Optimization and Characterization of plant-based functional biscuits incorporated with fenugreek leaf powder and flaxseeds using Response surface methodology

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THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF

Master of Science in Food Technology

Supervisor

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Submitted by

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Dear Sir,

I have great pleasure in forwarding the thesis entitled **"Optimization and Characterisation of plant-based functional biscuits incorporated with Fenugreek leaf powder and Flaxseeds using Response Surface Methodology"** submitted by ***Ms. Sonali Kanwar, I.D. No. 20412FST011*** in partial fulfilment of the requirements for the degree of **Master of Science in Food Technology,** from Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, BHU Varanasi.

I certify that the entire scheme of investigation, presented here in, was planned and carried solely by the candidate under my guidance. To the best of my knowledge, the data presented in the thesis are genuine and original.

Thanking you.

Forwarded Yours faithfully,

……..……

Prof. Anil Kumar Chauhan

(Supervisor)

(Head of the Department)

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By

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Date:……

Place: Varanasi **(SONALI KANWAR)**

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ABBREVIATIONS

ALA	:	Alpha-Linolenic Acid
AO	:	Antioxidant
AVA	:	Avenanthramide
BP	:	Blood Pressure
CA	:	Colour and Appearance
CB	:	Cocoa Butter
CCD	:	Central Composite Design
CFU	:	Colony forming unit
cP	:	Centi Poise
CVD	:	Cardiovascular Disease
DHA	:	docosahexanoic acid
DPPH	:	2,2-Diphenyl-1-picrylhydrazl
EPA	:	Eicosapentaoic acid
FLP	:	Fenugreek Leaf Powder
FS	:	Flaxseeds
GAE	:	Gallic Acid Equivalent
HCl	:	Hydrochloric acid
HDL	:	High Density Lipoprotein
HDL-C	:	High Density Lipoprotein Cholesterol
HMF	:	Hydroxy Methyl Furfural
HMF	:	Hydroxy Methyl Furfural
LDL	:	Low Density Lipoprotein

LDL-C	:	Low Density Lipoprotein Cholesterol
LDPE	:	Low Density Polyethylene
MDA	:	Malondialdehyde
OA	:	Overall acceptability
PDA	:	Potato dextrose agar
PUFA	:	Poly Unsaturated Fatty Acid
RPM	:	Rotations Per Minute
RSA	:	Radical Scavenging Activity
RSM	:	Response Surface Methodology
RVA	:	Rapid Visco Analysis
SDG	:	Secoisolariciresinol Diglucoside
STZ	:	Streptozotocin
TA	:	Taste and Aroma
TBARS	:	Thiobarbituric Acid Reactive Species
TGA	:	Thermo-Gravimetric Analysis
TPA	:	Texture Profile Analysis
TPC	:	Total Plate Count
VLDL-C	:	Very Low-Density Lipoprotein Cholesterol

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Chapter-I

INTRODUCTION

A biscuit is a baked edible product made of flour. It is a small, firm, flour-based product that is usually sweetened. **(Kumar *et al.*, 2016)**. Biscuits are a versatile bakery snack that has a significant place among bakery items due to their appealing qualities like long shelf life, varied taste, and texture, and are enjoyed by people of all ages. Biscuits are available in a multitude of forms, colors, toppings, and fillings, which makes them popular among the masses. Biscuits are frequently used as snacks, staple foods, luxury delicacies, foods for infants, dog and cat food, and dietary supplements. **(Chavan *et al.*, 2016)**

Commonly used ingredients and food additives for the manufacturing of biscuits include flour, sweeteners, shortenings, water, emulsifiers, antioxidants, leavening agents, salt, milk products, color, and flavor. The most significant variables in developing a nutritious biscuit are the ingredients and the conditions under which it is processed. Each ingredient has advantages and disadvantages, and they all play a part in the production of wholesome biscuits. During biscuit baking, water evaporates, browning reactions take place, protein is denatured, partial starch gelatinization occurs, Maillard reactions occur, and the dough is deformed. **(Arepally *et al.*, 2020)**

Wheat flour is typically used as the primary ingredient in biscuits. Flour plays the function of forming a viscoelastic network and holds all of the constituents in the dough in a uniform manner. Flour is a toughener that gives biscuits and biscuit-like foodstuffs their texture, shape, and hardness. Gliadins and glutenin are the main proteins found in wheat flour, and they form gluten when water is added, providing food structure. Non-gluten flours (rice, maize, etc.) could be used instead of wheat flour. Barley and millets can also be used to produce gluten-free biscuits. Sweeteners apart from providing sweetness, color and flavor also contribute to the texture of the biscuits. Shortenings or butter play role in shortening the dough and reducing its hardness. Leavening agents like baking powder and baking soda produce gas, uplift

the product's volume, and improve its texture. For toughening gluten and slowing down the rate of fermentation, a very little amount of salt is also added to baked products. Salt also contributes to improving the flavor. (**Chavan *et al.*, 2016)**

1.1 Fenugreek Leaves (*Trigonella foenum-graecum*)

Fenugreek is one of India's most miraculous herbs with numerous health benefits. Fenugreek *(Trigonella foenum-graecum)* is a legume that is used as a spice to improve the flavour and nutritional content of foods all over the world. Fenugreek has long been recognized as a potent herb in traditional medicine. Clinical experiments have demonstrated that fenugreek leaves and seeds significantly decrease the cholesterol levels and blood glucose levels in both human and animal participants. **(Wani *et al.*, 2017)**

The presence of a wide range of important phytochemicals (fenugreekine, trigonelline, diosgenin, 4-hydroxy isoleucine and galactomannan) imparts fenugreek its therapeutic properties **(Zandi *et al.*, 2017).** It has been reported that trigonelline from the fenugreek lowers the blood glucose level in rats and humans. (**Aldakinah *et al.*, 2017)** Fenugreek leaves contain a high content of choline, fiber thiamine, riboflavin, nicotinic acid, magnesium, and phosphorous. **(Srinivasan *et al.*, 2006)**

Antidiabetic effects, antioxidant activity, anticarcinogenic, hypocholesterolemic effects and immunological activities are among some of its therapeutic benefits. It is also employed in the development of numerous food products and their therapeutic significance. Apart from its therapeutic use, it is also utilized as a food stabilizer, adhesive, and emulsifying agent in the manufacture of numerous food products. Fenugreek has a lot of potential when it comes to enhancing the nutritional value of baked and extruded goods. (**Wani *et al.*, 2017)**

1.2 Flaxseeds (*Linum usitatissimum*)

Flaxseed, also known as flax or linseed, is a smooth surfaced oilseed, glossy hard shell that ranges in colour from deep amber to reddish-brown depending on the variety. It is a member of the *Linaceae* family. Flaxseed is classified as a functional food due to

its nutritional composition, which has a significant impact on disease prevention by providing health-beneficial components. **(Katare *et al.*,2012)**

Flaxseed contains a high concentration of polyunsaturated fatty acids (PUFA) omega-3 family, omega-6 family, soluble dietary fibers, lignans, α-linolenic acid, high-quality proteins, carbohydrates, and physiologically active components. Flaxseeds are particularly high in Omega-3 fatty acids, protein, and fiber. Flaxseed protein comprises an amino acid sequence that is similar to soybean protein, one of the most nutritious plant proteins to exist. Research suggests that flaxseed consumption is linked to several potential health benefits and could help in preventing many diseases such as diabetes, cardiovascular diseases, obesity disorders, cancer, and stroke. **(Katare *et al.*,2012)** Flaxseed is well-known for its nutritive and therapeutic effects, which are ascribed to the presence of high-quality omega-3 unsaturated fatty acids, alpha-linolenic acid (ALA), and antioxidants like phenolics, lignin, carotenoids, and tocopherols. **(Yasmeen *et al.*, 2018)** Flaxseed contains a variety of phytochemicals, including coumarins, flavonoids, alkaloids, phenol, steroids, saponins, and glycosides, highlighting flaxseed as a natural food supplement with significant antioxidant properties because of the high phenol content. Flaxseeds also contain betacyanin, saponin, quinones, steroids, coumarins, phenols, and terpenoids. **(Monica *et al.*, 2016)**

1.3 Stevia leaf (*Stevia rebaudiana*)

Stevia leaves produce diterpene glycosides (stevioside and rebaudioside), which are non-toxic, non-nutritive, high-potency sweeteners that are 300 times sweeter than sucrose and other synthetic sweeteners. **(Yadav *et al.*, 2010)**

Sweet-tasting compounds found in the leaves of *S. rebaudiana Bertoni* include stevioside, rebaudioside A, D, and E, and dulcosides A and B. Stevia rebaudiana includes stevioside, a sweetener that is calorie-free and is not metabolized by the human body. The main compounds are Stevioside and rebaudioside A. Rebaudioside A is the sweetest and most stable of the Stevia glycosides, with a milder flavor than stevioside. Because the sweet components of Stevia pass through the digestive tract without chemically breaking down, it is excellent for people who need to control their

blood sugar levels. **(Goyal *et al.*, 2010)** Alkaloids, Tannins, cardiac glycosides, saponins, sterols, and triterpenes are some of the phytochemical elements of stevia. Stevia contains calcium, sodium, phosphorus, potassium, magnesium, and sulfur in considerable levels. **(Tadhani *et al.*, 2006)**

With rising diabetes rates in India and worldwide, as well as growing worries about the safety of various chemical sweeteners, a natural non-caloric sweetener with acceptable flavor and health benefits is urgently needed. It has therapeutic qualities and uses in addition to its sweetening properties, such as being antihyperglycemic, anticancerous, and antihypertensive, as well as preventing dental cavities. **(Goyal *et al.*, 2010)**

1.4 Cocoa butter

The cocoa bean is the fatty seed that grows inside the cocoa pod, which is a fruit of the *Theobroma cacao* plant. The cocoa bean processing industry generates cocoa butter as a by-product and it is used as a key element in chocolate and other sweets. Cocoa butter is obtained by a step-by-step process comprising drying, roasting, cracking, de-shelling, grinding, and pressing of cocoa beans.

The main fatty acids found in it are palmitic acid, oleic acid, stearic acid, and linoleic acid, with modest quantities of lauric and myristic acid. Cocoa butter is high in saturated fat, which comes from starch and palmitic acid, as well as caffeine and theobromine in trace levels, are also present. It also provides fat-soluble antioxidants, such as vitamin E in the forms of -tocopherol, -tocopherol, and -tocopherol, which enhance the keeping quality and enhance remedial properties. **(Naik and Kumar 2014)**

1.5 Oats (*Avena sativa*)

Oats are one of the most nutrient-dense whole-grain cereals, with a nutritional profile that is well-balanced. It contains an appreciable amount of carbohydrates as well as high-quality protein with a well-balanced amino acid profile. A major portion of the oat diet is made up of lipids, particularly unsaturated fatty acids, minerals, vitamins, and phytochemicals. Oats contain one of the highest amounts of

soluble fibre beta-glucan in the diet **(Rasane *et al.*, 2013)**. The health benefits of dietary fibers present in the oat grain, such as beta-glucan, lipid, functional protein, and carbohydrate components, and phytochemicals, have contributed to a rise in oat consumption in the human diet. According to research, oat has anti-diabetic, anti-inflammatory and anti-carcinogenic characteristics.

Micronutrients present in oat include vitamin E, iron, folates, copper, zinc, selenium, manganese, choline, carotenoids, betaine, sulfur-containing amino acids, phytic acid, lignins, lignane, and carotenoids. **(Manolache *et al.*, 2019)** Phenolics, carotenoids, vitamin E, phytic acid, beta-glucan, sterols, and other vitamins, minerals, and bioactive substances are all abundant in whole grain oats. Oats contain beta-glucan, which lowers blood cholesterol and has hypoglycemic properties. The principal flavones present in oat flour are tricin, apigenin and luteolin. Flavonols such as quercetin and kaempferol have been identified in oats. Oats contain phenolic acids such as ferulic, sinapic, p-coumaric, and hydroxybenzoic acids. Avenanthramides, a class of hydroxycinnamoyl anthranilate alkaloids found only in oats, function as a phenolic antioxidant. Avenalins are the major proteins in oats. Prolamins (avenins) represent approximately 15% of total oat protein, while avenalins account for up to 80%. Oat proteins are regarded as safe and tolerable by gluten intolerance/coeliac disease patients (**Gangopadhyay *et al.*, 2015).**

Oat flour is a whole-grain flour that is made from rolled oats. It is rich in manganese, molybdenum, phosphorus, copper, biotin, vitamin B1, magnesium, chromium, and fibre. Oat flour is made by cleaning and milling oats first. Afterward, hulling and groat separation are performed followed by kilning. Finally, large groats are ground to obtain oats flour. Oats flour is used in biscuits, cookies, bread, muffins, and cakes **(Rasane *et al.*, 2013)**

1.6 Response Surface Methodology (RSM)

Response Surface Methodology (RSM) is a set of statistical and mathematical methods for creating, improving, and optimizing processes. It is useful for product development, design, and formulation as well as for upgrading existing product designs. The input variables, also known as independent variables, are investigated

for a variety of responses, and their relationship is assessed **(Myers *et al.*, 2016**). The independent variables (fenugreek leaf powder and flaxseeds concentration) influencing physical characteristics like hardness and sensorial attributes like colour and appearance, taste and aroma, texture and overall acceptability were optimised in this study using the central composite design (CCD) of Design-Expert software 7.0.0 Response Surface Methodology.

Nutrients and vitamins are deficient in traditional baked items. They provide empty calories to the body because their composition is high in sugar and fat. Keeping this in view, the present study is mainly targeted to develop and optimize a healthy and nutritive snacking alternative to the conventional biscuits available in the marketplace, with value addition in terms of higher nutritional content than conventional biscuits such as high dietary fiber content, ascorbic acid content and antioxidants. In the present study, plant-based sugar-free functional biscuits are developed, optimized, and characterized with the incorporation of fenugreek leaf powder and flaxseeds. The developed functional biscuits are sweetened using the natural sweetener stevia to make them sugar-free and suitable for consumption by diabetic patients and consumers who want to cut down on sugar. Unlike the typically used butter and refined wheat flour in biscuit production, the present research deals with the addition of cocoa butter and rolled oats flour, resulting in making the product completely gluten-free and plant-based. Furthermore, celiac patients can consume the product without risk. As compared to typically used refined wheat flour, oats flour as a base for biscuits is a better and healthier alternative as it is rich in dietary fiber, beta-glucan, and many other nutrients. It also provides the health benefits imparted by the functional compounds present in fenugreek leaves and flaxseeds.

The present research work is “Optimization and Characterization of plant-based functional biscuits incorporated with Fenugreek Leaf Powder and Flaxseeds using Response Surface Methodology.” has been carried out given the following objectives:

1. To optimize plant-based functional biscuits incorporated with Fenugreek Leaf Powder and Flaxseeds using Response Surface Methodology
2. To determine the proximate composition, physicochemical and textural properties of the product
3. To study FTIR spectra and TGA thermogram of the functional biscuits
4. To assess the optimized product's shelf life.
5. To study the rheological properties using Rapid Visco-Analysis

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Chapter-II

REVIEW OF LITERATURE

Biscuits are a popular food item because of its flavour, variety, ease of preparation, storage, appearance, and texture. With a rising market niche for healthier foods, using natural ingredients with functional qualities that provide unique health benefits in addition to regular nutrients is a very appealing strategy for developing new food items. The present study deals with the optimization and characterization of functional biscuits prepared with fenugreek leaf powder, flaxseeds, oats, stevia, and cocoa butter.

