# EFFECT OF FENUGREEK SEED FLOUR ON THE QUALITY OF BISCUIT


by

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## Approval Letter

This ***dissertation*** entitled ***Effect of Fenugreek Seed Flour on the Quality of Biscuit*** presented by **Manish Dahal** has been accepted as the partial fulfillment of the requirement for the **B. Tech. degree in Food Technology.**

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# Abstract

The main objective of this dissertation work was to study the effect of fenugreek seed flour on the quality of biscuit. The chemical composition, antioxidant activity and total phenolic content (TPC) were compared for raw and soaked fenugreek seed flour. Soaked fenugreek seed flour incorporated biscuit was prepared in lab by soft dough process with incorporation of soaked fenugreek seed flour at 0 part, 0.126 part, 0.252 part, 0.5 part, 0.748 part, 0.874 part and 1 part with wheat flour and the formulations were coded as A, B, C, D, E, F and G respectively. The sensory analysis of soaked fenugreek seed flour incorporated biscuit was carried. The obtained data were statistically analyzed using two way ANOVA (no blocking) at 5% level of significance. The chemical composition of best sample obtained from sensory analysis was compared with control (100% wheat flour biscuit) sample.

Sample D, the biscuit with the incorporation of 0.5 part of soaked fenugreek seed flour, was the best formulation and subjected for further physiochemical analysis. The moisture content was found to be 2.53% and crude protein, fat, fibre, total ash and total carbohydrate were found to be 10.74% (db), 20.93% (db), 0.59% (db), 1.6% (db) and 66.14% (db) respectively. Furthermore, the best formulated sample was subjected for iron and calcium content evaluation and were found to be 0.75 mg/100 g and 25.58 mg/100 g respectively. The total phenolic content of best formulated biscuit (0.5% concentration of fenugreek seed flour) was found to be 80 mg GAE/100g whereas the antioxidant activity was found to be 26.67%. These findings suggested that the fenugreek seed flour could be successfully incorporated in refined wheat flour up to the concentration of 0.5% without any adverse effect on sensory attributes. The calcium, iron, moisture, protein, crude fiber, ash and fat content were found to be higher in fenugreek seed flour incorporated biscuit in comparison to normal wheat flour biscuit. Similarly, Increment of antioxidant activity and total phenolic content was seen in fenugreek seed flour incorporated biscuit in comparison to normal wheat flour biscuit.

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# List of Abbreviations

| Abbreviation | Full form |
|---|---|
| ANOVA | Analysis of Variation |
| AOAC | Association of Analytical Communities |
| Ca | Calcium |
| CCT | Central Campus of Technology |
| DPPH | 2,2-diphenyl-1-picrylhydrazyl |
| FSF | Fenugreek Seed Flour |
| HDPE | High Density Polyethylene |
| K | Potassium |
| KMS | Potassium MetabiSulfite |
| MC | Moisture Content |
| Na | Sodium |
| SFSF | Soaked Fenugreek Seed Flour |
| SFSFIB | Soaked Fenugreek Seed Flour Incorporated Biscuit |
| SMP | Skimmed Milk Powder |
| TPC | Total Phenolic Content |

# Part I

## Introduction

### 1.1 General introduction

Biscuit belong to the flour confectionery. It is flat crisp and may be sweetened or unsweetened according to preference. Biscuit can be made from hard dough e.g. crackers, hard sweet dough e.g. rich tea and short or soft dough e.g. short bread and short cake (Lake and Worth, 1980). Biscuits are ready to eat, convenient and inexpensive food products containing digestive and dietary principles of vital importance (Agarwal, 1990). These biscuits become popular due to low cost, good nutritional quality and availability in different varieties, varied taste, easy availability and longer shelf life (Sudha *et al.*, 2007). The baked products have about 6-7% proteins. Biscuits owing to their long shelf life are considered useful for nutritional enrichment in feeding program (Agarwal, 1990). In recent years, consumption of biscuits has increased in most of countries as they served as important source of nutrients (Ranhota *et al.*, 1980). However several studies indicated that the nutritional, physical and sensory characteristic of biscuits depend on both physiochemical properties of the flour and processing method employed for flour preparation (Asifulalam *et al.*, 2014). Usually the production of biscuit has placed wheat flour as its sole composition, but addition of other valuable flour at a certain extent can be done for nutritional value addition of the produce without adversely affecting the must properties of the biscuit (Asifulalam *et al.*, 2014).

