Development & Process Optimization of Sugar Free Biscuit through Fenugreek Seed Powder and Natural Sweetener (Stevia)

काशी हिन्दू विश्वविद्यालय BANARAS HINDU UNIVERSITY

THESIS SUBMITTED IN THE PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Master Of Science

In

Food Science & Technology

Supervisor

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Dear Sir

I have great pleasure in forwarding the thesis entitled **"Development & Process Optimization of sugar free biscuit through fenugreek seed powder and natural sweetener (Stevia)"** submitted by **Mr. Sury Pratap Singh IDNo-16412FST020, Enrollment No.: 389424** in partial fulfilment of the requirements for the degree of **Master of Science** in **Food Science and Technology From Centre of Food Science and Technology, Faculty of Agriculture,** Institute of Agricultural Sciences, Banaras Hindu University, Varanasi and placing on record that he has completed the requisite residential requirements as contained in the ordinance of the University.

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

Thanking You

FORWARDED

(Anil Kumar Chauhan)

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TABLE OF CONTENTS

RESULTS AND DISCUSSION

CONCLUSION

LIST OF TABLES

LIST OF FIGURES

F	:	Flavour
C and A	:	Colour and appearance
OA	:	Overall Acceptability
CCRD	:	Central Composite Design
g	:	Gram
mg	:	Milligram
ml	:	Millilitre
min	:	Minutes
Sec	:	Second
RSM	:	Response Surface Methodology
TPA	:	Texture Profile analysis
◦C	:	Degree Celsius
HMF	:	Hydroxy Methyl Furfural
TBA	:	Thiobarbituric Acid
TPC	:	Total Plate count
FSP	:	Fenugreek Seed Powder
SMP	:	Skimmed Milk powder
F	:	Fracturability
H	:	Hardness
FT-IR	:	Fourier Transform Infrared
AAS	:	Atomic Absorption Spectroscopy
IC	:	Ion Chomatography
RVA	:	Rapid Visco analyser

CHAPTER-1

Introduction

INTRODUCTION

The term bakery products covers a variety of food products such as biscuit, breads, cookies, muffins, pastry, ban, cakes and pies etc.Bakery products like biscuits have a long history. Biscuit is an omani present product. Biscuit is a small baked unleavened cake, typically crisp, flat, and sweet food product. The composition and the manufacturing process is depends upon the variety of biscuits. Themain compositions of biscuits are wheatflour, sugar, baking powder, etc.

Biscuit is India's largest industry amongst food industries, with an estimated to annual turn-over about Rs. 3000 billion. Out of which the biscuit is contributesaccounting to turn over approximate 30% and 50% respectively of total bakery products produced in the country. The bakery industries have been established in organized and unorganized sectors. Biscuit is a diverse group of bakery product available in different varieties such as high and low in fat, high and low in sugar and more or less combination. It can be designed in different shape, size, and texture (Saghir and Mushir, 2014).

Wheat grain flour is the essential ingredient for baking process of biscuits. Biscuits is an unleavened crisp, sweet pastry made from wheat flour, shortening (hydrogenated fat) & sugar, and is usually made light by the addition of baking powder (a mixture of sodium carbonate, sodium bi-phosphate & cereal flour). Wheat flour constitutes the basic ingredient for biscuit production because of its gluten proteins, which are not present in other varities of millets and cereals flour. Gluten protein forms elastic dough during baking and gives high organoleptic quality to the finished product. Biscuits are very palatable bakery products of nutrition and energy point of view; because it is a good source of the goodness of flour, fat and sugar(Sharma *et al.*, 2003).

Diabetes is the very serious problem all over the world in general, particularly in India. Now days, there are no sugar free biscuitsavailable in Indian market. In the present research, sugar free biscuit was developed, fortified with fenugreek seed powder and sugar was replaced with natural sweetener like stevia, have potential benefits for diabetic patients. Fenugreek seed powders have a functional component "trigonelline" which helps in the insulin production.Trigonelline is present in many plants in less amountbut it present in more amount in fenugreek (*Trigonella foenum-graecum* L.) plant which is distributed widely throughout the world and belongs to the family *Fabacecae*. Fenugreek plants are already used for medicinal purposes all over the globe. Among different genus of fenugreek, *Trigonella* is most widely cultivated species. Various portions of fenugreek plants, including seeds, leaves, and extracts, have been extensively used as anti-diabetics.Sharma (1986) reported that in-vivo experiments showed possible hypoglycemic and hypolipidemic properties of fenugreek seed powder when taken orally. From the ancient time, fenugreek has been utilized as a traditional medicine to treat a wide range of diseases and result also showed that fenugreek seed powder decrease body fats and effective in obesity(Ptropoulos 2012).

Trigonelline content of the active pharmacological constituent of fenugreek and composite approximately 0.1-0.15% of the seed weight(Souvairet al., 1998).Talwalkar (1962) and Atal (1964) observed the other components of fenugreekViz. steroids, alkaloid, polyphenolic substances, volatile constituents, and amino acids. Trigonelline, a plant hormone that is widely distributed in plants within the subclass Dicotyledonae (Allred *et al.,* 2009) also exists in several animal species, such as arthropods, bryozoans, cnidarians, coelenterates, crustaceans, echinoderms, marine poriferans, mollusks, marine fishes, and mammals. Accumulation of trigonelline was also observed in the seeds of various legume species and coffee. Trigonelline has been reported to have hypoglycemic, hypolipidemic, sedative, anti-migraine, antibacterial, antiviral, and anti-tumour effects, and helps to improve memory retention and inhibit platelet aggregation (Zhou *et al.,* 2012).

Present research work resulted the sugar free biscuit containing non-nutritive sugari.e stevia (Steviol-glycoside) natural sweetener present in stevia.Stevia (*rebaudiana Bertoni)* belongs to *Asteraceae* family and reported as a substitute of artificial sweeteners for diabetic patients. Conventionally, Stevia is cultivated by seeds or stem cutting, but it was found that seed viability rate is very poor. For cultivation of stevia, callus induction and multiplication methods were reported to produce large number of calli in very short time. Surface sterilized nodal, leaf and root explants were cultured on Murashige and Skoog (MS) medium with different concentrations of plant hormone like, IBA (Indole Butyric Acid), kinetin, NAA, 2,4-

D, and NAA in combination with 2,4-D. Stevia cultivation deals with rapid callus multiplication for production of steviol glycosides in callus culture (Pratibha Gupta *et al*.,2010).

Stevia plant now days got more attention because the Food safety and standard authority of India (FSSAI) (2011) has allowed its utilization in various food products Viz. carbonated water, dairy based pastries and enhanced beverages, yoghurt , prepared to eat oats, organic product,nectors and jams as a sweetener.The sweetness in stevia comes from the chemical compound calledsteviolsglycode. More than 40 different steviol glycosides have been identified in the stevia plant so far while each of these steviol glycosides has its own unique taste profile and intensity of sweetness that can be up to 350 times sweeter than sugar.

Response surface Methodology (RSM) is collection of mathematical and statistical techniques for empirical model building. By careful design of experiments, the objective is to optimize a response (out variable) which isinfluenced byseveral independent variables (input variables). In the bakery industry it can be used to minimize the number of baking trials while gathering all information relating to ingredient interaction and quality characteristics.

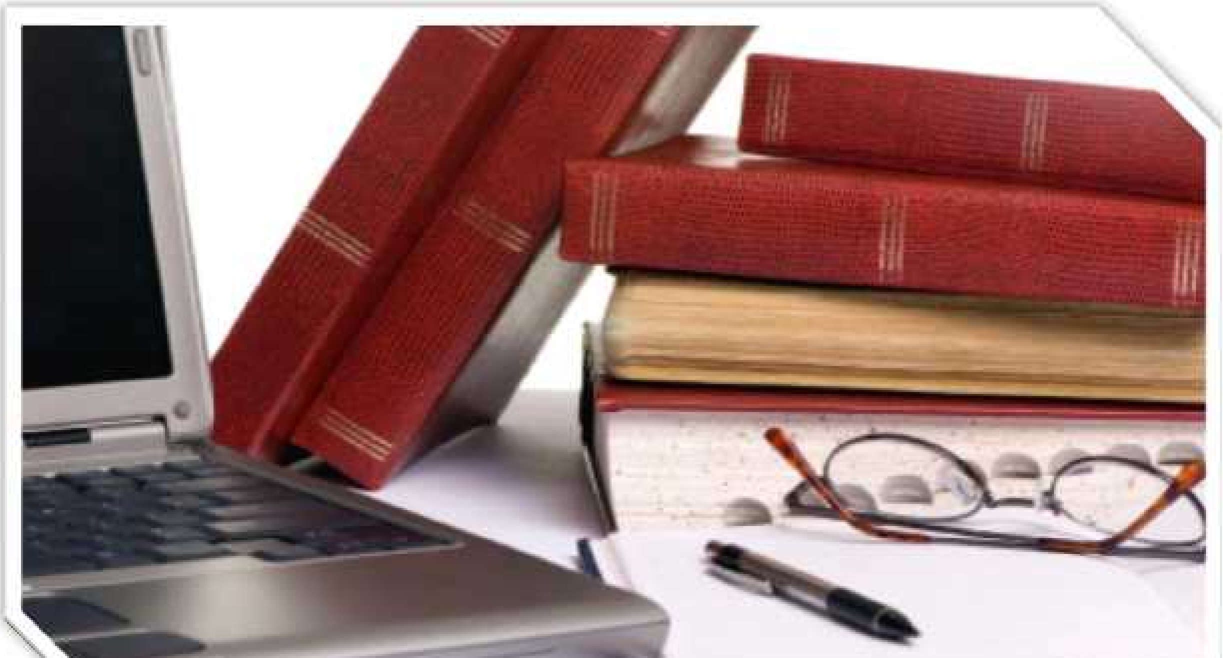
For the development of sugar free biscuits fortified with fenugreek seed powder and natural sweetener stevia were used. The sugar free biscuits composition was optimized by using RSM (Response Surface Methodology). For the optimization of sugar free biscuits, thirty-one trials were conducted by using RSM in which various parameter Viz. Colour and appearance, flavour, hardness, fracturability and DPPH Inhibition. To check the nutritive value and to analyze different functional component in sugar free biscuit various test were conducted like proximate analysis, sensory evaluation on 9-point Hedonic Scale, Texture Analysis, colour by Hunter color lab, digital bomb calorimeter for energy value calculation, mineral analysis by Atomic Absorption Spectrometry, Ion-Chromatography for calcium content, functional component by FT-IR (Fourier-transform infrared spectroscopy) and pasting property of starch by Rapid ViscoAnalyzer.

Keeping in view, the present research work **"Development & Process Optimization of sugar free biscuit through fenugreek seed powder and natural sweetener (stevia)"** has been carried under the following objectives:

1. Optimization of sugar free biscuits fortified with fenugreek seed powder and natural sweetener stevia by using Response Surface Methodology (RSM).
2. To determine the proximate analysis of optimized sugar free biscuit
3. Micronutrient analysis of sugar free biscuit by Ion Chromatography and Atomic Absorption Spectroscopy
4. To study the shelf life of the optimized product.

CHAPTER-2

REVIEW OF LITERATURE



REVIEW OF LITERATURE

Bakery Industryrepresents a fast growing segment of food in India because of consumer demands forconvenient and nutritious food products.The consumers demand has increased for the quality food products with taste, safety, convenience and nutrition. Thus nutrition has emerged as an added dimension in the chain of food product development (Shaghir and Mashir; *et al*., 2014). Biscuits are a popular foodstuff consumed by a wide range of population due to their varied taste, long shelf life and relatively low cost. Because of competition in the market and increased demand for healthy, natural and functional products, attempts are being made toimprove the nutritive value of biscuits and functionality by modifyingtheir nutritive composition.

Tyagi et al. (2006); Gallagher et al. (2003) observed by increasing the ratio of whole grain raw materials other than wheat or different types of dietary fibres in basic recipes with the attempt to increase biscuit's protein and mineral content for quality and availability or increase dietary fibre content and improve prebiotic characteristics of the final product.

2.1 **Bakery Industry in India**

Bakery industry is the one of the largest food industries in India with an annual turnover about Rs3000 billion. The biscuit industry has been growing at an average rate of 15% during the past 3 years and this is expected to be maintaining in coming years (IBMA,2010).

Serivastava, (2009) reported that biscuit consumption per capita in India is 2.1 kg, compared to more than 10 kg in the USA, UK and West European countries and above 4.25 kg in South-east Asian countries, e.g. Singapore, Hong Kong, Thailand, Indonesia, etc. China has per capita consumption of 1.90kg, while in the case of Japan it is estimated at 7.5kg.

The market in India is diverse and biscuit sector is divided into organized sector (40%) andunorganized sector (60%). The branded and organized biscuit sector is forecast annual growth of 17%. Major players in this segment include Britania, Parlae, ITC, Priya Gold etc. Growth in the un-branded biscuit sector, which currently has a smaller market, is much smaller at 8% per year.

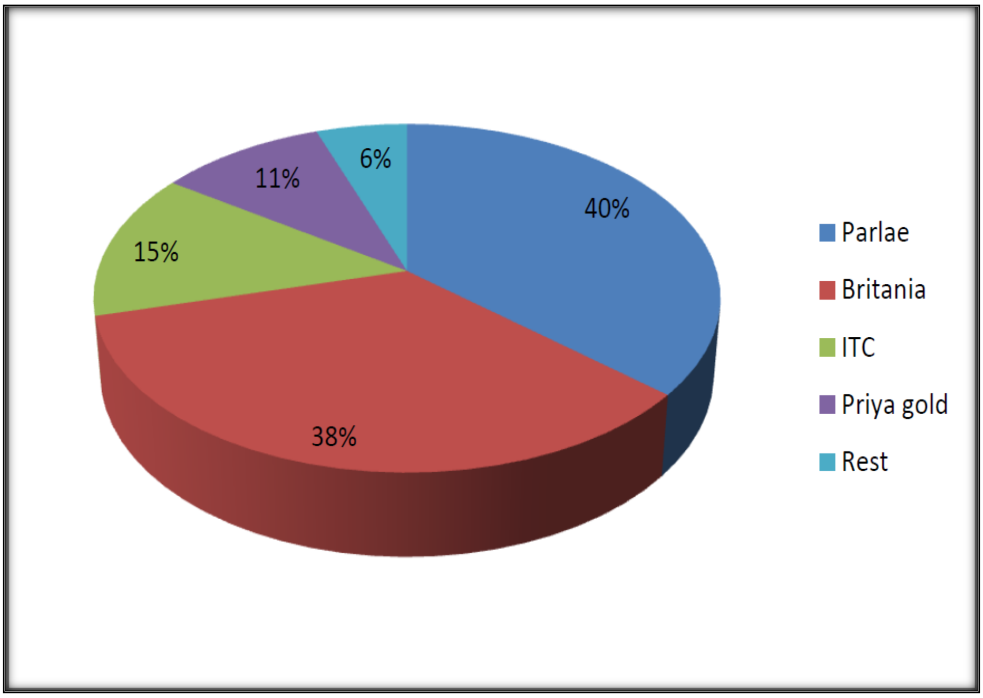


Figure2.1:source-Market share of biscuit (Brands wise) in India(2013)

Biscuit have been man's food since a long time. It is a processed convenience food ever produced andit is most widely acceptable. It is one of the few universal staples, which is complete in it and requires no additional preparation. Thus, for many, Biscuit becomes an important source of high molecular carbohydrates, vegetable proteins and some vitamins and minerals. But it is important to know that, as compared to refined wheat flour which is deficient in certain essential amino acids thus has a lost nutritional value. The nutritional value of biscuit can be enhanced by fortification and supplementation(Gandhi *et al.*, 1985).

2.2 Indian Scenario

India is the world's second largest producer of food next to China, and has the potential of being the biggest with its food and agricultural sector. The Indian bakery industry is one of the biggest sections in the country's processed food industry. Bakery products, which include bread and biscuits, form the major baked foods accounting for over 82 per cent of the total bakery products produced in the country. It provides a comparative advantage in manufacturing, with an abundant supply of primary ingredients required by the industry and is the third-largest biscuit manufacturing country after the United States and China (Saghir and Mushir, 2014).

2.3 Trends in Bakery Industry

- E-retailing of bakery products- for example, online florist and gift delivery sites like Florist X-press have gone into bakery items.
- Expanding foothold- for example, Florist Xpress has pan-India presence unlike many physical bakery shops.
- Improved packaging- for example, like experts at Florist Xpress has brought customization & personalization to a whole new level.
- Innovation in ingredients- for example, designer-cakes, photo-cakes, as-well as, eggless cakes are brought up by Florist Xpress to cater to the customer's needs.

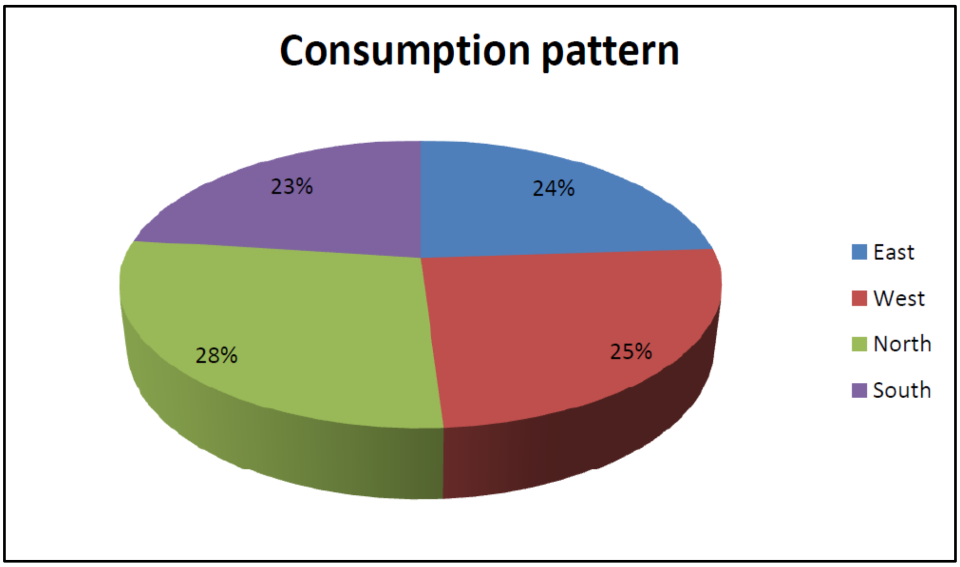


Figure2.2 :source Consumption pattern of biscuit in India(2013)

2.4 Bakery Product Range

Bakery products have vast range of product which contain high nutritive value and are manufactured from wheat-flour, sugar,baking powder, condensed milk, ghee (fat), salt, jelly, dry-fruits, various essences and flavouring etc. Different type of bakery products can be classified as:

2.4.1Dry Bakery Products

Dry bakery product mainly consists of biscuits such as soft biscuits, hard biscuits, cookies, crackers, fancy biscuits, cream waferbiscuits etc.

2.4.2 Moist Bakery Products

Moist bakery product range is large and it consist of bread like sweet bread, milk bread, masala bread, garlic bread, fruit bread etc. and buns such as fruit buns, hamburger buns, dinner rolls, crisp bread, pizza. Others moist bakery product includes cakes, pastries, doughnuts, muffins etc.

Table.2.1: Basic Characteristics for Dry Bakery Products

Properties	Basic Characteristics of Products
General	Foodstuffs for long storage
Physical-mechanical	Fragile
	Light
	Low resistance to moisture
	Variable sizes
Organoleptic	Crisp or crunchy texture
	Distinctive flavours
	Flavours that may change (loss of initial flavour or ingress of foreign flavour)
	Flavour that may deteriorate (go stale, soapy or bitter, etc.)
Physico-chemical	Low moisture content
	Hygroscopic
	Containing fatty matter
	Greasy surface
	Sensitive to:
	• Oxidation
	• Enzymatic reactions
	• Non-enzymatic browning
	• Light
Technical – economical	Industrial
	Low sales price

2.5 Wheat flour

Different types of wheat flour were reported by Barkeley Wellness University of California in 2016 on the basis of refined white flour consists of the ground endosperm of the wheat kernel. White flour is popular because it produces lighter baked goods than whole-wheat flour and has different ability to produce gluten. When the bran and germ are removed from the wheat kernel, vitamins and minerals are decreased, along with dietary fiber.

There are many types of white flours, including:

2.5.1 All-purpose flour (plain and white):

All-purpose wheat flour ismade up ofa blend of hard and soft wheat.This type of flour which constituent hard and soft wheat has a different protein and starch content that makes it suitable for either breads or cakes and pastries respectively.

2.5.2 Bleached flour:

Bleached flour is freshly milled flour which is slightly yellow. To whiten bleached flour, manufacturers allowed the flour to age naturally, but most of the time, this process is speed up by adding chemicals, such as benzoyl peroxide or acetone peroxide, to bleach it. This process gives the flour more gluten-producing potential, but naturally aged flours develop more gluten as well.

2.5.3 Bread flour:

Bread flour is entirely made up of hard wheat flour. High gluten content of bread flour helps bread to rise higher because the gluten traps and holds air bubbles as the dough is mixed and kneaded. It's also available in whole-wheat form.

2.5.4 Bromated flour:

Bromated flour is prepared by adding a maturing agent such as bromate to flour in order to further develop the gluten and to make the kneading of doughs easier. Other maturing agents include phosphate, ascorbic acid, and malted barley.

2.5.5 Cake flour:

Cake flour is made up of mainly soft wheat flour. Because of its low gluten content, it is especially well suited for soft-textured cakes, quick breads, muffins, and cookies.

2.5.6 Durum flour:

Durum wheat flour contains the highest protein than other type of flour. Durum flour can produce the maximum gluten. It is frequently used for pasta.

2.5.7 Gluten flour:

Gluten flour is made up of about twice the gluten strength of regular bread flour. It is basically used as a strengthening agent with other flours that are low in gluten-producing potential.

2.5.8 Instant flour (instant-blending, quick-mixing, granulated flour):

Instant flour is used for instant blending, quick mixing and as a granulated flour. Its pours easily and mixes with liquids more readily than other flours. It is used to thicken sauces and gravies, but is not appropriate for most baking because of its very fine, powdery texture and high starch content.

2.5.9 Pastry flour:

Pastry flour is also known as cookie flour or cracker flour. This flour has gluten content slightly higher than that of cake flour but lower than that of all-purpose flour, making it well-suited for fine, light-textured pastries.

2.5.10 Self-rising flour:

Self-rising flour is made up of soft wheat flour which contains salt, a leavening agent such as baking soda or baking powder, and an acid-releasing substance. However, the strength of the leavener in some flours deteriorates within two months. Self-rising flour should never be used in yeast-leavened baked goods.

2.5.11 Semolina:

Semolina is the coarsely ground endosperm (no bran, no germ) of durum wheat. Its high protein content makes it ideal for making commercial pasta, and it can also be used to make bread.