2.1 Fenugreek

Fenugreek is a dicotyledonous plant belonging to the Leguminosae family's *Papilionacea* subfamily. **(Acharya *et al.*, 2011)** It offers the human body natural dietary fiber and other vital nutrients. Its leaves and seeds are used in both Ayurvedic treatment and cooking. It is widely used in both culinary and medicinal purposes as an aromatic and flavourful spice. The leaves contain diosgenin glycosides, which are saponin compounds known as graecunins. Leaves are rich in minerals and vitamins such as phosphorus, riboflavin, iron, carotene, thiamine, calcium, and ascorbic acid. **(Jhajhria and Kumar 2016)** Clinical trials in humans and animals have demonstrated that they can significantly lower the cholesterol levels and blood glucose levels. Medicinally beneficial phytochemicals found in fenugreek seed and leaves include steroidal saponins namely diosgenin, galactomannan, alkaloid fenugreekine, and the amino acid 4-hydroxy isoleucine. Carbohydrates (45–60 percent) are found in mucilaginous fibre (galactomannans), lipids (5–10 percent) are found in fixed oil, proteins (20–30 percent) are rich in lysine and tryptophan, and pyridine type alkaloids (0.2–0.38 percent) are found in trigonelline; choline (0.5%), and other materials including carpaine and gentianine, flavonoids (apigenin, luteolin, quercetin, orientin, isovitexin, and vitexin) and 4-hydroxy isoleucine (0.09%), histidine, arginine, and lysine, calcium, iron, and saponins (0.6–1.7%), glycosides such as yamogenin, tigogenin, neotigogenin and diosgenin (generating steroidal sapogenins on hydrolysis); and cholesterol and sitosterol, vitamins (A, B1, C) and nicotinic acid; n-alkanes and sesquiterpenes

(0.015%) known as volatile oils. Fenugreek has been shown to have antioxidant and antibacterial properties. (**Zandi *et al.*, 2017**) Fenugreek green leaves are one of the oldest therapeutic herbs, with higher levels of b-carotene (19 mg/100 g), ascorbate (220 mg/100 g), iron, fiber, zinc, and calcium, than regular foods. 0 g), ascorbate (220 mg/100 g) **(Ahmad *et al.*, 2016)**

Fenugreek is well-known for being the plant's main source of soluble fiber. By lowering LDL and total cholesterol levels, dietary fiber has been shown to reduce the risk of cardiovascular disease and certain types of cancer**. (Zandi *et al.*, 2017)** Fenugreek fiber adds a balance of soluble and insoluble fiber to refined flours. Bakery goods with acceptable sensory qualities, such as pizza, bread, muffins, and cakes, have been made with flour supplemented with 8–10 percent fenugreek fiber. **(Srinivasan 2006)** The inclusion of fenugreek in flour allows for the manufacture of functional foods that are more likely to be accepted by customers who eat a western diet. **(Roberts 2011)** The husk of the fenugreek seed contains high amount of dietary fiber and vital minerals. This dietary fiber-rich functional component was used to make high-fiber muffins with twice the amount of dietary fiber, The fiber-rich muffins have a good volume, a soft texture, and a medium-fine grain. **(Srivastava *et al.*, 2012)** Fenugreek was incorporated in bread in a study conducted by **Losso *et al.*, (2009),** which revealed that fenugreek in food facilitates blood sugar reduction, albeit its application is limited due to its bitterness and strong odor. The wheat and fenugreek bread had no significant variations in colour, texture, proximate composition, hardness, or flavor intensity, but the fenugreek bread had lower glucose and insulin levels. Fenugreek's functional property of reducing insulin resistance was observed in the bread. As a consequence of this research, it's obvious that fenugreek can be used in baked products in an admissible amount to reduce insulin resistance and cure diabetes. **(Wani *et al.*, 2018**) Fenugreek flour has been utilized up to 10% in the formulation of biscuits without affecting the overall quality. In terms of physical, sensory, and nutritional properties, biscuits containing 10% germinated fenugreek flour were the best of all the composite fenugreek flour biscuits. As a result, not only will the production and use of such functional foods assist the general public's nutritional status, but it will also benefit persons suffering from ailments **(Hooda and Jood, 2005).**

2.2 Fenugreek enriched products

According to **Negu *et al.*, (2020**), cookies made with wheat flour with fenugreek and oat flours had considerably higher protein, fat, crude fiber, and calorie content, but lower carbohydrate content. In addition, the level of phytic acid and condensed tannin in fenugreek and oat enriched cookies increased moderately. The mineral content of fenugreek and oat-fortified cookies was also improved (Ca, Mg, Fe, and Zn). Although the influence of baking temperature on the individual response variables varied, 175° C was found to be a good combination. Cookies made with 70% wheat, 10% fenugreek, and 20% oat and cooked at 175°C had the maximum nutritional content and good sensory attributes, according to the findings. **(Negu *et al.*, 2020)**

Kay *et al.*, (2017) discovered that viscosity-matched soluble dietary fiber in a pudding matrix can decrease the acute postprandial peak glucose and insulin, as well as the risk of Type 2 diabetes when such pudding contains soluble dietary fiber from flaxseed mucilage, fenugreek gum and yellow mustard mucilage.

According to **Kasaye and Jha (2015),** fenugreek flour supplemented at 5, 10, and 15% with wheat flour for the production of biscuits and bread improved the protein, fiber, ash, magnesium, calcium, iron, and zinc content of the biscuits and bread.

Sakhare and Prabhashankar (2022) found that a 10% fenugreek fiber-rich in galactomannans composition used for chapati production had more minerals, soluble dietary fibre, insoluble dietary fibre, and minerals than the control.

According to **Hooda and Jood (2003),** the incorporation of fenugreek flour in wheat flour (raw, soaked, and germinated) increased the protein and fat contents of biscuits, bread, macaroni, and noodle and lowered the gluten contents. Among the enriched blends, those including germinated fenugreek flour showed higher protein levels (13.83–16.30 percent) by up to 20 percent.

Hussein (2007) utilized raw, soaked, and germinated fenugreek flour to partially replace wheat flour (72% extraction) in ratios of 5%, 10%, 15%, and 20 % for the preparation of biscuits. According to the findings, fenugreek flour is a rich source of protein, fat, fiber, and minerals (phosphorous, calcium, zinc, and iron). Arrival time,

water absorption, dough development time, and dough stability improved when wheat flour was replaced with prepared fenugreek flours, however mixing tolerance index and dough weakening decreased. According to baking quality, the colour characteristics, and organoleptic evaluation, wheat flour can be replaced with ten percent raw fenugreek, fifteen percent-soaked fenugreek, and twenty percent germinated fenugreek flours to produce acceptable and high nutritional value biscuits.

Kumar *et al.*, (2016) reported that biscuits made from germinated fenugreek seed flour in various proportions (0, 5, 10, 15, and 20 percent) and according to the sensory results, a maximum of 10% fenugreek flour can be used to make biscuits of satisfactory quality. At a 10% level of substitution, it was discovered that incorporating germinated fenugreek flour into wheat flour increased the content of protein, lysine, dietary fibre, total Calcium, and the total iron. According to the research, these biscuits can be safely stored in polypropylene bags for up to one month without losing their organoleptic qualities. **(Kumar *et al.*, 2016)**

2.3 Flaxseed

Flaxseed (*Linum usitatissimum L.*) contains a high amount of bioactive components that are beneficial to one's health **(Johnson and Pernilla, 2009)**. The four most common forms of flaxseed available for human ingestion are whole flaxseed, ground flaxseed, flaxseed oil, and partially defatted flaxseed meal **(Parikh *et al.*, 2018).** Flaxseed is renowned for containing high levels of alpha-linolenic acid, lignan, a phytoestrogenic molecule, as well as soluble fiber and insoluble fiber. **(Bhujangrao 2008)** It has the highest lignan concentration of any plant food, with tens to thousands of times more in the form of secoisolariciresinol diglucoside (SDG) **(Johnson and Pernilla, 2009).** SDG is the main lignan present in flaxseed, and it comes in the form of a linear ester-linked complex. **(Katare *et al.*, 2012)** Flaxseed also includes other phenolic constituents, such as flavonoids and phenolic acids, in addition to lignans. p-coumaric acid, gentisic acid, caffeic acid, ferulic acid, p-hydroxybenzoic acid, as well as syringic acid, sinapic acid, salicylic acid, and vanillic acid, are some of the free phenolic acids or bound phenolic acids contained in flaxseed. **(Johnson and Pernilla, 2009)** In addition to ALA and lignans, flaxseed contains cyclic peptides, cyanogenic glycosides, soluble and insoluble

fiber, and flax proteins. Some of the bioactive compounds found in flaxseeds are alpha-linolenic acid, docosapentanoic acid, linoleic acid, enterodiol, linustatin, enterolactone, docosahexanoic acid, eicosapentanoic acid and linamarin. **(Parikh 2018)** Flaxseed has a good amino acid ratio, including the limiting amino acids Lysine, tyrosine, and Threonine. Sulfur amino acids (Cysteine and Methionine) and branched-chain amino acids (Isoleucine, Leucine, and Valine) are also present. **(Oomah *et al.,* 2007)**

Fiber content ranges from 22 to 26 percent, more than double that of high fiber beans. Between 20% and 25% of ones, daily fiber needs are met by a half-ounce of dry whole flaxseed. Flaxseed contains a 20:80 to 40:60 ratio of soluble dietary fibres and insoluble dietary fibres. The major insoluble fibre fractions are cellulose and lignin, while the soluble fibre fractions are mucilage gums. **(Bernacchia *et al.,* 2014)** Flaxseeds contains great levels of lignan which is converted into enterolactone by microflora of intestine. Enterolactone has been considered as the primary active component mediating the anti-atherosclerosis activity. **(Fuchs *et al.,* 2007)**

In human trials, it has been demonstrated that flaxseed lowers total and LDL levels, decrease postprandial glucose absorption, lower various markers of inflammation, and raise serum levels of omega-3 fatty acids, eicosapentaenoic acid, and alpha-linolenic acid (**Katare *et al.,* 2012).** According to **Paschos *et al*. (2007),** dietary supplementation with 8 g of ALA per day for 12 weeks decreases both systolic BP and diastolic BP in dyslipidaemic men. According to a study conducted by **Saxena and Katare (2014)**, flaxseed supplementation resulted in a highly significant reduction in total cholesterol, triglycerides, and LDL-C, and VLDL-C levels, with simultaneous elevation in HDL-C levels. In both serum and myocardium, ALA was shown to improve cardiac function by improving the expression of the anti-inflammatory cytokine IL-10 and lowering the expression of the proinflammatory cytokines IL-6 and TNF-alpha. **(Xie *et al.,* 2011)**

2.4 Stevia

Stevia occurs in 150 varieties, all of which are native to South and North America. While the term "stevia" refers to the entire plant, the sweet components obtained and purified from stevia leaves are utilized as a sweetening agent. **(Smrity 2017)**

Stevia, in addition to its sweetening properties, has anti-hyperglycemic, anti-hypertensive, anti-inflammatory, anti-tumor, antidiarrheal, and diuretic properties. **(Gupta *et al.*, 2013)** As it's natural, has no calories, does not impact blood sugar levels like conventional sugar, and is heat stable up to 200°C, 2 non-fermentable, prevents cavities, and can be easily used in cooked or baked food, stevia has been identified to have multiple beneficial properties as a natural sugar substitute. **(Panpatil and Polasa, 2008)**

Steviol glycosides, which are up to 300 times sweeter than sucrose, are abundant in the leaves of *S. rebaudiana*. **(Brandle and Telmer, 2007**). The presence of eight glycosides provides stevia leaves their sweetness. Some of the glycosides present in stevia are stevioside, rebaudioside A, C, D, E, and F, dulcoside A and the steviolbioside (**Geuns 2003).** Ascorbic acid, beta-carotene, cobalt, chromium, iron, potassium, magnesium, riboflavin, phosphorus, thiamin, zinc, and other nutrients were found in Stevia. According to **Sharma *et al.*, (2006)**, other chemicals found in Stevia include avicularin, apigenin, austroinulin, b-sitosterol, daucosterol, caffeic acid, chorogenic acid, caryophyllene, , dulcosides A and B, campesterol, centaureidin, chlorophyll, cynaroside, di-terpene glycoside, foeniculin, gibberellic acid, luteolin, formic acid, gibberellin, indole-3-acetonitrile, isoquercitrin, isosteviol, kaempferol, kaurene, lupeol, polysatachoside, quercitrin, quercetin, scooletin, umbelliferone, stigmasterol, and xanthophylls. Stevia leaf extracts contain phytochemicals such as phenols, which are the major components responsible for the antioxidant activity of the extracts. **(Kim *et al.*, 2011).**

Asmei (2017) discovered that crystals derived from stevia leaves have anti-diabetic properties, as diabetic rats given these crystals at a dose of 500 mg/kg lost weight and had reduced blood glucose levels. The crystals also had a protective impact on the pancreas, mending structural damage to a minor amount, according to the histological analysis. **He *et al.*, (2019**) reported that a novel phenylethanoid glycoside named Steviophethanoside, isolated from stevia leaves extract, stimulated rat INS-1 islet cells significantly, suggesting that it could be a safe hypoglycemic agent. However, more research is needed to prove steviophethanoside's hypoglycemic activity and mechanism of action in diabetes. **(Ahmed *et al.*, 2020)** Aqueous extracts of stevia leaves were used

to treat rats with STZ-induced diabetes in a similar investigation. When compared to control rats, diabetic rats treated with stevia extract showed significant reductions in both random and fasting blood glucose, as well as glycosylated haemoglobin (HbA1c), following 8 weeks of treatment, while insulin and liver glycogen levels improved significantly. **(Ahmad and Ahmad, 2018).**

Another study divided individuals with diabetes into two groups: one was given a placebo, while the other was given stevia leaf powder. When compared to the control group, consuming stevia leaf powder for 60 days reduced fasting and postprandial blood glucose levels significantly in the intervention group. **(Ritu and Nandini, 2016)**

2.5 Stevia as an ingredient in food

Sucrose which is a common ingredient in baked goods such as cookies, biscuits, cakes, and muffins, is replaced fully or partially by stevia in several studies. **(Ahmad *et al.*, 2020**) The bitter aftertaste has been the only disadvantage of using stevia as a sugar substitute. **(Luo *et al.*, 2019)** According to **Gao *et al.*, (2016)** stevia successfully substituted up to 50% of the sucrose in muffins without altering the texture, as well as lowering the in vitro glycaemic index as compared to control which comprised only sucrose **(Gao *et al.*, 2016).** Oatmeal cookies were made with stevia aqueous extract in place of 25, 50, 75, and 100 percent sucrose, as well as 100 percent commercial stevia and 100 percent sucrose as controls. The judges gave cookies manufactured with stevia extract instead of sucrose a higher sensory acceptability rate in the sensory acceptance test, which evaluated appearance, taste, odour, texture, and overall impression. Cookies containing 75 and 100 percent stevia extract or 100 percent commercial stevia, on the other hand, had lower sensory acceptability**. (Salazar *et al.*, 2018)**

According to **Parimalavalli *et al.*, (2007),** stevia extract was used in biscuits and bread at levels of 1, 2, and 3% with regular ingredients, with sensory evaluations revealing that the one percent level was highly approved by the panel of judges. In terms of sensory attributes, the addition of extract to biscuits and bread was comparable to that of regular biscuits and bread. In terms of nutrient analysis, the nutrients found in the incorporated products were slightly greater than the standards.

2.6 Cocoa butter

Cocoa butter (CB) is a naturally occurring fat that comes from cocoa beans (*Theobroma cacao*). Due to its unique physical and chemical qualities, it is frequently utilized as a key ingredient in chocolate and other confectionery goods. At temperatures above 37°C, CB is liquid, whereas at temperatures below 25°C, it is solid. Palmitic acid (C16), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2) make up the majority of CB, with only a trace of lauric acid (C12) and myristic acid (C18:2) (C14). CB has a saturated fatty acid content of 57-64 percent and an unsaturated fatty acid content of 36-43 percent. Saturated fatty acids in CB include palmitic acid, stearic acid, lauric acid, and myristic acid, as well as arachidic acid, while unsaturated fatty acids include oleic acid, palmitoleic acid, linoleic acid, and -linolenic acid. The quantities of palmitic acid, stearic acid, and oleic acid are higher, while lauric acid, myristic acid, palmitoleic acid, arachidic acid, and linoleic acid are lower. **(Naik and Kumar, 2014)**

Triacylglycerols make up the majority of cocoa butter, with mono and diacylglycerols, free fatty acids, phospholipids, and other complex lipids making up the rest. CB is known to have nearly similar levels of palmitic (20-26%), stearic (29-38%), and oleic acids (29-38 percent). 1,3-desaturated-2-oleoyl- glycerol is the most common kind of triacylglycerol found in CB. Two other classes of triacylglycerol present in significant amounts are monosaturated dioleoylglycerols and desaturated-2-linoleoyl-glycerols. These contribute to the liquid phase of CB as they have a lower melting point than 1,3-desaturated-2-oleoyl- glycerol. **(Smith 2001)** It also contains fat-soluble antioxidants including vitamin E in the forms of α-tocopherol, β-tocopherol, and γ-tocopherol, which aid in its preservation and therapeutic qualities. It has β-tocopherol in higher amounts followed by α-tocopherol and γ-tocopherol **(Erickson *et al.,* 1983)**

Many epidemiological studies have connected cocoa and chocolate consumption to a lower risk of chronic diseases, and the antioxidant and anti-inflammatory characteristics of cocoa components have been related to a variety of human health advantages. **(Ellam and Williamson, 2013)** Cocoa contains a significant amount of flavonoids, notably flavanols, and is a rich source of polyphenolic compounds. **(Scapagnini *et al.,* 2014)** According to **Lee *et al.,* (2003)**, cocoa's phenolic and flavonoid levels, as well as total antioxidant activity, are higher than those of other

phytochemical-rich foods. Cocoa components' antioxidant capabilities may influence insulin resistance, reduce diabetes risk, or boost redox-sensitive signalling pathways involved in endogenous antioxidant defense gene expression. **(Katz *et al.*, 2011)**

2.7 Oats

Oat has been regarded as a healthful food since ancient times due to its nutritional characteristics. This cereal is high in carbohydrates, particularly starch, as well as dietary soluble fibre, lipids, well-balanced proteins, and several B-vitamins. Oat products have been associated to lower serum cholesterol levels, a lower risk of cardiovascular disease (CVD), as well as the prevention of cancer, diabetes, and gastrointestinal diseases. **(Villaluenga and Pens, 2017)**. Oatmeal is high in essential amino acids, fiber, unsaturated fatty acids, vitamins, minerals, and antioxidants. The high total dietary fiber and beta-glucan content of oats is their principal health advantage. The Food and Drug Administration (FDA) has specified that oat-glucan ingestion can lower the serum cholesterol levels in persons with excessive cholesterol, lowering the proneness to cardiovascular disease. **(Manolache *et al.*, 2019)**

Oats include a unique group of antioxidants known as avenanthramide (AVA), which has also been discovered in cereals. In oat, the AVAs 2p, 2c, and 2f are the most prevalent. The letters c, p, and f denote the types of hydroxycinnamic acids, which are p-coumaric, caffeic, and ferulic acids, respectively. **(Rasane *et al.*, 2015)** AVAs have ten to thirty times the antioxidant activity of other phenolic antioxidants as vanillin and caffeic acid, according to **Dimberg *et al.*, (1993).** According to preliminary study, AVAs may have anti-inflammatory and anti-atherogenic properties because they block monocytes from adhering to human aortic endothelial cells and macrophages from generating pro-inflammatory chemicals. **(Liu *et al.*, 2004).** They also play a role in blood pressure regulation because they synthesize nitric oxide, which dilates the blood vessels. **(Rasane *et al.*, 2015)**

Oats have a lower carbohydrate level than other cereals, but they have a higher protein and fat content. Oats contain more oleic acid and less linoleic acid than other cereals and are high in mono- and di-unsaturated fatty acids. The lipid-soluble vitamins and lipid-soluble vitamins are prone to oxidation because the oat grain is abundant in unsaturated lipids and lipolytic enzymes like lipoxygenase and lipase. Phenolic amino

acids, tocopherols, thiol, L-ascorbic acid, and phenolic compounds are all biologically active components found in oats. The major antioxidants in oats are vitamin E (tocols), phytic acid, and phenolic compounds such avenanthramides, but flavonoids and sterols are also present. **(Ryan 2007)**

Oats have been shown to provide a wide range of therapeutic effects in humans, including reduced diabetes symptoms **(Tapola *et al.*, 2005)** and obesity **(Zdunczyk *et al.*, 2006).** Beta-glucan is the main component of oat that is assumed to be responsible for these health benefits, but other antioxidant chemicals and phenolic compounds in oat also play a role. Oats' antioxidant activity is boosted by tocotrienols, tocopherols, phytic acid, flavonoids, and non-flavonoid phenolic chemicals such as AVAs. Oats are high in lipids. It has far more lipids than other cereals, giving it an excellent source of energy and unsaturated fatty acids. **(Rasane *et al.*, 2015)**

2.8 Functional Biscuits

Gouveia *et al.*, (2008) tested the colour, texture, and fatty acid profile of ordinary butter biscuits enhanced with *I. galbana* biomass (1 percent and 3 percent). The biscuits had excellent texture properties, good colour and texture stability, and a desirable polyunsaturated fatty acid profile, with an emphasis on EPA and DHA, according to the study.