Biscuit has certain advantages, as it is easy to use during travel or at home because of its availability in the variety in the pack sizes suiting to individual tastes. A biscuits apart from offering good taste is a snack items with substantial energy having wholesome and nutritious quality at affordable prices. Further biscuit have, in general, a good shelf-life, which is longer than all other snack item. Thus biscuit have important role to play as a diet supplement for adult and children alike. It is no more viewed as a luxury tea time snacks, but essentially daily food component for an average Nepalese household (Shrestha, 1995).

Fenugreek seed is pleasantly bitter in taste but it is a good source of many nutrients and has medicinal properties. Studies have shown various physiological health benefits such as anticancer, antidiabetic, antiparasitic, lactation stimulant and hypocholesterolaemic effects.

Fenugreek currently attracts research and commercial attention mainly due to high content of protein (20–25%), lysine (5–6%). Fenugreek possesses higher amounts of dietary fiber than cereals and legumes, thus making it an interesting raw material for the development of fiber-rich bakery products. Flour fortified with 8-10% fenugreek flour has been used to prepare bakery foods like pizza, bread, muffins, cookies and cakes with acceptable sensory properties (Agrawal and Syed, 2017). In the present study the efforts have been made to develop functional biscuits, their sensory and chemical characteristics were also studied.

## 1.2 Statement of the problems

Today foods are not intended to only satisfy hunger and to provide necessary nutrients for humans but, also to prevent nutrition related diseases and improve physical and mental well being of the consumers. In this regard, functional foods play an outstanding role (Hassan *et al.*, 2012). With the incorporation of fenugreek during bread preparation total phenolic content, anti-oxidant activity and flavanoids were reported to increase (Afzal *et al.*, 2016). The farinograph analysis showed that the supplementation of wheat flour with fenugreek flour was satisfactory in producing better flour for bread production. However, the gluten content decreased with increasing proportion of fenugreek flour. The protein, fiber and ash as well as minerals content of products increased as a result of the addition of fermented fenugreek flour (Kasaye and Jha, 2015).

It has been reported that fenugreek seeds flour could be incorporated up to a 10% replacement level in the formulation of biscuits without affecting their overall quality. The physical, chemical and sensory characteristics, in general, revealed that biscuits containing 10% germinated fenugreek seeds flour were the best among all the composite FSF biscuits (El-Naggar, 2019). It has been found that addition of soaked or germinated FSF to wheat flour decreased markedly phytic acid content of produced biscuits by ranges between 20 to 44%.(Ibrahium and Hegazy, 2009). Since, almost all researches of fenugreek seed flour incorporated biscuit were done with germinated or fermented fenugreek seed flour, the study of biscuit incorporated with only soaked fenugreek seed flour has not been done yet. So, this study would reveal the effect of incorporation of soaked fenugreek seed flour in biscuit.

## 1.3 Objectives of study

### 1.3.1 General objective

The general objective of this dissertation work was to study the effect of fenugreek seed flour on the quality of biscuit.

### 1.3.2 Specific objectives

The specific objectives of the study were:

1. To compare iron and calcium content of raw and soaked fenugreek seed flour.
2. To compare antioxidant activity and total polyphenol content of raw and soaked fenugreek seed flour.
3. To determine the effect of incorporation of fenugreek seed flour on sensory quality of biscuit.
4. To compare chemical composition of best biscuit sample with control biscuit sample.
5. To calculate the cost of prepared biscuit.

## 1.4 Significance of the study

In the field of functional food preparation, the development of functional biscuits in modern time is futuristic scientific effort that could enhance the medicinal value of food without compromising human health. The diets are seen healthy when using the plants parts in it.

Biscuits are most common snack consumed which lack in different minerals and other nutrients due to the its consituents flour and sugar. . Fenugreek has a high protein content (25%), lysine (5.7 g/16 g N), soluble (20%) and insoluble (28%) dietary fiber besides being rich in calcium, iron and beta-carotene (Hooda and Jood, 2005a). Fenugreek seeds contain 20% soluble fiber (gum), which can act as functional agent in wheat dough.

The study will further show the comparison between the nutrition present in raw seed powder of fenugreek and soaked fenugreek seed powder and suitability of using plant materials in biscuit preparation that will improve nutritional standard of biscuit. The study will also point in the right path for choosing plant components in the production of functional biscuits.