2.6 Fenugreek

Srinivasan, (2006) reported that fenugreek (*Trigonella foenum graecum*) is an annual plant belongs to the family *Leguminosae*. It is very famous spice in human food. The seeds and green leaves of fenugreek are used in food as well as in medicinal application that is the old practice of human history. It has been used to increase the flavoring and color, and also modifies the texture of food materials. Seeds of fenugreek spice have medicinal properties such as hypocholesterolemic, lactation-aid, antibacterial, gastric stimulant, for anorexia, anti-diabetic agent, hepatoprotective effect and anticancer. These beneficial physiological effects including the anti-diabetic and hypocholesterolemic effects of fenugreek are mainly attributable to the intrinsic dietary fiberconstituent which have promising nutraceutical value.

The *trigonella foenum-graecum* cold water extract, known as fenugreek tea, has been traditionally used against respiratory infections and since it nourishes the body during illness. The fenugreek herb has also been used to reduce fever, when taken with lemon and honey. *Trigonella foenum-graecum* has been found to have potential health benefits effects such as hypoglycemic, antihypertensive, and hypolipidemic activities (Micallef andGarg, 2009).

Table:2.2Percentage of Carbohydrate, Protein, and Fat in Fenugreek Leaves and Seeds

Plant Parts	Carbohydrate	Protein	Fat	References
Seed	62.48	28.55	4	(Sulieman*et al.*, 2008)
Seed	45−60	20–30	5−10	(Mehrafarin*et al.*, 2010)
Seed	42.3	9.5	7.5	(Hemavathy and Prabhakar, 1989)
Seed	42.3	25.4	7.9	(Meghwal and Goswami, 2012)
Leaf	6.6	4.4	0.9–1	(Meghwal and Goswami, 2012), (Snehlata and Payal, 2012)
Seed		20–30	6.53	(Sheikhlar, 2013)
Seed	44	24.7	7.6	(Abdel Aal*et al*., 1985), (Shankaracharya and Nalarjan, 1973)
Seed	58	23−26	6−7	(Lu *et al*., 2008)

2.6.1 Phytochemistry

Khare(2004) reported that Fenugreek contains a number of chemical constituents including steroidal sapogenins. Diosgenin component has been found in the oily embryo of fenugreek. There are two furastanol glycosides, F-ring opened precursors of diosgenin that have been reported in fenugreek also as hederagin glycosides. Alkaloids such as trigocoumarin, nicotinic acid, trimethylcoumarin and trigonelline are present in stem. The mucilage is a standing out constituent of the seeds

2.6.2 Fenugreek Seed

Fenugreek is known for its pleasantly bitter, slightly sweet seeds. The seeds are available in any form whether whole or ground form is used to flavor many foods mostly curry powders, teas and spice blend. Fenugreek seed has a central hard and yellow embryo which is surrounded by a corneous and comparatively large layer of white and semi-transparent endosperm (Betty, 2008).

2.6.3 Hypoglycemic effect of fenugreek seed

Dietary fiber from fenugreek blunts glucose after a meal. The mechanisms hypoglycemic effects have not been fully elucidated before. Fenugreek seeds contain 45.4% dietary fiber (32% insoluble and 13.3% soluble), and the gum is composed of galactose and mannose. The mannoseis mainly associated with reduced glycemic effect. The hypoglycemic effect of fenugreek has been especially documented in humans and animals with type 1 and type 2 diabetes mellitus (Roberts, 2011).

Sauvaire et al. (1989) demonstrated in vitro the amino acid 4-hydroxyisoleucine in fenugreek seeds increased blood glucose and insulin levels. This study suggests that fenugreek seed extract and diet; exercise may be equally effective strategies for attaining glycemic control in type 2 diabetes.

2.6.4 Antioxidant activity of fenugreek seed

Bukhariet al.(2008) reported that fenugreek seed extract with methanol, ethanol, dichloromethane, acetone, hexane and ethyl acetate has a radical scavenging activity.

Bhatia et al. (2006) reported protective effect of fenugreek, on lipid peroxidation and on enzymatic antioxidants.

Naidu *et al*. 2010) reported that the proximate composition of fenugreek seeds,husk and cotyledons had the highest saponin and protein content.

2.6.5 Application of fenugreek seed powder in the treatment of diabetes

2.6.5.1 Type 1 Diabetes

Sharma et al. (1996) conducted a randomized, controlled, crossover trial in 10 patients with type 1diabetes.The person, above 25 Year, provided 100 g meals of fenugreek seed powder for a period of 10-day, (lunch and dinner). At the study’s end, significant improvement was noted in the fenugreek group in several parameters, including a 54-percent reduction in 24-hour urine glucose levels and mean reductions in glucosetolerance test values and fasting serum-glucose levels. This study suggests fenugreek may aid with insulin secretion, as suggested by animal studies, since typically these patients have little or no endogenous insulin production. More studies in people with type 1 diabetes are warranted.

Table:2.3 Chemical constituents of fenugreek.

S. No	Chemical constituent of fenugreek
Alkaloids	Trimethylamine, Neurin, Trigonelline, Choline Gentianine, Carpaine and Betain
Amino acids	Isoleucine, 4-Hydroxyisoleucine, Histidine, Leucine, lysine, L-tryptophan, Arginine
Saponins	Graecunins, fenugrin B, fenugreekine, trigofoenosides A–G
Steroidal sapinogens	Yamogenin, diosgenin, smilagenin, sarsasapogenin, tigogenin, neotigogenin, gitogenin, neogitogenin, yuccagenin, saponaretin
Flavonoids	Quercetin, rutin, vitexin, isovitexin
Fibers	Gum, neutral detergent fiber
Lipids	Triacylglycerols, diacylglycerols, monoacylglycerols, phosphatidylcholine phosphatidylethanolamine, phosphatidylinositol, free fatty acids. (Chatterjee. *et al*., 2010)
Other	Coumarin, lipids, vitamins, minerals. 28% mucilage; 22% proteins; 5% of a strongerswelling, bitter fixed oil.

Source: Yadav*et al*. 2011; Sowmya and Rajyalakshmi, 1999

2.6.5.2 Type 2 Diabetes

Gupta et al. (2001) observed that fenugreek seedsfound to lower fasting serum glucose levels in animal and several small, human trials have been, both acutely and chronically. The results of a small randomized, controlled, double-blind trial to evaluate the effects of fenugreek seeds on glycemic control. Above age 18, twenty-five patients with newly diagnosed type 2 diabetes received either 1 g daily of a hydro-alcoholic extract of fenugreek seeds or “usual care” (dietary discretion and exercise). After two months, mean fasting blood glucose levels were reduced in both groups without significant differences between groups. There were no significant differences between groups in mean glucose tolerance test values at the study’s end. This study suggests that fenugreek seed extract and diet/exercise may be equally effective strategies for attaining glycemic control in type 2 diabetes.

2.6.6 Toxicology:

Toxicological evaluation was observed on 60 diabetic patients who provided powdered fenugreek seeds at a dose of 25 g per day for 24 weeks disclosed no clinical hepatic or renal toxicity and no hematological abnormalities. In an animal study, the acute oral LD50 was found to be >5 g/kg in rats, and the acute dermal LD50 was found to be >2 g/ kg in rabbits.38 In another animal study, fenugreek powder failed to induce any signs of toxicity or mortality in mice and rats who received acute and sub-chronic regimens. Moreover, there were no significant haematological, hepatic, or histopathological changes in weanling rats fed fenugreek seeds for 90 days (Gupta *et al.*, 2001).

2.6.7 Nutritional Value of fenugreek

The edible part of fenugreek leaves contains moisture (86.1%), carbohydrate (6%), protein (4.4%), minerals (1.5%), fibre (1.1%), and fat (0.9%), while the contents of carbohydrate, protein, fat, fibre, and moisture in fenugreekseeds are 44.1, 26.2, 5.8, 7.2, and 13.7%, respectively.

Moradi and Moradi, (2013)analyse the mineral content minerals (Ca, P, Fe, Zn, and Mn) and vitamins such as A, B1, C, and nicotinic acid in fenugreek seed powder. Meghwal and Goswami (2012) reported that fresh 100 g fenugreek leaves contain ascorbic acid, 220.97 mg and β-carotene, 19 mg while Srinivasan (2006) reported vitamin C, β-carotene, thiamine, riboflavin, nicotinic acid, and folic acid contents as 52 mg, 2.3 mg, 40μg, 310 μg, 800 μg, 0 (zero) μm in leaves, and 43 mg, 96 μg, 340 μg, 290 μg, 1.1 mg, 84 μg in seeds respectively.

Shakuntalaet al.(2011) concluded in a research study that germinated endosperm, un-germinated endosperm, germinated sprouts, germinated seed coat, and un-germinated seed coat contain 39.25, 48.20, 36.12, 10.51, and 7.35% of protein, respectively, and 11.44, 12.26, 6.65, 1.75, and 1.22% of fat, respectively.

2.7 Stevia

Stevia (*Stevia rebaudiana*) is a small shrub native to subtropical and tropical South America and Central America (North to Mexico, Paraguay and Brazil). Native Indians of the Guarani Tribe appear to have used the leaves of this herb as a sweetener since pre-Columbian times. It is also called as sweet leaf or sugar leaf and is a genus of about 150 species of herbs and shrubs. It grows well in the sandy soil of elevated land and may grow to a height of 80 cm when it is fully mature. Stevia does produce seeds, but only a small percentage of them germinate. Planting cloned stevia is a much more effective method of reproduction. In 1887, a South American natural scientist named Antonio Bertoni first discovered it. Different glycosides extracted from the stevia were named steviosides, rebaudiosides and dulcoside(Pomaret and Lavieille, 1931).

2.7.1 Stevioside

Kennelly (2002), Starratet al. (2002) reported that stevia leaves contain a complex mixture of sweet deterrence glycosides, including stevioside, steviolbioside, rebaudiosidesand dulcoside. Stevioside is isolated and purified from *stevia rebaudiana bertoni* leaves after multiple and selective extractions followed by recrystallization, resulting in a stevioside purity greater than 95% and with rebaudioside A as the main impurity (less than 2%). Refined steviosides and rebaudiosides are the sweetest forms of stevia. Stevioside constitutes 5-15% of the dried leaves of stevia (Lima Filho andMalavolta, 1997).

2.7.2 Teratogenicity study:

Teratogenicity of stevioside (purity, 95.6%) was examined in rats. Stevioside dissolved in distilled water was given to pregnant Wistar rats by gavage once a day from days 6 to 15 of gestation at doses of 0, 250, 500 and 1000 mg/kg/day. The rats were sacrificed on 20th day of pregnancy and their fetuses were examined for malformation. Stevioside caused no increased incidences of fetal malformation, and no toxic signs in the pregnant rats and the fetuses. It was concluded that stevioside has no teratogenicity in rats when given by gavage (Takanaka*et al.*, 1991, Usami*et al.*, 1995).

2.7.3 Benefits of stevia

Stevia have no calories, and it has no effect on blood sugar levels like common sugar. Stevia is100% naturalnon-nutritive sweetener and it is 250 to 300 times sweeter than sugar and it is heat stable up to 200°C and non-fermentable. Stevia enhances the flavour, prevents cavities, recommended for diabetics and reported non-toxic and can be used in natural state.Pure stevioside extract from stevia leaves can be cooked and found no-bitterness, used as a non-addictive sweetener mainly for children(Melis and Sainati.,1991).

2.7.3.1 Diabetes:

Suttajitet al. (1993) reported that stevia leaves have been used as herbal teas by diabetic patients in Asian countries. No side effects have been observed in these patients after many years of continued consumption. Furthermore, studies done by Curiet al.(1986) have shown that stevia extract can actually improve blood sugar levels. Stevioside helps in insulin secretion and acts directly on pancreatic beta cells to secret insulin (Jeppesen*et al.* 2000). Stevioside had no effect on gluconeogenesis or oxygen uptake in isolated Wistar rat (Yamamoto *et al*., 1985).

2.7.3.2 Blood pressure:

Geunset al.(2007) reported that three days of oral stevioside administration (750 mg/day) affected neither the systolic or diastolic blood pressure nor the fasting plasma glucose and insulin concentrations of healthy volunteers. Melis and Sainati (1991) resulted that high dose of stevioside to rats resulted in reduction of blood pressure as well as an increased elimination of sodium.

2.7.3.3 Biological effects

Nakayama et al.(1986) studied absorption, distribution and excretion after oral application of radio-labeledstevioside, 1.5% of the radioactivity was excreted in the urine of intact rats, whereas in rats with a ligated bile duct 96% of the radio-activity was excreted in the urine. This indicates enterohepaticcirculation of stevioside and its metabolites with an elimination half-life of 24 h. After oral administration of stevioside to the rat, a major part seems to be degraded by the gut flora to steviol.

2.7.4 Comparison between stevia and other artificial sweeteners

Comparativestudy was conducted by the Department of Food and Nutrition, FCF-UNESP in Araraquara, Brazil for the relative sweetness of stevia to that of aspartame, a cyclamate/saccharin combination and 10% sucrose concentration. An equivalent dose of stevia, aspartame, cyclamate/saccharin combination and 10% sucrose concentration all had practically the same potency (Cardello*et al*. 1999). Stevia has lower calorie and glycemic index compared to common sugar. Stevia has good source of protein, ash and crude fiber which are essential for good health (Savita*et al*., 2004).

Okpalaand Egwu(2015) reported that biscuits were produced from five broken rice and cocoyam flour blends in which 100% cocoyam flour, 25% cocoyam flour ,75% broken rice flour, 50% cocoyam flour 50% broken rice flour, 75% cocoyam flour; 25% broken rice flour and 100% broken rice flour. Biscuits made with 100% wheat served as the control. Functional properties of the flour blends were analysed while proximate composition, physical properties and sensory quality of the biscuits produced were also determined. Significant differences in the functional properties existed among the flour blends. Proximate content of biscuit is such as protein: 7.18–10.54%, ash: 1.46–1.75%, fat: 11.17–18.52%, moisture: 6.56–7.89% ,fibre: 1.15–1.23% and carbohydrates: 58.71–65.97%.

Jariyahet al. (2013) conduct a research on utilization of mangrove fruit for food and found that products is still very low, especially mangrove fruit types of pedada (*Sonneratiacaseolaris*). The flour products from this fruit were given a name Pedada Fruit Flour (PFF). It contains antioxidant and dietary fiber is high enough, and proved to have hypoglycemic and hypocholesterolemic properties.

Jariyahet al. (2014) found that the PFF has potential to develope into functional food products, as a biscuits. This products classified a dry food product that made with baking dough containing basic ingredients of flour, fats, and improvers with the addition of ingredients such as sugar, salt, milk, shortening by heating process, it can be consumed among all age groups in many countries, (Adebowale*et al*.,2012; Cauvain and Young, 2000).

2.8Physical properties and chemical composition of biscuit

In AOAC method (1995), the physical properties of biscuit such as Spread ratio was measured from width (cm) and thickness (cm) of biscuit (Adebowale*et al*., 2012), color of biscuit (Yuwono andSusanto, 1998), breaking strength, moisture, fat, ash, protein contents, and carbohydrates of biscuit were analyzed

2.9 Response surface Methodology (RSM)

Experimentation plays an important role in science, engineering and industry. It is an application of treatments to experimental units, and measurement of one or more responses. It requires observing and gathering information about how process and system works. In an experiment, some input X's transform into an output that has one or more observable response variables y. Therefore useful results and conclusions can be drawn by experiment.

In order to obtain an objective conclusion and experimenter needs to plan and design the experiment and analyse the results. There are many types of experiment used in real world situations and problem. When treatment are from a continuous range of values then the true relationship between y and x's might not be known. The approximation of the response function $y = f(x_1, x_2 \ldots x_q) + e$ is called Response Surface Methodology.

Response surface Methodology (RSM) is collection ofmathematical and statistical techniques for empirical model building. By careful design of experiments, the objective is to optimize a response (output variable) which is influenced by several independentvariables (input variables). Originally,RSM was developed to model experimental responses (D.C. Montgomery.,2005) and then migrated into modelling of numerical experiments. The difference is a type of error generated by the response. In physical experiments, inaccuracy can be due, for example, to measurement errors while in computer experiments, numerical noise is a result of incomplete convergence of iterative processes, round –off errors or the discrete representation of continuous physical phenomenon (Rowe df.et. al.,). In RSM, the errors are assumed to be random.

The application of RSM to design optimization is aimed at reducing the cost of expensive analysis methods (e.g finite element method or CFD analysis) and their associated numerical noise. The problem can be approximated with smooth functions that improve the convergence of the optimization process because they reduce the effects of noise and they allow for the use of derivative – based algorithms.

The surface represented by $f(x_1, x_2)$ is called a response surface. The response can be represented graphically,either in the three –dimentional space or as contour plots that help visualise the shape of the response surface. Contours are of constant response drawn in the x_i, x_j plane keeping all other variables fixed. Each contour corresponds to a particular height of the response surface.

RSM is important in designing formulation, developing and analysing new scientific studying and products. It is also efficient in the improvement of existing studies and products. The most common applications of RSM are in industrial, biological and clinical science, social science, food science, and physical and engineering science. In the bakery industry it can be used to minimize the number of baking trials while gathering all information relating to ingredient interaction and quality characteristics.

CHAPTER-3

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1 Prologue

This chapter deals with various materials and methodologies employed in the presence investigation relating to technological, analytical, sensory and statistical aspects.

3.2 Materials

3.2.1 Stevia

Stevia was purchased from Dhanad MedicalStore local market of Varanasi, India

3.2.2 Fenugreek seed powder

Fenugreek seed was purchased from local market of Varanasi, India.

3.2.3 SMP, (Skimmed milk powder)

SMP (Anikspray) was procured from local market of Varanasi, India.

3.2.4 Wheat flour (Maida)

Very finally grainded wheatflour (Ashirwad)was purchased from local market of Varanasi, India.

3.2.5Butter

Butter (Amul) was purchased from local market of Varanasi, India

3.2.6Others

Other materials such as Baking powder, water, were purchased from the local market of Varanasi, India.

Figure:3.1 – A- Stevia leaves B 3.2- Crytalline granulated stevia powder

Figure:3.3 – A. Fenugeek seed B 3.4- Fenugreek seed powder

Figure:3.5 Skim milk powder

Figure: 3.6 Fine wheat flour (Maida)

Table: 3.1 Instruments used for manufacturing and analysis of sugar free biscuit

S.No.	Name	Company, Model and Country
1.	**Electronic weighing Balance**	Metller Toledo, JBI 603-CIF act, Switzerland
2	**Texture profile analyse**	TA.XT plus texture profile analyser, stable Micro systems, UK
3	**Vortex shaker**	Macro scientific works Pvt. Ltd, Delhi
4	**Hot air oven**	Perfit, 992110, India
5	**Laminar air flow**	Labtech LCB 1201v, Daihan Pvt. Ltd, India
6	**Centrifuge machine**	Sigma, 3-30k, Germany
7	**High pressure steam sterilizer (Vertical Autoclave)**	Tomy, SX-500, japan Pelican
8	**Incubator**	Remi, India
9	**Kel plus Apparatus**	Pelican kel plus Chennai
10	**Spectrophotometer**	Model:3375 Input voltage :AC220V/50Hz, SN:YH211705012
11	**Hot plate**	TARSONS ,SPINOT MODEL MC-09, CAT.6030, S.NO. 1601120, 220V 50Hz 4.9Amps, 1170 Watts
12	**FT-IR**	Company; Thermo Scientific ; Model Nicolet i S5
13	**AAS**	Agilent, S240
14	**OIN Chromatography**	Metrohm (930 compact IC, Switzerland) Column used: Metrosep C4 (for cation)Metrosep A Supp.5 (for anion)
15	**Digital Bomb Calorimeter**	Model CC01/M2
16	**Rapid Visco Analyzer (RVA)**	Tec master

Figure:3.7 Electronic oven

Figure:3.8Ion Chromatography

Figure:3.9 Atomic Absorption spectroscopy

Figure:3.10 Spectrophotometer

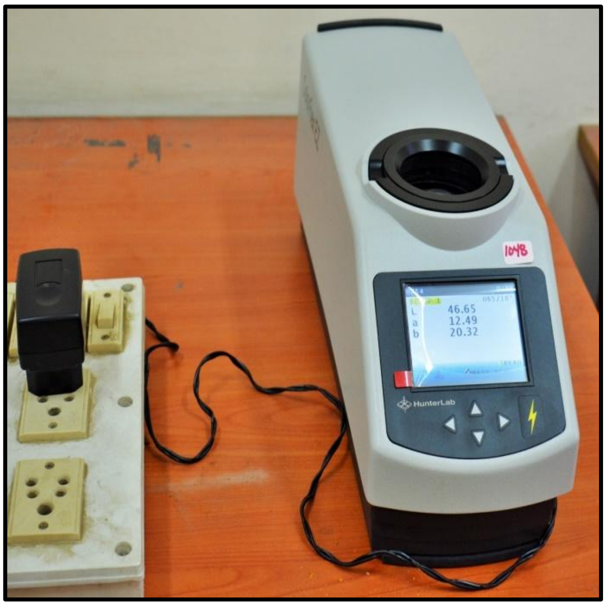


Figure: 3.11 FT-IR

Figure:3.12 Hunter Lab (Colorflex)

Figure:3.13 Digital Bomb Calorimeter

Figure:3.14 Rapid Visco Analyser (RVA)

3.3 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.

3.4 Pastry Board

A pastry board was purchased from the local market of Varanasi for making sugar free biscuit of uniform thickness.

3.5 Biscuit Cutter

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

3.6 Method for Preparation of Sugar Free Biscuit

An Electric Beater was used to beat the butter for about a minute, after which the stevia gradually added and beating was done till it was light fluffy for 5 minutes. The fenugreek seed powder, SMP, and baking powder were then added and beating was done for 30 second until a smooth wet mixture was formed.

The wheat flour was weighed, sieved and mixed well. Gradually the wet mixture was added to the dry flour mixture in a bowl and kneaded gently to make soft non sticky dough. The dough was then cut into equal round shapes with uniform thickness using a biscuit cutter and placed prior preheated oven to bake at 180 ^{o}Ċ for 30 minutes. After baking the biscuit were taken out of the oven and allowed to cool.

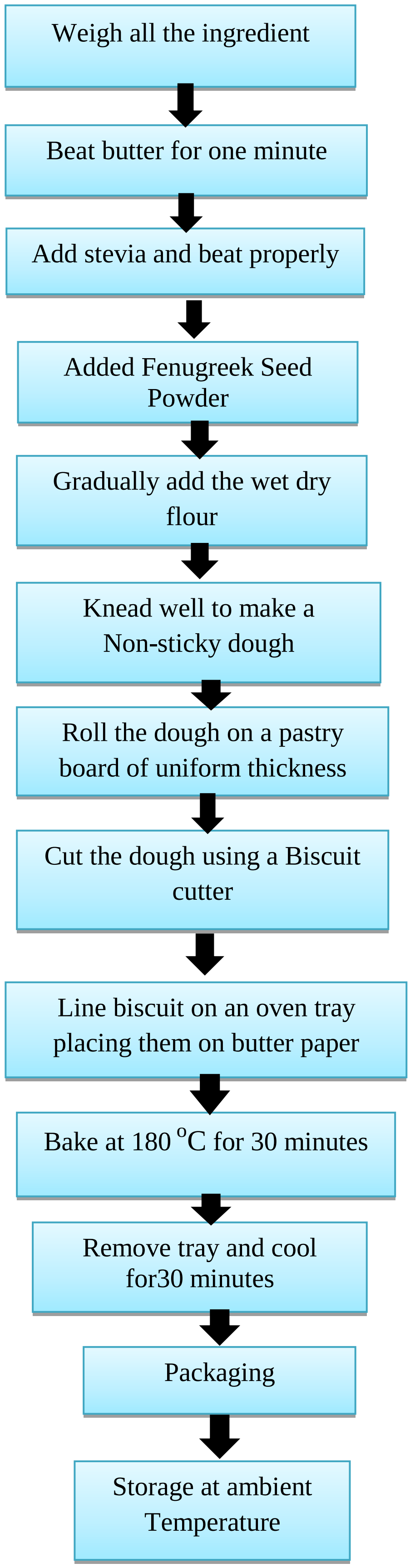


Figure:3.15 Flow Diagram of manufacturing Sugar free biscuit inreached with Stevia and Fenugreek Seed Powder

3.7 Methods of Analysis

3.7.1 Textural Profile analysis

Hardness and fracturability of sugar free biscuitwas measured by Texture Analyser (TA.XT Plus texture profile analyser stable Micro System, UK). The peak force (g) and force mean distance at (mm) were recorded.