According to **Pasqualone *et al.*, (2015)** anthocyanin-rich biscuits from purple wheat were developed which showed that the total anthocyanin content was 13.86 mg/kg cyanidin 3-O-glucoside, and the antioxidant activity was greater as compared to control. Purple biscuits contained lower levels of lipid-derived carboxylic acids and higher levels of alcohols and aldehydes in their volatile chemical profile than control biscuits, indicating that the lipid part had been less oxidatively destroyed.

Čukelj *et al.*, (2017) investigated the nutritional (antioxidant activity, lignans, phenolic acids omega-3), physical (texture and spreading), and sensory attributes of biscuits prepared with oats, whole wheat, rye, barley, and milled flaxseed added (10 percent on flour basis), as well as oxidative stability during storage. The concentration of lignans in flaxseed biscuits was thirty times higher (final range 101–117 mg/kg) than in non-enriched whole-wheat biscuits (3.6 mg/kg), and the baking did not influence concentration. The addition of flaxseed increased the spread factor and the kind of

phenolic acids, but not the total phenolic acids. Sensory acceptance was equivalent to white flour biscuits when cereal flours and flaxseed were used in the right proportions. Whereas barley-flaxseed was the least liked, oat flour performed best with flaxseed, but its acceptability fell rapidly over time.

Hassan *et al.*, (2012) supplemented barley, mustard, defatted mustard, flaxseed meal, and flaxseed oil to develop functional prebiotic biscuits for lowering blood lipids. The biscuit formulas were developed by replacing wheat flour with various plant meals at 5, 10, 15, 20, 25, and 30% levels, or shortening with flaxseed oil at 25, 50, 75, and 100% levels. The control samples included biscuit samples with 15% mustard and flaxseed meals, 10% defatted mustard meal, 30% barley meal, and 100% flaxseed oil. The protein content of the biscuit samples with 10% defatted mustard meal and 15% mustard meal was 1.37 and 1.25 times higher than the control. Biscuits with 30% barley flour and 15% flaxseed meal contained 2.84 and 3.31 times more total dietary fibres than the control, respectively. The alpha-linolenic acid content of flaxseed oil biscuits was 42.76 percent, but the linoleic acid content was lower (13.52 percent). Histopathological analysis demonstrated that feeding hypercholesterolemic rats diet supplemented with flaxseed, flaxseed oil, and barley meals reduced the severity of heart and liver tissue lesions. The biological evaluation of hypercholesterolemic rats fed various functional prebiotic biscuit diets for 8 weeks discovered that the diets significantly reduced serum total cholesterol, triglycerides, LDL, VLDL, and ratios of total cholesterol/HDL cholesterol, LDL/HDL cholesterol, atherogenic index, and raised HDL.

Santosh *et al.*, (2019) reported that the functional biscuits made by substituting 20 percent processed bamboo shoot paste for wheat flour exhibited a substantial increase in dietary fiber content, protein, phenols, phytosterols, and vitamin C content when compared to control biscuits. The high sensory score and general acceptability of the bamboo shoot paste supplemented biscuits lead to the conclusion that bamboo shoot supplementation in biscuits is an effective strategy to include nutrients and health-promoting phytochemicals into diets.

Adegoke *et al.*, (2017) reported that composite biscuits were made using wheat flour, soya bean, and turmeric as functional components, with the optimal mixes containing

72.88 percent, 26.63 percent, and 0.5 percent wheat flour, soybean flour, and turmeric, respectively. It was found that 6.59 percent moisture, 13.01 percent protein, 21 percent fat, 0.74 mg GAE/g phenolic, 48.77 percent DPPH, 1.59mg/ml reducing power and, 3.78mg GAE/g total antioxidant, were the optimal combinations. According to the conclusions of this study, adding turmeric to composite flour biscuits will boost their nutritional composition and functionality.

According to **Rani *et al.*, (2020),** functional biscuits were created by incorporating orange peel powder (5, 10, 15, and 20%) and it was discovered that the total dietary fibres, soluble dietary fiber, and insoluble dietary fibres, radical scavenging activity and, total polyphenols contents were significantly higher in orange peel powder supplemented biscuits when compared to controls. In orange peel powder supplemented biscuits, the levels of total, insoluble, and soluble dietary fibre ranged from 8.33 to 13.33 percent, 5.43 to 7.36 percent, and 2.82 to 6.00 percent, respectively, whereas they were 2.70, 1.74, and 0.95 percent in control biscuits.

Alongi *et al.*, (2019) created short dough biscuits by enriching them with apple pomace and partially replacing wheat flour (10 and 20% w/w) to lower their glycemic index. According to the findings, apple pomace had a large level of dietary fibre of almost 40 percent, which was primarily represented by insoluble fibre of more than 25 percent. The glycemic index of the developed biscuits was significantly reduced after enrichment. By replacing 10% and 20% of the wheat flour in biscuits with apple pomace, the glycemic index was reduced to 65 and 60, respectively, making the product of intermediate glycemic index.

Pasqualone *et al.*, (2014) discovered that biscuits made with grape marc extracts had better antioxidant activity and phenolic content than control biscuits (without the addition of extract). They also had higher sensory scores for colour (as indicated by increased a* and 100-L* values), fruity odour, and sour taste, as well as reduced friability. The amounts of Maillard and lipid oxidation volatiles in the supplemented biscuits were high.

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Chapter-IV

MATERIALS AND METHODS

The materials used and the methods employed in this investigation are presented in this chapter. The following studies were carried out at the food technology laboratory of the Department of Dairy Science and Food Technology, Banaras Hindu University.

3.1 Materials

3.1.1 Glassware

The "Borosil" make glassware were used for chemical and microbiological analysis. Prior to use, all the glassware were thoroughly washed using detergent, rinsed with distilled water, air-dried and sterilized in a hot air oven at 160º C for 90 minutes. Properly cleaned stainless steel utensils were used for handling of ingredients, processing and preparation of biscuits.

3.1.2 Ingredients used for biscuit preparation

For the preparation of functional biscuits following ingredients were purchased: oats, stevia, fenugreek leaves, flaxseeds from local market and cocoa butter from Flipkart. For the preparation of control biscuits following ingredients were used: oats, sugar, butter purchased from local market of Banaras.

3.1.3 Tools used for biscuit preparation

3.1.3.1 Pastry Roller

A wooden pastry roller was used to roll the dough on a pastry board. It was purchased from the local market of Varanasi, India.

3.1.3.2Pastry Board

A pastry board was purchased from the local market of Varanasi for making functional biscuits of uniform thickness.

3.1.3.3 Biscuit Cutter

A round-shaped cutter made of tin was used to cut the dough to shape out the functional biscuits. It was purchased from the local market of Varanasi, India

3.2 Equipments Used

Table 3.1 List of equipments used

Name of Instrument	Company, Model and Country
Electronic weighing balance	Labtech LCB 1201v Daihan Lab Tech India Pvt. Ltd
Vortex shaker	MSW-308 (Deluxe Model) Macro Scientific works Pvt. Ltd, Delhi
Hot air oven	992 Perfit, India
Laminar air flow	Lab tech lcb 1201v, daihan pvt. lmt., India
Centrifuge machine	3-30K Sigma, Germany Water bath VS-1205 wp Vision scientific co., Ltd, Korea
Incubator	LTI-700 EYELA, China
Soxhlet Apparatus	SOCS PLUS, SCS-4, Chennai
Spectrophotometer	UV-1800 Shimadzu corporation, Japan
Muffle Furnace	SNOL 8,2/1100-1LZ PagamintaLietuvoje, Lithuania
Autoclave	High-Pressure steam sterilizer (Autoclave) SX-500 TOMY
Texture Profile Analyser	CT 3 Texture Analyzer (Brookfield Engineering Labs, Middleboro, MA, USA),
FTIR	Model Nicolet iS 10, Thermo Fisher Scientific, USA
RVA	Rapid Visco Analyzer (RVA, Newport Scientific RVA-4SAPty. Ltd., Warriewood, NSW, Australia)
Thermogravimetric analyser	thermo-gravimetric analyzer TGA7 (Perkin Elmer, Norwalk, CT, U.S.A.)

3.3 Preparation of Raw Ingredients

Fresh fenugreek leaves (Kauri methi) were procured from local market of Varanasi. The leaves were properly washed and spread in the trays for drying. The leaves were dried at 55° C for 2-3 hours in a tray dryer. The dried leaves were made into powder of uniform particle size using a mixer grinder to obtain fenugreek leaf powder (FLP) that will be easy to blend with wheat flour. Flaxseeds were roasted at 180°C for 10 min and then cooled. Rolled oats were ground and made into flour.

Fig 3.1 Fresh fenugreek leaves procured from the market

(a) **(b)**

Fig 3.2 (a) Dried fenugreek leaves (b) Roasted Flaxseeds

3.4 Method for Preparation of Functional Biscuits

All the dry ingredients were weighed accurately. An Electric Beater was used to beat the cocoa butter for 1 to 2 minutes, after which the stevia was gradually added, and beating was done till it was light fluffy for 5 minutes. The oats flour, FLP, FS, baking soda, and baking powder were then added to it and mixed properly. Gradually water was added into the dry mixture and kneaded gently to make a soft non-sticky dough. The dough was then kept for proofing for 10-15 minutes. The dough was then cut into equal round shapes with uniform thickness using a biscuit cutter and placed prior preheated oven (preheated for at 160°C for 10 minutes) to bake at 160° C for 24 minutes. After baking the biscuit were taken out of the oven and allowed to cool. Afterward, the biscuit samples were separated for different analyses and properly packed in metalized polyester/polyethylene (LDPE) laminate and stored. For the shelf-life study, properly labeled samples were kept at room temperature and 10°C.

(a) **(b)**

Fig 3.3 Functional biscuits (a) before baking (b) after baking

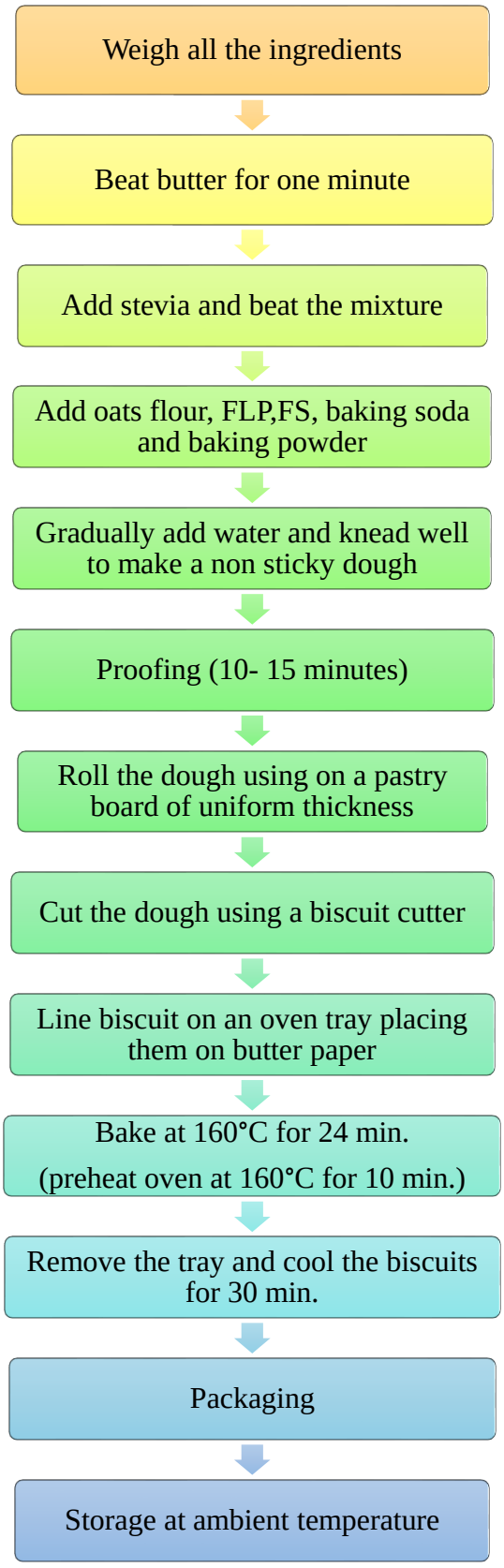

Fig 3.4 Flow Diagram of manufacturing Functional biscuits incorporated with FLP and FS

3.5 Control Sample Biscuits

Refined wheat flour biscuits, made using butter, milk powder, refined wheat flour, sugar and baking powder, procured from the local market of Varanasi, were used as the control sample. The functional biscuits prepared in the present study were compared with the market biscuits in terms of nutritional quality and physical characteristics.

Fig 3.5 Market biscuits as the Control Sample

3.6 Methods of Analysis

3.6.1 Texture Profile analysis

Textural properties such as Hardness, cohesiveness, gumminess, springiness, and chewiness of FB and CB were measured by CT 3 Texture Analyzer (Brookfield Engineering Labs, Middleboro, MA, USA). The peak force (g) and force mean distance at (mm) were recorded. A 5 mm (5P) cylinder probe was used for the analysis and the T.A. settings are shown in Table 3.2

Table 3.2 Parameter used for measurement of textural properties by Texture Analyser

S. No.	T.A. Setting	Measure Force in Compression
1	Pre-test speed	2 mm/sec
2	Test speed	1 mm/sec
3	Post-test speed	1 mm/sec
4	Target mode	Distance
5	Distance	7 mm
6	Trigger type	Auto force
7	Trigger force	5 g
8	Break	Off
9	Stop plot	Start position
10	Tare mode	Auto
11	Advance option	On

3.6.2 Physical characteristics of functional Biscuits

3.6.2.1 Diameter

The diameter of biscuits was determined by placing four biscuits edge to edge. The total diameter was measured in centimeters with the help of Vernier calipers. The biscuit was rotated at an angle of 90º for duplicate readings. This process was repeated thrice to get an average value and results were reported in centimeters.

3.6.2.2 Thickness

The thickness of the biscuit was determined by placing four biscuits stacked on one another. The thickness was measured in centimeters with the help of a Vernier caliper. This process was repeated thrice to get an average value and results were reported in centimeters.

3.6.2.3 Spread ratio

The spread ratio was calculated as diameter (length) to thickness ratio (Shrestha and Noomhorm, 2002).

$$Spread\ ratio = \frac{Diameter}{Thickness}$$

3.6.2.4 Bulk Density

The bulk density of the biscuits was measured by the method of **Marak *et al*. (2019).** Two gram of flour samples were added into an empty 10 mL graduated cylinder which was further kept on a vortex vibrator for 1 min. The volume was then recorded. The ratio of the mass of the powder to the volume occupied in the cylinder determines the bulk density value in g/ml using the following equation:

$$Bulk\ Density\ \left(\frac{g}{ml}\right) = \frac{W}{V}$$

where W = grams of Sample; V = measuring volume

3.6.2.5 Colour measurement

L*, a*, b* values were analysed using hunter lab Color Flex. L* (100 for perfect lightness or zero for perfect darkness), a* (Redness/-Greeness) and b* (Yellowness/ -Blueness)

3.6.3 Sensory Evaluation

The sensorial qualities of the functional biscuit sample were judged by sensory panelists. The sample of each trial was evaluated for sensory attributed viz. color and appearance, taste and aroma, texture, and Overall acceptability with the help of 9 points Hedonic scale.

3.6.4 Proximate Analysis of Functional Biscuit

3.6.4.1 Protein

3.6.4.1.1 Principle:

Protein was esteemed by the AOAC method (2004). In the Kjeldahl process, the protein and other organic food components are digested with concentrated H_2SO_4 in the presence of a catalyst (1:5 cupric sulfate: sodium sulfate). The total nitrogen is converted to Ammonium sulfate. The digest is diluted with water. Alkali containing NaOH is added to neutralize H_2SO_4. The ammonia formed is distilled into an H_2SO_4 solution containing a methyl red indicator.

3.6.4.1.2 Digestion

Two grams of biscuit sample were weighed in a Kjeldahl digestion flask and a 15g digestion mixture (Na_2So_4/K_2So_4+ 1g $CuSo_4$) was added to 25 ml conc. H_2So_2 was also added. The content was boiled vigorously until the appeared clear or transparent. Heating was continued for 2-3 hours.