## 1.5 Limitations of the study

The limitations of the study were:

1. The shelf life of the product had not been studied.

2. Texture profile analysis of the product wasn't carried out.

# Part II

## Literature review

### 2.1 Introduction to biscuit

Biscuits are the low cost, processed food which offers good taste along with nutritional values at affordable price with convenient availability. Biscuits have in general, a good shelf life in comparison to most of the other snacks items. Hence it's obvious that it is convenient to be used as a travel snacks item. It is no more only viewed as luxury item snack, but as an essential daily food composition of an average Nepalese household. Since biscuit is a kind of dry food having a long shelf life, the deterioration is very low in comparison to other bakery products (Shrestha, 1995).

The word biscuit is derived from the Latin "biscoctus" or old French "bescoit" word (Stevenson and Waite, 2011), meaning twice cooked, a reference to practice of first baking the product in hot oven and then transferring it to a cooler oven to complete drying process. Biscuits are very popular item prepared on regular basis and these too vary in size, shape, filling and type of recipe used. While they are simple to make, they require care, attention and understanding necessary to produce a standard product (Sultan, 1989). Generally the term biscuit is used in European countries and cookies in the USA. Biscuits and biscuit like products have been made and eaten by man for centuries (Hoseney, 1985).

Biscuits are ideal for nutrient availability, palatability, compactness and convenience. They differ from other baked products like bred and cakes because of having low moisture content, comparatively free from microbial spoilage and long shelf life of the product (Wade and Peter, 1988). Although the first biscuit were dried-out rusks, useful as long-life food for sea journeys, early cooks making confections wit fat and sugar would have found that if little dough pieces are baked in a typical hot oven and taken out when they have a good color and a stable structure they would not have been dry enough to be entirely crispy. Putting them back into somewhat cooler oven to dry them out improved their eating qualities and also their shelf life. Baking from the start in a cooler oven for a longer period allows drying but results in less coloration and structure development. However, the term biscuit was applied originally to dried bread pieces. These were also sweetened and flavored with spices. Other products like our modern biscuits were made but called by more cake-like names for example: shortcake and shortbread etc. (Manley, 2000).

Now biscuit are made mostly in factories on large production plants, these plants are large and complex and involve considerable mechanical sophistication. Forming, baking and packaging are largely continuous operations but metering ingredients and dough mixing are typically done in batches (Manley, 1998b).

All flour confectionery is developed from human skill in baking and very much research has been directed to improving our knowledge of the science of what happens when flour is hydrated, mixed with other materials and baked. It is this research that has been the main driving force in the development of the biscuit industry. It is therefore correct to say that without science there is no innovation and innovation there is no competitiveness (Manley, 2000).

## 2.2 Classification of biscuits

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

### 2.2.1 Soft dough biscuits

Soft dough biscuits are generally sweet, thin and possess smooth surface with dimensions that are much more regular and consistence than the hard dough biscuits. Generally they contain higher fat content (25%-35%) and sugar (30%-45%) and are low in moisture. Soft dough biscuits are less versatile because of the inherent nature of the dough (Manley, 1998a). The higher gluten network development should be avoided which can be achieved by:

a) Weak flour
b) Lower moisture content
c) Short mixing time
d) Less aerating agents

### 2.2.2 Hard dough biscuit

Hard dough biscuits generally contain less fat (10%-20%) and low sugar content (10%-18%). The dough adheres due to its higher water content and relatively lower fat content. This type of formulation produces an extensive gluten structure. The long mixing time develops the gluten and the mixer action stretches and orients the gluten strands to a point where much of the elasticity is destroyed. The water content varies in accordance with the flour strength, which might be as high as 20% of the flour weight (George, 1981). Further according to the variance in composition of one or more parameters hard dough biscuits can be further divided into:

#### 2.2.2.1 Semi sweet biscuits

The flour used in this type of biscuits should be as weak as possible. Its higher water content and relatively low amount of sugar and fat produces an extensive gluten system and structure. Many flour formulations contain cornstarch or arrowroot to an extent of 10% of the flour weight in order to weaken the flour strength. Further to prevent excessive gluten development, a long mixing time followed by addition of sodium metabisulfite is carried out. Rapid cooling should be avoided as these types of biscuits are highly susceptible to checking (Dunn, 1928).

