation and physical properties evaluation. In the physical analysis; the height volume, muffin height and baking loss decreases with increase of flaxseed in the formulation whereas the density increases with the substitution. Sensory evaluation was carried out based on color, flavor, taste, texture, sponginess and overall acceptability and the data obtained were statistically analyzed using two-way ANOVA (no blocking) at 5% level of significance. Sample B (25% flaxseed) got the highest mean sensory score and proximate analysis for moisture, crude protein, crude fat, crude fiber, total ash and carbohydrate were found to be 17.84%, 7.10%, 16.8%,0.7%,1.583%, 73.83% of sample B respectively. Calcium and iron content were 28.33 mg/100g and 1.79mg/100g of sample B respectively. The phytic acid content, HCN content, total phenolic content the anti-oxidant activity (RSA) of sample B were found to be 0.000089 g/kg,0.00623 mg/kg, of were found to be 128.4 mg GAE/100g and 8.57% respectively.

The acid value and peroxide value of sample B at day 0 was found to be 0.154 mgKOH/g and 0.732 meq/kg respectively which reached be 0.630 mgKOH/g and 11.35 meq/kg at day 5. Coliform was nil. No colony of yeast and mold were found till day 4. Total plate count was 0 till day 4. Thus, the product was chemically and microbiologically safe till day 4.

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Appendices

Appendix A

Sensory evaluation score sheet of flax-egg substituted muffin

Name of the panelist: Date:

Name of the product: Muffins with eggs replaced by flax-egg

Dear panelist, you are provided with 5 samples of muffins with eggs replaced by soaked flaxseeds (flax-egg) on each proportion with variation on flax-egg content. Please test the following samples of muffin 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

Parameters	Sample code				
	A	B	C	D	E
Color					
Flavor					
Taste					
Texture					
Sponginess					
Overall acceptability					

Any comments:

Signature:

Appendix B

Sensory evaluation of flax-egg muffin

Table B.1 Mean sensory score for different variety of flaxseed muffin

Sample	Color	Taste	Flavor	Texture	Sponginess	Overall acceptability
A	7.8^{b}±0.4	8.1±0.3	7.7^{b}±0.45	8.1±0.3	8.1^{ab}±0.3	8.3^{ab}±0.45
B	8.4^{a}±0.48	8.1±0.3	8.5^{a}±0.5	8.1±0.3	8.6^{a}±0.45	8.4^{a}±0.48
C	7.100^{c}±0.3	7.9±0.3	7.2^{c}	7.9±0.3	8^{abc}±0.15	7.7^{abc}±0.4
D	6.000^{d}	7.8±0.4	7.2^{c}	7.7±0.45	7.8^{ab}±0.4	7.6^{bc}±0.48
E	5.200^{e}±0.4	7.7±0.45	7^{c}±0.44	7.7±0.44	7.4^{c}±0.8	7^{c}±0.53

ANOVA results of sensory analysis

Table B.2 ANOVA(no blocking) for color of flax-egg muffin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Sample	4	68	17	235.29	<.001	0.3312
Panelist	9	1.7	0.1889	1.40	0.218	0.4684
Residual	36	4.8	0.1333			
Total	49	74.5				

Table B.3 ANOVA (no blocking) for taste of flaxseed muffin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Sample	4	1.2800	0.3200	2.44	0.064	0.2916
Panelist	9	1.6800	0.1867	1.42	0.215	0.4123
Residual	36	4.7200	0.1311			
Total	49	7.6800				

Table B.4 ANOVA (no blocking) for sponginess of flaxseed muffin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Sample	4	4.6800	1.1700	4.16	<.001	0.4464
Panelist	9	0.8800	0.0978	0.35	0.335	0.6313
Residual	36	10.1200	0.2811			
Total	49	15.6800				

Table B.5 ANOVA (no blocking) for flavor of flaxseed muffin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Sample	4	13.9200	3.4800	14.77	<.001	0.3284
Panelist	9	13.9200	0.4356	1.85	0.093	0.4644
Residual	36	8.4800	0.2356			
Total	49	26.3200				

Table B.6 ANOVA (no blocking) for texture of flaxseed muffin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Sample	4	1.6000	0.4000	2.25	0.083	0.3824
Panelist	9	0.5000	0.0556	0.31	0.966	0.5408
Residual	36	6.4000	0.1778			
Total	49	8.5000				

Table B.7 ANOVA (no blocking) for overall acceptability of flaxseed muffin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Sample	4	13.0000	3.2500	10.26	<.001	0.5104
Panalist	9	1.6000	0.1778	0.56	0.819	0.7218
Residual	36	11.4000	0.3167			
Total	49	26.0000				