3.6.4.1.3 Distillation and Titration

The digested sample was taken into a conical flask filled with 25ml 4% Boric acid (neutralized with a mixture of methyl red and in a ratio of (5:7) and then the flask was placed in a distillation chamber. After that, the sample was diluted and alkali was added till the sample changed the color to brown, then the distillation chamber was allowed to run for 10 minutes. After completion of 10 minutes, the conical flask was taken out from the distillation chamber and titrated against 0.1N HCL. The titer value was noted down.

$$\boldsymbol{Nitrogen}\ \% = \frac{\boldsymbol{TV\ (S-B) \times N \times 14 \times 100}}{\boldsymbol{W \times 1000}}$$

Where, TV =Titre value

B= Blank

S = sample

N= Normality of HCL

W= Weight of sample

3.6.4.2 Fat Analysis (AACC, 2000):

3.6.4.2.1 Procedure:

Five grams of biscuit sample was taken in a thimble and then placed in a previously weighed soxhlet beaker. The beaker was then placed in the extractor. After that extractor was filled with petroleum ether and its top were covered with cotton plugs. The soxhlet apparatus was then switched on with a set temperature of 70°C for half an hour. after completion of extraction, temperature was increased upto 130°C for 10 minutes. For the complete removal of moisture. The cooled beaker were then removed from the apparatus and cooled in dessicator. The beaker were then weighed.

3.6.4.2.2 Calculation

$$\%\, Fat\ = \frac{Weight\ of\ residue}{Weight\ of\ sample} \times 100$$

Where,

Weight of residue = Weight of beaker after drying – Weight of empty beaker

3.6.4.3 Ash Content (AACC,2004)

3.6.4.3.1 Procedure:

Five-gram biscuit sample was completely homogenized and then taken in a silica crucible. The crucibles were then placed on a hot plate at 130° C till smoke disappeared. The crucibles were then placed in to muffle furnace at 550° C (2-3 hours). Weight of the cooled crucibles was then noted down.

3.6.4.3.2 Calculation:

$$\%\, Ash = \frac{Weight\ of\ residue}{Weight\ of\ sample}$$

3.6.4.4 Moisture (AACC,2004)

In washed, preheated, cooled, and weighed empty silica crucible, 2 grams of samples were weighted in duplicate. The crucibles were then placed in a preheated, hot air

oven at 100±5°C for 24 hours. After drying, the crucible was cooled in the desiccator and weighed.

$$\%\ Moisture\ content\ = \frac{Weight\ after\ drying - Initial\ weight}{Weight\ of\ Sample} \times 100$$

3.6.4.5 Crude Fibre (AACC, 2004)

The crude fiber was estimated according to the procedure as outlined in (AACC,2000). It was carried out by taking 3 g of each fat-free flour sample and digesting first with 1.25% H2SO4, washed with distilled water and filtered. Then ignited the sample residue by placing the digested sample in a muffle furnace maintained for 3-5 hour at temperature of 550-650oC till or white ash was obtained. The percentage of crude was calculated after igniting the sample according to the expression given below.

$$Crude\ fiber\ \%\ = \frac{Weight\ loss\ in\ ignition}{Weight\ of\ biscuit\ sample}$$

3.6.4.6 Carbohydrate (AOAC, 1995)

The carbohydrate content was determined by difference method that is by subtracting the measure

protein, fat, ash, moisture and Crude fibre from 100 g of food.

Total carbohydrate = 100- (Weight in gram [moisture +fat+protein+ ash +crude fibre] in 100 gram

of food

3.6.5 Physicochemical Analysis of Functional biscuits

3.6.5.1 Antioxidant activity by DPPH inhibition (Radical Scavenging Activity)

For DPPH inhibition, Brand (1995) slightly modified method was used.

3.6.5.1.1 Procedure

The biscuit sample was dried in an oven (40ºC) for 24 hrs. The dried material was grounded and sieved through a 60-mesh screen to obtain in powdered biscuit. The powdered biscuit was extracted with 80% aqueous ethanol (1gm per 10gm) for 2hrs of shaking at 37ºC. The samples were then centrifuged at 10000 rpm for 15 min. The supernatant collected was used in the essay. DPPH radical solution was prepared by dissolving 10mg of DPPH in 25ml of 80% ethanol blank was prepared by 250µl ethanolic DPPH solution and 2.1 ml of 80% ethanol. 100µl of biscuit extracts were taken and to it 250µl of DPPH solution and 2.0ml of 80% ethanol was added. The mixture was shaken vigorously and allowed to stand in the room temperature for 20 minutes. The decrease in absorbance of the resulting solution was monitored spectrophotometrically at 517 nm. Percentage inhibition or % of decoloration was calculated as follows:

$$\%\ Inhibition = \frac{\{A\ (Blank) - A\ (Sample)\}}{A\ (Blank)} \times 100$$

3.6.5.2 Ascorbic acid Content

The dye-titration method used was essentially that of the AOAC procedure (AOAC, 1984). Metaphosphoric acid extracts of the foods were prepared and pH adjusted to about 1.2. The reducing capacity of the extracts was then measured by titrating with 2, 6-dichlorophenolindophenol (DCIP). In this oxidation-reduction reaction, ascorbic acid in the extract was oxidised to DHAA and the indophenol dye reduced to a colourless compound. End point of the titration was detected when excess of the unreduced dye gave a rose-pink colour in acid solution.

3.6.5.3 Dietary Fiber Estimation

The total dietary fiber contents (% dw) of the biscuit samples were estimated by the enzyme gravimetric method 991.43 (AOAC 2000) with the commercial enzyme kit Megazyme.

3.6.6 Pasting Properties of functional biscuits by Rapid Visco Analyser

The Rapid Visco-Analyser was used to assess the quality of functional biscuit. The pasting properties of starch and starch-containing products are readily assessed in the RVA. During the test, the starch is gelatinized with a consequent rise in viscosity, subject to high temperature and controlled shear during which its stability is revealed and then cooled to provide an indication of setback during gelation. Samples can be assessed for pasting temperature, peak paste viscosity, time to peak, the temperature at peak, hot and cold paste viscosity, breakdown, setback, final viscosity, and other parameters.

3.6.6.1 METHOD

- Switched on the RVA and allowed 30 min warm-up. Switch on the associated computer, run the RVA control software, and select the desired profiles.
- Measured 25.0 ± 0.1 ml of distilled water (corrected to compensate for 14% moisture basis correction of the sample) into a new canister.
- Finely, the grounded sample was taken (3.5 ± 0.01 g) (14% moisture basis) into a weighing vessel and transferred sample onto the water surface.
- A paddle was placed into the canister and vigorously stirred through the sample up and down 10 times. If any lumps remain on the water surface or adhere to the paddle then repeat the jogging action.
- Place the paddle into the canister and insert the canister into the instrument. Initiate the measurement cycle by depressing the motor tower of the instrument. Remove canister on completion of test and discard.
- From the pasting curve, the pasting temperature, peak viscosity, time to peak, breakdown, minimum viscosity, setback and final viscosity may be measured.

3.6.7 Characterisation of Functional Biscuits

3.6.7.1 FTIR Analysis

FTIR spectroscopy was carried out by using a FTIR spectrometer (Model Nicolet iS 10, Thermo Fisher Scientific, USA) from 4000 to 500 cm^{-1} to measure any changes in the spectra.

3.6.7.2 Thermogravimetric analysis

The weight loss of biscuits during baking was measured with a Thermal gravimetric analyser under a nitrogen atmosphere as per the procedure described by **Ayed *et al.*, (2021).** In an aluminium pan, 5.730 mg of sample was placed and sealed with a pierceable lid. The lid was perforated immediately before measurement, and the sample was heated at a rate of 10°C/min from 30 to 600°C. The moisture loss at different stages and the overall percentage weight loss were calculated.

3.6.8 Physicochemical changes during storage (Shelf-life testing)

Shelf-life study for functional biscuit was conducted at different temperature for 1 month at the interval of ten days. Samples were packed, sealed and kept at 10, 25and 37 °C for storage study. The freshly prepared biscuit after being cooled were packed and analyzed for different parameters to be shelf stable. Thiobarbituric acid, Hydroxy methyle furfural content and moisture of the product were observed at the interval of ten days.

3.6.8.1 Hydroxy Methyl Furfural (HMF) Content

Total HMF in the functional biscuit was determined by taking 0.5 g of sample which was then thoroughly mixed with 9.5ml distilled water, 5 ml 3 N oxalic acid was added and the tube were cooled and 5 ml of 40% Trichloroacetic acid solution was added. The precipitated mixture was filtered through Whatman filter paper No.42.0.5 ml of the filtrate was pipetted out into a 5 ml test tube and added with 3.5 ml of distilled water and 1 ml of 0.05 M Thiobarbituric acid solution and mixed well.

The tubes were then kept in a water bath at 40 °C for 50 minutes. After cooling to room temperature absorbance was measured at 443 nm. A blank test was carried out in the same manner as above substituting distilled water for functional biscuit.

A standard curve of HMF concentration and optical density at 443 nm was made using a standard stock solution (10 µmole/ml HMF concentration). The dilutions were treated same as the sample for HMF estimation. From the standard curve, the HMF content in the samples was determined using the following regression equation:

Total HMF (µ mole/100 gram) = (Absorbance -0.55) ×87.5×0.4

3.6.8.2 Thiobarbituric Acid Assay

TBARS value was determined by the procedure described by **Bajaj *et al.*, (2016).** Ground biscuit samples (10 g) were homogenized with distilled water (25 ml) and 10% TCA (25 ml) and filtered. To 1ml aliquot, 3 ml of 0.67% TBA solution and 0.05 N H_2SO_4 were added and boiled on water bath for 30 min at 95°C. It was cooled in ice water for 5 min and n-butanol (4 ml) was added and centrifuged for 10 min at 1500 RPM. The organic layer was pipetted out and absorbance measured at 532 nm in a spectrophotometer.

3.6.8.3 Moisture content:

The protocol is the same as the described in the section3.6.4.4

3.6.8.4 Determination of Microbial Population

3.6.8.4.1 Preparation of the sample (Serial dilution):

1 g of sample was taken and transferred to the test tube with 9 ml of normal saline solution (0.9% NaCl). The sample was serially diluted up to 10^{-10} dilution. The test tube containing samples were homogenized for proper mixing.

3.6.8.4.2 Total plate count

Total plate count (TPC) was used for determination of bacterial count.

3.6.8.4.2.1 Method

The prepared media was autoclaved for 15 minute at 15 psi and temperature at 121°C.All glassware's and necessary item were properly autoclave to avoid contamination. Pouring was done in the laminar- air flow chamber. A flame was

lighted and petri-dishes were slightly opened near the flame and the media was poured in the petri-dishes and kept for solidification in incubator. Inoculation was done aseptically in laminar air flow chamber by taking 0.1 gm of the sample suspended in saline solution from 10^{-2} dilution and transferred to a petri dish with label 10^{-2} of nutrient agar media. Similarly, all the samples were taken and transferred into their respective petri-dishes of nutrient agar media. Duplicate sample was taken for each dilution, a control of nutrient agar media was also kept without inoculation. The inoculated petri dishes were incubated in incubator for 24 hours at 37 ±1°C temperature. Total plate count was noted after 24 hours.

TPC (CFU/ml) = No. of colonies / dilution factor ×0.1

Where,

CFU= Colony Forming Unit

Amount Plated =0.1 g

3.6.8.4.3 Coliform count:

Violet red bile agar was used for coliform count.

3.6.8.4.3.1 Method:

In laminar air flow, the media was poured in sterile petri-dishes in hot condition and kept till it solidified. The plates were marked according to the sample in duplicate. 100 mg of sample was weighed in a sterilized beaker added to the first dilution tube and mixed thoroughly, which was then serial diluted till 10^{-10} was achieved. The 10^{-10} sample was then plated on solidification agar plates using spread plate technique. The plates were then incubated at 37°C for 48 hours in inverted position. The colonies were then counted.

TPC (CFU/ml) = No. of colonies /dilution factor ×0.1

Where;

CFU= colony forming unit

Amount plated =0.1 g

3.6.8.4.4 Yeast and mold:

PDA (Potato dextrose agar) was used to determine the yeast and mold in the biscuit. The prepared media was heated for 15 min an autoclave maintained at 15 psi for sterilization at 121°C. All glassware's and necessary item were properly autoclaved to avoid contamination. Pouring was done in the laminar–air flow chamber. The flame was lighted and petri-dishes were slightly opened near the flame and the media was poured in the petri-dishes and kept for solidification.

Inoculation was done aseptically in laminar air flow chamber by taking 0.1 g of the sample suspended in saline solution from 10-2 dilution and transferred to petri-dishes with label 10-2of nutrient agar media. Similarly, all the samples were transferred to the respective petri-dishes of nutrient agar media. Duplicate sample were taken for each dilution and a control of nutrient agar media was also kept without inoculation. The inoculated petri-dishes were incubated in incubator for 72 hours at 25° C temperature. Colony was counted after 72 hours.

TPC (CFU/ ml) = No.of colonies /dilution factor ×0.1

Where,

CFU= Colony Forming Unit

Amount plated = 0.1 g

3.6.9 Statistical Optimization

The study employed the response surface methodology, which entails the design of experiments, the selection of levels of variables in experiment runs, the fitting of mathematical models, and finally the selection of levels of variables by optimization of the response **(Khalil et al.,1999).** The trials were designed using the Central Composite Rotable Design (CCRD), which included three separate processing parameters or factors **(Lorezen et al., 1993**). 13 Trials were conducted for the optimization of different composition of sugar free biscuits. There were six experiments at centre point to calculate the repeatability of the method. The experiments were conducted in randomized order to minimize the effect of

unexpected variability in the observed responses because of extraneous factors. The experimental design and the codes for the processing parameter or factor are reported in (Table:3.4). The variables considered for the Response surface analysis are amount of fenugreek leaf powder and flaxseeds. The lower and Upper Limit has been shown below

Table 3.3 Lower and Upper Limits of amount of functional ingredients used in trials

S.No.	Name	Low	High
1	Fenugreek leaf powder (FLP)	2.5 %	10 %
2	Flaxseeds (FS)	5%	10%

The rest of the ingredients used in manufacturing of sugar free biscuit were kept constant and their quantities are 100g Oats flour, 25 g Cocoa butter, 2g Stevia,1.25g Baking soda, 1.75g Baking powder and 25ml water.

3.6.10 Statistical Analysis

All experiments were undertaken in triplicate. Data were statistically analysed by ANOVA. Values were considered significantly different at $p < 0.05$, and all values were expressed as average ± standard deviation.

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Chapter-IV

RESULTS AND DISCUSSION

The present study was undertaken to optimize and characterize functional biscuits incorporated with fenugreek leaf powder (FLP) and flaxseeds (FS). In the initial stages of the study, trials were conducted to optimize the concentration of fenugreek leaf powder and flaxseeds using the Response Surface Methodology. Further, proximate analysis, texture profile analysis, FTIR, TGA, Rapid Visco-Analysis, shelf-life study, and characterization of antioxidant activity, dietary fiber, ascorbic acid content, and physical parameters of the optimized product were performed.

4.1 Experimental design and Optimization of functional biscuits fortified with fenugreek leaf powder and flaxseeds using Response Surface Methodology (RSM)

Response surface Methodology (Design Expert 7.0.0) was applied for the optimization of functional biscuits and the factors taken into consideration were fenugreek leaf powder (FLP) and flaxseeds (FS). The levels of fenugreek leaf powder and flaxseeds for a CCD and RSM were determined on the basis of preliminary experiments carried out and varied from 2.5 – 10 % and 5- 10 %, respectively. The experimental design used for the analysis was a CCD with two factors and four responses. The responses considered were taste and aroma, color and appearance, and overall acceptability and hardness of the biscuits. The equation used was a second-order polynomial model. The determination coefficient R^2, the fraction of variation explained by the model, and analysis of variance (ANOVA) were used to assess the model's fitness. The F-test was applied to confirm whether the variance evaluated by the regression model was significantly larger than the variance of the residual and to

analyse the model lack-of-fit. All the models exhibited statistical significance as indicated by the F-Value, R^2 -value for all the models was near 1, C.V. % was less than 10% and lack of fit was also found to be not significant. Table 4.1 shows the central composite rotatable design (CCRD) for the optimization of functional biscuits. The effects of two factors (FLP and FS content) and their interactions on the sensory and textural properties of biscuits were displayed in surface and contour plots.

The experiments consisted of 13 runs. The effect of the two independent variables (FLP content and FS content) on the responses (Y_n, Y_1—Color and appearance CA, Y_2—Taste and Aroma (TA), Y_3—Overall Acceptability, Y_4—Hardness) were modeled using a polynomial response surface. The second-order response function for the experiments was predicted by the following equation:

$$Y_n = \beta_0 + \beta_1 FLP + \beta_2 FS + \beta_{11} FLP^2 + \beta_{22} FS^2 + \beta_{12} FLP \times FS$$

Where, Y_n is one of the four responses; FLP and FS represent the independent variables; β_0 is the constant; β_1, β_2 are the linear-term coefficients; β_{11}, β_{22} are the quadratic-term coefficients; and β_{12} is the cross-term coefficient.