#### 2.2.2.2 Fermented dough biscuits

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

#### 2.2.2.3 Puff dough biscuit

This hard dough biscuit is leavened with well layered fat between the dough sheets. The dough and the fat should possess nearly the same flow properties and care must be taken that the fat doesn’t become the part of the homogenous dough phase as it will not contribute to layering but instead reduce the elasticity of the dough and might give undesirable properties. During preparation the dough is mixed for 15 min and then relaxed for 30 min then after 60% of the puff dough margarine is applied and sheeted. Rest of the fat is applied after the sheet is laid off for 15 minutes (George, 1981).

## 2.3 Types of biscuits

### 2.3.1 Cream crackers

These have a simple recipe of flour, fat and salt, which is always fermented with yeast and the dough is laminated prior to cutting and baking. These have characteristic flaky and variously blistered biscuits (Manley, 1998a).

### 2.3.2 Soda crackers

It is a square biscuit about 50 × 50 mm and 4 mm thick. The biscuits are produced with scrap less cutters so the edges are white and broken after baking. The fermentation is usually in two stages with a wet sponge lying for 18 h followed by a dough stage, which is left to ferment for about 4 h. It has alkaline reaction after baking hence the name soda crackers (Manley, 1998a).

### 2.3.3 Savory crackers

These are variously salted, flavored and fat sprayed after baking. Depending upon their size, because they are made in a very wide range of shapes and sizes, they can be regarded as Savory snacks (Manley, 1998a).

### 2.3.4 Water biscuits and matzos

Water biscuits have a simple recipe of flour, fat, salt and water in the ratio 100:6.5:1:29. The dough is undeveloped and crumbly or in balls after mixing. Matzos are a Jewish product and recipe is about 100 parts of flour to 38 parts of water (Manley, 1998a).

### 2.3.5 Puff biscuits

Puff biscuits are all made from puffed dough in which there is a non-homogeneous distribution of fat. When this dough is laminated the fat causes discontinuities between the layers of dough and during subsequent baking these layers separated to give a flaky structure.

The dough is not fermented and is invariably cold and under developed. Puff biscuits are eaten cold so the fat used must not have waxy tail after eating (Manley, 1998a).

### 2.3.6 Short dough biscuits

These are made from cohesive dough that lacks extensibility and elasticity without the formation of gluten stands from the wheat flour. The texture of the baked biscuit is attributable to starch gelatinization and super cooled sugar rather than a protein or starch structure (Manley, 1998a).

### 2.3.7 Deposited soft dough and sponge drop biscuits

Short dough, which are soft enough to be just pourable, are referred to as soft dough. The biscuits are rich in fat or based on egg whites whipped to stable foam (Manley, 1998a).

### 2.3.8 Wafers

They are formed from a batter, which is baked between pairs of heated metal plates. The majority of wafer biscuits are based on the large flat sheets. The wafer sheet itself is baked from a simple batter containing little or no sugar. It is a tasteless product, which has a smooth surface and a very open cellular structure within (Manley, 1998a).

### 2.3.9 Miscellaneous biscuit-like products

These include crisp breads, pizza bases, sausage rusk etc. (Manley, 1998a).

## 2.4 Chemical composition of biscuits

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

**Table 2.1** Chemical composition of biscuit

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

Source: Kyte (2007)

### 2.5 Raw materials for biscuit making

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

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

Salt, skimmed milk powder (SMP), ammonium bi-carbonate, sodium bi-carbonate, coloring agents, flavoring agents, emulsifiers, fortifying agents, improvers etc. are used in small amounts and aren’t a must for all sort of biscuits. These ingredients are used for developing the taste, texture, flavor and aesthetic value of the product. Therefore these minor ingredients are also known as the product improvers (Shrestha, 1995).

All these ingredients are individually important to obtain more palatable and satisfactory products. The raw materials are found in the form of solid, liquid and paste (Shrestha, 1995).

### 2.5.1 Major ingredients

#### 2.5.1.1 Flour

Flour is the basic raw material for biscuit making responsible for the major bulk of biscuit (Whitely, 1971). The flour used in biscuit and cracker vary in strength and baking characteristics (Bohn, 1957). Wheat grain is the only grain naturally capable of producing flour capable of being made into a low density baked product (Kent and Amos, 1983). Soy flour is used in dough due to emulsifying property and higher level of protein content (Whitely, 1971).