Appendix C

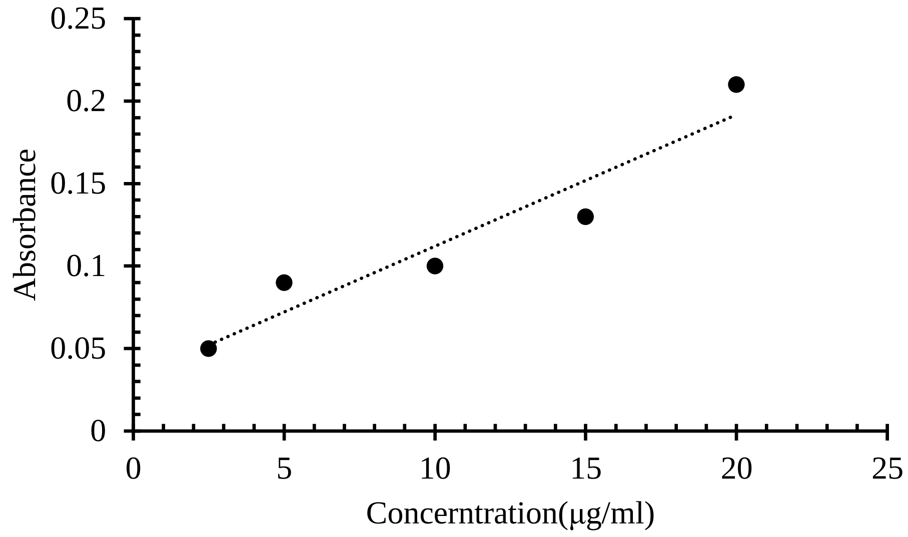

Fig C.1 Standard curve of Ferric nitrate

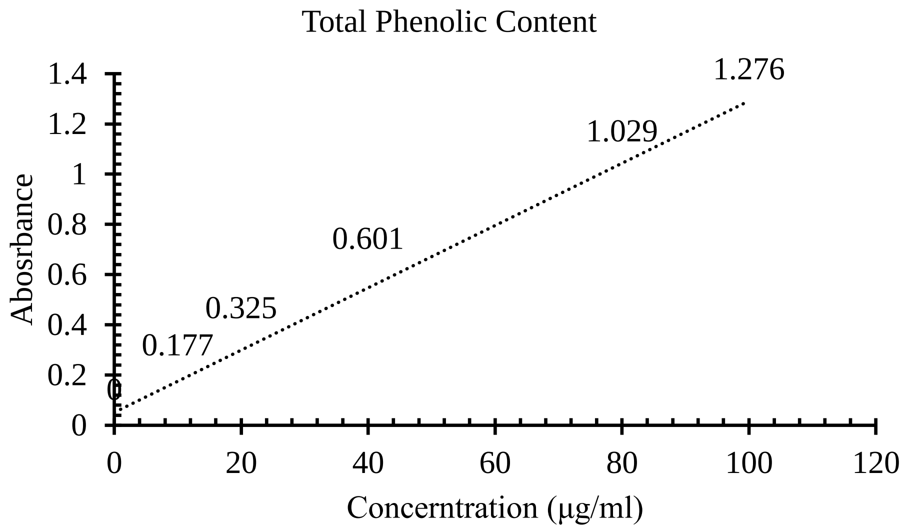

Fig C.2 Standard curve for total phenolic content

Appendix D

Table D.1 Cost calculation of the product (FM)

Particulars	Cost (NRS/kg)	Weight in a lot(g) (0%)	Weight in a lot(g) (25%)	Weight in a lot(g) (100%)	Cost (NRS) (0%)	Cost (NRS) (25%)	Cost (NRS) (100%)
Wheat flour	70	100	100	100	7	7	7
Egg	380	112.5	84.375	0	42.75	32.06	0
Flaxseed	180	0	7.03	28.12	0	1.26	5.06
Water	20	0	21.09	84.375	0	0.42	1.687
Fat	700	105	105	105	73.5	73.5	73.5
Sugar	90	82.5	82.5	82.5	7.425	7.425	7.425
Baking powder	125	1.875	1.875	1.875	0.24	0.24	0.24
Milk powder	1050	37.5	37.5	37.5	39.37	39.37	39.37
Raw material cost					170.29	161.18	134.4
Processing and labor cost(10% of raw material cost)					17.029	16.18	13.44
Profit (10%)					17.029	16.175	13.44
Grand total cost					204.35	193.41	161.28
Average weight of FM (g)			31.6				
Total no. of FM formed			12				
Total weight of FM(g)			379.2				
Total cost of FM(NRs/100g)					53.84	51	42.53