Table:3.2 Parameter used for measurement of hardness and fracturability by Texture Analyser

S.No.	T.A. Setting	Measure Force in Compression
1	Pre-test speed	1.50mm/sec
2	Test speed	5mm/sec
3	Post-test speed	10.00mm/sec
4	Target mode	Distance
5	Distance	5.00 mm
6	Trigger type	Auto force
7	Trigger force	50.0g
8	break	Off
9	Stop plot	Start position
10	Tare mode	Auto
11	Advance option	On

3.7.1.1Hardness

Hardness is defined as the maximum peak force during the first compression of cycle and often been substituted by term firmness.

3.7.1.2 Fracturability

Fracturability is defined as the distance at the point of break i.e. a sample that breaks at a very short distance has a high fracturability.

3.7.2 Physical characteristicsof Sugar free Biscuits

3.7.2.1 Diameter

Diameter (D) of biscuit was determined by placing four biscuit edge to edge. The total diameter was measured in centimetre with help of a Vernier callipers .The Biscuit were rotated at an angle of $90^{o}$for duplicate readings. This process was repeated thrice to get an average value and results were reported in centimetre.

3.7.2.2 Thickness

The thickness (T) of the biscuit was determined by placing four biscuit stacking on one another. The thickness was measured in centimetre with the help of a Verniercalliper. This process was repeated thrice to get an average value and results were reported in centimetre.

3.7.2.3 Spread ratio

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

Spread ratio = Diameter/ Thickness

3.7. 3 Sensory Evaluation

The physical quality of Sugar free biscuit sample was judge by a panel of five judges. The sample of each trial was evaluated for sensory attributed viz. colour, appearance and Overall acceptability by an experienced panel comprising of five judges selected from the Centre of Food Science and Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi with the help of 9 point Hedonic scale (Annexture-1).

3.8 Statistical Optimization

Response surface Methodology which involves designing of experiments, selection of levels of variables in experiments runs, fitting mathematical models and finally selection levels of variables by optimization the response was used in the study (Khalil et al.,1999). Central Composite Rotable Design (CCRD) was used to design the experiments comprising three independent processing parameters or factors(Lorezen *et al.*, 1993) (Table:3.4). 31Trials 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 (Montgomery, 2001). The experiments were conducted in randomized order to minimize the effect of unexpected variability in the observed responses because of extraneous factors. Theexperimental design and the codes for the processing parameter or factor are reported in (Table:3.4).Response surface analysis required coding of the values of the processing parameters are fenugreek seed powder, stevia,SMP and Butter. There lower and Upper Limit has been shown below (Table:3.3)

Table:3.3 Factors of Response surface Methodology (RSM)

Factor	Name	Low	High
A	Fenugreek seed powder	0.5	6.5
B	Stevia	1	4
C	SMP	0.5	6.5
D	Butter	10	50

The rest of the ingredients used in manufacturing of sugar free biscuit were kept constant and their quantities are as given below**:**

Baking powder- 1gram

Maida-100 gram

Water – As per requirements

Table:3.4 Experimental design for manufacturing of Sugar Free Biscuit by using RSM

	Factor 1	Factor 2	Factor 3	Factor 4
Run	**SMP (%)**	**Stevia (%)**	**Butter (%)**	**F. S .P (%)**
1	2.0	1.0	40	2.0
2	3.5	2.5	30	3.5
3	2.0	4.0	40	5.0
4	0.5	2.5	30	3.5
5	5.0	1.0	20	5.0
6	3.5	2.5	50	3.5
7	3.5	2.5	30	3.5
8	5.0	1.0	40	2.0
9	5.0	1.0	40	5.0
10	3.5	2.5	30	0.5
11	2.0	1.0	40	5.0
12	3.5	5.5	30	3.5
13	3.5	0.5	30	3.5
14	6.5	2.5	30	3.5
15	5.0	4.0	40	5.0
16	3.5	2.5	30	3.5
17	5.0	4.0	40	2.0
18	2.0	4.0	20	5.0
19	3.5	2.5	30	3.5
20	2.0	1.0	20	2.0
21	3.5	2.5	30	6.5
22	2.0	4.0	20	2.0
23	5.0	4.0	20	2.0
24	3.5	2.5	30	3.5
25	3.5	2.5	30	3.5
26	2.0	1.0	20	5.0
27	5.0	1.0	20	2.0
28	5.0	4.0	20	5.0
29	3.5	2.5	30	3.5
30	2.0	4.0	40	2.0
31	3.5	2.5	10	3.5

Abbreviation: Skimmed milk Powder (SMP); Fenugreek Seed powder (FSP)

3.9 Statistical Analysis of data

The experimental data obtained was analysed with the help of Minitab 17 software (e-Academy). After that each individual experiment, responses were analysed to assess the effect of independent parameters or factors on them .The second order polynomial equation of the following form was fitted to the responses:

$$YK= \sum\beta o+\sum\beta i\, Xi+\sum\beta ii+X2 +\sum\beta ijXiXj$$

Where:

Y= Responses

Bo, Bi, Bii and Bij= constant, linear, quadratic and cross product regression coefficient

X= are the actual value of the independent variables

3.9.1 Optimization

The processing parameters or factors were optimized with respect to the responses viz. Colour and appearance (C and A), Flavour, Overall Acceptability (OA), Hardness and Fracturability of the biscuit. Numerical optimization technique of the Minitab 17 software was used for simultaneous optimization of the multiple responses. The desired goals for each processing parameter or factor and response were chosen. The goals may be applicable to either processing parameter or factor and responses. The possible goals or constraints are; Maximize,Minimize Target and within Range and set to an exact value (for processing parameters or factors only). In order to search a solution, called the desirability function; the maximumvalue of the function is unity. The response surfaces help to understand the effect of varying the processing parameter or factors upon response, i.e. in which direction the response is increasing or decreasing. Response surfaces were generated with the help of statistical package (Minitab 17).

Figure :3.16(A)Before baking (B)3.17 Ready for baking sugar free biscuit

Figure:3.18 Final Baked Fenugreek and Stevia Enriched Sugar Free Biscuits

3.10 Chemical Analysis of Sugar Free Biscuit

3.10.1 Protein

3.10.1.1Principle:

Protein was esteemed by AOAC method (2004). In the Kjeldahl process the protein and other organic food components are digested with concentrated H_2So_4in the presence of catalyst (1:5 cupric sulphate: sodium sulphate). The total nitrogen is converted to Ammonium sulphate .The digest is diluted with water. Alkali containing NaOH is added to neutralizeH_2SO_4 .The ammonia formed is distilled into H_2SO_4 solution containing methyl red indicator .

3.10.1.2 Digestion –

Two grams of biscuit sample was weighed in Kjeldahl digestion flask and 15g digestion mixture (Na_2So_4/K_2So4+ 1g $CuSo_4$) was added.25 ml conc. $H_2So_2$was also added.The content was boiled vigorously, until the appeared clear ortransparent.Heating was continued to 2-3 hours.

3.10.1.3 Distillation and Titration

The digested sample was taken in to a conical flask filled with 25ml 4% Boric acid (neutralized with a mixture of methylred and in ratio of (5:7) and then flask was placed in distillation chamber. After that, the sample was diluted and alkali was added till the sample changethe colour 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 titre value was noted down.

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

Where: TV =Titre value

B= Blank

S = sample

N= Normality of HCL

W= Weight of sample

3.10.2 Fat Analysis (AACC, 2000):

3.10.2.1Procedure:

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

3.10.2.2 Calculation

$$\% \text{ Fat} = \frac{Weight\ of\ Residue}{Weight\ of\ Sample} \times 100$$

Where

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

3.10.3 Ash Content(AACC,2004)

3.10.3.1.Procedure:

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

3.10.3.2 Calculation:

$$\% \text{ Ash} = \frac{Weight\ of\ Residue}{Weight\ of\ sample} \times 100$$

3.10.4Reducing Sugar Estimation

Reducing sugar content in sugar free was determined by Miller et al.(1959).

3.10.4.1Reagent required

- Sodium Potassium Tartarate
- 2 N Sodium Hydroxide
- Dinitro Salicylic Acid (DNS)

3.10.4.2 Preparation of DNS and Rochellesalt

- DNS prepared by dissolving 1 gm sodium salt of DNS 200 mg phenol, 50 mg Sodium Sulphide 100 ml 2N NaOH.
- Rochelle salt: 40% Na-K Tartarate dissolved in 100 ml of distilled water.

3.10.4.3 Protocol

- Prepared standard D-Glucose solution of 100 mg in 100ml of water.
- Make five dilution tube according to given concentration.

Table: 3.5 Standard glucose concentration solution:

S.No.	Standard glucose (ml)	Water (ml)
1.	0.6	2.4
2	1.2	1.8
3	1.8	1.2
4	2.4	0.6
5	3.0	0.0

- Added 3 ml DNS reagent in each test tube mixed and kept in boiling water bath until yellow colour turns down to brick red color.
- Cooled to room temperature added 1 ml of Rochelle salt in each test tube.
- Measure the absorbance at 540 nm in a UV-VIS spectrophotometer against suitable blank.

3.10.5 Moisture (AACC,2004)

3.10.5.1 Procedure

In washed, preheated, cooled and weighed empty silica crucible,2 gram of sampleswere weighted in duplicate. The crucibles were then placed in preheated, hot air oven at 100±5^{o}Cfor 24 hours. After drying, the cruciblewere cooled inthe desiccatorand weighed.

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

3.10.6 Crude Fibre(AACC, 2004)

The crude fibre was estimated according to the procedure as outlined in (AACC,2000). It was carried out by taking 3 g of each fat free flour sampleand digested first with 1.25% H_2SO4, 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 550-650^{o}C till or white ash was obtained. The percentage of crude was calculated after igniting the sample according to the expression given below.

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

3.10.7 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

3.11 Measurement of Energy value of sugar free biscuits by bomb calorimeter

3.11.1 Principle:

The gross energy is the amount of heat produced from unit feed when it is completely burnt down to its ultimate oxidation products (CO_2 and H_2O). The feed is burnt is a closed container (Bomb calorimeter) and heat produced from it is measured. A bomb calorimeter is used to measure the heat created by a sample burned under an oxygen atmosphere in a closed vessel (bomb), which is surrounded by water, under controlled conditions. The measurement result is called the Combustion-Calorific- or BTU-value.

3.12 Antioxidant analysis

3.12.1 Free Radical Scavenging activity by DPPH inhibition

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

3.12.2 Procedure

Biscuit sample was dried in an oven (40^oC) for 24 hrs. The dried material as 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 37^oC. 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 were added. 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:

$$\% \text{ inhibition } = \frac{\{A(Blank) - A(sample)\}}{A(Blank)} \times 100$$

3.13 Determination of mineral content by Atomic Absorption Spectroscopy (AAA) and Ion Chromatography

3.13.1 Digestion Procedure for mineral content in sugar free biscuit

1gram dried and powdered biscuit sample (20 mesh) was taken in a 50 mL digestion tube and 10 mL di-acid mixture (9:4 v/v HNO_3: $HClO_4$) was added to it and was kept overnight. It was then digested on a block digester till a colourless solution was obtained. The volume of acid was reduced till the flask contained only moist residue. The flask was cooled and 25 mL of distilled water was added. The solution was filtered into a 50 mL volumetric flask and diluted up to mark.

3.13.2 Estimation of mineral

The mineral content in biscuit was determined by using atomic absorption spectrophotometer (ELICOS SL 194) as per procedure outlined by Lindsay and Norwell 1978

3.13.3 Calculation

Dilution factor = (50/1) = 100 times

Elemental concentration (mg kg^{-1}) = AAS reading x 100

3.14 Pasting Properties of sugar free biscuits by Rapid Visco Analyser

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

3.14.1 METHOD

- Switched on the RVA and allowed 30 min warm up. Switch on 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 sample) into a new canister.
- Finely, grounded sample was taken (3.5 ± 0.01 g) (14% moisture basis) into a weighing vessel and transfer 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.

3.15 Physicochemical changes during storage

Shelf life study for sugar free 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 ^{o}C for storagestudy. 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.

3.15.1 Hydroxy Methyle Furfural (HMF)

Total HMF in the sugar free 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 Thiopbarbituric acid solution and mixed well.

The tubes were then kept in a water bath at 40 ^{o}C for 50 minutes. After cooling to room temperatureabsorbance was measured at 443 nm (Shimadzu Corporation, Japan). A blank test was carried out in the same manner as above substituting distilled water for sugar free 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 thestandard curve, the HMF content in the samples was determinedusing the following regression equation:

$$\text{Total HMF } (\mu \text{ mole/100gram}) = (\text{Absorbance} - 0.55) \times 87.5 \times 0.4$$

3.15.2 Thiobarbituric Acid(TBA)

The extent of oxidation of fat in the sugar free biscuit was measured in terms of increase in TBA value. For TBA value determination, about 2 gram sample of biscuit was taken and blend with 50 ml of 20 % TCA (Trichloroacetic acid) and 50 ml of distilled water and left undisturbed for 10 minutethen the contents were filtered through whatman No. 1 filter paper .The filtrate (5ml) was then pipeted out in a test tube and added with 5ml of 0.01M 2-Thiobarbituric acid. Colour was developed by incubating the tube in boiling bath for 30 minutes at 100^{o}C .The content were then cooled to room temperature and absorbance was determined at 532 nm (Shimadzu corporation Japan). Blank determinationwere made sing distilled water in place of sample TBA value was expressed at absorbance at 532 nm.

3.15.3 Moisture content:

The protocol is the same as the described above (3.10.5).

3.16 Determination of Microbial Population

3.16.1 Preparation of the sample (Serial dilution):

1 g of sample was takenand transferred to the test tube with 9 ml of normal saline solution

(0.9% NaCl). The samplewas serially diluted up to 10^{-10}dilution. The test tube containing samples were homogenized for proper mixing.

3.16.1.1 Total plate count

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

3.16.1.2Method

3.16.1.3 Sterilization:

The prepared media was autoclaved for 15 minute at 15 psi and temperature at 121 ^{o}C.All glassware’s and necessary item were properly autoclave to avoid contamination.

3.16.1.4 Pouring:

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.

3.16.1.5 Inoculation of sample:

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 hour at 37 ±1 ^{o}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

3.16.2 Coliform count:

Violet red bile agar was used for coliform count.

3.16.2.1 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 usingspread plate technique.

The plates were then incubated at 37 ^{o}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

3.16.3 Yeast and mold:

PDA (Potato dextrose agar) was used to determine the yeast and mold in the biscuit.

3.16.3.1 Method:

3.16.3.2 Sterilization:

The prepared media was heated for 15 min an autoclave maintained at 15 psi for sterilization at 121 ^{o}C. All glassware’s and necessary item were properly autoclaveto 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.

3.16.3.3 Inoculation of sample:

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^{-2} of nutrient agar media.Similarly, all the samples were transferred to the respective petri-diishes 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 ^{o}Ctemperature. 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

CHAPTER-4

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

The present study was conducted for the "**Development & Process Optimization of sugar free biscuit through fenugreek seed powder and natural sweetener (Stevia)".** In the initial stages of the research work, preliminary trials were conducted to screen the various ingredients for the manufacturing of sugar free biscuit. Later the levels of these ingredients were optimized using Response Surface Methodology (RSM) which evaluated the individual and interactive effects of the independent variables. Finally, the product was assessed for proximate composition, textural properties, mineral analysis, pasting properties of starch, flavonoid component analysis by FT-IR, energy value measurement by bomb calorimeter and storage stability as well as its physical characteristics and microbial load.

4.1 Optimization of sugar free biscuits fortified with fenugreek seed powder and natural sweetener (stevia) by using Response Surface Methodology (RSM)

Response surface Methodology (Minitab 17, e-academy software) was applied for the optimization of sugar free biscuits and the parameters taken into consideration were Stevia, SMP, Fenugreek seed powder, and Butter. The equation used was second order polynomial models. All the models exhibited statistical significance as indicated by the F-Value, R^2 -value for all the model was more than 80%, c.v was less than 10% and lack of fit was also found to be not significant. Table 4.1 shows the central composite rotable design (CCRD) for the optimization of sugar free biscuit.

Table 4.1 Central composite Design (CCRD) by RSM for the optimization of sugar free biscuits

Run Order	SMP (%)	Stevia (%)	Butter (%)	FSP (%)	C&A	Flavor	OA	Hardness *(Kg/cm$^{2)}$*	Fracturability *(mm)*	DPPH Inhibition(%)
1	2	1	40	2	7.6	6.6	7	3.0165	43.9065	35.7142
2	3.5	2.5	30	3.5	7.4	7	7.5	2.655	41.4915	3.1476
3	2	4	40	5	7.8	7.8	8	4.2525	41.8485	10.8958
4	0.5	2.5	30	3.5	7	7.2	7.6	1.159	42.3765	2.7845
5	5	1	20	5	7.2	7	7.8	7.378	44.053	19.6125
6	3.5	2.5	50	3.5	6.2	7.4	7	0.593	42.21	6.4164
7	3.5	2.5	30	3.5	7.8	8	8	4.316	43.8925	54.2372
8	5	1	40	2	7.6	7.3	7	5.0725	44.1305	37.4092
9	5	1	40	5	8	7.2	7	1.795	42.0575	28.6924
10	3.5	2.5	30	0.5	7.6	7.6	7.4	2.7395	41.5265	21.0653
11	2	1	40	5	8.5	8	8	2.1745	43.1655	23.7288
12	3.5	5.5	30	3.5	7.8	7.4	7.5	3.2745	43.5265	30.6295
13	3.5	-0.5	30	3.5	8	8	8	3.6295	44.4765	41.7719
14	6.5	2.5	30	3.5	7.8	7.2	7	6.3955	42.48	36.8038
15	5	4	40	5	8	7	7	1.656	43.7465	33.6561
16	3.5	2.5	30	3.5	7.6	7	7	1.622	43.0375	37.5302
17	5	4	40	2	8	7.5	7.4	1.5145	43.6165	46.0048
18	2	4	20	5	7.8	7.4	7.6	1.7555	43.603	36.8038
19	3.5	2.5	30	3.5	8	7.4	8	3.394	44.8695	51.937
20	2	1	20	2	7.6	7	7.2	7.628	43.8575	34.0193
21	3.5	2.5	30	6.5	8	7.7	8	4.074	41.1045	64.5278
22	2	4	20	2	8	8	7.8	4.4055	42.6185	28.2082
23	5	4	20	2	8	7.4	7.3	7.831	43.062	38.0145
24	3.5	2.5	30	3.5	7	6	6	4.5675	41.6825	36.5617
25	3.5	2.5	30	3.5	7.5	7.2	7	5.059	41.653	19.7336
26	2	1	20	5	7	6	6.5	6.5915	41.529	1.6949
27	5	1	20	2	6.8	6.5	6.8	4.6335	43.9815	1.5738
28	5	4	20	5	8	7.6	7	5.5805	41.7925	41.888
29	3.5	2.5	30	3.5	8	7.8	8	7.535	42.663	33.8983
30	2	4	40	2	8.5	8	8	1.417	42.29	45.6416
31	3.5	2.5	10	3.5	6.5	6.4	6	6.869	42.5315	29.1767

4.2 Effect of Ingredient on DPPH Inhibition of Sugar Free Biscuit

The average DPPH inhibition of sugar free biscuit varied from 2.7845 to 64.5278 (Table 4.1)The minimum and maximum DPPH inhibition was obtained for experiment number 4 and 21 respectively .In experiment number 4 , the level of SMP , stevia, butter and fenugreek seed powder 0.5, 2.5, 30, 3.5, respectively while in experiment number 21 the level of SMP, Stevia, butter and fenugreek seed powder was 3.5,2.5, 30,6.5 respectively . The data fitted the following Quadratic Model.

4.2.1 DPPH Inhibition

= -31.3 - 0.0 SMP (%) + 6.1 Steviia (%) + 3.83 Butter (%)- 5.1 Fenugreek Powder (%) - 1.58 SMP (%)*SMP (%)+ 0.25 Steviia (%)*Steviia (%) 0.0405 Butter (%)*Butter (%)
+ 0.98 Fenugreek Powder (%)*Fenugreek Powder (%)+ 1.27 SMP (%)*Steviia (%)+ 0.123 SMP (%)*Butter (%)+ 1.98 SMP (%)*Fenugreek Powder (%)0.322 Steviia (%)*Butter (%)
+ 0.01 Steviia (%)*Fenugreek Powder (%) 0.275 Butter (%)*Fenugreek Powder (%)

Figure: 4.1(a) show the response surface plot for DPPH as influenced by the level of butter and skimmed milk powder (SMP) . From the figure it can be observed that with increase in SMP there is an increase in the DPPH. There is a little effect on DPPH due to butter.

Figure: 4.1(b) show the response surface plot for DPPH as influenced by the level of stevia and butter . From the figure it can be observed that with increase in stevia there is increase in the DPPH there is very little effect on DPPH due to butter.

Figure: 4.1(c) show the response surface plot for DPPH as influenced by the level of stevia and fenugreek . From the figure it can be observed that with increase in Fenugreek seed powder there is an increase DPPH and there is little effect on DPPH due to Stevia.

Figure: 4.1(d) show the the response surface plot for DPPH as influenced by the level of SMP and Fenugreek seed powder . from the figure it can be observed that with increase Fenugreek seed powder there is an increase DPPH and there is very little effect on DPPH due to SMP.