**a. Wheat flour**

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

Glutenin gives solidity to the product whilst gliadin is the binding agent imparting the soft sticky character to the gluten. Gliadin is soluble in 70% alcohol and may be extracted from flour whereas glutenin is soluble in alcohol and water (Gorinstein *et al.*, 2002).Gluten is elastic, cohesive and rubbery and holds together and holds together the various ingredients of the dough. It has the property of holding the gases given off during fermentation and during baking. It sets in oven to form the firm, porous, open texture during baking which are necessary in the production of biscuits and crackers. Thus gluten is the necessary framework, forming the sustaining wall of the whole structure of baked products (Bohn, 1957).

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

The strong flour protein has long links with few bonds while weak flour protein has short links with many bonds. During biscuit making weak and easy to stretch, soft wheat flour is found to be better (Kim and Kim, 1999). Beside the natural quality of flour, the modifications in the flour strength can be done by various treatments. Treatment of the flour with Sulphur dioxide reduces the flour strength. Heat treated flour added to untreated flour is claimed to strengthen the flour. According to Kent and Amos (1983), improvers have some effect upon the nature and character of the gluten and cause it to behave, during fermentation, like the gluten of the stronger flour.

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

**Table 2.2** Requirements for the flour characteristics

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

Source: Arora (1980)

**b. Corn flour**

Corn flour is a yellowish power, the direct result of mixing sound corn, and containing about 2.5% of fat. Corn starch is the nearly pure starch obtained from the maize kernel. Because of its high starch content; it can be used to weaken flour which is too strong (Smith, 1972).

**c. Rice flour**

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

#### d. Oat flour

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

#### e. Soy flour

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

#### f. Arrowroot flour

The flour is obtained from the root stock of arrowroot plant. This flour is mainly used in biscuit making due to its easily digestible starch and proved to be beneficial for teething children. It also works as a flour weakener an adjunct to pallet smoothness (Smith, 1972).

### 2.5.1.2 Fat or shortening

Fat is one of the major ingredients in biscuit making. Shortening function of fat during biscuit making is a must step without which the baked product will be a solid mass held firmly together by strands of gluten (Schober *et al.*, 2003) Fat itself being insoluble in water prevents the extra cohesion of the gluten strands during mixing. The greatest attribute a shortening can possess is that it should have a plastic nature over a wide range of temperatures as it is likely to be encountered in its use for biscuit making (Smith, 1972).

The main action of the fat or shortening during mixing is to avoid the gluten forming proteins to come in contact with water by insulating the gluten forming protein molecules due to its hydrophobic nature. Hence, less tough dough with desired amount of gluten formation can be obtained. Thus shortened baked products possess less hard, crispier nature and can easily melt in mouth (Mukhopadhyay, 1990).

During mixing fat also helps in entrapment and retention of the air, which is highly necessary for a product for its good texture. Fat also lubricates the formed gluten molecules to distribute it to various sites during sheeting and hence preventing the agglomeration of the gluten molecules. Fat also plays a vital role in the softness, texture palatability and keeping quality of the product (Manley, 1998a).

In the earlier days of biscuit making animal lard was used for biscuit making which has now been totally replaced by hydrogenated vegetable oils. The molecules that exist in oil are built up of unsaturated fatty acid chains, some of which are loosely joined together by double bonds which are weak bonds hence, making the fat prone to easy oxidative rancidity of the oil. During hydrogenation the added hydrogen replaces the double bonds present to convert it to single bonds hence a stable solid fatty acid molecule is formed from weak bonded liquid fatty acid molecule (Smith, 1972).

Hydrogenated Vegetable fat are superior to the lards recently used in various aspects. Furthermore in order to obtain the best product the hydrogenated vegetable oil to be used for biscuit making should possess the following properties (Mukhopadhyay, 1990):

1. It should possess good white to creamy color.
2. After keeping the fat at 50˚ c for 24 h and filtering, its color should be comparable with the control sample of oil.
3. The fat should have a smooth, uniform texture, free from any oil separation and large grains.
4. The fat should have a bland ‘clear’ odour and taste.
5. The fat should have a wide plastic range to suit particular production techniques and the product.
6. The crystalline structure of fat should be stable during mixing and after baking.
7. The fat should possess reasonable shelf life on its own without the addition of antioxidants. The acid value and peroxide value of the extracted fat should not exceed 0.5 mg KOH/gm oil and 10 meqv peroxide/kg fat respectively.
8. The fat should be prepared from the blend of oils, which will not cause fat bloom during the storage of biscuit.