Table 4.1: Central composite Design (CCRD) by RSM for the optimization of functional biscuits

Run order	**FLP** (%)	**FS** (%)	**Colour & Appearance (CA)**	**Taste & Aroma (TA)**	**Overall Acceptability (OA)**	**Hardness (g)**
T1	6.25	7.50	7.2	6.1	6.8	1136
T2	6.25	7.50	7.2	6.1	6.8	1136
T3	10.00	5.00	6.6	6.2	6.4	2335
T4	6.25	11.04	6.6	6.4	6.5	1258
T5	0.95	7.50	8.0	7.0	8.0	1356
T6	6.25	3.96	7.4	6.6	6.7	1706
T7	6.25	7.50	7.2	6.2	6.8	1136
T8	10.00	10.00	6.2	5.8	6.2	1320
T9	6.25	7.50	7.2	6.2	6.8	1136
T10	2.50	5.00	8.2	7.4	8.0	1660
T11	2.50	10.00	7.6	7.2	7.6	1242
T12	11.55	7.50	6.4	5.6	6.0	1834
T13	6.25	7.50	7.2	6.2	6.8	1136

Abbreviations: FLP= Fenugreek leaf powder; FS= flaxseeds

4.1.1 Effect of functional ingredients on the Colour and Appearance of Functional biscuits

The values of color and appearance varied from 6.2 to 8.2 with the average value being 7.15. the minimum and maximum values for color and appearance were observed in experiments 8 and 10 respectively. In trial 8 the levels of fenugreek leaf powder and flaxseeds were10 g and 10 g respectively. In trial 10, the level of fenugreek leaf powder was 2.5g and flaxseeds were 5g.

Figure 4.1 shows the response surface plot for Colour and Appearance as influenced by the level of Fenugreek leaf powder and flaxseeds. From the figure, it can be observed that with an increase in the amount of fenugreek leaf powder and flaxseeds, the color and appearance values are decreasing. Furthermore, a higher decrement is observed in color and appearance with the increasing levels of fenugreek leaf powder as compared to flaxseeds.

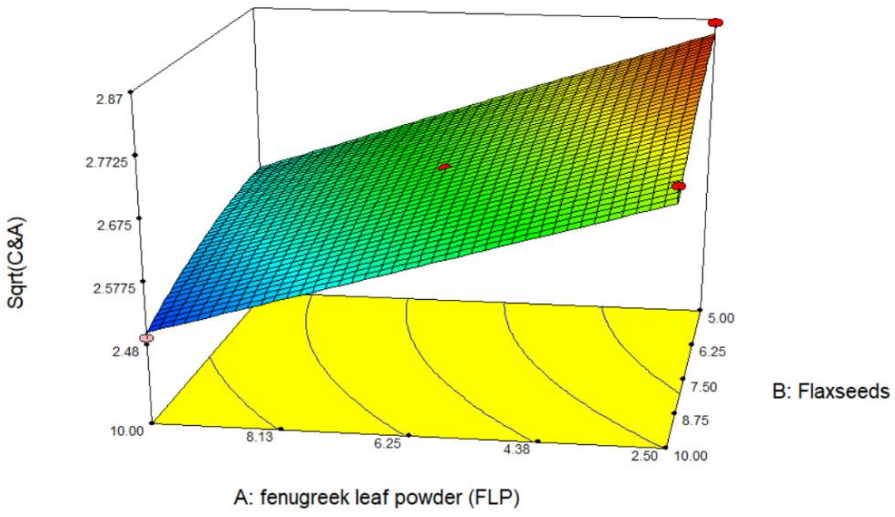

Fig. 4.1 Effect of FLP and FS on Color and Appearance

4.1.2 Effect of functional ingredients on the Taste and Aroma of Functional biscuits

Figure 4.2 shows the response surface plot for taste and aroma as influenced by the level of Fenugreek leaf powder and flaxseeds. From the figure, it can be observed that with an increase in the amount of fenugreek leaf powder and flaxseeds, the taste and aroma values are decreasing. Moreover, a higher reduction is observed in taste and aroma with the increasing levels of fenugreek leaf powder as compared to flaxseeds.

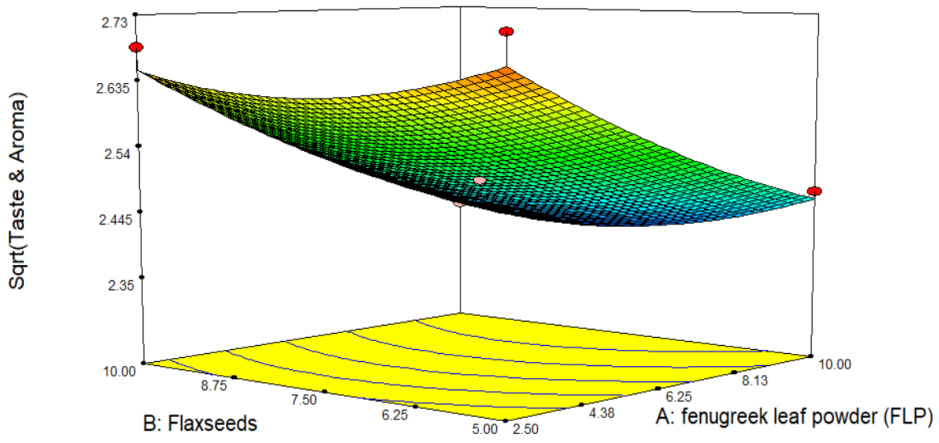

Fig. 4.2 Effect of FLP and FS on Taste and Aroma

4.1.3 Effect of functional ingredients on the Hardness of Functional biscuits

Figure 4.3 shows the response surface plot for hardness as influenced by the level of Fenugreek leaf powder and flaxseeds. It is observed that with the increase in the flaxseeds concentration, the hardness levels of the biscuit samples first decrease slightly, and then it increases continually. The hardness values first decrease up to the addition of 6.5% of fenugreek leaf powder and then increase further raising the levels of fenugreek leaf powder up to 10%.

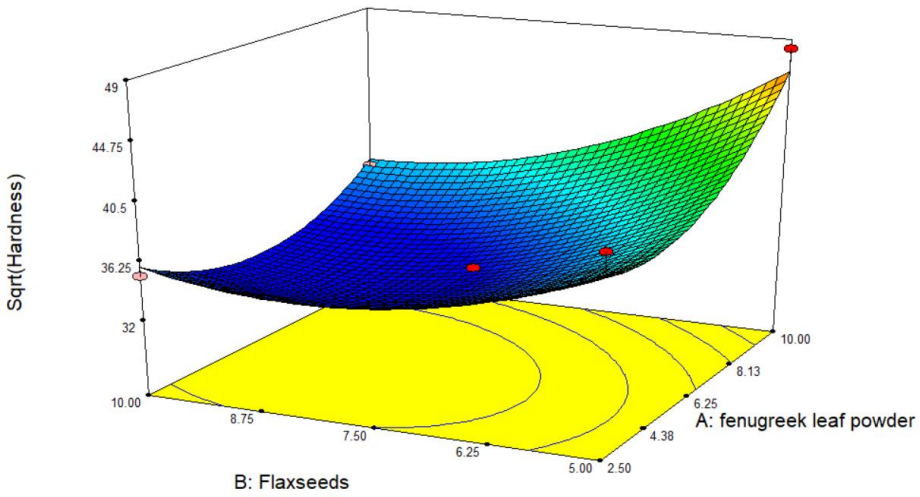

Fig. 4.3 Effect of FLP and FS on Hardness

4.1.4 Effect of functional ingredients on the Overall Acceptability of Functional biscuits

Figure 4.4 shows the response surface plot for Overall Acceptability as influenced by the level of Fenugreek leaf powder and flaxseeds. From the figure, it can be observed that with an increase in the amount of fenugreek leaf powder and flaxseeds, the overall acceptability values are decreasing. Moreover, a higher reduction is observed in overall acceptability with the increasing levels of fenugreek leaf powder as compared to flaxseeds.

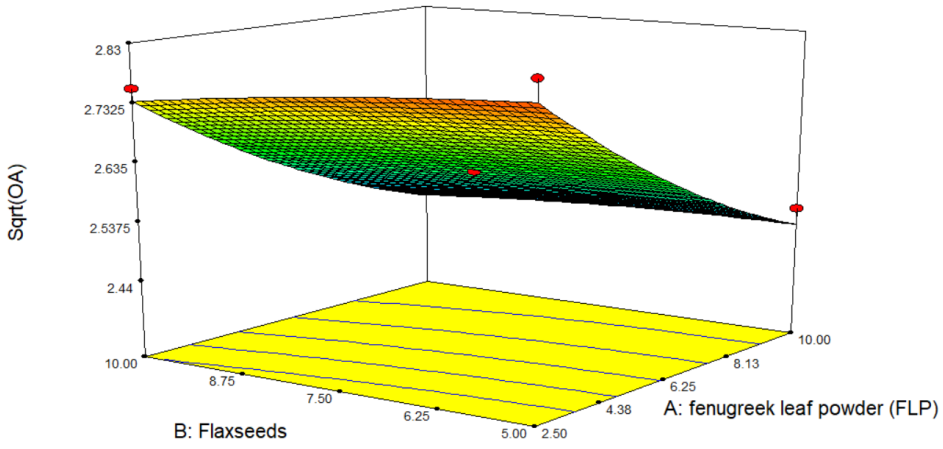

Fig. 4.4 Effect of FLP and FS on Overall Acceptability

4.1.5 Optimization of the product

For the optimization of the functional biscuits, the numerical optimization method of Design-Expert software was utilized and the constraints of the optimized solution were obtained as shown in the table 4.2.

Table 4.2 Constraints of the Optimized Solution

S.No.	Response	Goal
1	Colour and appearance	Maximum
2	Taste and aroma	Maximum
3	Overall acceptability	Maximum
4	Hardness	Minimum

As shown in Table 4.3, the Design-Expert software suggested the optimized parameters on the basis of the constraints presented. Desirability of 0.846 was obtained for the optimized parameters.

Table 4.3 Optimized Parameters

FACTORS		**RESPONSES**			
Fenugreek Leaf Powder (%)	**Flaxseeds (%)**	**Colour and appearance**	**Taste and aroma**	**Overall acceptability**	**Hardness (g)**
2.50	6.12	8.0219	6.9873	7.76404	1336.71

4.2 SENSORY EVALUATION

As shown in figure 4.5, the score for the color and appearance was the highest for T10 (FLP=2.5% and FS= 5%) and the minimum for T8 (FLP= 10% and FS=10%). The score for the taste and aroma was the highest for T10 (FLP=2.5% and FS= 5%) and the minimum for T8 (FLP= 10% and FS=10%). The score for the texture was the

highest for T5 (FLP=0.95% and FS= 7.50%) and the minimum for T12 (FLP= 11.55% and FS=7.50%). The overall acceptability of the functional biscuits was the highest for T10 (FLP=2.5% and FS= 5%) and the minimum for T12 (FLP= 11.55% and FS=7.50%). According to the results of the sensory evaluation, the most liked sample is T10 which contains FLP=2.5% and FS= 5%.

The data obtained by sensory evaluation in the present study were analyzed for the significant difference in the trials by subjecting to the analysis of variance (ANOVA) technique (ONE WAY ANOVA). Statistical analysis of sensory data for color and appearance, taste and aroma, texture, and overall acceptability reveals that their significant difference ($p < 0.05$) in sensory scores of the different samples of functional biscuits.

Table 4.4 Mean scores for the different trials of functional biscuits

Trials	**Colour & Appearance**	**Taste and Aroma**	**Texture**	**Overall Acceptability**
T1	7.2 ±0.05	6.1±0.05	7.1±0.06	6.8±0.05
T2	7.2 ±0.04	6.1±0.04	7.1±0.06	6.8±0.04
T3	6.6±0.01	6.2±0.02	6.4±0.05	6.4±0.05
T4	6.6±0.02	6.4±0.05	6.5±0.04	6.5±0.04
T5	8±0.05	7±0.06	9±0.06	8±0.06
T6	7.4±0.01	6.6±0.04	6.1±0.04	6.7±0.04
T7	7.2±0.02	6.2±0.02	7±0.04	6.8±0.03
T8	6.2±0.06	5.8±0.03	6.6±0.08	6.2±0.05
T9	7.2±0.01	6.2±0.05	7±0.06	6.8±0.05
T10	8.2±0.08	7.4±0.05	8.4±0.04	8±0.06
T11	7.6±0.05	7.2±0.06	8±0.01	7.6±0.05
T12	6.4±0.02	5.6±0.03	6±0.01	6±0.03
T13	7.2±0.03	6.2±0.04	7±0.04	6.8±0.04

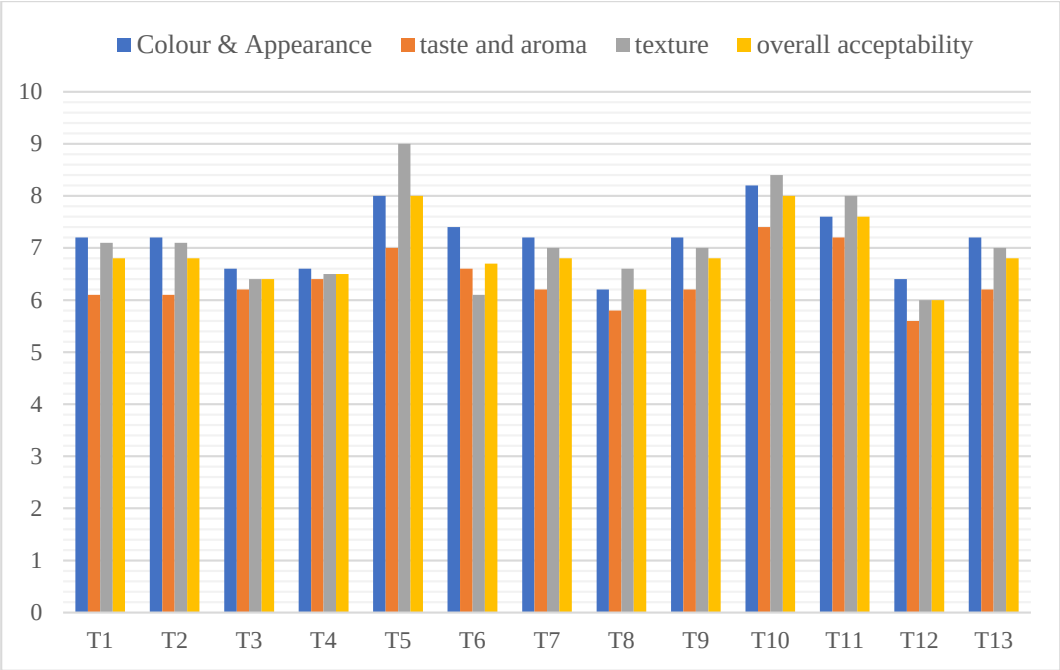

Fig. 4.5 Graphical representation of Sensory Evaluation of Functional biscuits

4.3 Physical properties of functional biscuits

Table 4.5 Physical characteristics of optimized and control sample

Physical characteristics	**Functional Biscuits**	**Control**
Diameter (cm)	5.26±0.05	4.43±0.05
Thickness (cm)	0.63±0.05	0.59±0.10
Spread ratio	8.35±0.68	9.11±1.8
Bulk density (g/ml)	0.54±0.01	0.48±0.01

Values are expressed as Mean ± Standard Deviation of 3 Replicates

From table 4.5, it is observed that the functional biscuits have a greater diameter and thickness as compared to the control sample. Furthermore, the spread ratio of the functional biscuits is higher than the control sample. A higher value of spread ratio i.e., higher diameter and lower thickness are considered a positive characteristic in biscuits. In accordance with our results, other studies have reported a decrease in the spread ratio of the biscuits supplemented with oats bran and fenugreek flour **(Hooda and Jood (2005); Cuklej *et al.*, (2017).** This is due to the presence of more hydrophilic sites in whole grain flours like oats flour, which bind free water in the dough, resulting in increased dough viscosity and a lower spread ratio. Moreover, **Hussain *et al.*, (2006)** reported that flaxseed flour addition at a 5-30% level reduces the spread factor. Similarly, **Rajiv *et al.*, (2012)** achieved a lower spread ratio by replacing wheat flour with toasted flaxseed at a 5-20% level. The bulk density was found to be 0.54g/ml for functional biscuits and 0.48 g/ml for the control biscuits. The fluffy texture of biscuits is indicated by their bulk density. The fluffier the texture of biscuits, the lower the bulk density. **(Guo *et al.*, 2014)** Based on the results, the bulk density of the functional biscuits was found to be greater than that of control biscuits.

4.4 Color characteristics

The color of functional biscuits was measured by Hunter lab (colour-flex). The final optimized functional biscuit is L*- 47.18 ± 0.02, a*-6.64± 0.10, b*-40.15±0.36 and the control sample is L*- 60.04 ± 0.30, a*-11.16 ±0.01, b*-32.80± 0.56. The L value is lowered in the functional biscuits as compared to the control and the b* value is greater in the functional biscuits due to the addition of fenugreek leaf powder and flaxseeds. A similar lowering of the L* value of cookies with the addition of flaxseed flour was reported by **Kaur *et al.*, (2017).** In accordance with our results, **Paramesha *et al.*, (2021)** observed a lowering of L* and a* value, and an increase in the b* value when fenugreek leaf powder was incorporated in bread.