Figure: 4.1(e) show the response surface plot for DPPH as influenced by the level of butter and stevia. From the figure it can be observed that with increase in stevia there is an increase DPPH and there is little effect on DPPH due to butter

Figure: 4.1(f) show the response surface plot for DPPH by the level of butter and fenugreek seed powder . from figure it can be observed that with increase in butter there is an increase DPPH and with incsrese of fenugreek seed powder there is little effect on DPP

Figure: 4.1 Response surface plots showing effect on the DPPH of sugar free biscuit

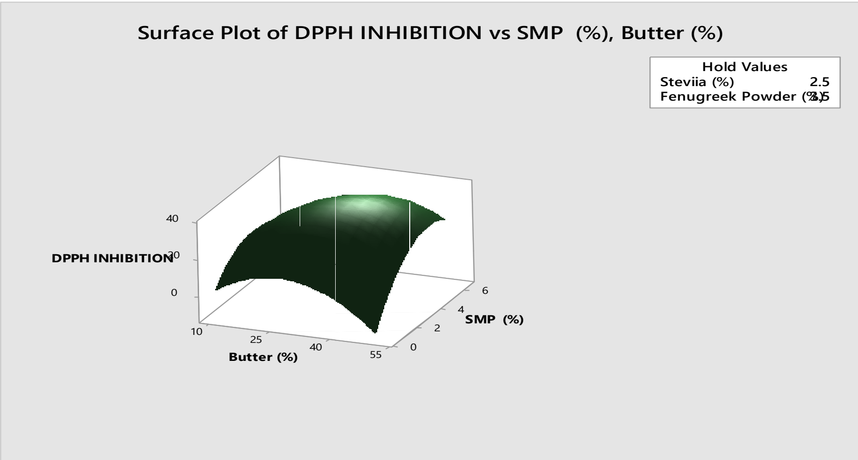


Figure: 4.1(a) Effect of butter and skimmed milk powder

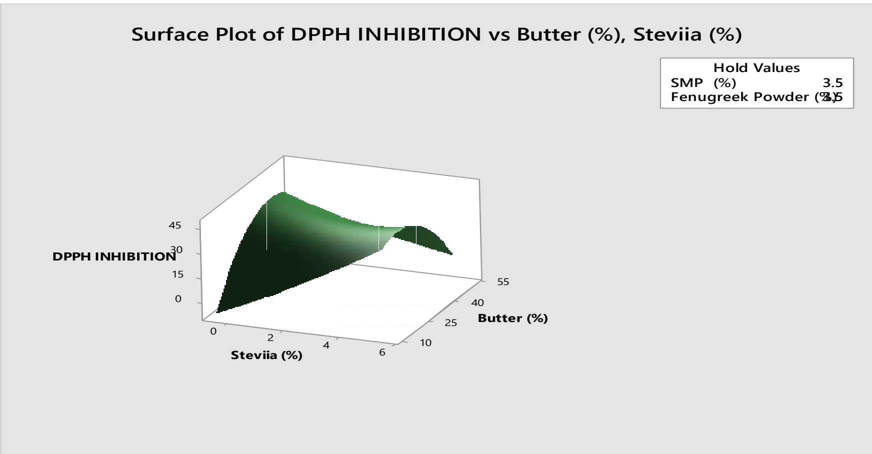


Figure: 4.1(b) Effect of stevia and butter

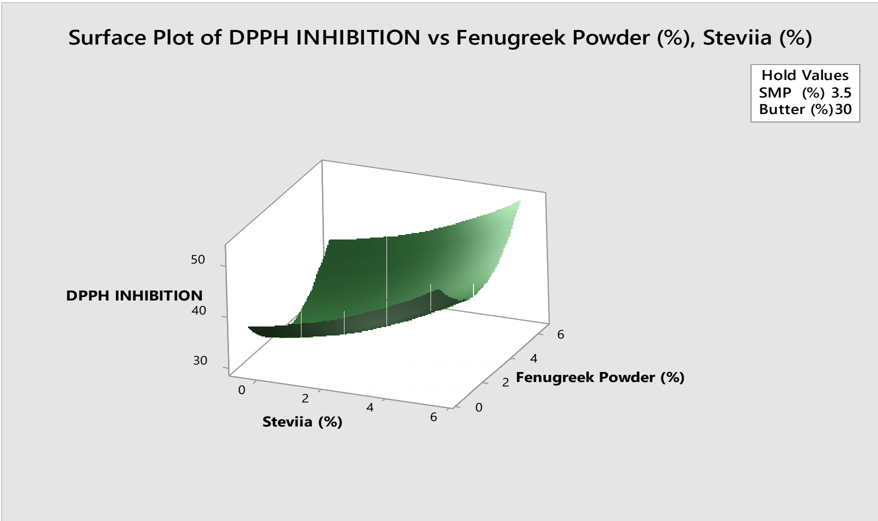


Figure: 4.1(c) Effect of stevia and Fenugreek seed powder

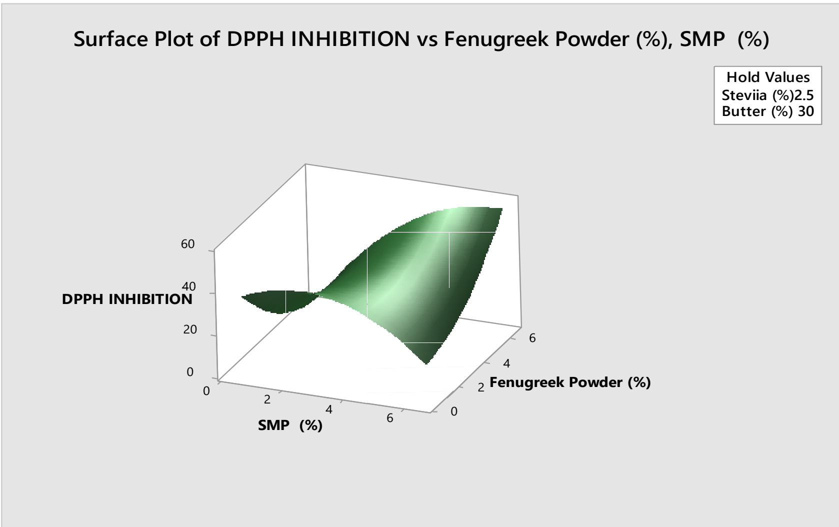


Figure: 4.1(d) Effect of skimmed milk powder and fenugreek seed powder

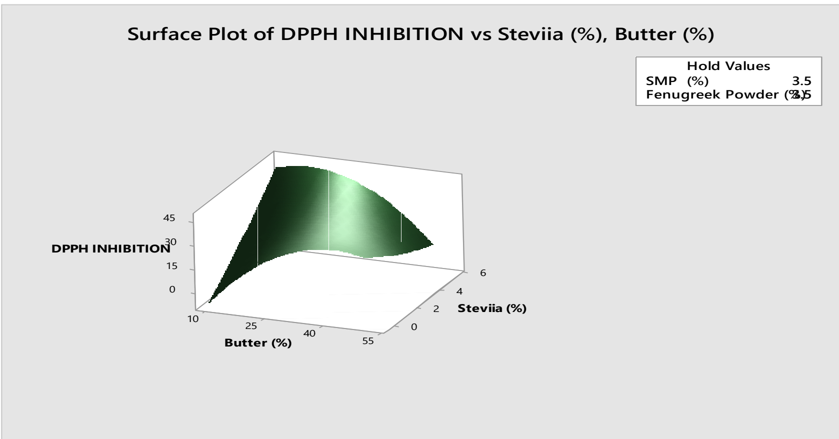


Figure: 4.1(e) Effect of Butter and stevia

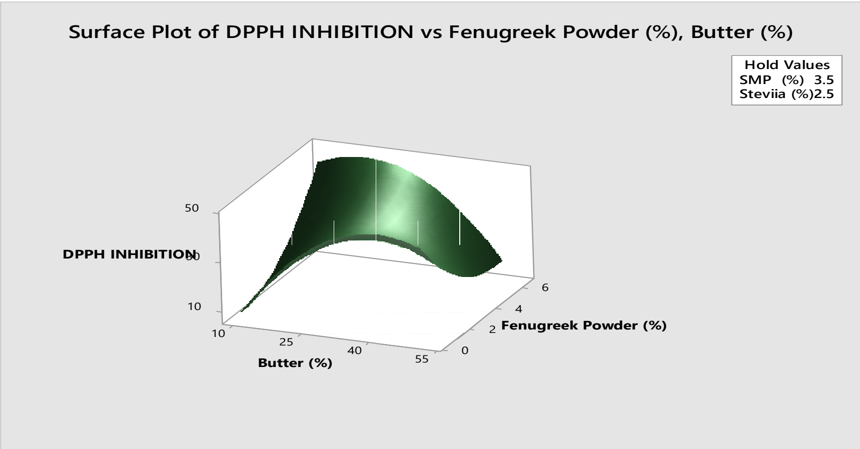


Figure: 4.1(f) Effect of butter and fenugreek seed powder

4.3 Effect of ingredient on fracturability of sugar free biscuit

The average Fracturability of sugar free biscuit varied from 41.1045 to 44.1305 (Table 4.1)The minimum and maximum Fracturability was obtained for experiment number 21 and 8 respectively .In experiment number 21 , the level of SMP , stevia, butter and fenugreek seed powder 3.5, 2.5, 30, 6.5 respectively while in experiment number 8 the level of SMP, Stevia, butter and fenugreek seed powder was 5, 1, 40, 2 respectively . The data fitted the following Quadratic Model.

4.3.1 Fracturability =

44.44 - 0.00 SMP (%) - 1.580 Steviia (%) - 0.012 Butter (%)+ 0.48 Fenugreek Powder (%)
+ 0.0043 SMP (%)*SMP (%)+ 0.1791 Steviia (%)*Steviia (%)
0.00005 Butter (%)*Butter (%)-
0.1193 Fenugreek Powder (%)*Fenugreek Powder (%)+ 0.003 SMP (%)*Steviia (%)
+ 0.0044 SMP (%)*Butter (%)0.017 SMP (%)*Fenugreek Powder (%)+ 0.0024 Steviia (%)
*Butter (%)+ 0.124 Steviia (%)*Fenugreek Powder (%) -
0.0024 Butter (%)*Fenugreek Powder (%)

Figure: 4.2(a) show the response surface plot for fracturability as influenced by the level of Skimmed milk powder SMP and stevia . From figure it can observed that with increase in SMP there is an increase in the fracturability and there is very little effect on fracturability due to stevia

Figure: 4. 2 (b) show the response surface plot for fracturability as influenced by the level of SMP and butter . From figure it can be observed that with increase in SMP there is an increase in the fracturability and there is little effect on Fracturability due to butter.

Figure: 4. 2 (c) show the response surface plot for fracturability as influenced by the level of SMP and fenugreek seed powder . From figure it can be observed that with increase in SMP there in an increase fracturability and there is little effect on fracturability due to fenugreek seed powder.

Figure: 4. 2 (d) show the response surface plot for fracturability as influenced by the level of butter and stevia . From figure it can be observed that fracturability increased due to stevia and there in very little effect on fracturability due to butter

Figure: 4. 2 (e) show the response surface plot for fracturability as influenced by the level of fenugreek seed powder and stevia . from figure it can be observed that with increase in stevia there is an increase in the fracturability and there is very little effect on Fracturability due to fenugreek seed powder

Figure: 4. 2 (f) show the response surface plot for the fracturability as influenced by the level of fenugreek seed powder and butter . from figure it can be observed that with increase fenugreek seed powder there is an increase in the fracturability and there is very little effect on fracturability due to butter

Figure: 4. 2 Response surface plots showing effect on the fructurability of sugar free biscuit

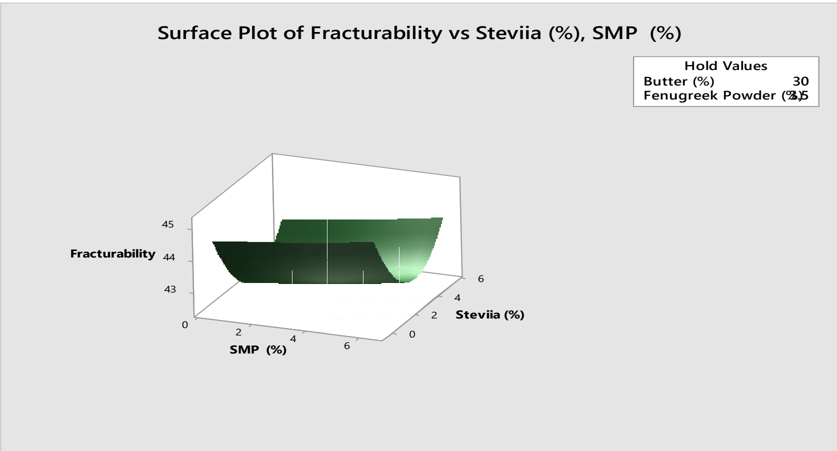


Figure: 4.2 (a) Effect of SMP and Stevia

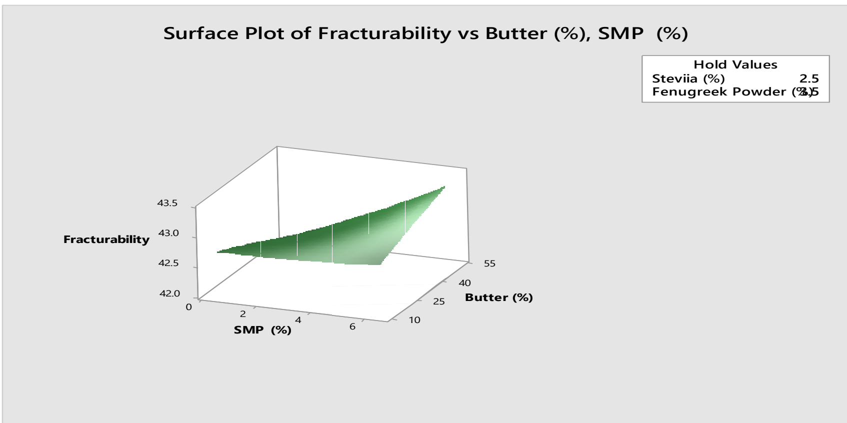


Figure: 4.2 (b) Effect of SMP and Butter

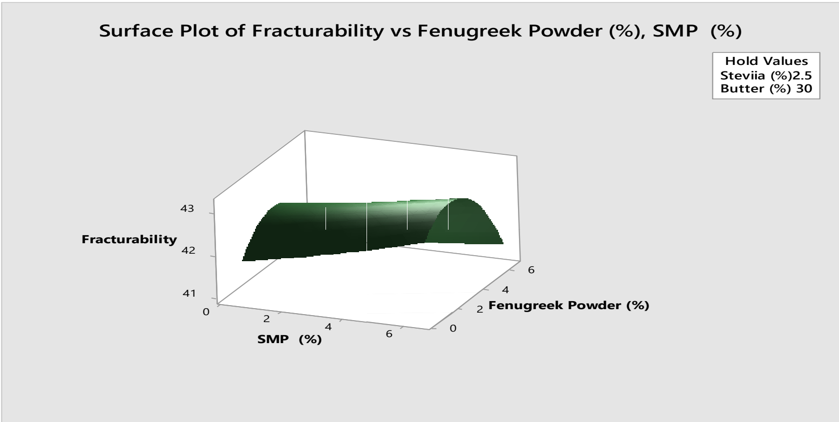


Figure: 4.2(c) Effect of SMP and Fenugrek seed powder

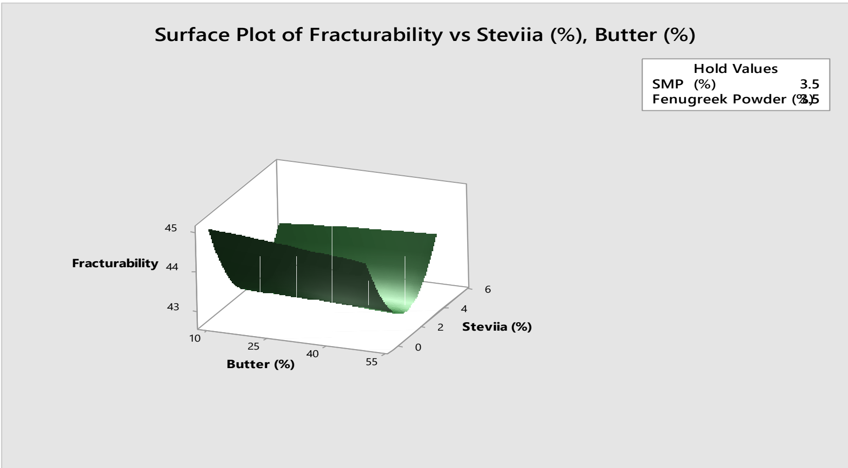


Figure: 4.2 (d) Effect of Butter and Stevia

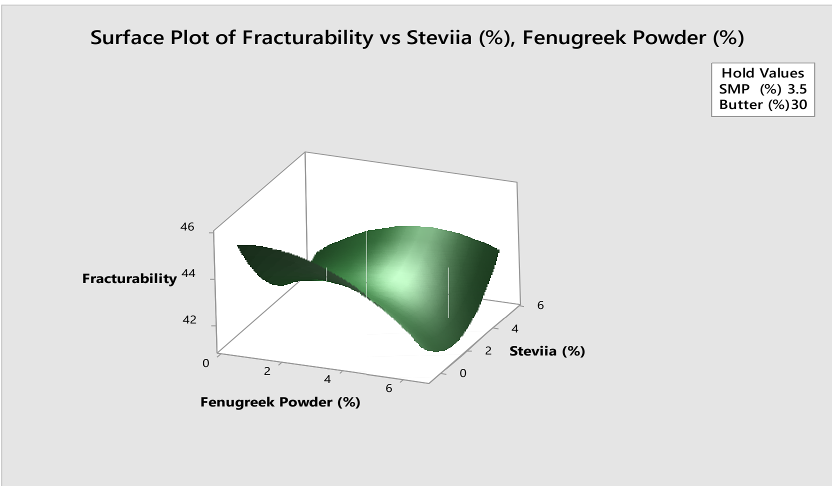


Figure: 4.2(e) Effect of Fenugreek seed powder and stevia

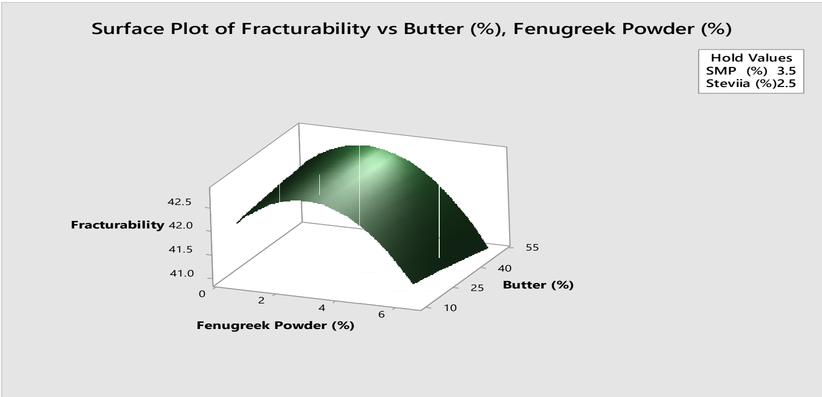


Figure: 4.2 (F) Effect of Fenugreek seed powder and Butter

4.4 Effect of ingredients on hardness of sugar free biscuit

The average hardness of sugar free biscuit varied from 0.593 kg/cm^{2}to 7.831kg/cm^2(Table 4.1)The minimum and maximum hardness was obtained for experiment number 6 and 23 respectively .In experiment number 6 , the level of SMP , stevia, butter and fenugreek seed powder 3.5,2.5, 50,3.5,respectively while in experiment number 23 the level of SMP, Stevia, butter and fenugreek seed powder was 5, 4, 20, 2, respectively . The data fitted the following Quadratic Model.

4.4.1 Hardness

7.95 + 0.87 SMP (%) - 1.10 Steviia (%) - 0.129 Butter (%)+ 0.05 Fenugreek Powder (%) -
0.000 SMP (%)*SMP (%)- 0.036 Steviia (%)*Steviia (%) - 0.00012 Butter (%)*Butter (%)
- 0.041 Fenugreek Powder (%)*Fenugreek Powder (%)+ 0.147 SMP (%)*Steviia (%)-
0.0244 SMP (%)*Butter (%) -
0.026 SMP (%)*Fenugreek Powder (%)+ 0.0143 Steviia (%)*Butter (%)+ 0.014 Steviia (%)
*Fenugreek Powder (%)+ 0.0085 Butter (%)*Fenugreek Powder (%)

Figure: 4.3 (a) show the response surface plot for hardness as influenced by the level of Skimmed milk powder (SMP) and stevia . From figure it can observed that with increase of SMP there is increase in hardness and there is little effect on hardness due to stevia.

Figure: 4.3(b) show the response surface plot for hardness as influenced by the level of SMP and butter . From the figure it can be observed that with increase SMP there is an increase in the hardness and there is vely little effect on hardness due to butter.

Figure: 4.3(c) show the response surface plot for hardness as influenced by the level of SMP and fenugreek seed powder. From the figure it can be observed that with increase SMP there is increase in the hardness and little effect on hardness due to fenugreek seed powder.

Figure: 4.3(d) show the response surface plot for hardness as influenced by the level of stevia and fenugreek seed powder. From the figure it can be observed that with increase fenugreek seed powder there is increase in the hardness and little effect on hardness due to stevia.

Figure: 4.3(e) show the response surface plot for hardness as influenced by the level of stevia and butter. From the figure it can be observed that with increase stevia there is increase in the hardness and little effect on hardness due to butter.

Figure: 4.3(f) show the response surface plot for hardness as influenced by the level of butter and fenugreek seed powder. From the figure it can be observed that with increase fenugreek seed powder there is increase in the hardness and little effect on hardness due to butter.