#### 2.5.1.3 Sweetening agents

Sugar is another major ingredient in biscuit making. Sugar generally used in biscuit making is obtained from sugarcane and sugar beet. The sugarcane consists of 16-22% of sucrose while sugar beet consists of 8-9% of sucrose. During biscuit making various forms of sugar namely crystalline, pulverized, liquid, brown or soft sugar are used as per product requirement. Generally most commonly used form of sugar in biscuit making is pulverized sugar. The reason behind this may be due to its readily soluble characteristic which causes the palate to be deceived in sweetness. The crystalline size also has effect on sweetness, shortness and better spread capacity of biscuit (Whitely, 1971).

Use of crystal size varies according to the final product. Medium fine powdered sugar, with or without very fine granulation is more suitable for rotary doughs; while a coarser sugar is tolerable in hard semi-sweet doughs because of the larger quantities of water used, the longer mixing time and higher final dough temperature. Use of coarser sugar gives fissured tops or cracks which is desirable in case of crunchier and ginger biscuits. Apart from these sugar types, lactose sugar from milk, and brown sugar which gives both color and delicious flavor to the product also are used.

Another type of sugar used in biscuit making is invert sugar syrup or simply invert syrup. Due to its lower caramelization temperature compared to sucrose the crust coloration of the biscuit takes on a browner appearance much quickly which is desirable in many products. If the crust coloration becomes too darker than requirement then a part of invert syrup can be replaced with glucose syrup. Experiments also show that use of invert syrup also reduces the baking time. Invert syrup also helps in preventing checking problem in biscuits. It also helps in moisture retention in biscuits. As a whole, sugar may be of any form helps in imparting sweetness, increasing tenderness, maintaining volume, crust color development, flavor improvement, moisture retention and proper spread of the biscuit (Smith, 1972).

### 2.5.2 The minor ingredients

#### 2.5.2.1 Emulsifying agents

Emulsifying agents are surface-active agents promoting the formulation and stabilization of emulsions during biscuit making. It helps proper mixing of lipid and aqueous fraction and helps in maintaining good texture of the product. The unifying property of emulsion is due to the presence of a hydrophobic and a lipophilic group on the same molecule.

Various recipes in biscuit making include those from high fat recipe to low fat recipe, with low water and high water respectively. In the low fat recipes, process problems are associated with gluten development and dough machinability but in high fat recipes, there is more concern for the fat, to give maximum textural effects, dough stickiness and control of spread while baking (Manley, 1998a).

Most commonly used emulsifiers are lecithin, eggs, mono and diglycerides etc. In creaming stage where the fat, sugar etc. are combined with all or no part of water, lecithin does exert an emulsifying action to give a smooth homogeneous mixture. Lecithin may be much more useful as an antioxidant also (Smith, 1972).

#### 2.5.2.2 Leavening agents

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

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

Source: Smith (1972)

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

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

Ammonium bicarbonate → Ammonia + Carbon dioxide + Water

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

Ammonium carbonate → Ammonia + Carbon dioxide + Water

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

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

Sodium bicarbonate + Acid → Carbon dioxide + Water

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

Cream of tartar → Sod. Pot. Tartarate

Both sodium and ammonium bicarbonate react with acidic ingredients if any, in the dough. Use of an excess ammonium bicarbonate makes the dough more alkaline and thereby may affect the protein structure of the dough. Use of an excess sodium bicarbonate will affect the crumb and crust color often with an accompanying unpleasant or soapy taste, unless any acidic material is used to neutralize the residual sodium carbonate (Bohn, 1957).

#### 2.5.2.3 Milk and milk solids

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

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

#### 2.5.2.4 Salt (Sodium chloride)

Use of salt during biscuit making is not mainly to increase saltiness except in some salty biscuits. It helps to enhance the natural or other added flavors. Salt can reduce the sourness of acids and increase the sweetness of sugars in their effect in the palate (Fabriani, 1977). In fermented dough salt helps to develop the gluten of the flour besides acting as a fermentation rate controller. Flours which lack a bit of ageing can be readily used with good gluten fermentation by use of a little bit higher dosage of salt. Salt to be used during biscuit making should be magnesium and calcium chloride free as the minerals may cause rancidity. Use of salt in the range of 1.0-1.5% of the flour weight is thought to be best but above 2.5% it may become objectionable or even nauseous (Mukhopadhyay, 1990).