4.5 Texture profile analysis

Table 4.6 Texture Profile Parameters for biscuits

S. No.	Parameter	Functional Biscuit	Control
1	Hardness (g)	1660 ±30	3670±10
2	Cohesiveness	0.48±0.03	0.64±0.01
3	Gumminess (N)	8.22±0.01	23.36±0.59
4	Chewiness (mJ)	55.26±0.95	194.72±0.42
5	Springiness (mm)	6.71±0.04	8.15±0.05

From table 4.6, the hardness of the functional biscuits is observed to be 1660g and the control is 3670 g. The functional biscuits have a lower hardness as compared to the control sample. The values for cohesiveness, which indicates about how well the product withstands a second deformation relative to the first deformation, was found to be 0.48 for functional biscuits and for the control is 0.64. The gumminess of the functional biscuits is 8.22 N and the control is 23.36 N. The chewiness of the functional biscuits is observed to be 55.36 mJ and the control is 194.72 mJ. The functional biscuits have a springiness value of 6.71mm and for control is 8.15 mm. Springiness values shows the recovery behaviour of the dough between the two strokes. It is observed that upon incorporation of fenugreek leaf powder and flaxseeds in functional biscuits, lower cohesiveness, gumminess, chewiness and springiness are obtained as compared to the control sample. The decrease in the hardness and cohesiveness values of the functional biscuits could be attributed to the presence of flaxseed fat and cocoa butter. The lubricating function is more at high-fat content; hence less water is required and a softer texture is obtained. **(Sujirtha, N., and Mahendran, 2015).**

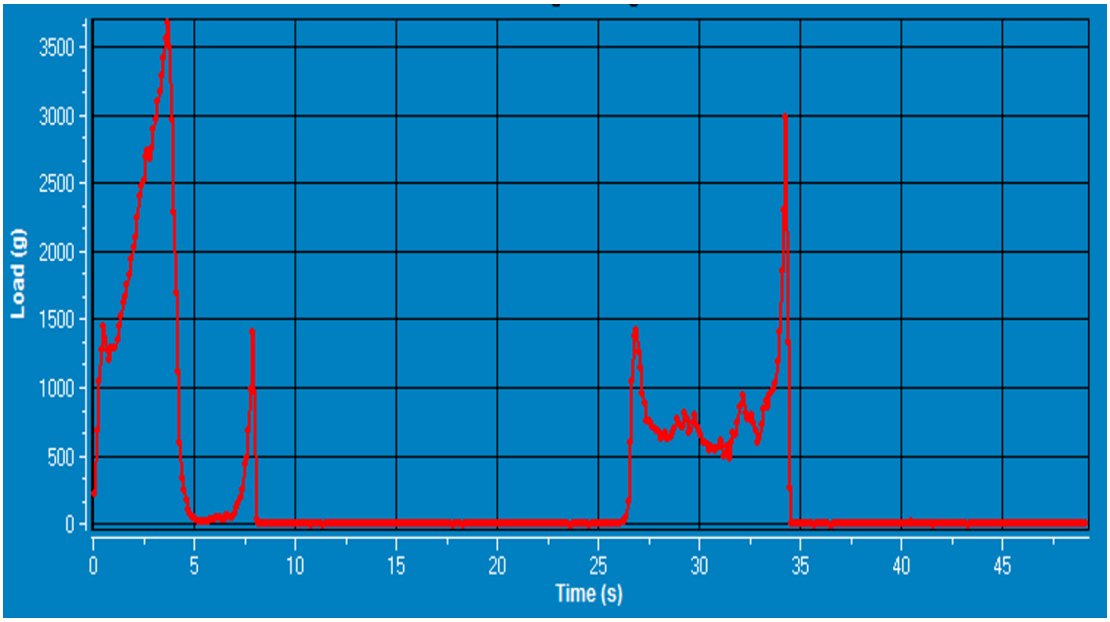

Fig. 4.6 Texture profile of Functional biscuits

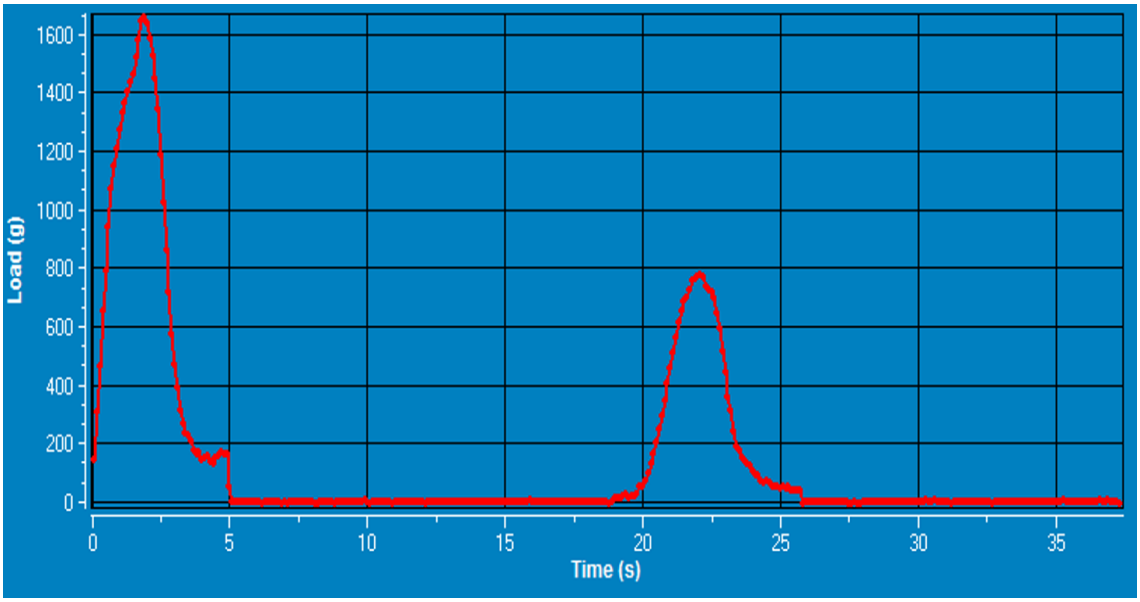

Fig. 4.7 Texture profile of control biscuits

4.6 Proximate Analysis of Functional Biscuits

Table 4.7 Proximate analysis of Functional biscuits and control

S. No.	Constituents	Functional biscuits	Control
1	Ash (%)	3.70 ± 0.10	1.30 ± 0.26
2	Moisture (%)	3.17 ± 0.06	2.60± 0.10
3	Protein (%)	12.93± 0.74	7.02± 0.23
4	Fat (%)	15.07± 0.25	13.60± 0.20
5	Crude Fiber (%)	5.07± 0.25	1.36± 0.60
6	Carbohydrate (%)	59.97± 0.90	73.64± 0.37

Values are expressed as Mean ± Standard Deviation of 3 Replicates

Table 4.7 shows the results of the proximate analysis of functional biscuits and control biscuits. The moisture content in the functional biscuits is 3.17 % and of control is 2.60 %. The $p < 0.05$ show a significant difference in the moisture content of functional biscuits and the control sample. In comparison with control biscuits, the moisture content of functional biscuits increased. This can be ascribed to flaxseed fiber (gum mucilage), which has a greater moisture retention property. **(Cui and Mazza, 1996).** Additionally, the difference in the moisture content of the functional biscuits may be due to the difference in water uptake during the kneading stage to obtain optimum dough consistency for biscuit preparation.

The ash content in the functional biscuits is 3.70 % and of control is 1.30 %. The $p < 0.05$ show a significant difference in the ash content of functional biscuits and control sample. The ash content of the biscuits also increased as compared to the control. This is due to the higher mineral content of flaxseeds and fenugreek leaves.

The protein content of functional biscuits is found to be 12.93 % and in control sample is 7.02%. The p <0.05 show significant difference in the protein content of functional biscuits and control sample. The fat content of functional biscuits is found to be 15.07 % and in control sample is 13.60 %. The p <0.05 indicates a significant difference in the fat content of functional biscuits and control sample. The crude fiber content of functional biscuits is observed to be 5.07 % and in control sample is 1.36%. The p <0.05 indicates a significant difference in the crude fiber of functional biscuits and control sample. The protein, fat, and crude fiber content is increased by the addition of fenugreek leaf powder and flaxseeds. This could be due to the fact that flaxseed has considerably more fat, protein, and crude fiber than refined wheat flour. (Ganorkar and Jain, 2014). Similar findings were obtained by **Chetana *et al.*, (2010); Gambus *et al.*, (2004); Ganorkar and Jain (2014); Masoodi and Bashir (2012).** The carbohydrate content of functional biscuits is found to be 59.97 % and, in the control, sample is 73.64%. The p<0.05 indicates a significant difference in the carbohydrate content of functional biscuits and the control sample. The control sample has greater carbohydrate content as compared to the functional biscuits.

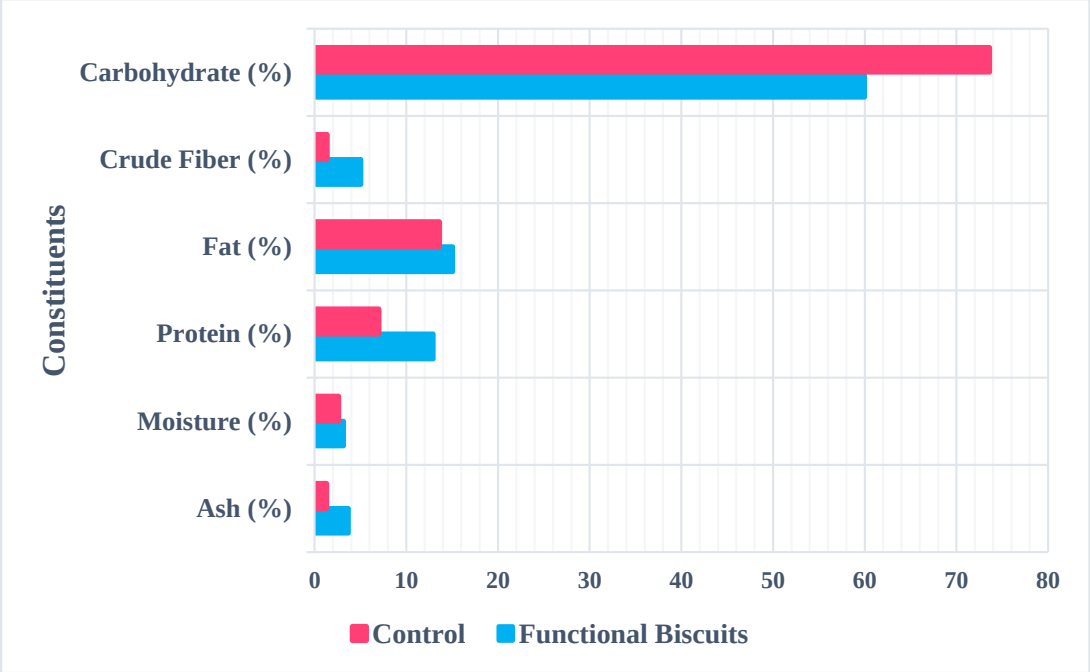

Fig. 4.8 Graphical Representation of Proximate Composition of Functional Biscuits and control

4.7 Antioxidant activity

The functional biscuits were found to exhibit DPPH (% RSA) of 68.40± 5.51 % and the control sample exhibited 15.71±0.55%. There is a significant difference between the antioxidant activity of the functional biscuits and the control as indicated by $p<0.05$. The incorporation of flaxseeds, fenugreek leaf powder, stevia, and oats in biscuits increased the antioxidant activity significantly as compared to that of control biscuits made with refined flour. This gain can be explained by the fact that flaxseed contains a rich antioxidative system and is particularly high in lignans, such as secoisolariciresinol diglucoside (SDG), that are also found in flaxseed oil. **(Akl *et al.*, 2020)** Moreover, fenugreek leaf also possesses significant antioxidant activity which plays a role in enhancing the DPPH% of the functional biscuits. (**Premanath *et al.*, 2011)**

Man *et al.*, (2021) reported that biscuits with 10 % roasted flaxseed flour in wheat flour exhibited a DPPH (%RSA) of 18.89 %. Moreover, **Kaur *et al.*, (2017)** observed that cookies added with 5% raw flaxseed flour to 95 % wheat flour showed DPPH% of 7.93 %. The difference between the antioxidant activity observed in these two studies may be due to the different levels of flaxseed added to the product and the form in which flaxseed is added (roasted or raw flaxseed flour). It is observed that even though the roasting of flaxseeds reduces antioxidant activity slightly, the baking process results in a considerable increase in antioxidant activity due to the Maillard pigments, which are known for their antioxidative properties **(Man *et al.*, 2021).** Likewise, in the present study, flaxseeds were added in the roasted form which may be responsible for the greater antioxidant activity. **Meral and Dogan (2013)** observed that the addition of flaxseed in whole wheat flour bread greatly increased the AO activity. DPPH % of flaxseed fortified wheat flour bread with flaxseed concentrations of 4% and 8% were found to be 35 and 37%.

Fenugreek leaf also possesses significant AO activity. **Dhull *et al.*, (2020)** found that the percent inhibition against the DPPH radical of kasoori methi was 83.3 % in ethanolic extract and 84.4% in methanolic extracts.

The replacement of refined wheat flour with oats flour also accounts for the high AO activity observed. Oats are abundant in antioxidants such as tocopherol, phytic acid, phenolic compounds, and avenanthramide. **(Peterson 2001)** Additionally, **Ragaee *et al.*, 2011** observed that the incorporation of whole-grain flours in the baked product can be beneficial in improving the AO activity. When compared to the control recipe without enrichment, bread enriched with 30 g/100 g wholegrain flours had a 2-fold increase in DPPH scavenging capability.

4.8 Dietary Fiber Content

The dietary fiber content of the control sample is found to be 4.29 ±0.40% and dietary fiber in the functional biscuits is 20.55 ± 0.73%. The fiber content is approximately four times the amount found in the control sample because of the incorporation of fiber-rich ingredients namely oats flour, flaxseeds, and fenugreek leaf. **Ragaee *et al.*, (2011**) reported that the addition of oats flour in bread (30 g/100 g substitution of oats flour with white flour) resulted in dietary fiber content of 7.5g/100g. In comparison with the study by **Ragaee *et al.*, (2011),** it is reasonable to find an increase in the dietary fiber content of the functional biscuits in the present study as it contains a higher amount of oats flour (100g) and fiber-rich ingredients like flaxseed and fenugreek leaf. Similar observations were obtained by **Kurek *et al.*, (2016).** Additionally, in accordance with our results, reported that the dietary fiber content of fenugreek leaf-enriched *parathas* (Indian flat bread) was 20.4 %.

4.9 Ascorbic acid Estimation

The ascorbic acid content of functional biscuits was found to be 14.33±0.57 mg/100g. Ascorbic acid was not detectable in the control sample as it did not contain any vitamin C-rich ingredients. The high ascorbic acid present in functional biscuits is attributed to the fenugreek leaves which are rich in Vitamin C. **Yadav and Sehgal (1998)** reported that fresh fenugreek leaves have an ascorbic acid content of 220.97 mg/100g.

4.10 Characterization of Functional Biscuits

4.10.1 Pasting properties of Functional Biscuits by Rapid Visco-Analyser (RVA)

A Rapid Visco Analyzer was used to test the pasting properties of functional biscuits. The RVA's pasting parameters provide a relative measurement of starch gelatinization, gelling ability, and swelling properties. One of the most crucial aspects of combining functional ingredients into biscuits is to analyze the effect of these ingredients on the rheological and textural properties of the biscuits. The RVA heating-cooling cycles are shown in the graphical representation (Figures 4.8 and 4.9) for evaluating changes in gelling and melting temperature, the viscosity of functional biscuits, and control and evaluation of pasting time and temperature.

As evident from Figures 4.8 and 4.9, the Peak viscosity is found to be 564 cP for the functional biscuits whereas the peak viscosity for the control sample is 569 cP. The peak viscosity is an indication of the water holding capacity of the sample. The final viscosity of the functional biscuits is 1700 cP and the control sample is 1221 cP. Final Viscosity denotes a material's ability to form a viscous paste or gel after heating and cooling. **(Cozzolino 2016)** The pasting temperature, which indicates the minimum temperature required for the cooking of a sample, was found to be 71.10°C for the functional biscuits and 93.70°C for the control. The breakdown value for the functional biscuits was found to be 97 cP and 128 cP for control. The pasting time and temperature for functional biscuits were 6.53 minutes and 71.10°C and for control were 5.67 minutes and 93.70°C. The pasting properties of functional biscuits and control were found to differ significantly ($p<0.05$)

Table 4.8 Pasting Properties measured with Rapid Visco Analyser

RVA Property	Functional biscuits	Control
Peak viscosity (cP)	564±0.02	569±0.01
Trough (cP)	467±0.04	441±0.20
Breakdown (cP)	97±0.08	128±0.10
Final (cP)	1700±0.55	1221±0.42
Setback (cP)	1233±0.21	780±0.28
Pasting time (min)	6.53±0.34	5.67±0.39
Pasting Temperature (T°C)	71.10±0.20	93.70±0.25