Appendix E

Royal horticulture color chart

white - brown - grey - black colours

Back

RHS	sRGB				CIE Lab D65 / 10°			CIE LCh D65 / 10°		
	Out of RGB	R	G	B	L	a	b	L	C	h
155A		241	231	220	92	2	6	92	6	71
155B		246	235	228	94	3	4	94	5	52
155C		239	232	229	93	2	2	92	3	32
155D		250	236	230	94	4	4	94	5	42
N155A		226	220	238	89	6	-9	89	11	302
N155B		242	226	238	92	8	-5	92	9	329
N155C		244	224	231	91	8	-2	91	8	348
N155D		245	229	233	92	6	-1	92	6	352
NN155A		248	237	232	95	3	3	94	4	41
NN155B		248	238	241	95	4	-1	95	5	344
NN155C		247	238	248	95	5	-5	95	7	318
NN155D		251	242	252	96	5	-5	96	7	318
156A		201	188	175	77	3	7	77	8	70
156B		211	199	189	81	3	6	81	6	64
156C		213	202	194	82	3	5	82	5	58
156D		224	213	208	86	3	3	86	4	42
157A		228	222	201	88	-1	10	88	10	96
157B		230	225	209	90	0	8	89	8	95
157C		237	231	218	92	0	6	92	6	88
157D		244	237	228	94	1	5	94	5	74
158A		245	221	189	89	4	18	89	19	78
158B		251	232	206	93	3	14	93	14	79
158C		250	232	216	93	4	9	93	10	67
158D		252	235	224	94	4	7	94	8	56
159A		247	214	186	88	8	17	88	19	66
159B		249	219	196	89	7	14	89	16	63
159C		243	222	207	90	5	10	90	11	60
159D		249	232	221	93	4	7	93	8	56
160A		218	200	119	81	-4	42	80	42	96
160B		225	204	138	82	-2	35	82	35	93
160C		225	209	159	84	-2	26	84	26	94
160D		225	210	175	85	0	18	84	18	90
161A		214	180	119	75	5	35	75	35	83
161B		225	190	127	79	4	36	79	36	83
161C		230	200	155	82	5	26	82	26	80
161D		236	210	175	86	4	20	86	20	78
162A		222	183	99	76	5	47	76	47	85
162B		229	195	122	80	3	40	80	41	86
162C		227	197	141	81	3	31	81	32	84
162D		229	206	163	84	2	24	84	24	85
163A		202	136	45	62	18	56	62	58	72
163B		216	165	58	71	9	60	71	60	81
163C		225	184	105	77	6	45	77	45	83
163D		232	201	148	82	4	30	82	30	83
N163A		202	100	50	54	38	45	54	59	51
N163B		221	120	50	61	35	53	61	64	57
N163C		231	145	34	68	25	66	67	70	69
N163D		228	156	40	70	18	65	70	68	74

159D		249	232	221	93	4	7	93	8	56
160A		218	200	119	81	-4	42	80	42	96
160B		225	204	138	82	-2	35	82	35	93
160C		225	209	159	84	-2	26	84	26	94
160D		225	210	175	85	0	18	84	18	90
161A		214	180	119	75	5	35	75	35	83
161B		225	190	127	79	4	36	79	36	83
161C		230	200	155	82	5	26	82	26	80
161D		236	210	175	86	4	20	86	20	78
162A		222	183	99	76	5	47	76	47	85
162B		229	195	122	80	3	40	80	41	86
162C		227	197	141	81	3	31	81	32	84
162D		229	206	163	84	2	24	84	24	85
163A		202	136	45	62	18	56	62	58	72
163B		216	165	58	71	9	60	71	60	81
163C		225	184	105	77	6	45	77	45	83
163D		232	201	148	82	4	30	82	30	83
N163A		202	100	50	54	38	45	54	59	51
N163B		221	120	50	61	35	53	61	64	57
N163C		231	145	34	68	25	66	67	70	69
N163D		228	156	40	70	18	65	70	68	74
164A		177	113	73	54	21	32	54	38	56
164B		204	150	94	66	14	37	66	40	69
164C		220	173	115	74	10	36	74	37	74
164D		239	205	171	85	7	21	84	22	70
165A		125	87	73	41	14	14	41	20	45
165B		178	119	73	55	19	34	55	39	61
165C		212	157	109	69	15	33	69	36	65
165D		234	189	149	80	11	26	80	28	67
166A		115	76	72	37	17	9	36	19	28
166B		154	89	73	45	25	20	45	32	39
166C		183	111	83	54	26	27	54	38	46
166D		195	131	97	60	21	28	60	35	53
167A		200	124	63	59	24	44	59	50	61
167B		212	135	78	63	25	42	63	48	60
167C		220	144	88	67	23	41	66	47	60
167D		225	155	101	70	21	38	70	43	61
N167A		173	111	55	53	20	40	52	45	64
N167B		181	108	56	53	25	40	53	47	58
N167C		213	143	83	65	21	42	65	47	63
N167D		207	139	90	64	21	36	64	41	60
168A		214	108	70	57	39	40	57	56	45
168B		215	119	72	60	34	41	60	53	51
168C		228	143	85	67	28	43	67	51	58
168D		235	172	120	75	18	35	75	39	64
169A		201	88	56	51	43	40	51	59	42
169B		213	103	61	56	41	43	56	59	46
169C		220	117	60	60	36	48	60	60	53
169D		230	130	69	64	34	49	64	59	55
170A		213	108	71	57	39	39	57	55	45
170B		219	121	80	61	35	39	61	52	48
170C		228	146	102	68	27	36	68	45	53
170D		243	168	130	75	24	30	75	38	52
N170A		180	101	65	51	29	34	51	44	49
N170B		197	127	89	60	24	31	60	39	53
N170C		220	152	133	69	23	20	69	31	40
N170D		232	183	172	78	17	12	78	20	35

Color Plates

P1. Prepared muffin samples

P2. Preparation of muffin

P3. Analysis of muffin