Figure: 4.3 Response surface plots showing effect of Hardness on sugar free biscuit

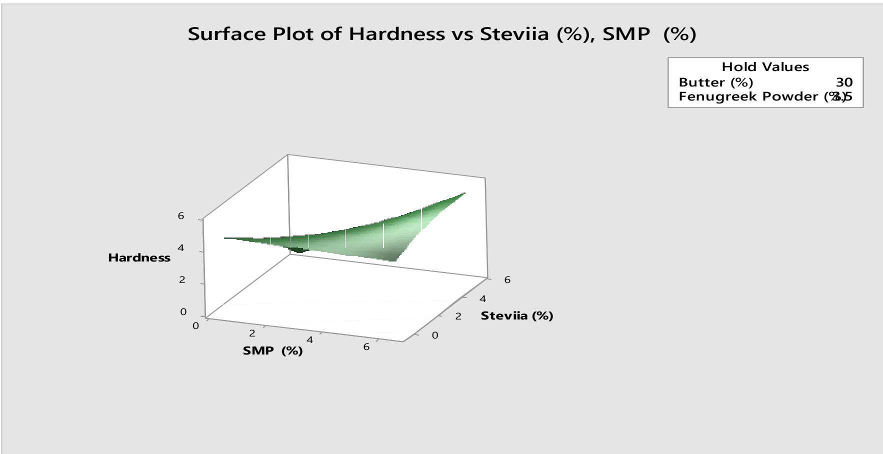


Figure: 4.3 (a) Effect of SMP and Stevia

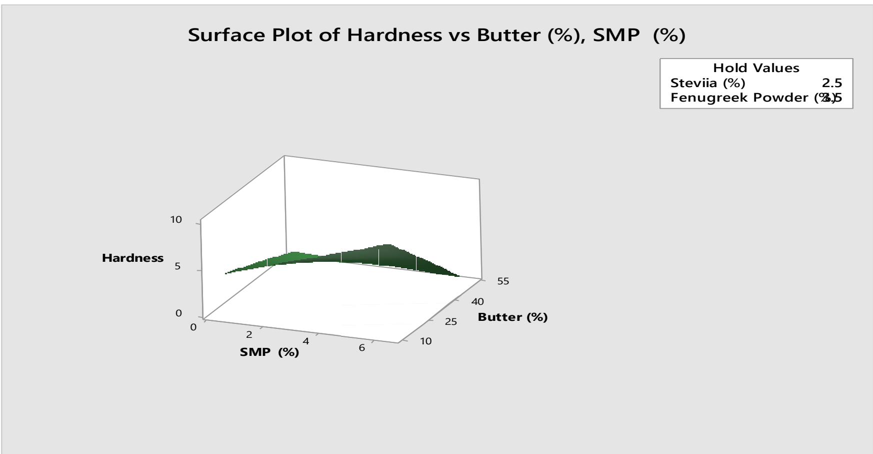


Figure: 4.3 (b) Effect of SMP and Butter

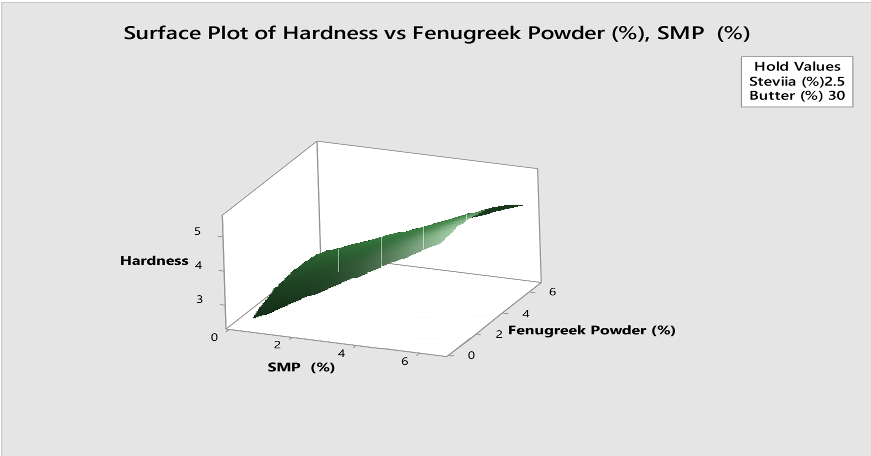


Figure: 4.3 (c) Effect of SMP and Fenugreek seed powder

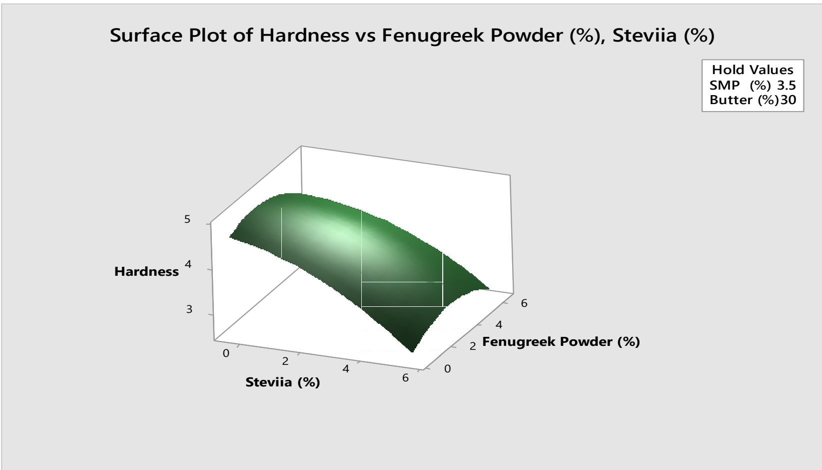


Figure: 4.3 (d) Effect of Stevia and Fenugreek Seed powder

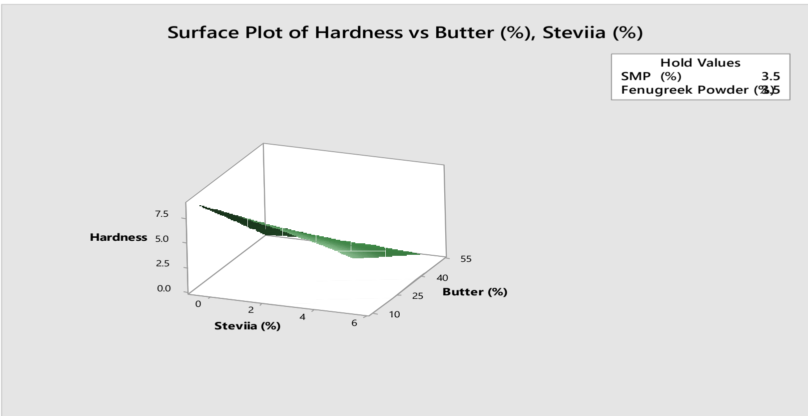


Figure: 4.3 (E) Effect of Stevia and Butter

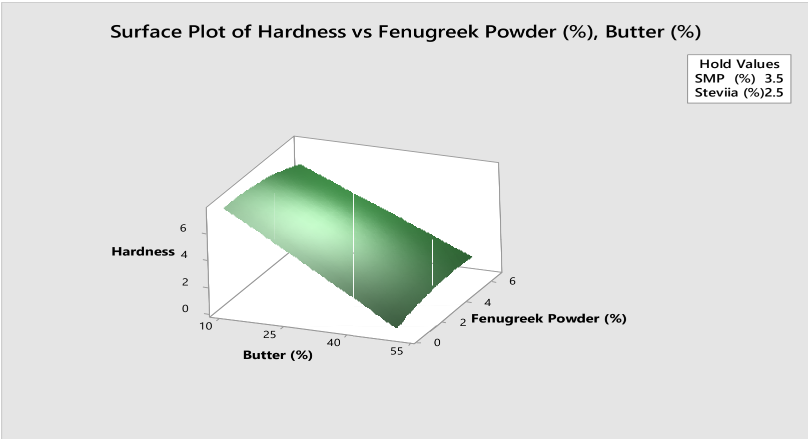


Figure: 4.3 (F) Effect of Butter and Fenugeer seed powder

4.5 Effect of ingredients on colour and appearance of sugar free biscuit

The average colour and appearance of sugar free biscuit varied from 6.2 to 8.5(Table 4.1)The minimum and maximum colour and appearance was obtained for experiment number 6 and 30 respectively .In experiment number 6 , the level of SMP , stevia, butter and fenugreek seed powder 3.5,2.5,50,3.5,respectively while in experiment number 30 the level of SMP, Stevia, butter and fenugreek seed powder was 2, 4, 40,2,respectively . The data fitted the following Quadratic Model.

4.5.1 Colour and appearance = 5.42 - 0.192 SMP (%) + 0.205 Steviia (%)
+ 0.1792 Butter (%)- 0.453 Fenugreek Powder (%)
+ 0.0067 SMP (%)*SMP (%)+ 0.0623 Steviia (%)*Steviia (%) -
0.002473 Butter (%)*Butter (%)+ 0.0512 Fenugreek Powder (%)*Fenugreek Powder (%)+ 0.027
8 SMP (%)*Steviia (%0.00167 SMP (%)*Butter (%)+ 0.0389 SMP (%)*Fenugreek Powder (%)
– 0.01083 Steviia (%)*Butter (%)- 0.0556 Steviia (%)*Fenugreek Powder (%)
+ 0.00417 Butter (%)*Fenugreek Powder (%)

Figure: 4.4(a) show the response surface plot for colour and appearance as influenced by the level of SMP and stevia. From the figure it can be observed that with increase stevia there is increase in the colour and appearance and little effect on colour and appearance due to SMP.

Figure: 4.4(b) show the response surface plot for colour and appearance as influenced by the level of SMP and butter. From the figure it can be observed that with increase butter there is increase in the colour and appearance and little effect on colour and apperance due to SMP.

Figure: 4.4(c) show the response surface plot for colour and appearance as influenced by the level of SMP and fenugreek seed powder. From the figure it can be observed that with increase fenugreek seed powder there is increase in the colour and appearance and little effect on colour and appearance due to SMP.

Figure: 4.4 (d) show the response surface plot for colour and appearance as influenced by the level of stevia and butter. From the figure it can observed that with increase butter there is increase in the colour and appearance and little effect on colour and appearance due to stevia .

Figure: 4.4 (e) show the response surface plot for colour and appearance as influenced by the level of stevia and fenugreek seed powder. From the figure it can be observed that with increase stevia there is increase in the colour and appearance and little effect on colour and appearance due to fenugreek seed powder.

Figure: 4.4 (f) show the response surface plot for colour and appearance as influenced by the level of butter and stevia. From the figure it can be observed that with increase butter there is increase in the colour and appearance and little effect on colour and appearance due to stevia

Figure: 4.4 Response surface plots showing effect on the colour and appearance of Sugar free biscuit

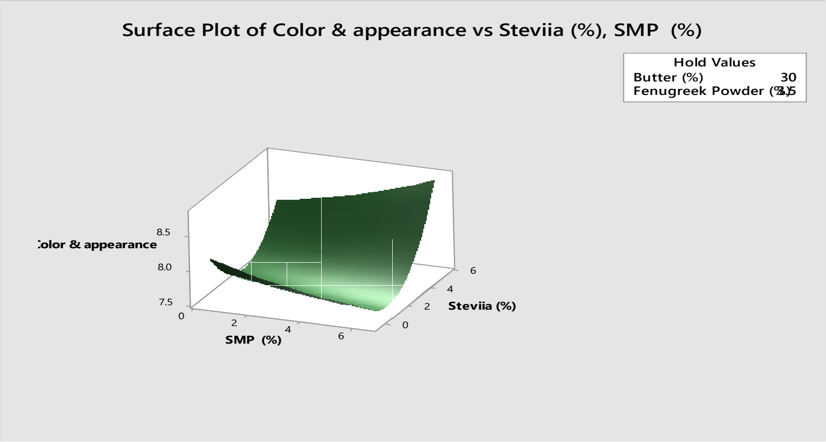


Figure: 4.4 (a) Effect of SMP and Stevia

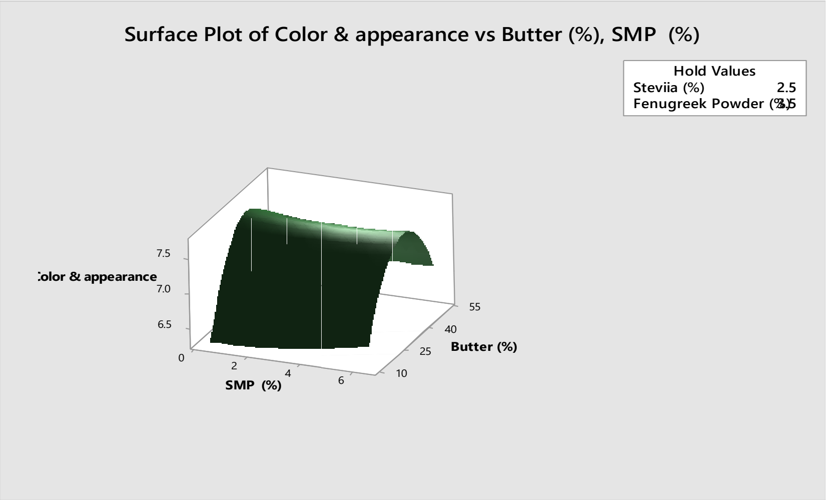


Figure: 4.4 (b) Effect of SMP and Butter

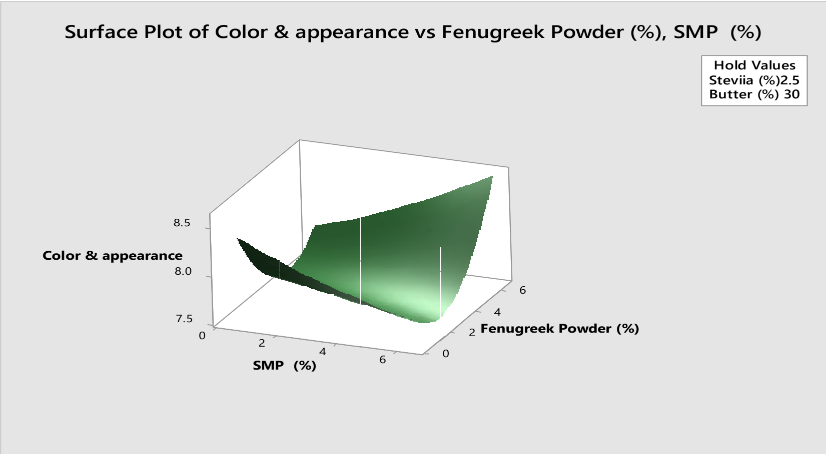


Figure: 4.4 (c) Effect of SMP and Fenugreek seed powder

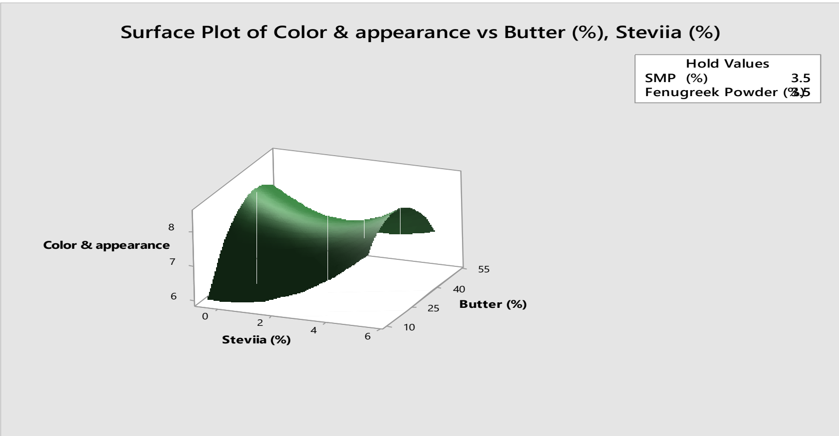


Figure: 4.4 (d) Effect of Stevia and Butter

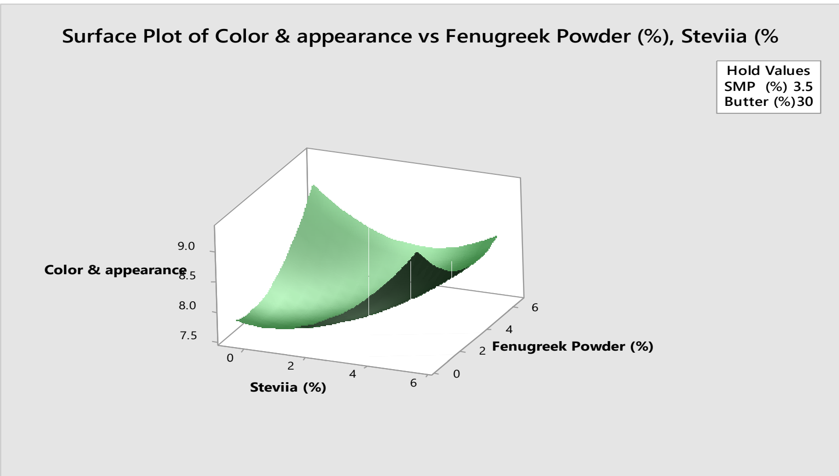


Figure: 4.4 (e) Effect of Stevia and Fenugreek Seed powder

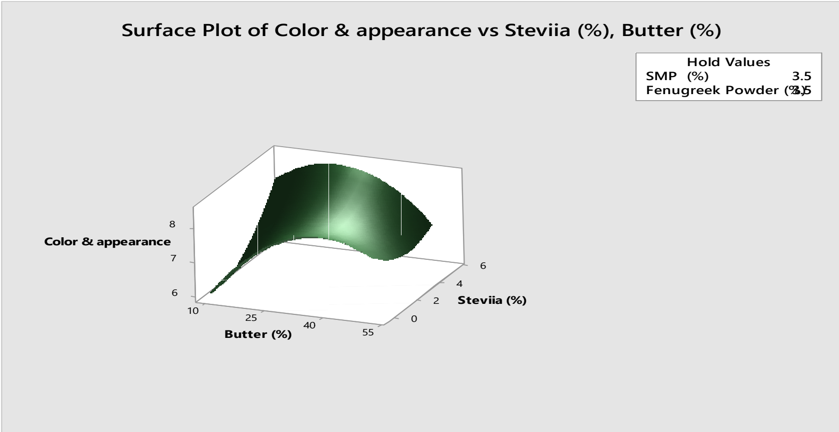


Figure: 4.4 (F) Effect of Butter and Stevia

4.6 Effect of ingredient on flavour of sugar free biscuit

The average Flavour of sugar free biscuit varied from 6 to 8 (Table 4.1)The minimum and maximum Flavour was obtained for experiment number 31 and 30 respectively .In experiment number 31 , the level of SMP , stevia, butter and fenugreek seed powder 3.5,2.5,10,3.5 respectively while in experiment number 30 the level of SMP, Stevia, butter and fenugreek seed powder was2, 4, 40,2 respectively . The data fitted the following Quadratic Model.

4.6.1 Flavour = 4.79 + 0.297 SMP (%) + 0.592 Steviia (%) + 0.1012 Butter (%)- 0.408 Fenugreek Powder (%) - 0.0069 SMP (%)*SMP (%)+ 0.0486 Steviia (%)*Steviia (%) - 0.00091 Butter (%)*Butter (%)+ 0.0431 Fenugreek Powder (%)*Fenugreek Powder (%)0.0583 SMP (%)*Steviia (%)0.00625 SMP (%)*Butter (%)+ 0.0139 SMP (%)*Fenugreek Powder (%)- 0.01125 Steviia (%)*Butter (%) - 0.0528 Steviia (%)*Fenugreek Powder (%)+ 0.00625 Butter (%)*Fenugreek Powder (%)

Figure: 4.5 (a) show the response surface plot for flavour as influenced by the level of SMP and stevia . From the figure it can be observed that with increase SMP there is increase in the flavour and little effect on flavour due to stevia.

Figure: 4.5 (b) show the response surface plot for flavour as influenced by the level of SMP andbutter. From the figure it can be observed that with increase butter there is increase in theflavour and little effect on flavour due to SMP.

Figure: 4.5(c) show the response surface plot for flavour as influenced by the level of SMP and fenugreek seed powder. From the figure it can be observed that with increase SMP there is increase in the flavour and little effect on flavour due to fenugreek seed poeder.

Figure: 4.5 (d) show the response surface plot for flavour as influenced by the level of stevia andbuter. From the figure it can observed that with increase butter there is increase in the flavour and little effect on flavour due to stevia.

Figure: 4.5 (e) show the response surface plot for flavour as influenced by the level of stevia and fenugreek seed powder. From the figure it can be observed that with increase stevia there is increase in the flavour and little effect on flavour due to fenugreek seed powder.

Figure: 4.5 (f) show the response surface plot for flavour as influenced by the level of butter and fenugreek seed powder. From the figure it can be observed that with increase butter there is increase in the flavour and little effect on flavour due to fenugreek seed powder

Figure: 4.5 Response surface plots showing effect on flavour of sugar free biscuit