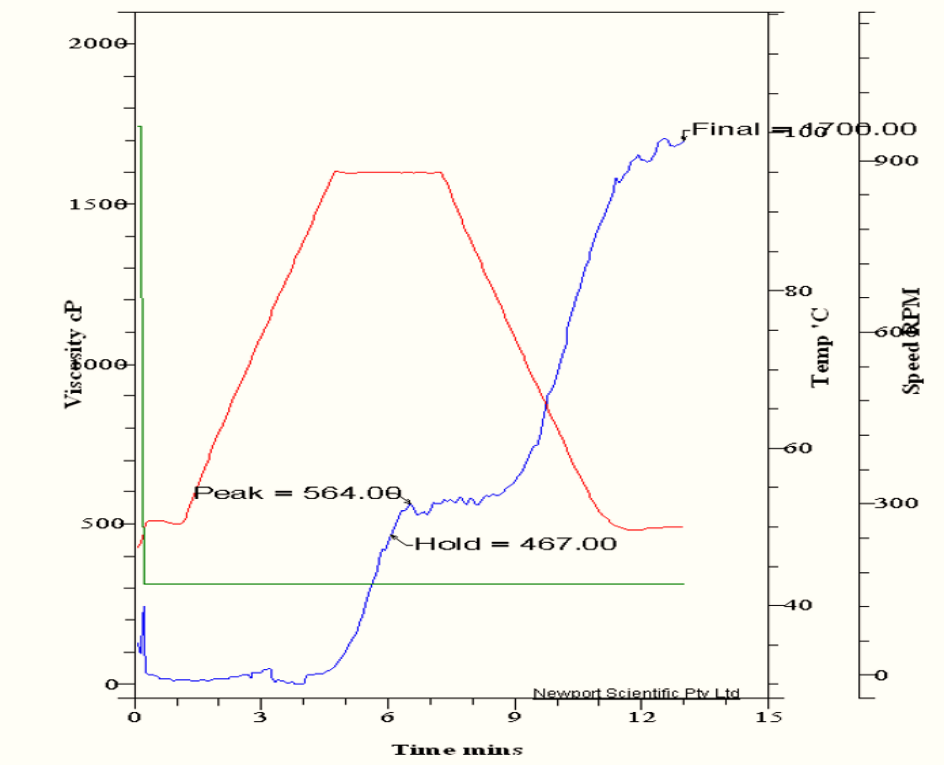

Fig. 4.9 RVA Pasting profile of functional biscuits

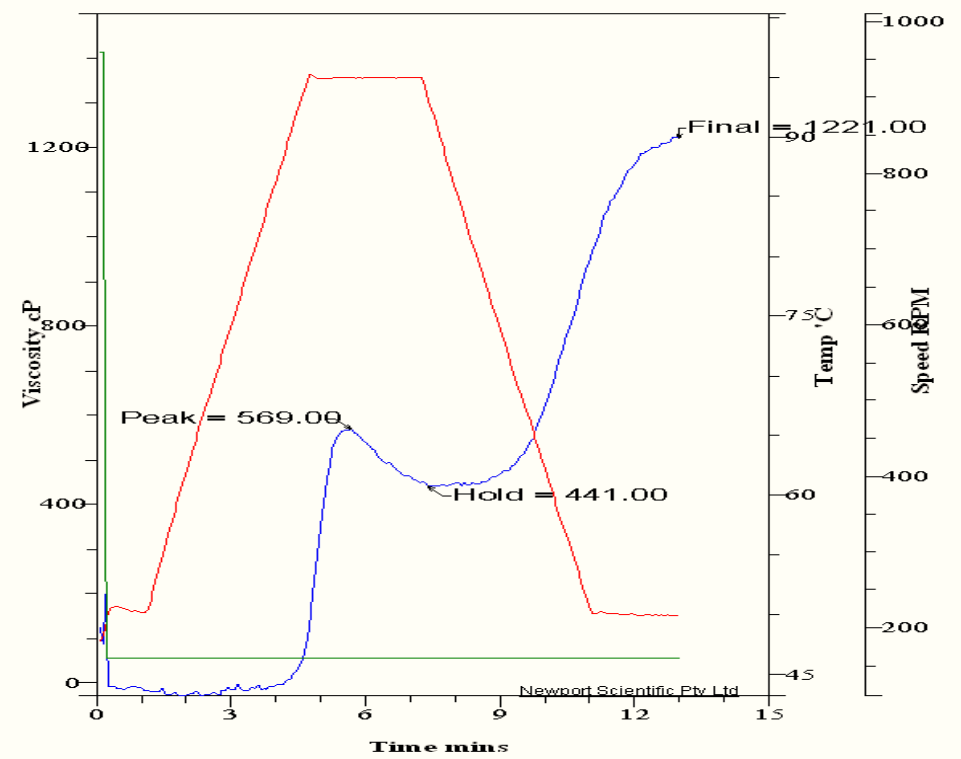

Fig. 4.10 RVA Pasting profile of control sample

4.10.2 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR analysis was used to analyze the chemical structure of the functional biscuit samples and the FTIR spectra obtained are given in the figure 4.10. FTIR spectra are useful for providing structural information that helps identify the functional components in fortified foods. It was identified that the first characteristic region of vibrations observed in the spectra in the figure, i.e., the peak formation at 3367 cm^{-1}, corresponds to the –NH stretching frequency of the free -NH groups present in the sample. The low intense peak identified at 3012 cm^{-1} has been assigned to the stretching frequency of aromatic C-H groups. The peaks identified at 2926 cm^{-1} and 2855 cm^{-1} correspond to the CH and CH_2 stretching aliphatic groups. This could be due to the presence of carbohydrates. The C=O fatty acid group is observed at a broad peak of 1745 cm^{-1} which signifies the presence of a considerable amount of fat in the sample. The C=C stretching is observed at the wavelength of 1653 cm^{-1}. A weak peak observed at 1469 cm^{-1} corresponds to the presence of the aromatic C=C group. This

signifies the presence of aromatic compounds present in fenugreek leaf powder and roasted flaxseeds. A weak peak may indicate the loss of these aromatic compounds during high temperatures of baking. The peaks at 1160 cm^{-1} and 1027 cm^{-1} correspond to the C-N stretching of the aliphatic amines. This could be attributed to the presence of peptide bonds of proteins in the sample. Similar findings of the presence of C=O fatty acid group, C-N stretching, –NH stretching were obtained by **Acari (2021).**

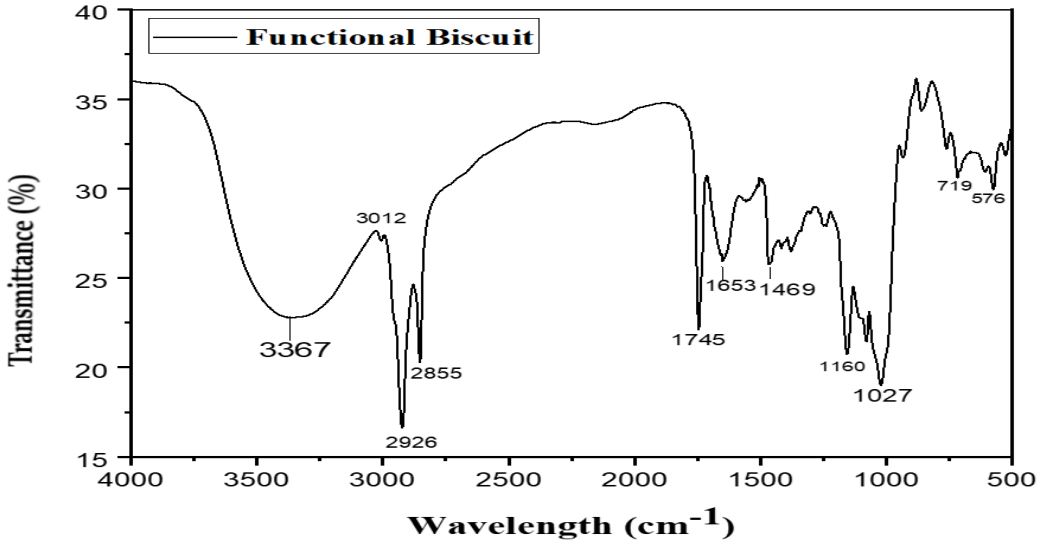

Fig. 4.11 FTIR Spectra of functional biscuits

4.10.3 Thermo Gravimetric Analysis (TGA)

The thermal properties of the biscuit samples were determined by TGA. The functional biscuits and control samples were subjected to TGA and were heated from 30°C to 600°C at 10° C /min. The TGA analysis's findings performed within the scope of the study are presented in the figures. It is observed that the control biscuits and functional biscuits samples gave slightly different thermograms. Three different mass losses can be identified in the TGA thermogram of the functional biscuit sample. The first mass loss is observed approximately between 30°C and 240°C in the functional biscuit samples. The mass loss resulting in this phase is 13.613 %. The second mass

loss of 44.842 % is observed approximately between 240°C and 370°C. the final mass loss of 27.382% is approximately between 370°C and 590°C.

In the control sample thermogram, three mass losses can be observed. Initial mass loss of 10.501% occurred approximately between 40°C to 260°C. Further, a rapid second mass loss of 46.507% occurred approximately between 260°C and 350°C. The final mass loss was observed between 350°C and 590°C. The mass loss found in this phase was 26.216%. The findings are harmonious with the results obtained by **Acari (2021)**

The initial mass loss may be due to the removal of structural moisture in the biscuits. The second mass loss may be caused by the breakdown of carbohydrates and starch molecules in the biscuit structure. The final mass loss could be attributed to the thermal decomposition of complex structures. **(Acari 2021)** A greater mass loss is observed in functional biscuits as compared to control as it contains ingredients like flaxseeds and fenugreek leaves, stevia, and oats flour which have complex structural components such as lignan, beta-glucan, protein, etc.

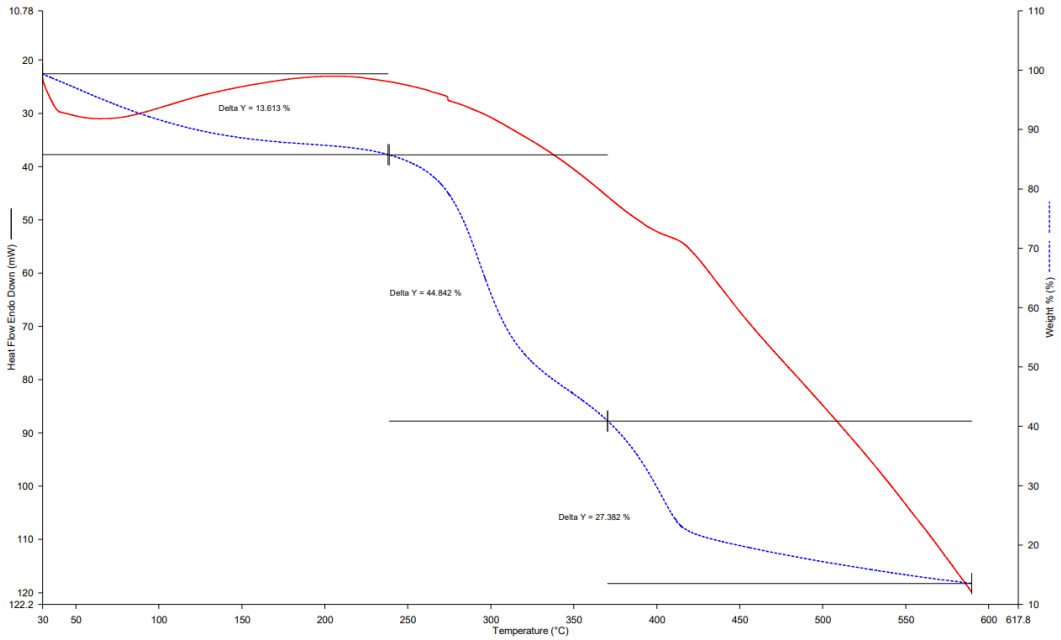

Fig. 4.12 TGA thermogram for functional biscuits

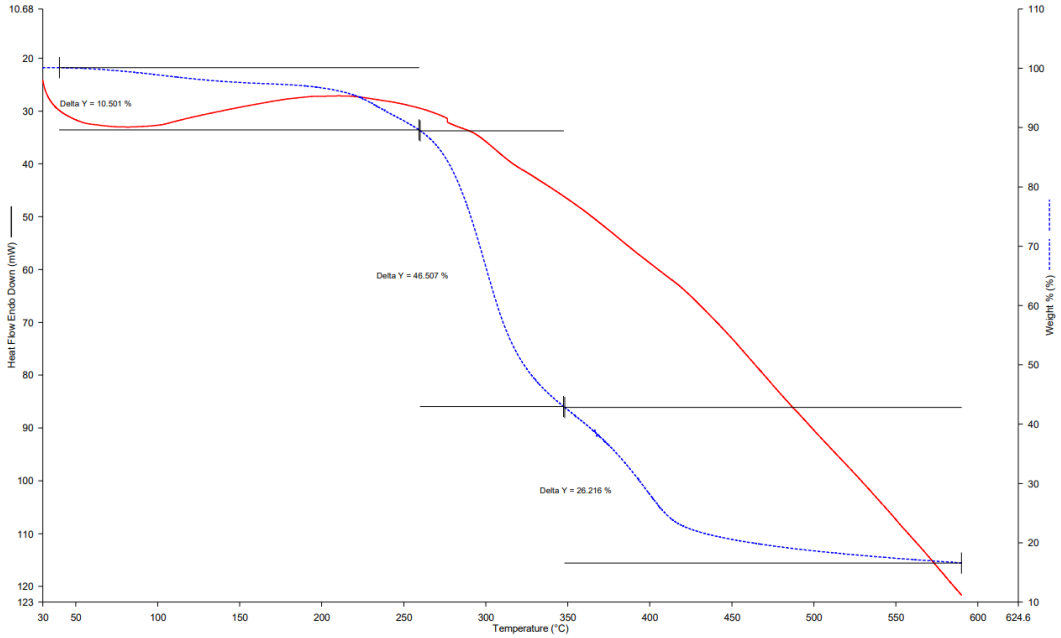

Fig. 4.13 TGA thermogram for control sample

4.11 Shelf-life study of functional biscuit

4.11.1 Changes in HMF content during Storage period

In fresh functional biscuit, HMF level was found to be 4.68 µmol/100g which increased to 4.83 µmol/100g after storage of 10 days at 10°C and increased to 5.49 µmol/100g after 10 days storage at 37°C. After 20 days of storage, the HMF increased to 5.54 µmol/100g and 6.12 µmol/100g at 10° C and 37°C respectively. Further, storage at 10°C and 37° C for 30 days, increased the HMF value to 6.10 µmol/100g and 6.58 µmol/100g respectively.

From figure 4.14, it is evident that the HMF content is found to increase rapidly at a higher temperature and with the increase in storage time. The increase in HMF content is greater at 37°C as compared to 10°C. The findings are in accordance with **Ameur *et al.*, (2007)** who reported that an increase in temperature results in greater levels of HMF formation in cookies. Similar observations were obtained by **Giovanelli and Cappa (2021)**, wherein HMF concentrations in bread increased with the increase in temperature and time.

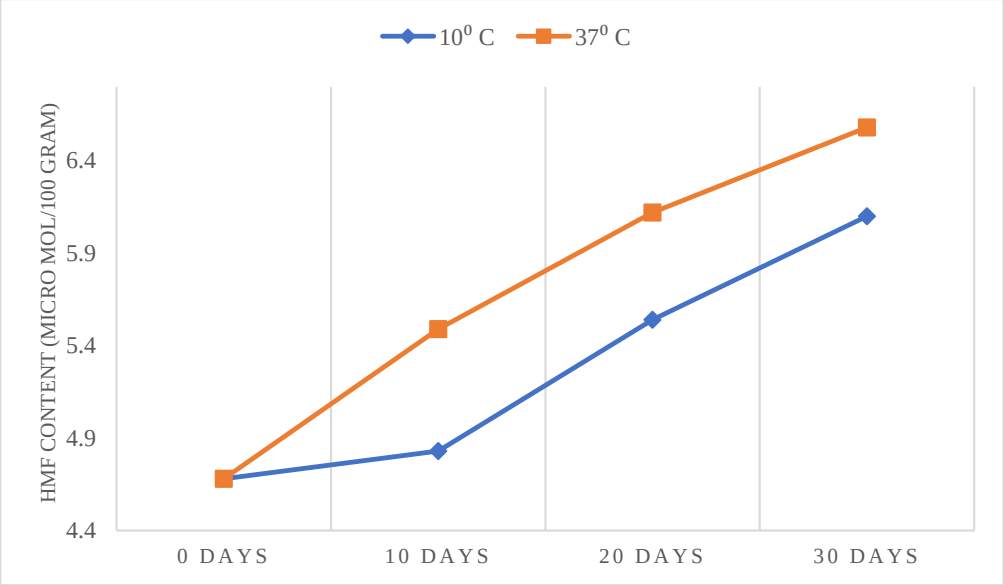

Fig. 4.14 Changes in HMF content during shelf-life study

4.11.2 Changes in TBARS during storage period

In fresh functional biscuits, the TBARS value was found to be 1.02 MDA µg/gram which increased to 1.24 MDA µg/gram and 1.32 MDA µg/gram after storage for 10 days at 10°C and 37° C respectively. Further storage for 20 days, resulted in TBA values of 1.31 MDA µg/gram and 1.43 MDA µg/gram at 10°C and 37°C respectively. The TBA values further increased to 1.39 MDA µg/gram at 10°C and 1.51 MDA µg/gram at 37°C after storage for 30 days. The figure 4.15 shows that the rate of formation of TBA is greater at 37°C as compared to 10°C and the TBA value continues to increase with storage time. A similar increment in the TBA values of fortified biscuits during storage was reported by **Mohamed *et al.*, (2014).** TBA values less than 0.576 mg / kg^{-1} sample are not considered rancid, whereas values between 0.65 and 1.44 mg / kg^{-1} sample are considered rancid but acceptable, and values over 1.5 mg / kg^{-1} sample are deemed rancid and objectionable. **Bajaj *et al.*, (2016)** The TBARS values at the end of the shelf-life study were found to be 1.39 MDA µg/gram at 10°C and 1.51 MDA µg/gram at 37°C which are in the acceptable range. The less lipid peroxidation in biscuits may be due to the high antioxidant activity of the flaxseeds, fenugreek leaf, and oats flour. Additionally, a similar increase in the TBARS values on storage was observed by **Bajaj *et al.*, (2016)** in biscuits added with mint powder.