#### 2.5.2.5 Flavoring and coloring agents

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

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

#### 2.5.2.6 Water

Water is one of the most important ingredients during biscuit making. Quality of water used has a great effect on the product. Dissolved minerals and organic matters present in water can affect the flavor, color and physical attributes of the finished baked product (Smith, 1972).

The water used in the baking product should be potable and odorless if required, although no significant effect has been noticed due to the hardness, but demineralization is recommended if the mineral content is too higher which might cause a adverse in product color (Arora, 1980).

#### 2.5.2.7 Anti-oxidants

Anti-oxidants act as a retarding or inhibiting agent in the onset of oxidation rancidity. As biscuit is rich in nutrients and fat content, it is highly prone to oxidative rancidity, so role of antioxidant is essential for prolonging the shelf life of the product. There are a number of naturally occurring substances as well as many man-made chemicals which possess antioxidant properties which can be used during biscuit making. Use of antioxidants should be done in the early stage of biscuit making as antioxidants cannot hide or remove the incipient rancidity (Smith, 1972).

Most commonly used antioxidants are, BHA (Butylated hydroxyl anisole), BHT (Butylated hydroxyl toluene), PG (Propyl gallate), NGA (Nordihydro guaiaretic acid). Nearly all the added antioxidants are added with the shortenings for use. An antioxidant should possess the following properties:

- Non-toxic
- Very little or effect on color, flavor or odor of the fat or the product.
- Be readily incorporated- soluble in fat and oil.
- Be effective in as low a concentration as possible.
- Be stable to baking or frying temperatures.
- Be stable to heat, even in alkaline media, such as biscuit doughs.

Apart from all these major and minor ingredients sodium metabisulfite and potassium metabisulfite are used as conditioning agents. Special fortifying agents like protein, vitamins, fruits, nuts, chocolates etc. can also be mixed with biscuit (Smith, 1972).

### 2.6 General specification of biscuit as published by NBS

Biscuit should be properly baked, crisp and uniform in texture and appearance. They should not possess rancid flavor, fungal infection, off odour and any insect infestation. For filled biscuits any of the fillers like jam, jellies, marshmallow, cream, caramel, figs, raisins, etc. can be used. The biscuits may be coated with caramel, cocoa, or chocolates. Use of antioxidants as well as permitted preservative can be done not exceeding the maximum dosages. The general specification of biscuits as decribed by Nepal Bureau of Standards (NBS) is given in Table 2.3

**Table 2.3** General specification of biscuits

| S. No | Characteristics | Requirements |
|---|---|---|
| 1 | Moisture | 6.00% max |
| 2 | Acid insoluble ash (on dry basis) | 0.05% max |
| 3 | Acidity of extracted fat (as oleic acid) | 1.00% max |

Source: NBS (2040)

### 2.7 Nutritive value of biscuits

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

**Table 2.4** Nutritive value of biscuit (values per 100 g)

| Weight per serving in gram | 100 g |
|---|---|
| Calories (kcal) | 480 |
| Protein (g) | 5.2 |
| Fat (g) | 20.2 |
| Carbohydrate (g) | 71.0 |
| Calcium (g) | 0.04 |
| Phosphorous (g) | 0.16 |
| Iron (mg) | 1.8 |
| Vitamin A value (I. U) | - |
| Thiamine (mg) | 0.03 |
| Riboflavin (mg) | 0.04 |
| Nicotinic acid (mg) | 0.8 |

Source: Swaminathan (1991)

## 2.8 Technology involved in biscuit making

Technology is the factor which enables easy doing of something by significantly reducing the labour, time, expenditure and increasing the quality. Technology is always beneficial until it is under control. Hence the skill to handle the available technology is the fore most need during an operation. Not only the technology controller or his department is responsible with the machinery control but with the total control from ingredients purchase to sales (Kyte, 2007). The technology of biscuit production is shown in Fig. 2.1.

Preparation of ingredients

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

↓

Creaming and mixing

(Performing is done for hard dough and fermentation dough biscuits)

↓

Laying the dough

↓

Dough forming

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

↓

Baking

(Temperature 150°C to 250°C for 6 to 7 min)

↓

Cooling

(For 6 to 7 min)

↓

Wrapping and packaging

↓

Labelling and storage

(RH 40 to 50% at temperature of 23 to 27°C)

**Fig. 2.1** Flow sheet of manufacturing process biscuit

Source: Smith (1972)

### 2.8.1 Dough mixing

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

In the context of biscuit dough, biscuit sandwich creams and batters, the "mixing" covers a number of distinct operations. It includes:

a. The blending of ingredients to form a uniform mass.
b. The dispersion of a solid in a liquid, or liquid in liquid.
c. The solution of a solid in a liquid.
d. The kneading of mass to impart development of gluten from flour proteins which have been hydrated at earlier stage of the mixing.
e. Build-up of temperature as a result of work imparted.
f. Aeration of a mass to give a lower density.