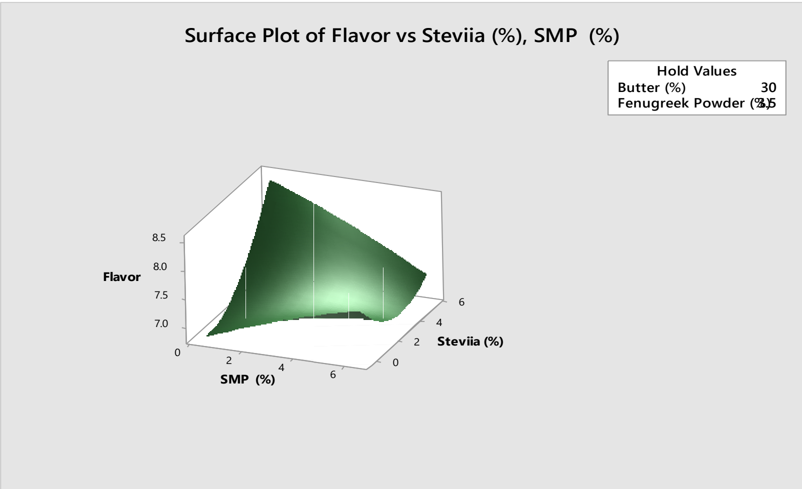


Figure: 4.5 (a) Effect of SMP and Stevia

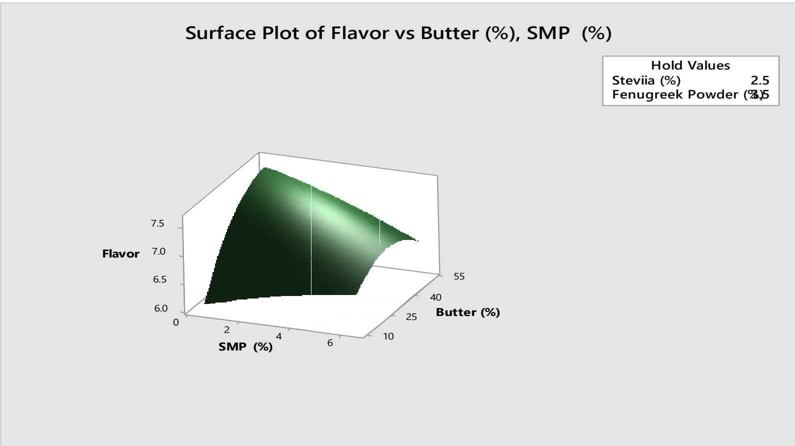


Figure: 4.5 (b) Effect of SMP and Butter

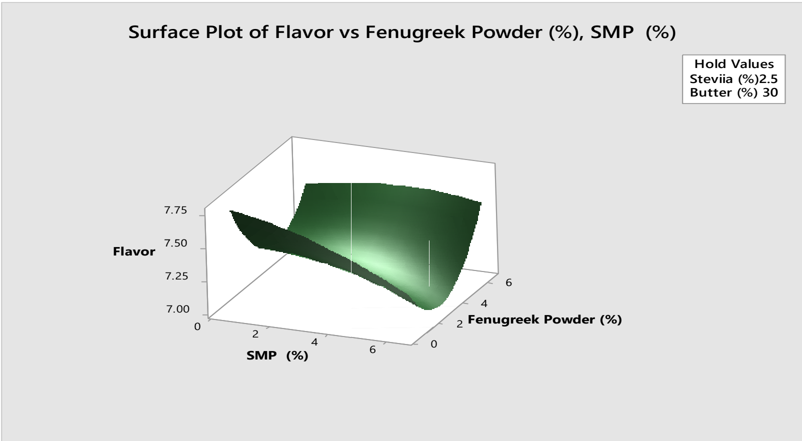


Figure: 4.5 (c) Effect of SMP and Fenugreek seed powder

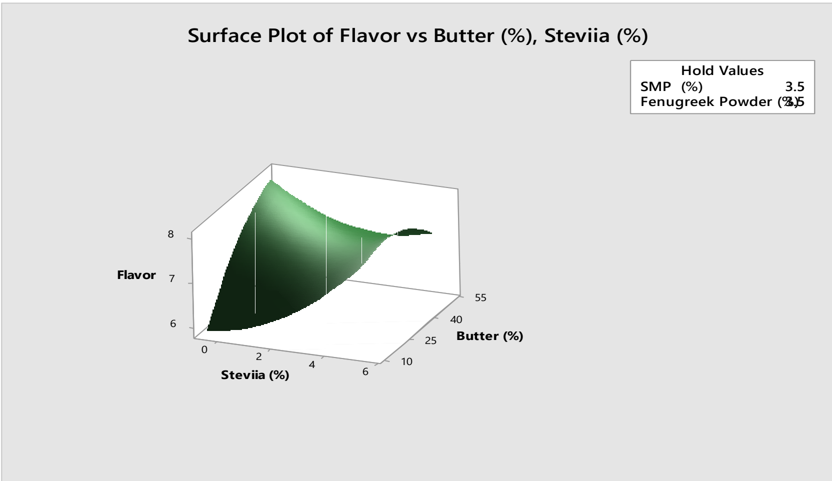


Figure: 4.5 (d) Effect of Stevia and Butter

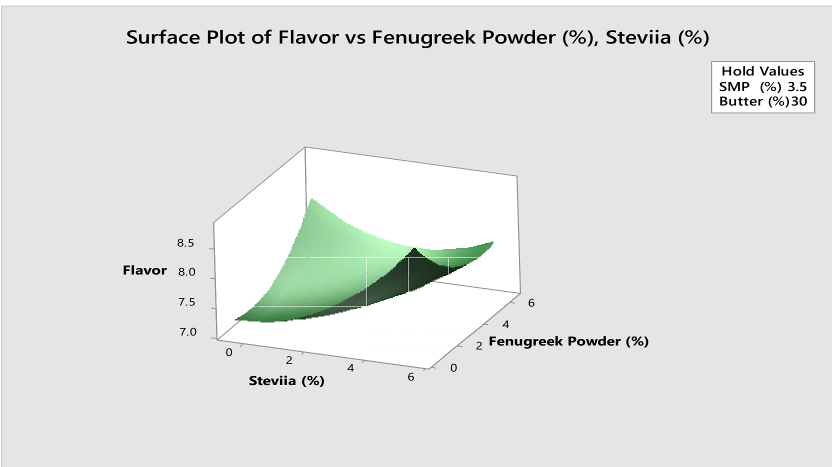


Figure: 4.5 (e) Effect of Stevia and Fenugreek seed powder

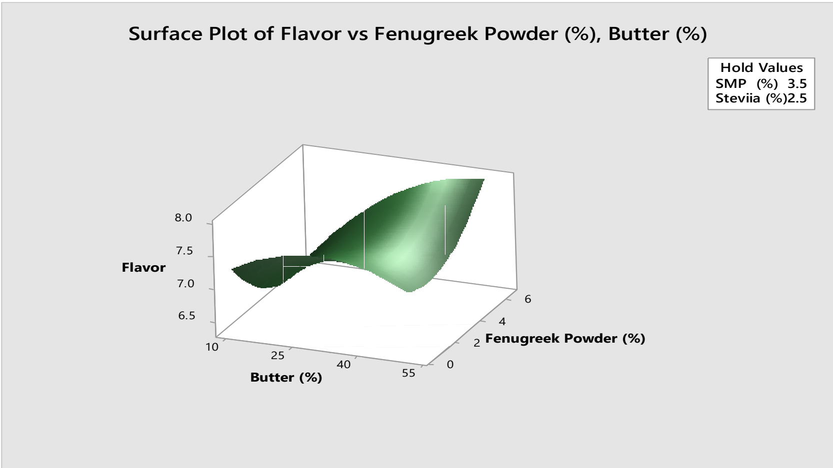


Figure: 4.5 (f) Effect of Butter and Fenugreek seed powder

4.7 Effect of ingredient on overall acceptability of sugar free biscuit

The average overall acceptability of sugar free biscuit varied from to 6.5to 8(Table 4.1)The minimum and maximum overall acceptability was obtained for experiment number 6 and 30 respectively .In experiment number 6 , the level of SMP , stevia, butter and fenugreek seed powder 3.5,2.5,50,3.5, respectively while in experiment number 30 the level of SMP, Stevia, butter and fenugreek seed powder was 2,4, 40,2, respectively . The data fitted the following Quadratic Model.

4.7.1 Overall acceptability=4.07 + 0.381 SMP (%) + 0.289 Steviia (%) + 0.1636 Butter (%)-
0.202 Fenugreek Powder (%) - 0.0045 SMP (%)*SMP (%) + 0.0455 Steviia (%)*Steviia (%) -
0.00210 Butter (%)*Butter (%) + 0.0399 Fenugreek Powder (%)*Fenugreek Powder (%)-
0.0722 SMP (%)*Steviia (%) -
0.01000 SMP (%)*Butter (%)+ 0.0056 SMP (%)*Fenugreek Powder (%)-
0.00000 Steviia (%)*Butter (%)-
0.0611 Steviia (%)*Fenugreek Powder (%)+ 0.00333 Butter (%)*Fenugreek Powder (%)

Figure: 4.6(a) show the response surface plot for overall acceptability as influenced by the level of SMP and stevia. From the figure it can observed that with increase SMP there is increase in the overall acceptability and little effect on the overall acceptability due to stevia.

Figure: 4.6(b) show the response surface plot for overall acceptability as influenced by the level of SMP and butter. From the figure it can observed that with increase butter there is increase in the overall acceptability and little effect on overall acceptability due to SMP.

Figure: 4.6 (c) show the response surface plot for overall acceptability as influenced by the level of SMP and fenugreek seed powder. From the figure it can be observed that with increase SMP there is increase in the overall acceptability and little effect on overall acceptability due to fenugreek seed powder.

Figure: 4.6(d) show the response surface plot for overall acceptability as influenced by the level of stevia and butter . From the figure it can be observed that with increase butter there is increase in theoverall acceptability and little effect on overall acceptability due to stevia.

Figure: 4.6(e) show the response surface plot for overall acceptability as influenced by the level of stevia and fenugreek seed powder. From the figure it can be observed that with increase stevia there is increase in the overall acceptability and little effect on overall acceptability due to fenugreek seed powder.

Figure: 4.6 (f) show the response surface plot for overall acceptability as influenced by the level of butter and fenugreek seed powder. From the figure it can be observed that with increase butter there is increase in the overall acceptability and little effect on overall acceptability due to fenugreek seed powder

Figure: 4.6 Response surface plots showing effect on the overall acceptability of sugar free biscuit

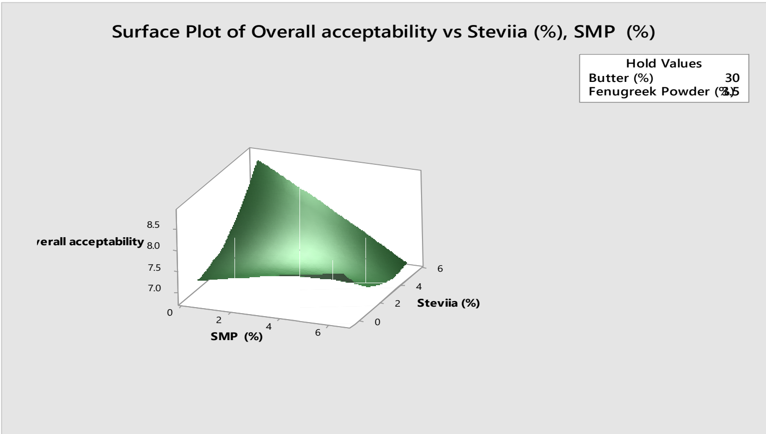


Figure: 4.6 (a) Effect of SMP and Stevia

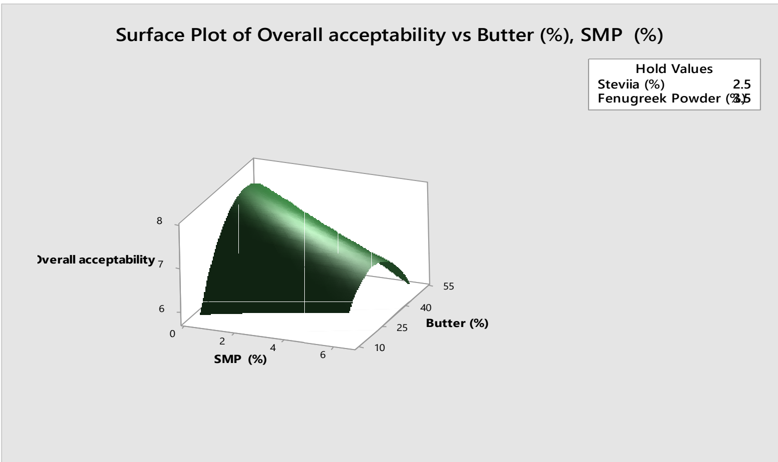


Figure: 4.6 (b) Effect of SMP and Butter

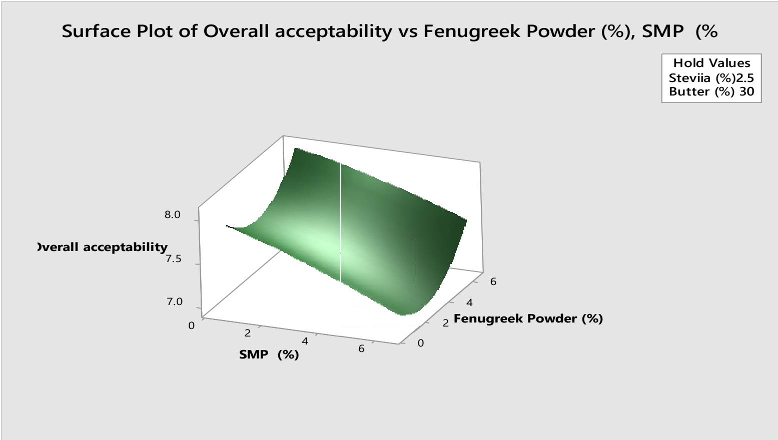


Figure: 4.6 (c) Effect of SMP and Fenugreek seed powder

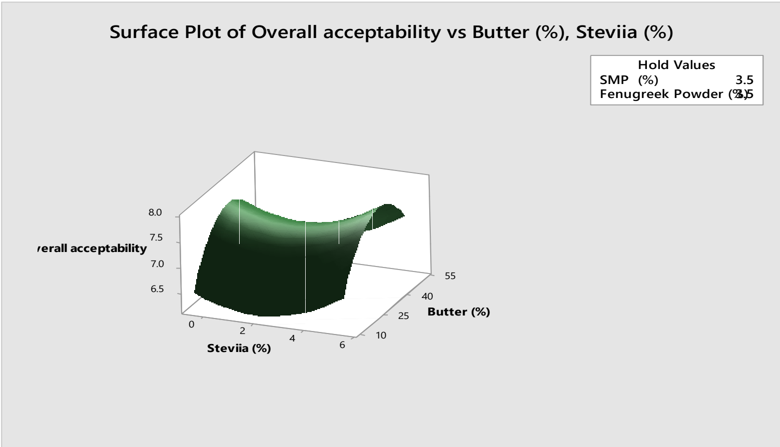


Figure: 4.6 (d) Effect of Stevia and Butter

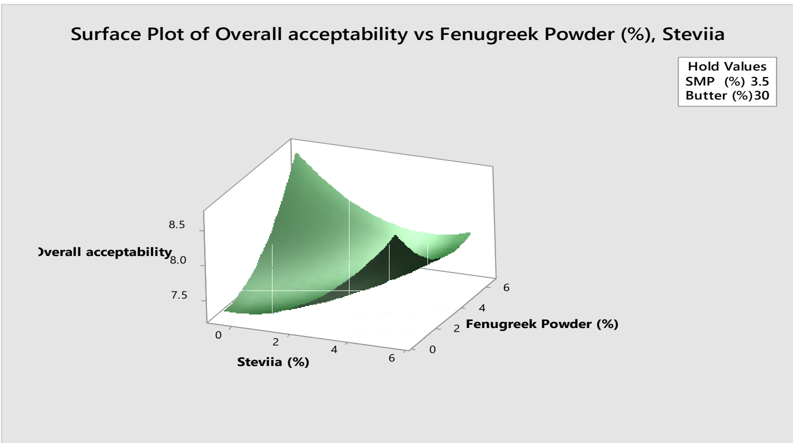


Figure: 4.6(e) Effect of Stevia and Fenugreek seed powder

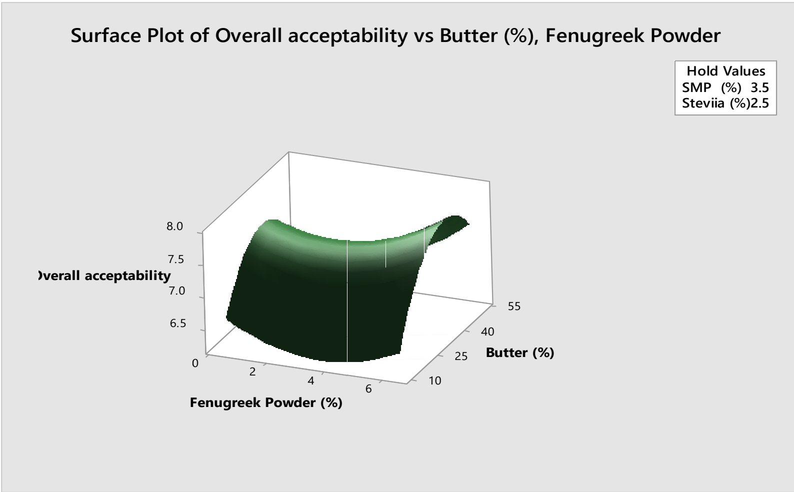


Figure: 4.6 (f) Effect of Fenugreek seed powder

The method adopted for the process optimization was based on numerical method. Table 4.2 shows the constraints for the optimized solution.

Table 4.2: **Constraints of optimized solution**

Sl. No	Response	Goal
1	Colour and Appearance(C and A)	Maximum
2	Flavour	Maximum
3	Overall Acceptability (OA)	Maximum
4	Hardness	Minimum
5	Fracturability	Minimum
6	DPPH Inhibition	Maxiomum

On the basis of constraints suggested, Minitab 17 software selected the following solution as given in Table 4.3 with desirability 0.8951

Table:4.3 Optimized parameter by RSM for the development of sugar free biscuits

Factors				Responses					
SMP	stevia	butter	FSP	C and A	Flavour	OA	H	F	DPPH Inhibition
1.7727	4.3485	37.8788	0.50	7.6	8.4197	8.6465	0.9788	41.8247	40.8247

Abbreviation: OA= Overall Acceptability, C and A= Colour and Appearance,

F= Fracturability, H= Hardness, FSP= Fenugreek seed powder

4.8 Analysis of the Optimized sugar free biscuit

4.8.1 Physical characteristics

Data for physical analysis of sugar free biscuit is presented in the Table 4.4 spread ratio or diameter of nsugar free biscuit has long been used to determine the quality of flour for producing biscuit (Gaines,1990)

Table: 4.4 Physical characteristics of sugar free biscuit

S.No	***Physical characteristics***	***Value (cm)***
1	Diameter	5.04
2	Thickness	0.82
3	Spread ratio	4.92

4.8.1.1Frcturability of sugar free biscuit-

The Fracturability of final optimized sugar free biscuit was found 39.20 mm by Texture profile analyser using Biscuit cutting set knife Probe.

4.8.1.2 Hardness of sugar free biscuit –

The hardness of final optimized sugar free biscuit was found 3.04 Kg by Texture profile analyser using Biscuit cutting set knife Probe.

4.9 Mineral analysis of Sugar Free Biscuit by Ion Chromatography

For the analysis of minerals in sugar free biscuits were compared with blank. The blank sample was prepared from the di-acid mixture 9:4 (v/v HNO_3: $HClO_4$) and the mineral content found in blank such as anion, chloride was 1.87 ppm concentration (Table: 4.5) and (Figure: 4.7)whereas cations Viz. Sodium, potassium, magnesium, calcium concentration in blank sample was 3.075, 0.699, 1.526, and 10.309 ppm respectively (Table: 4.5, Figure:4.8).

The mineral content was also analysed in sugar free biscuit and found more in concentration than blank sample. The anion content in sugar free biscuits like chloride, bromide, sulphate was 0.961 ,3.236, and 4.897 ppm respectively (Table:4.6, Figure:4.9) and whereas cations viz. magnesium, and calcium was 6.041, 13.257 ppm respectively (Table4.6 , Figure:4.10)

The result from mineral analysis by Ion Chromatography, it is recommended that the developed sugar free biscuit, fortified with fenugreek seed powder and natural sweetener stevia, rich in calcium and magnesium and their concentration 294.8 mg/100 g and 451.5 mg/100 g respectively . Rao et. al. (2017) also reported the similar result for biscuit from wheat flour have calcium concentration 98.66mg/100g.