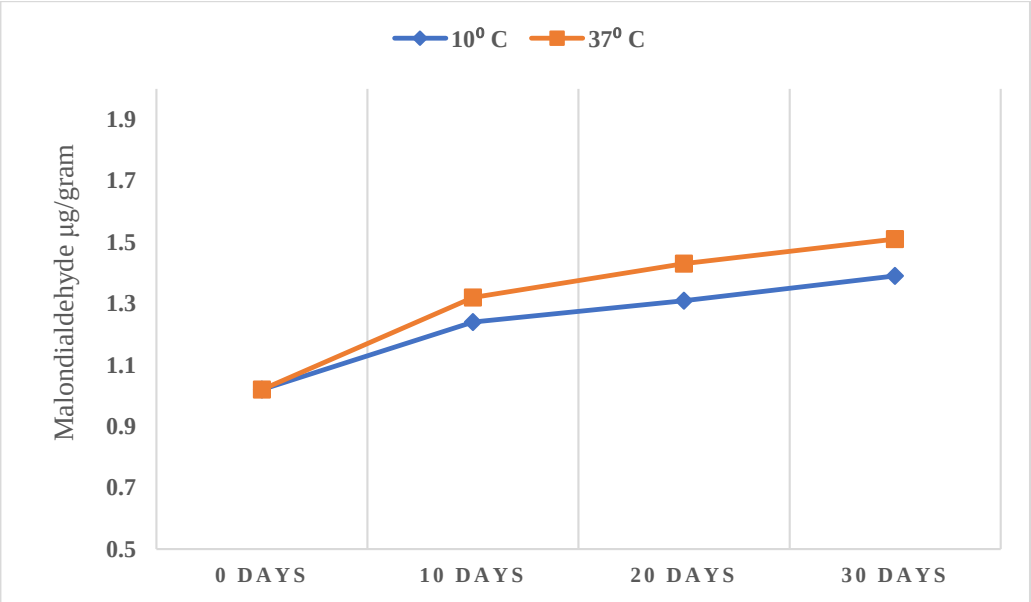

Fig. 4.15 Changes in MDA µg/gram of functional biscuits during storage

4.11.3 Changes in moisture content during the storage period

The gain in moisture content of the functional biscuits samples is presented in the figure. At 10°C, the initial moisture content of 3.17 % increased to 3.7 % after 10 days, 4.4 % after 20 days, and 4.8 % after 30 days of storage. The moisture content increased to 4.1% after 10 days, 4.7% after 20 days, and 5.3 % after 30 days of storage at 37°C.

The increase in the moisture content of the functional biscuits could be attributed to the presence of lignans in flaxseeds which have high moisture retention properties. (**Ganorkar and Jain 2014)** Similar findings of an increase in moisture content of biscuits during the storage period were reported by **Romani *et al.*, (2016)** and **Nadarajah and Thevaki (2015)**. The increase in the moisture content is greater during the first 10 days of storage and a comparatively lesser moisture gain is observed during further storage of 20 days and 30 days. This behaviour may be caused due to the higher diffusivity of water in the more porous and less hydrated matrix of fresh biscuits. **(Guillard *et al.*, 2004; Roca *et al.*, 2006)** The moisture gain is more at a higher temperature of 37°C compared to storage at10°C which is harmonious with the findings of **Sury (2018).**

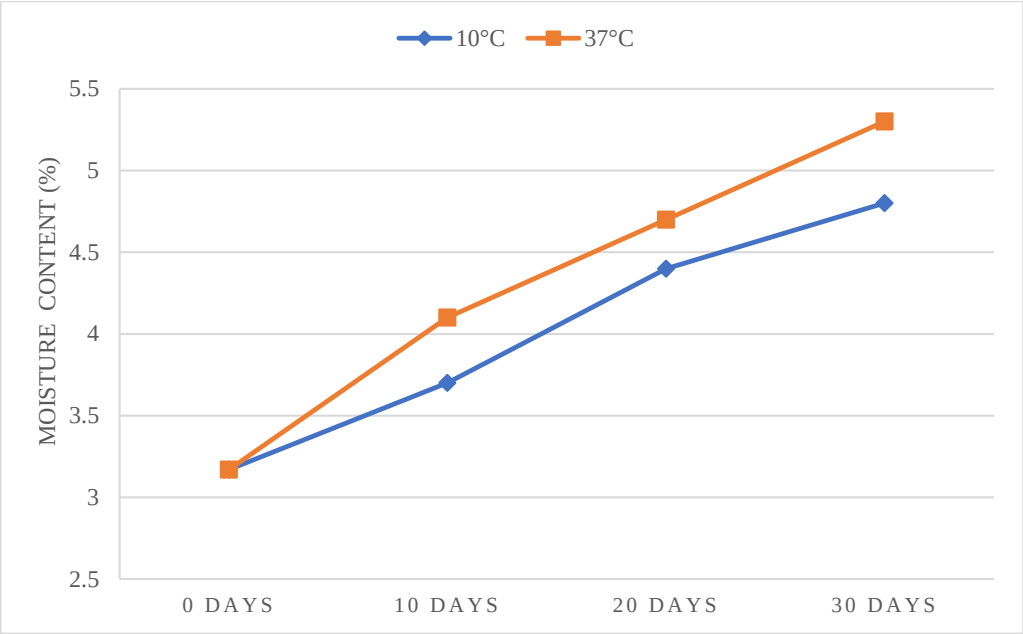

Fig. 4.16 Changes in moisture content of functional biscuits during storage

4.11.4 Microbiological Testing

The Total Plate Count, Yeast and Mould Count, and Coliform Count of the functional biscuits were analyzed in order to check the duration of storage and shelf life of the biscuits. The microbiological study was performed for a month at two different temperatures and the growth of culture was observed and calculated as CFU/g as shown in Table.

No microbial growth was observed in the functional biscuits stored at 10°C and 37°C except after 30 days of storage at 37°C, Total Plate Count of 1.0×10^{1} CFU/g was observed which is acceptable according to the prescribed standards. This absence of microbial load may be due to almost all microorganisms being eliminated during the baking process since biscuits are baked at high temperatures as well as a low amount of water activity in biscuits which suppresses the growth of micro-organisms. The study suggested that the overall microbial load was low in the biscuits and thus it could have a long shelf life and stability.

Table 4.9 Microbiological testing of biscuits

S.No.	Microbial Analysis	0 Days	Temperature 10°C			Temperature 37°C		
			10 d	20 d	30 d	10 d	20 d	30 d
1	TPC (CFU/g)	ND	ND	ND	ND	ND	ND	1.0×10^{1}
2	Yeast and Mold count (CFU/g)	ND	ND	ND	ND	ND	ND	ND
3	Coliform count	ND	ND	ND	ND	ND	ND	ND

d= days; ND= Not Detectable

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Chapter-V

SUMMARY AND CONCLUSION

The present research work "Optimization and Characterisation of plant-based sugar-free functional biscuit incorporated with fenugreek leaf powder and flaxseeds using RSM" was carried out taking two factors viz. Fenugreek leaf powder (FLP), and flaxseeds (FS) and the responses were analysed by Response Surface methodology for optimization. Sensory attributes such as colour and appearance, taste and aroma, texture, and overall acceptability were taken into account. Hardness was also considered a factor as it is important in the evaluation of biscuit quality. The interaction influence of various variables on sensory qualities and physical properties of functional biscuits was analysed using the responses collected after each trial. Furthermore, proximate analysis, texture profile analysis, FTIR, TGA, Rapid Visco-Analysis, and characterization of antioxidant activity, dietary fiber, ascorbic acid content, and physical parameters of the optimized product were performed. The shelf life of the developed functional biscuits was studied after 10 days, 20 days, and 30 days of storage at two different temperatures (10°C and 37°C). The storage stability was judged on the basis of changes in the HMF content, TBARS content, moisture content, and the microbiological quality (Bacterial, yeast and mold, and coliform growth) of optimized biscuits during different storage periods.

5.1 Selection of the level of ingredients for functional biscuits

After conducting preliminary trials and reviewing the relevant literature, the numerical ranges for the two factors (FLP and FS) were determined. The ranges were 2.5g to 5g FLP and 2.5g to 10g flaxseeds. Taking into account, the two factors were optimized for 4 responses viz. CA, TA, OA, and hardness by using RSM design sets of experiments CCRD (Central Composite Rotatable Design) conducting a total of 13 trials/runs. The functional biscuits were optimized at FLP and FS levels of 2.5 % and 6.5 % as per the numerical optimization by RSM.

5.2 Effect of FLP and FS on properties of functional biscuit

The response surface plots showed that with the increasing levels of FLP and FS, the color and appearance, taste and aroma, and overall acceptability scores decrease. A greater decrement is observed in these factors when the levels of FLP are increased in comparison to levels of FS. The hardness values first decrease up to the addition of 6.5% of fenugreek leaf powder and then increase further raising the levels of fenugreek leaf powder up to 10%.

5.3 Sensory Evaluation

Sensory evaluation using a 9-point Hedonic rating scale revealed that the score for the color and appearance varied from 6.2 to 8.2, taste and aroma from 5.8 to 7.4, texture from 6.0 to 9.0, and overall acceptability varied from 6.0 to 8.0. According to the results of the sensory evaluation, the most liked sample is T10 which contains FLP=2.5% and FS= 5% as it showed the best results for all the sensory parameters.

5.4 Physical properties of functional biscuits

The diameter, thickness, spread ratio, and bulk density of the functional biscuits were found to be 5.26 cm, 0.63 cm, 8.35, and 0.54 g/ml respectively. The functional biscuit exhibited colour values L*, a*, and b* of 47.18, 6.64, and 40.15 respectively.

5.5 Texture profile analysis of functional biscuits

The Texture profile analysis revealed that the functional biscuits exhibited a hardness of 1660 g, the cohesiveness of 0.48, gumminess of 8.22 N, chewiness of 55.26 mJ, and springiness of 6.71 mm.

5.6 Proximate Composition of functional biscuits

The optimized biscuit had a proximate composition of 12.93 % protein, 3.17 % moisture, 5.07 % fiber, 15.07 % fat, 3.70 % ash, and 59.97 % carbohydrate which was significantly different than the proximate composition of the control. (7.02 % protein, 2.60 % moisture, 1.36% fiber, 13.60 % fat, 1.30 % ash, and 73.64 % carbohydrate).

5.7 Rheological properties of functional biscuits

Rapid Visco analysis of functional biscuits revealed that the peak viscosity was 564 cP, Final Viscosity of 1700 cP, pasting temperature of 71.10°C, pasting time was for 6.53 minutes and breakdown value was 97 cP, trough of 467 cP, and setback value of 1233 cP. The pasting properties of functional biscuits and control were found to differ significantly.

5.8 Physico-chemical properties of functional biscuits

The functional biscuits were found to exhibit DPPH (% RSA) of 68.40 % and the control sample exhibited 15.71%. The higher antioxidant activity of the functional biscuits is due to the various antioxidants present in FLP, FS, oats flour, and stevia. The dietary fiber content of the control sample was found to be 4.29 % and dietary fiber in the functional biscuits was 20.55 %. The high dietary fiber content of the developed functional biscuits makes them suitable for consumption by people suffering from digestion problems like constipation. The ascorbic acid content was found to be 14.33 mg/100g whereas the control sample was devoid of ascorbic acid.

5.9 Characterization of Functional Biscuits

The thermal properties of the biscuit samples were determined by TGA. The first mass loss of 13.613 % is observed approximately between 30°C and 240°C, the second mass loss of 44.842 % is observed approximately between 240°C and 370°Cand the final mass loss of 27.382% is approximately between 370°C and 590°C. The mass loss could be attributed to the loss of moisture during baking and the breakdown of complex compounds. The FTIR spectra of functional biscuits showed the presence of several functional groups and nutrients like proteins, carbohydrates, and fatty acids.

5.10 Shelf-life study

The shelf-life studies of the optimized product found that the shelf life of functional biscuits was comparatively lower at 37°C than at 10°C. The HMF content, TBA content, and moisture content increased during the storage period. The HMF increased from 4.68 µmol/100g to 6.10 µmol/100g and 6.58 µmol/100g at 10°C and 37°C

respectively. The TBA also increased from 1.02 MDA μg/gram to 1.39 MDA μg/gram at 10°C and 1.51 MDA μg/gram at 37°C after storage for 30 days. A gain in moisture content was observed from 3.17% to 4.8% at 10°C and at 37°C after 30 days of storage. No objectional microbial load was observed during microbiological testing of the product. The product was found to be completely safe for consumption at the end of 30 days of storage.

5.11 Conclusion

From the present study, it can be inferred that the functional biscuits developed are more nutritionally rich as compared to the conventionally refined wheat flour biscuits available in the market. The developed functional biscuits are rich in protein, dietary fiber, antioxidants, carbohydrates, minerals, and ascorbic acid content and have good sensory acceptability in terms of color, appearance, taste, aroma, and texture.

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APPENDIX

APPENDIX-I

SENSORY SCORE CARD- HEDONIC RATING SCALE

PRODUCT: Functional Biscuits

DATE:

TIME:

NAME OF PANELIST:

Instruction:

Given below are the samples of "functional biscuits". You are requested to judge the sample on the 9-point hedonic scale for the parameters listed below: -

SAMPLE	Color and appearance	Taste and Aroma	Texture	Overall acceptability

Key:

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

SIGNATURE: **REMARK:**

APPENDIX-II

ANOVA for Ash content

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	8.64	1	8.64	216	0.000124726	7.708647
Within Groups	0.16	4	0.04			
Total	8.8	5				

APPENDIX-III

ANOVA for Moisture content

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.481666667	1	0.481666667	72.25	0.001050578	7.708647
Within Groups	0.026666667	4	0.006666667			
Total	0.508333333	5				

APPENDIX-IV

ANOVA for Fat content

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.226666667	1	3.226666667	62.4516129	0.001386977	7.708647
Within Groups	0.206666667	4	0.051666667			
Total	3.433333333	5				

APPENDIX-V

ANOVA for Protein content

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	52.33306667	1	52.33306667	172.5929753	0.000193871	7.708647
Within Groups	1.212866667	4	0.303216667			
Total	53.54593333	5				

APPENDIX-VI

ANOVA for Crude Fiber content

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	20.57202	1	20.57202	98.29744366	0.000580994	7.708647422
Within Groups	0.837133	4	0.209283			
Total	21.40915	5				

APPENDIX-VII

ANOVA for Carbohydrate content

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	280.4401	1	280.4401	587.9041	0.00001716	7.708647
Within Groups	1.908067	4	0.477017			
Total	282.3481	5				

APPENDIX-VIII

ANOVA for Bulk density

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.0054	1	0.0054	54	0.001826	7.708647
Within Groups	0.0004	4	0.0001			
Total	0.0058	5				

APPENDIX-IX

ANOVA for Thickness

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.026667	1	0.026667	4	0.116117	7.708647
Within Groups	0.026667	4	0.006667			
Total	0.053333	5				

APPENDIX-X

ANOVA for spread ratio

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.852702	1	0.852702	0.444078	0.541628	7.708647
Within Groups	7.68065	4	1.920163			
Total	8.533352	5				

APPENDIX-XI

ANOVA for Diameter

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.041667	1	1.041667	312.5	0.0001	7.708647
Within Groups	0.013333	4	0.003333			
Total	1.055	5				

APPENDIX-XII

ANOVA for Springiness

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.124817	1	3.124817	1499.912	0.000003	7.708647
Within Groups	0.008333	4	0.002083			
Total	3.13315	5				

APPENDIX-XIII

ANOVA for Gumminess

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	343.5267	1	343.5267	1970.705	0.000002	7.708647
Within Groups	0.697267	4	0.174317			
Total	344.2239	5				

APPENDIX-XIV

ANOVA for Hardness

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.036817	1	0.036817	58.13158	0.001588887	7.708647
Within Groups	0.002533	4	0.000633			
Total	0.03935	5				

APPENDIX-XV

ANOVA for Cohesiveness

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.036817	1	0.036817	58.13158	0.001588887	7.708647
Within Groups	0.002533	4	0.000633			
Total	0.03935	5				

APPENDIX-XVI

ANOVA for Chewiness

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	29170.85	1	29170.85	53893.67	0.0000000021	7.708647
Within Groups	2.165067	4	0.541267			
Total	29173.01	5				

APPENDIX-XVII

ANOVA for Colour and Appearance

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.086666667	2	0.043333333	0.124631268	0.883201611	3.259446306
Within Groups	12.51692308	36	0.347692308			
Total	12.60358974	38				

APPENDIX-XVIII

ANOVA for texture

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.148205	2	0.074103	0.085478	0.918259	3.259446
Within Groups	31.20923	36	0.866923			
Total	31.35744	38				

APPENDIX-XIX

ANOVA for Taste and Aroma

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.073846154	2	0.036923077	0.133766837	0.875226786	3.259446306
Within Groups	9.936923077	36	0.276025641			
Total	10.01076923	38				

APPENDIX-XX

ANOVA for Overall Acceptability

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.086667	2	0.043333	0.110566	0.89563	3.259446
Within Groups	14.10923	36	0.391923			
Total	14.1959	38				

APPENDIX-XXI

ANOVA for Sensory Evaluation Scores

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	18.81231	12	1.567692	8.002618	0.00000029	2.010183
Within Groups	7.64	39	0.195897			
Total	26.45231	51				

APPENDIX-XXII

ANOVA for Dietary fiber

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	396.4188	1	396.4188	724.2952	0.0000113	7.708647
Within Groups	2.189267	4	0.547317			
Total	398.6081	5				

APPENDIX-XXIII

ANOVA for Ascorbic acid

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	308.1667	1	308.1667	1849	0.000002	7.708647
Within Groups	0.666667	4	0.166667			
Total	308.8333	5				

APPENDIX-XXIII

ANOVA for RVA Parameters

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3498096	6	583016	18.6854	0.000555	3.865969
Within Groups	218411.7	7	31201.68			
Total	3716508	13				

APPENDIX-XXIV

ANOVA for Color characteristics

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2035.315	2	1017.658	25.45921	0.013124	9.552094
Within Groups	119.9163	3	39.97208			
Total	2155.232	5				

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