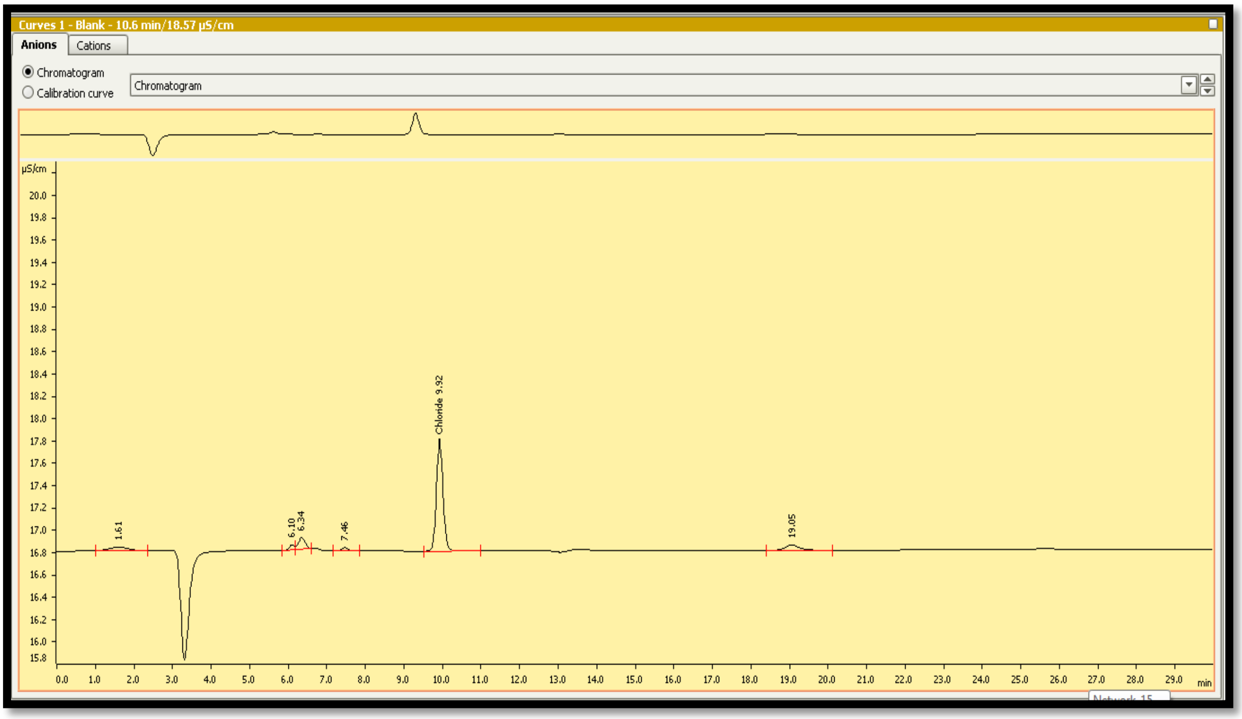


Figure: 4.7 Anion content in blank sample

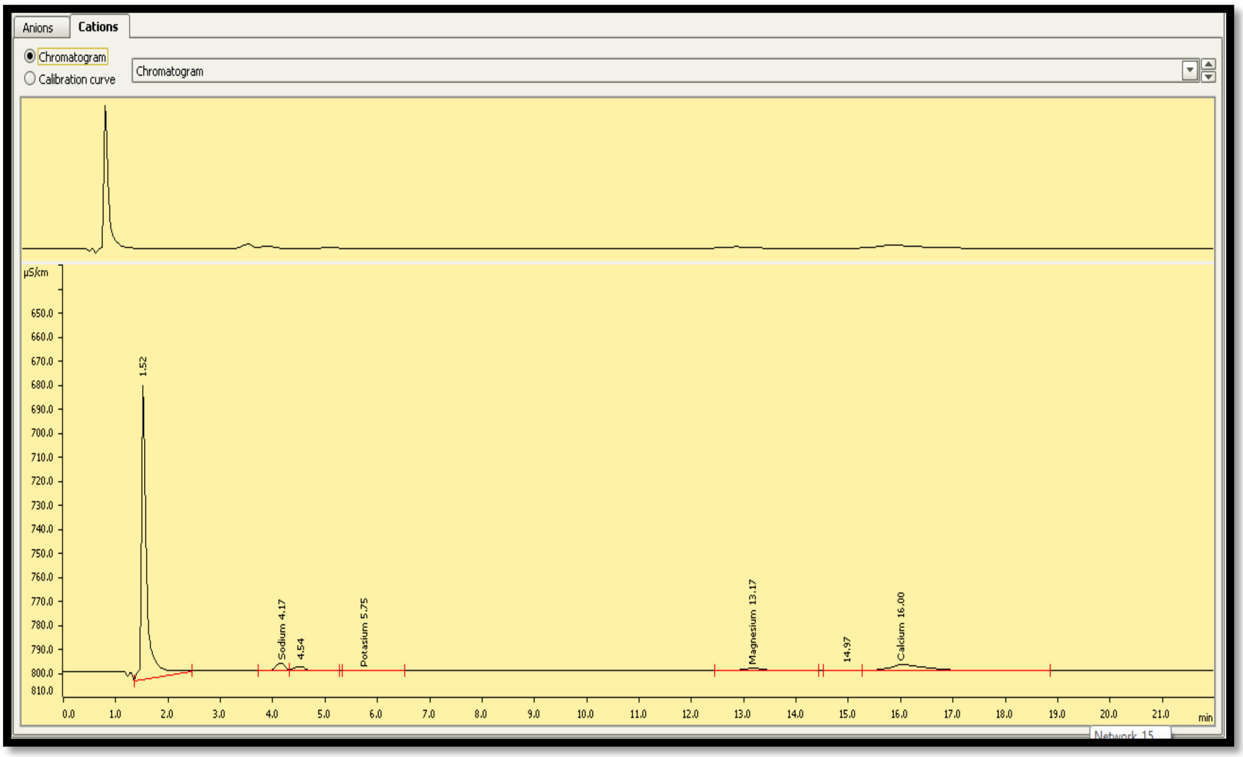


Figure: 4.8 Cation content in blank sample

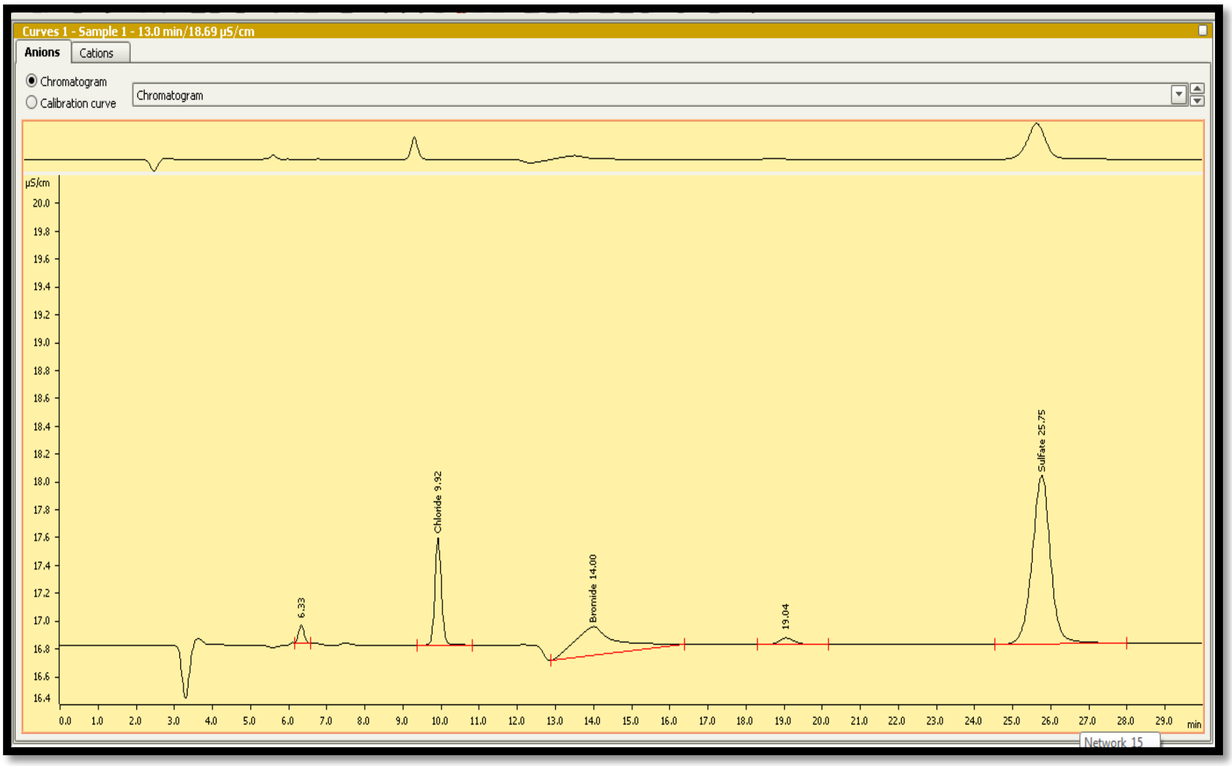


Figure: 4.9 Anion content in sugar free biscuit

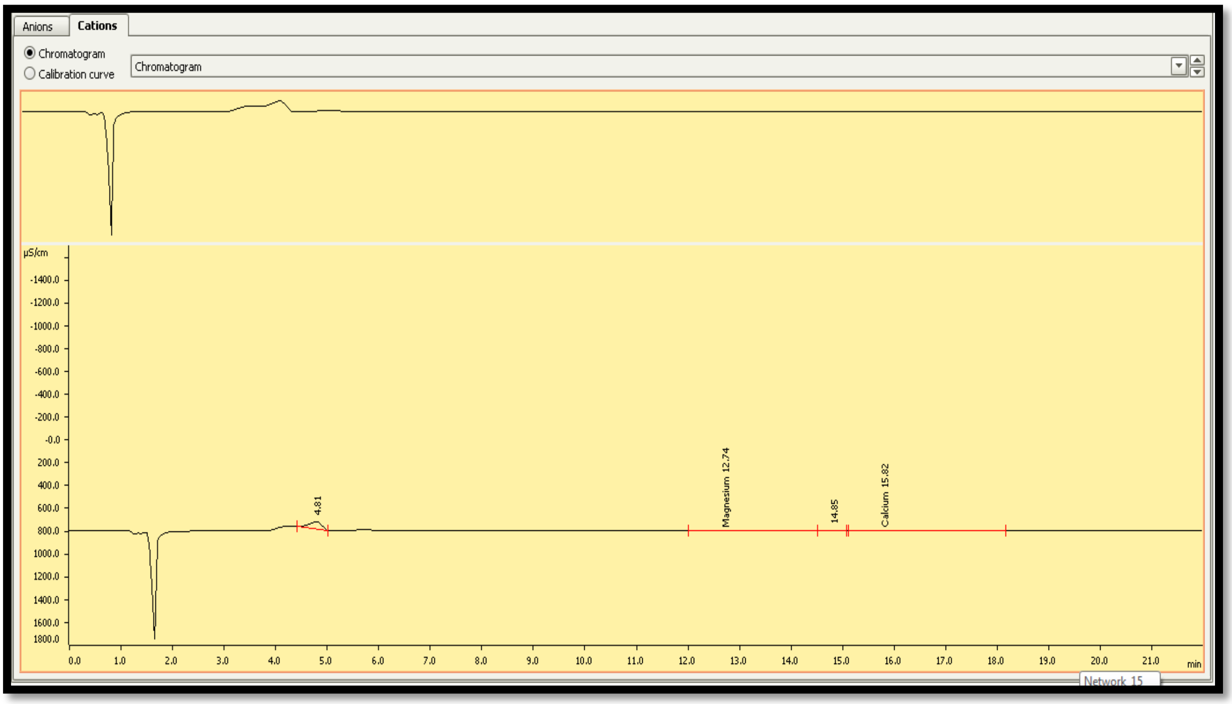


Figure: 4.10 Cation content in sugar free biscuit

Table: 4.5 Mineral content in blank sample

Anion					
Sl. No	Component name	Retention time(min)	Height (µs/cm)	Area[(µs/cm)×min]	Concentration (ppm)
1	Chloride	9.92	1.011	0.198	1.87
Cation					
2	Sodium	4.17	3.326	0.677	3.075
3	Potasium	5.75	0.326	0.090	0.699
4	Magnesium	13.17	1.147	0.545	1.526
5	Calcium	16.00	2.716	2.140	10.309

Table: 4.6 Mineral content in sugar free biscuit

Anion					
Sl. No	Component name	Retention time(min)	Height (µs/cm)	Area[(µs/cm)×min]	Concentration (ppm)
1	Chloride	9.92	0.772	0.150	0.961
2	Bromide	14.00	0.205	.262	3.236
3	Sulphate	25.75	1.214	.677	4.897
Cation					
4	Magnesium	12.74	3.782	2.293	6.041
5	Calcium	15.82	3.249	2.759	13.257

4.10 FT-IR analysis of Sugar Free Biscuits

FT-IR spectroscopy was used to analyse the chemical structure and functional group. Figure 4.11 showed the FT-IR spectra at around 2923.80 cm-1 the intensity of peak absorbance which is C-H group ,around 1745 cm-1 which is C=O, 1164 cm-1 C-O group is present in the sugar free biscuit similarly reported in (Abi.et.al.,(2017).

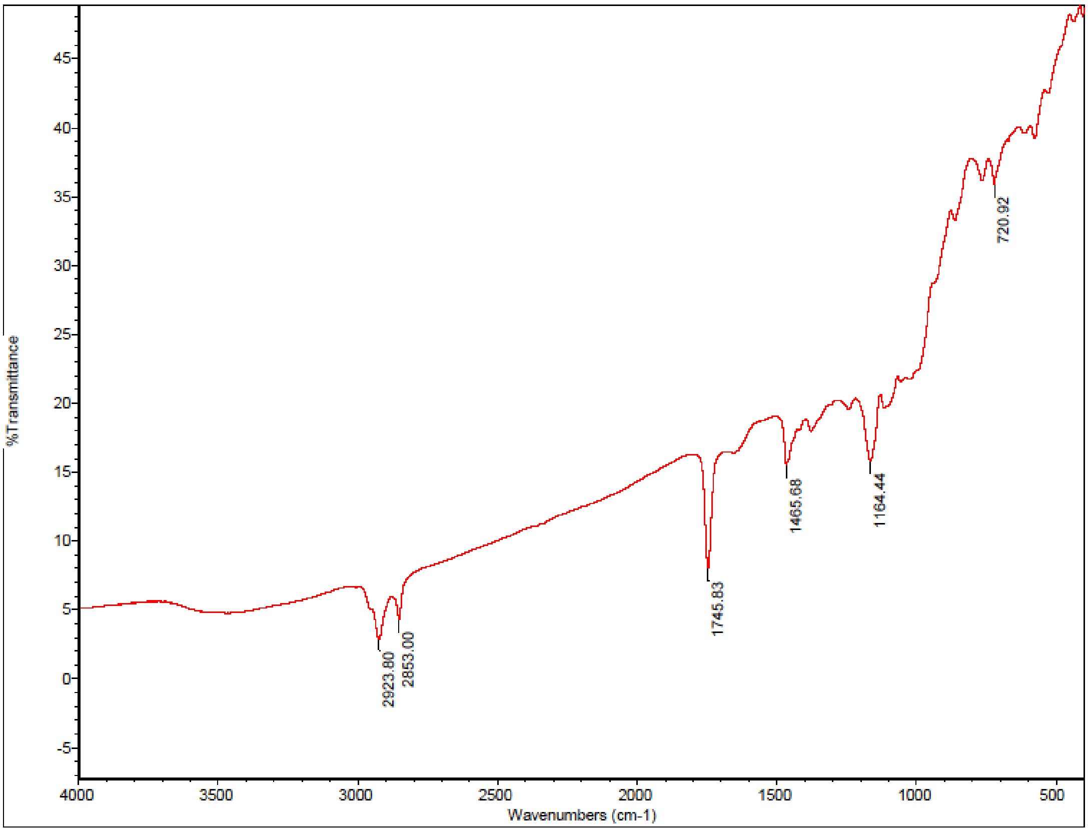


Figure: 4.11 FT-IR analysis of sugar free biscuit

4.11 Pasting properties of Sugar Free Biscuits and Refined wheat flour by Rapid Visco Analyser (RVA)

4.11.1 Pasting properties of Sugar Free Biscuits

There is very little gelatinization was found in sugar free biscuit as shown in (**Figure:4.12)**. There is no clear peak viscosity or hot paste viscosity evident was found. Peak viscosity is an indicator of amylose content and water absorption by the starch. A higher peak indicates a good proportion of amylose present which gives a greater cooking or hot paste viscosity. This pattern of **fig** was obtained because of the fact that the biscuit sample being tested here was a pre-cooked sample at a high temperature, where most of the starch and protein would have got gelatinized and denatured respectively.

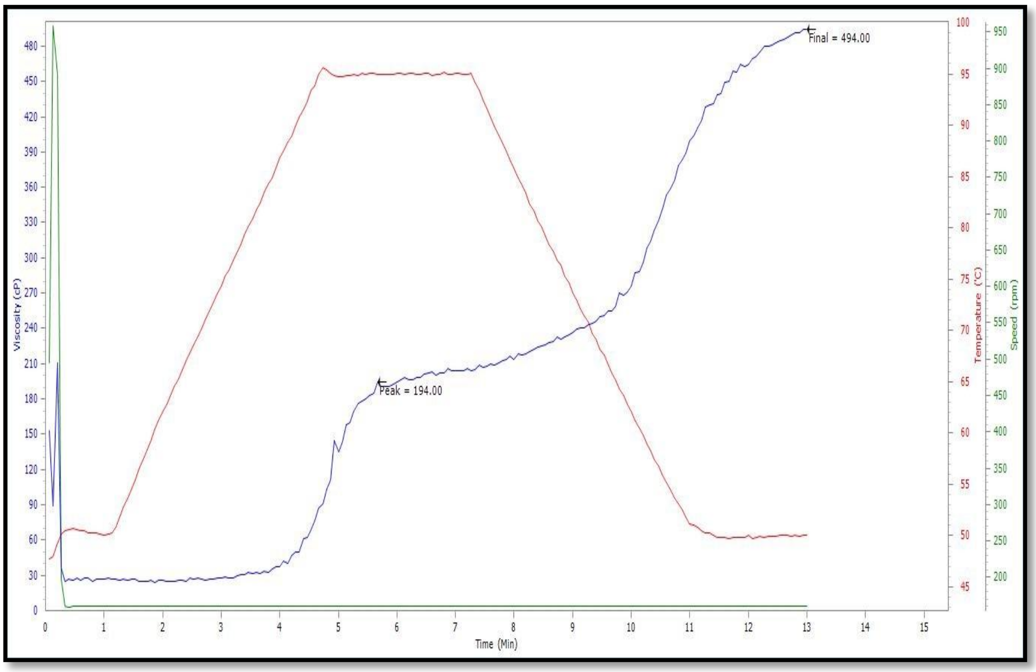


Figure: 4.12 Graph of pasting properties, peak viscosity and breakdown viscosity of sugar free biscuits

The peak viscosity mentioned in the report was merely the value of viscosity obtained, at a point just before the shear sets in. There was no hold viscosity found and the peak viscosity being reported was the viscosity found at the end of test. The final viscosity at large depends upon the amylopectin component of starch being tested. This is because; amylopectin is resistant to water uptake and shear forces. Amylopectin, thus, is responsible for cool paste viscosity or retrograded viscosity. There was no breakdown also reported. Breakdown points indicates the sample's ability to resist shearing stress. It mainly is the result of amylose content of the starch which readily takes up water and swells up. The graph that has been obtained was most likely due to the presence of resistant starch that escapes any high heat treatment or because of the relative effect of various components present in the biscuit.

4.11.2 Pasting properties of refined wheat flour

Refined wheat flour (Maida) sample shows relatively low pasting temperature, indicating some modifications has been done (**Figure)**. It high peak viscosity with high trough values indicates that the sample has good proportion of amylose and thus has high water absorption and swells too many times their original size. This gives a greater cooking property. The high breakdown value indicates the sample was unable to resist shear stress, because of the high amylose content in it. It's nearly same final viscosity as its peak viscosity; show the partial waxy nature of the sample, implying that the proportion of amylose and amylopectin in the sample was nearly similar.

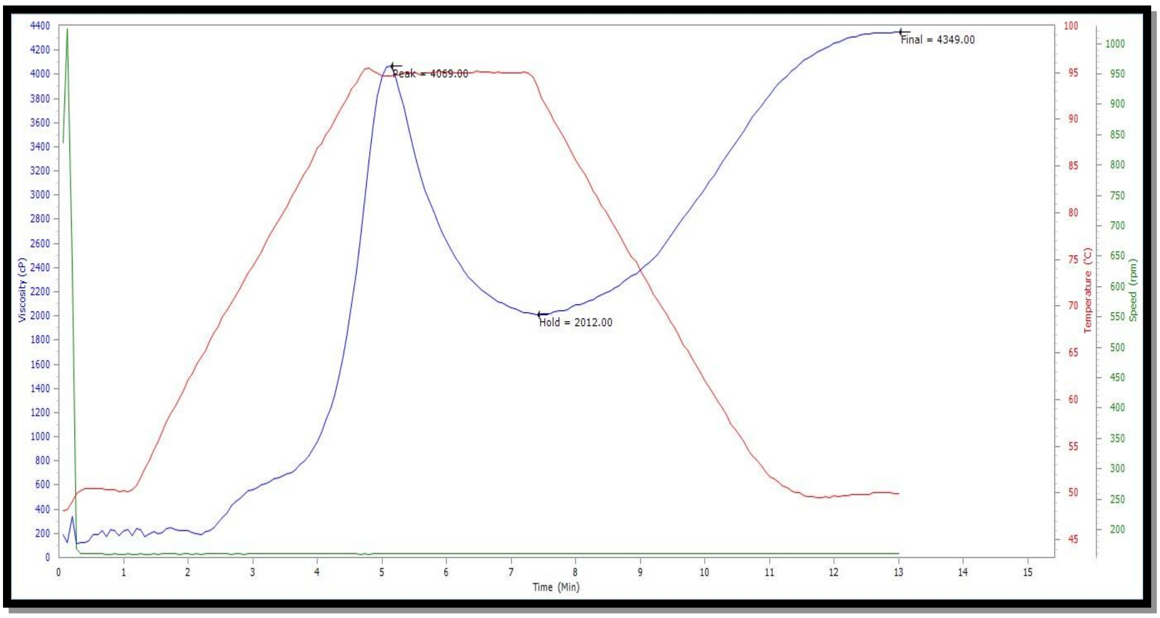


Figure: 4.13 Graph of pasting properties, peak viscosity and Breakdown viscosity of refined wheat flour

4.12 Proximate analysis of sugar free biscuit

The proximate analysis of sugar free biscuits was performed for the composition of carbohydrate, protein, fat, ash, moisture and fibre (Table). During analysis of sugar free biscuits carbohydrate (76.75%), protein (5.90%), fat (14.85%), ash (~1%), fibre (1.5%) and moisture (4%). Carbohydrate percentage is very high in the sugar free biscuit due to refined wheat flour. Similar finding were also reported by Kamal et al. (2013) fortified biscuit carbohydrate (73%), fat (14%), protein (7.65%) and moisture (3%).

Table:4.7 Proximate composition of Optimized sugar free biscuit

No.	Constituents	Amount (g/100)
1	Protein	5.90
2	Fat	14.85
3	Ash	~1.00
4	Moisture	4
5	Carbohydrate	76.75
6	Fibre	1.5

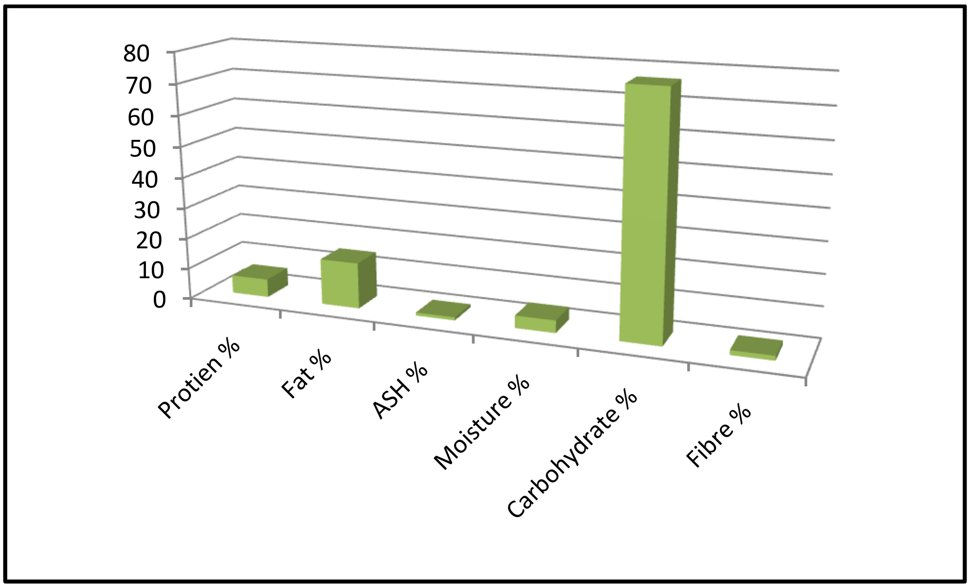


Figure: 4.15 Proximate composition of sugar free biscuit

4.13 Determination of mineral content in sugar free biscuit by Atomic Absorption Spectroscopy

Iron, copper, magnesium and zinc content were analysed in sugar free biscuits by Atomic absorption spectroscopy. It was found that sugar free biscuit contain iron, 1.93mg/100g, copper, 0.065mg/100g, and zinc, 0.325 mg/100g. Similar finding were also reported by Rao et al. (2017) where biscuits fortified with spinach have iron content 2.85mg/100g.

Table: 4.8 Mineral content in sugar free biscuit by AAS

Sl. No	Sugar free biscuit	Mineral content in mg/100g
1	Iron	1.93
2	cu	0.065
3	Zn	0.325

4.14 Energy value

Energy value of sugar free biscuit was measured by the digital bomb calorimeter and reported 484 kcal /100 g.

4.15 Reducing sugar

In the final optimized sugar free biscuit not detected reducing sugar .

4.16 Antioxidant properties of sugar free biscuit

4.16.1 DPPH inhibition

The DPPH inhibition of final optimized sugar free biscuit was found 38% and similar finding was reported Rao et. al. (2017) where the plain biscuits have DPPH inhibition 36 %. it can be concluded that in the sugar free biscuit antioxidant properties is more than other normal biscuit which is available in the market.

4.17 Colour characteristics

The colour of sugar free biscuits was measured by Hunter lab (colour-flex). The final optimized sugar free biscuit is L- 46.40, a-12, b-20.29 and similar finding reported Rao et. al. (2017) where L-66.15, a-11.86 and b-33.95. The developed sugar free biscuit have L and b value was low due to the fortification of fenugreek seed powder and as well as using of skimmed milk powder (SMP) and stevia.

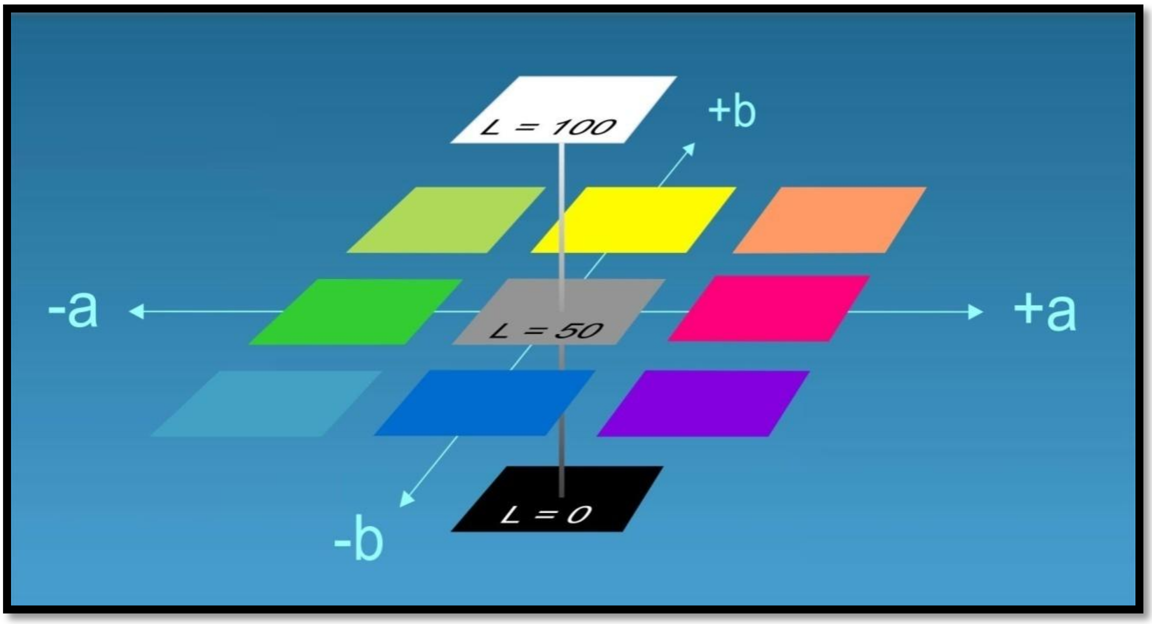


Figure: 4.16 Colour flex chart for L, a and b value

4. 18 Shelf life study of sugar free biscuit


Figure: (A) Working on Texture analyser for Hardness and fracturability of sugar free biscuits

Figure: (B) Working in Laminar air-flow

4.18.1 Changes in Hydroxy Methyl Furfural (HMF) content during Storage

Hydroxymethyle furfural (HMF) in an organic compound derived from dehydration of certain sugars. HMF has been identified in wide variety of baked goods. It is practically absent in fresh food, but is naturally generated in sugar – containing food during heat – treatments like drying or cooking. Along with many other flavour and colour related substances, HMF is formed in the Maillard reaction as well as during caramelization. In these foods it is also slowly generated during storage and with increasing time (Lorenzo et al., 2010).

The increase in HMF value is presented in figure. In fresh sugar free biscuit HMF value recorded was 5.1 mol/100g which increased to 5.34 mol/100g after storage of 10 days at 10 ^{o}C and 5.63 and 5.71 mol/100g at 25 and 37 ^{o}C respectively . After 20 days of storage the HMF increased to 5.81, 5.91, 5.96 mol/100g at 10 ^{o}C, 25 ^{o}C , 37 ^{o}C respectively . After 30 days of storage the HMF increased to 6.1, 6.3, 6.5 mol/100g at 10, 25 , 37 ^{o}C respectively .

It was observed that the formation of HMF was lesser at 10 ^{o}C as compared to 25 and 37 ^{o}C .It can therefore be concluded that with increase in storage time and temperature the formation of HMF increses . At 10 ^{o}C, 25 ^{o}C, 37 ^{o}C, the total HMF increased to 6.1 , 6.3, 6.5 mol/100g respectively after 30 day

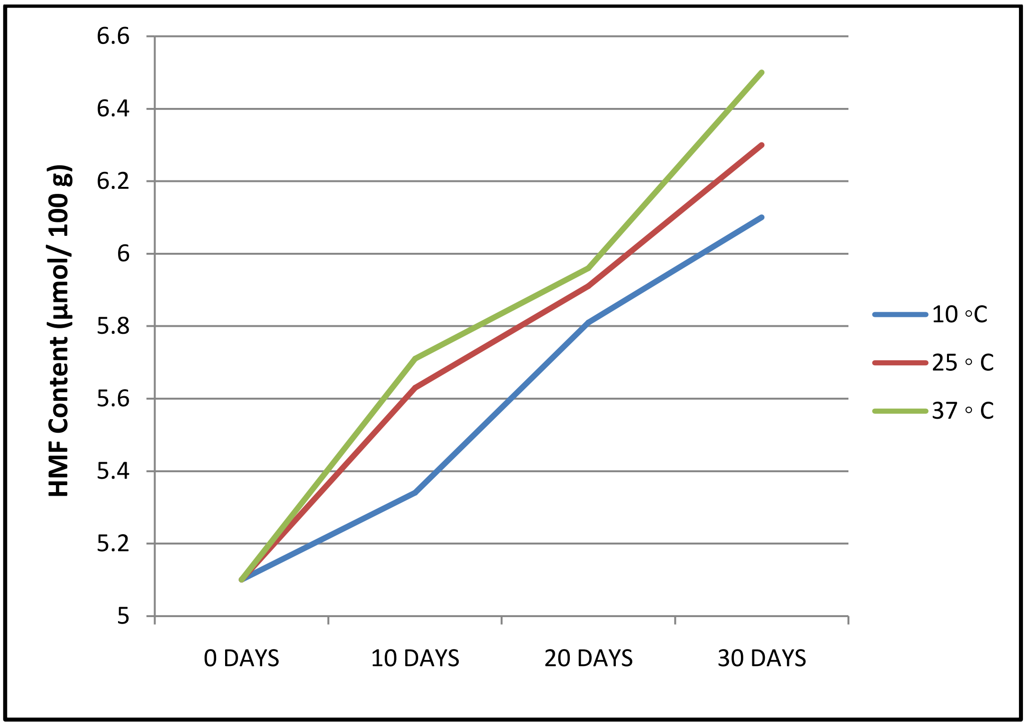


Figure: 4.17 Changes in HMF

4.18.2 Changes in Thiobarbituric acid (TBA) during storage

During storage fat or shortening used in bakery products such as biscuit, cookies, cakes etc undergo auto-oxidation which is often followed by storage odours. The TBA value is widely used for estimating peroxidation and rancidity in foods. The increase in (TBA) values is presented in Figure. In Fresh sugar free biscuit TBA value recorded was 0.178 which increased to 0.183 after storage 10 days at 10 °C and increased to 0.196 in 10 days at 25 °C and increased to 0.203 in 10 days at 37 °C after 20 days of storage the TBA increased to 0.212, 0.224 and 0.243 at 10, 25 and 37 °C respectively. After 30 days of storage the TBA increased to 0.254, 0.265 and 0.286 at 10 °C, 25 °C and 37 °C respectively.

It was observed that the formation of TBA was lesser at 10 °C as compared to 25 °C and 37 °C. It can therefore be concluded that with increase in storage time and temperature the formation of TBA increases. At 10°C, 25°C and 37 °C the total TBA increased to 0.254, 0.265, 0.286 respectively after 30 days of storage.

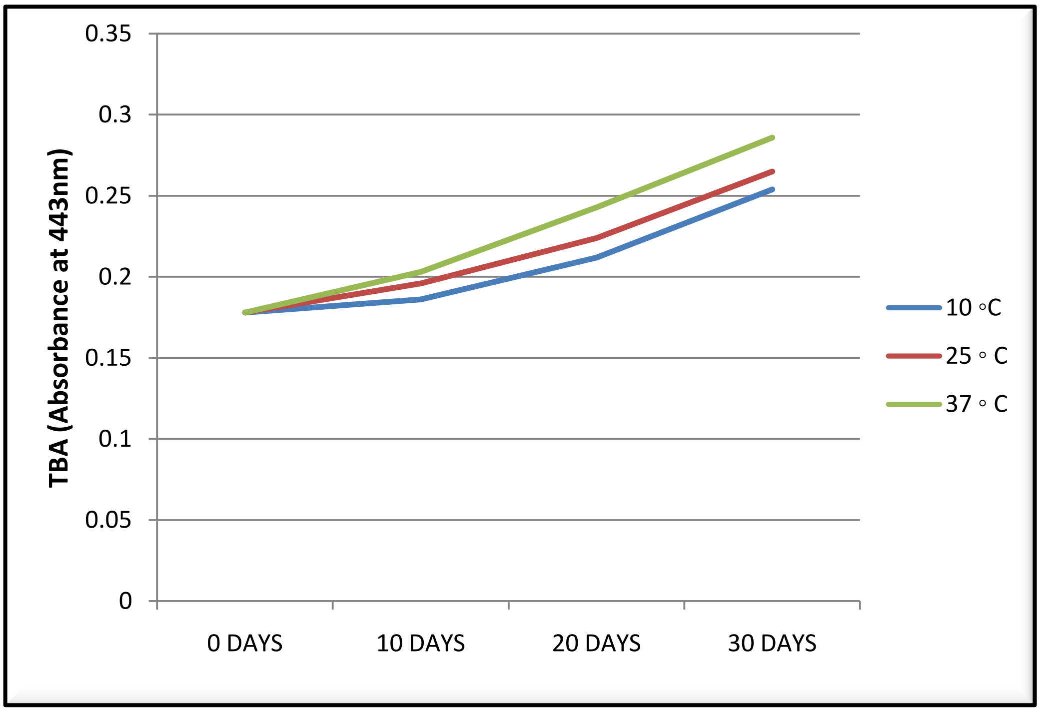


Figure: 4.18 Changes in TBA value

4.18.3 Changes in moisture during storage

Fegure shows the moisture gain in sugar free biscuit during storage .The moisture content in the biscuit sample initially was 4 % which increase to 4.21 and 4.32, 4.43 % at 10 , 25, 37 ^{o}C respectively in 10 days .In 20 days the moisture increased to 4.50, 4.58, 4.62 , at 10 ^{o}C , 25 ^{o}C , and 37 ^{o}C respectively . The moisture increased after 30 days to 5.1, 5.25., 5. 5% at 10 ^{o}C, 25 ^{o}C, 37 ^{o}C respectively .

It is observed that the increase In moisture content was lesser at 10^{o}C as compared to 25 ^{o}C and 37^{o}C. It included that with increase in storage temperature and time the moisture content increased .

This phenomenon of moisture absorption during storage is also supported by (wade , 1988), (leelawathi and Rao,1993) and (Rao et al..,1995).

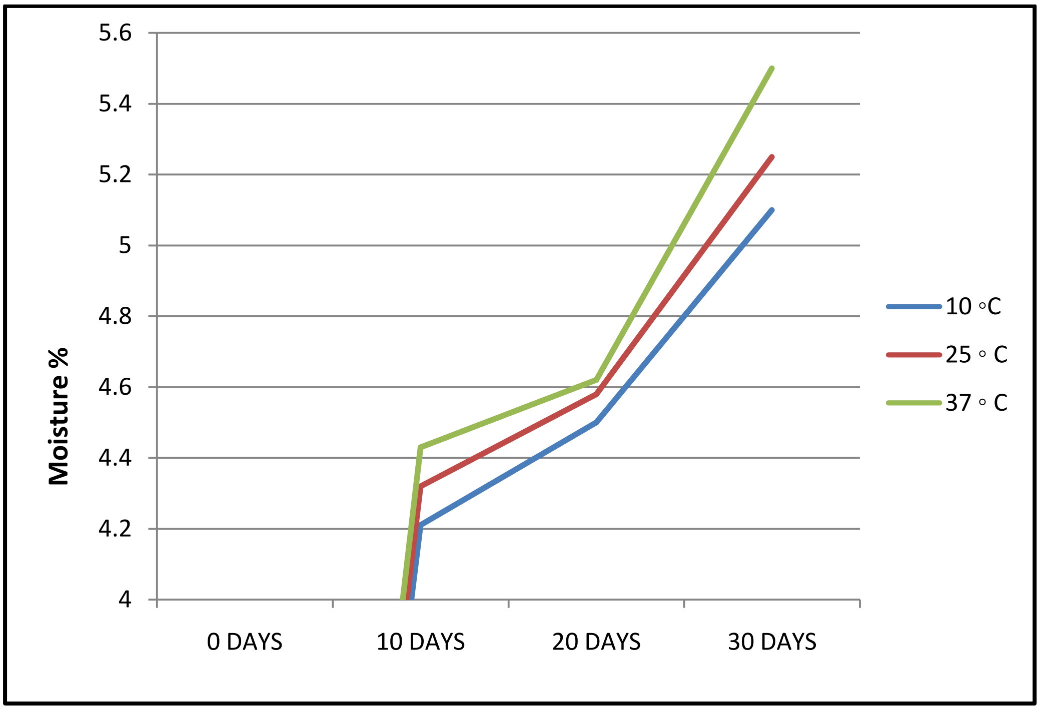


Figure: 4.19 Changes in moisture

4.19.4 Microbial Analysis

Microbiological studies were conducted for yeast and mould, total plate count and coliform. It was found that total plate count in fresh and after 10 days and 20 days storage period was not detected but after 30 days of storage the total plate count was found 3×10 cfu/ml which was well below the permissible limits . On the basis of these finding, it could be concluded that the product is safe to consume due to the high heat treatment and proper hygiene consideration during preparation of sugar free biscuit . The microbiological analysis is given below in Table .

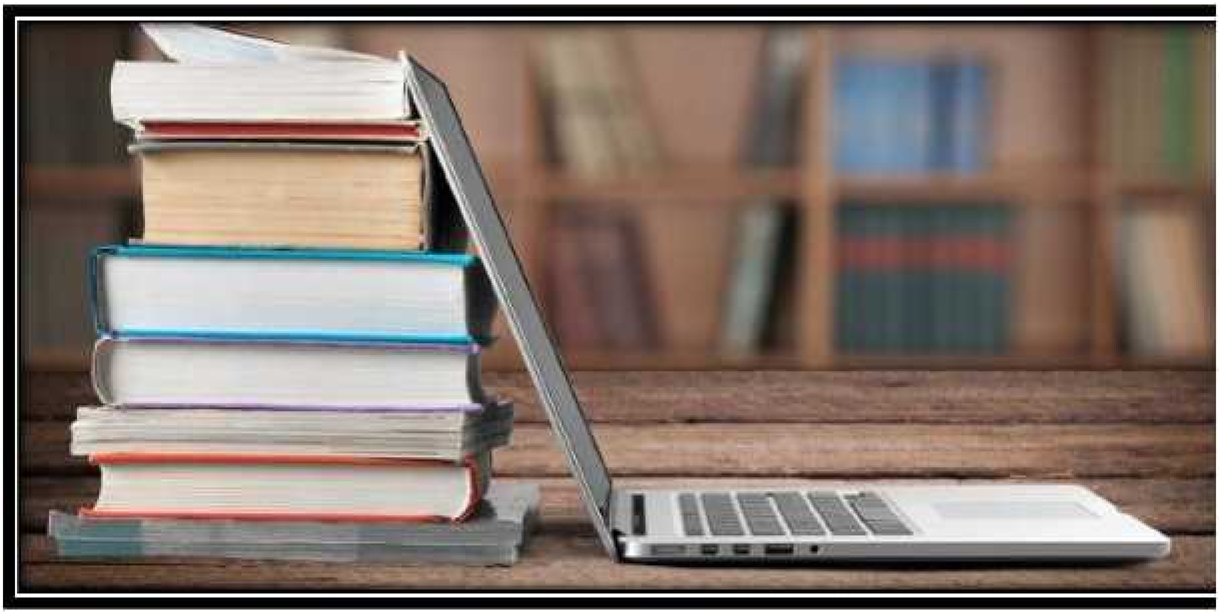
Table:4.9 Microbial Analysis

Sl.No.	*Parameter*	*0 Days*	*10 Days*	*20 Days*	*30 Days*
1	TPC (cfu/ml)	ND	ND	ND	3×10
2	Yeast and mould (cfu/ml)	ND	ND	ND	ND
3	Coliform count	ND	ND	ND	ND

ND- Not Detected

CHAPTER-5

CONCLUSION



CONCLUSION

The present research work"Development & Process Optimization of sugar free biscuit through fenugreek seed powder and natural sweetener (stevia)" was carried out and 31 trials were performed with taking four factors Viz. Skimmed milk powder (SMP), Stevia, butter, and fenugreek seed powder responses and analysed by Response Surface methodology for optimization of developed sugar free biscuits. Sensory attributes as well as textural properties of the sugar free biscuits were studied during the study.Responses obtained after each and every trial were analysed to visualize the interactive effect of various parameters on sensory attributes and physical properties of sugar free biscuit. After optimizing, proximate analysis,textural properties, micronutrient analysis, sensory evaluation and storage periods were studied up to 30 days of the developed sugar free biscuits.

5.1 Selection of the level of ingredient for the sugar free biscuit

The numerical ranges of the four factors were set after performing preliminary trials and studying the relevant literature. The ranges were 0.5 g to 6.5g SMP, 0.5g to5.5 g stevia, 10 to 50g butter and fenugreek seed powder 0.5 to 6.5. Taking into account, these four factors were optimized by using RSM design sets of experiments CCRD (Central Composite Rotable Design).

5.2 **Effect of SMP, Stevia, Butter and Fenugreek seed powder on properties of sugar free biscuit**

The responses were experimented to compare effect on DPPH inhibition, Fracturability, hardness, colour and appearance, flavour and overall acceptability. The desired goals for each factor and responses were chosen.

5.2.1 **Sensory properties**

The sensory evaluation was done by using 9-point hedonic scale (Annexure 1). The average score of the sensory characteristics colour and appearance varied from 6.2 to 8, flavour 6 to 8and overall acceptability varied from 6 to 8.

It is concluded from the sensory evaluation score that butter plays the major role in acceptability of the sugar free biscuit. It was observed that with increase in butter the colour and appearance , flavour as well as the overall acceptability increases whereas with increase in stevia and fenugreek the major effect was seen in the flavour.

5.2.2 Textural characteristics

From studying the effect of the responses, it was found that Skimmed milk powder (SMP) was the major ingredient responsible for hardness and fracturability and while increasingthe butter amount decreases the fracturability and hardness.

5.3 Optimizationof levels of Skimmed milk powder (SMP), stevia, butter and fenugreek seed powder of sugar free biscuit

The ingredients of sugar free biscuits were optimized by RSM. The optimized parameters for developed sugar free biscuits includes SMP (1.7727 %), Stevia (4.3485 %), Butter (37.8788 %) and fenugreek seed powder (0.5 %) that gave value of responses of sensory characteristics: DPPH inhibition (40.8247%), Fracturability (41.5894 mm), hardness (0.9788kg/cm^2), overall acceptability (8.6465) and flavour 8.4197.with and excellent desirability of 0.8951 .

The average DPPH inhibition of sugar free biscuitvaried from 2.7845 to 64.5278 %, fracturability 41.1045 to 44.1305 mm and hardness 0.953 kg/cm^{2}to 7.831 was obtained.

5.4 Physical characteristics

The optimized sugar free biscuits weretested to measure its physicals characteristics such as diameter, thickness and spread ratio. The diameter, thickness and spread ratio was found 5.04cm, 0.82 cm, and 4.92 cm,respectively. On the basis of this it can be concluded that sugar free biscuit had good spreadratio which makes it desirable to the consumers.

5.6 Proximate composition

The proximate analysis of optimized sugar free biscuits was performed and it was found that the final optimized product contain carbohydrate (76.75 %), protein (5.90 %), fat (14.85), ash (~ 1%),moisture (4%) and crude fibre (1.5 %). On the basis of proximate composition of prepared sugar free biscuit it could be concluded this product was fortified with fenugreek seed powder, non-nutritive natural sweetener stevia so recommend for diabetic patients.

5.7 Colour properties of sugar free biscuit

The colour of sugar free biscuit was recorded by hunter colour flex and the L*, a*,b*, value was46.40, 12, 20.29, respectively which is similar to other biscuits available in the market and acceptable by consumer.

5.8 Micronutrient in sugar free biscuit

Developed sugar free biscuit was rich in calcium and magnesium and their concentration were observed 294.8mg/100g and 451.5mg/100g respectively by ion chromatography.

Atomic absorption spectroscopy of developed sugar free biscuits was also performed for mineral analysis and reported thatit contains iron(1.93mg/100g), copper (0.065mg/100g), and zinc (0.325 mg/100g).

5.9 **Shelf life study**

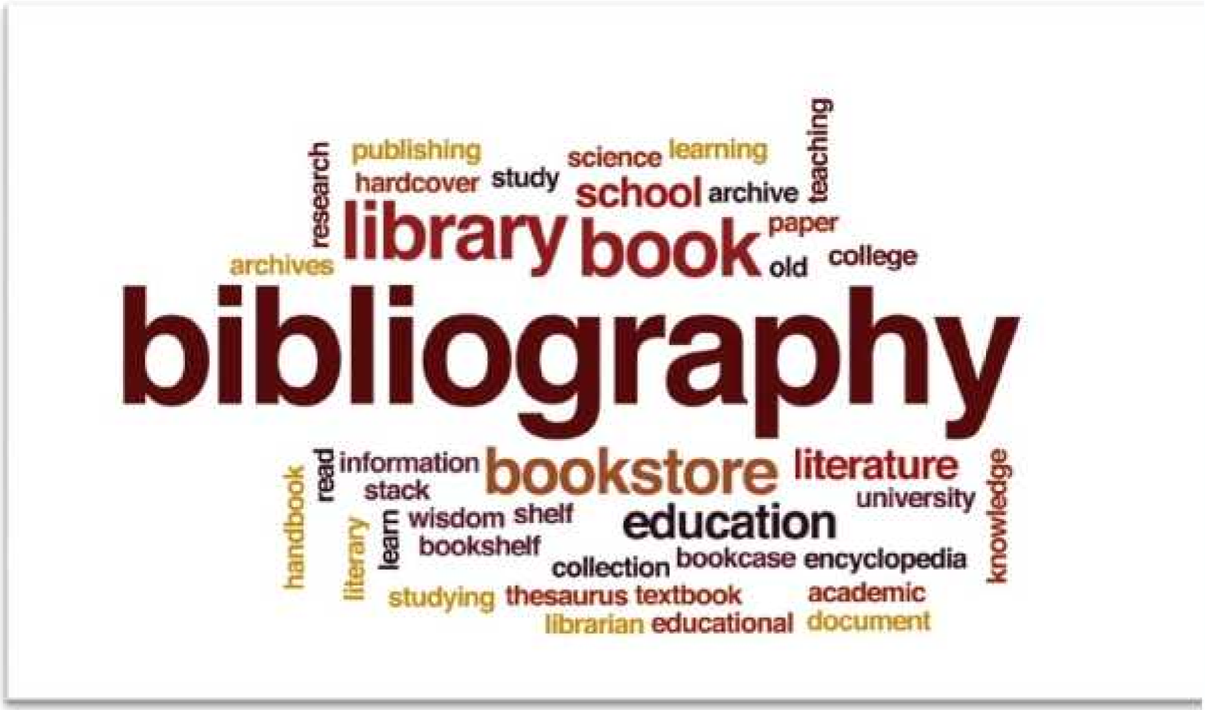
The shelf life studies of the optimized product found that the shelf life of sugar free biscuit was lower at 37^{o}Cthan at 10^{o}C and 25^{o}C. The HMF (Hydroxy Methyl Furfural) content which indicates the mallard reaction at a higher temperature and with increasing days of storage, the HMF concentration was also increased . The HMF increased to 6.1,6.3, 6.5 µmol/100g at 10, 25, 37^{o}C respectivelyafter 30 days from 5.1 mol/100g which was the HMF value initially. The TBA (Thiobarbituricacid)also increased themost at 30 days storage at 10 ^{o}C as compared to 25 ^{o}C and 37^{o}C,TBA value was0.254, 0.265 and 0.286 respectively. The same result was seen in moisture content 5.1, 5.25., 5. 5% at 10^{o}C, 25^{o}C, 37^{o}C respectively from 4 % of moisture initially content.

The Microbiological studies concluded that the product was safe with no yeast and mould growth and coliform was also not detected. Only total plate count after 30 days was found 3×10CFU/ml. The product was found safe for consumption even after 30 days of storage.

So from the above study, it can be concluded that fenugreek seed powder is the good source of mineral content and functional component trigonelline. The developed sugar free biscuit was also enriched with natural sweetener stevia. From the above data, it can be recommended that the developed sugar free biscuit have promising health benefits for diabetic patient.

5.10 Epilogue

Further, research needs to be carried out to increase the trigonelline content in sugar free biscuit which is present in fenugreek seed powder with use of other legumes or other source of trigonelline.*In-vivo* studies can also be performed to check the effect trigonelline content. Work can also be done for the development of multigrain biscuits enriched with trigonelline content for diabetic patient which is a life-style disease now days.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APPENDICES

APPENDICS 1

9 Point Hedonic Scale Score Card

Product: Sugar Free Biscuit

DATE:

TIME:

NAME OF PANELIST:

Instruction:

Given below are the samples of “Sugar free biscuit”. You are requested to judge the sample on the 9 point hedonic scale for the parameters listed below:

Sample	***Colour and Appearence***	***Flavour***	***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:

APPENDICS 2

The graph shows the desirability of the product optimized by the software Minitab 17,