One or more of these actions is required in the formulation of dough for the very many types of products that are called biscuits (Manley, 1998b).

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

#### 2.8.1.1 Creaming up method

This method of mixing up of dough mixing includes two steps, during first step the sugar and fat are blended together to fine dissolution after that other ingredients like milk powder, water, invert syrup, lecithin, color, essence, salt are mixed up for around 3-5 min. to form a homogeneous cream. Now the flour along with the aerating agents is mixed up with the cram and mixed at slow speed in the mixing machine for around 10min. If other type of flour are to incorporated than care must be taken that they must be pre-mixed into the shortenings and the water before adding the other ingredients if the true attribute of thus added flour is to be achieved (Smith, 1972).

This type of mixing method holds the water in a more or less stable state so that it is prevented from making wide spread attack on the flour to form any significantly higher amount of gluten network. Mostly short cake rotary and wire cut dough are mixed by this method, in order to control flow and volume during baking. A significant factor in such mixing is the amount of water used (Whitely, 1971).

#### 2.8.1.2 All in one method

As the name suggests, all the ingredients are mixed together and fed in the mixing machine. This method is straight forward where all the ingredients along with major part of water is fed into the mixing machine which some part of water is used to dissolve the aerating chemicals, flavors, colors and salt which is alter on mixed with the dough and is mixed until a satisfactory dough is produced. This type of mixing method is widely applied with hard, semi-sweet dough. Due to the relatively higher water content in these dough it results in very satisfactory gluten production and formation.

In fermented dough an important step apart from the above described methods is used which is known as punch back or knock back. This helps to break down the pockets formed by the carbon dioxide during fermentation. The accumulated carbon dioxide might otherwise be poisonous for the yeast cells (Smith, 1972).

### 2.8.2 Laying the dough

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

### 2.8.3 Forming and performing

The shaping or forming of the dough into various shapes and to the required thickness before baking is one of the major steps in biscuit making. In case of hard dough and fermented dough forming is done by using sheeters and laminators, which reduces the thickness of the dough to convert into a thin sheet. Occluded air is eliminated from the dough. It also helps in proper spread of fat, salts which helps in producing a product with short eating and layered appearance. Soft dough is directly fed into moulding or embossment disc which cuts them into required size, shape and appearance (Shrestha, 1995).

### 2.8.4 Baking

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

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

Every oven used till date consists of four basic parts:

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

During baking the dough undergoes gradual changes physically as well as chemically (Smith, 1972).

Physical changes include:

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

Chemical changes include:

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

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

**Table 2.5** Temperature related changes in biscuit during baking

| Temperature (°C) | Changes occurred |
|---|---|
| 32-37 | Top crust skin formation (evaporation of surface moisture). |
| 32-48 | Evolution of $CO_2$ with crumb (less solubility of $CO_2$) |
| 32-65 | Increase in volume due to $CO_2$ |
| 32-99 | Gas expansion ($CO_2$ and steam) |
| 51-99 | Starch gelatinization (Biscuit structure) |
| 76-88 | Evaporation of alcohol, yeast action ceases. |
| 76-121 | Coagulation of protein (irreversible). |
| 176-204 | Caramelization (Combustion of sugars like maltose, fructose and glucose). |
| 188-204 | Dextrinization (surface gloss). |

Source: Mukhopadhyay (1990)

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

### 2.8.5 Cooling

Cooling is one of the most important part of biscuit production. As biscuits emerge from the oven they are very hot nearly at a range of 99-101˚C, very soft and moist. Therefore cooling of the biscuit prior to packaging must be done to maintain the proper structure of the biscuits. Immediately after the biscuits are released from the oven they possess a very high moisture content which signifies that the flour starch is still in some form of gelatinous paste and the dextrin still in partial solution. Similarly the sugar as well as fat will also be in its liquid form, protein although firmer than other ingredients is also pliable. Hence, almost all ingredients are in unset state. Cooling helps in consequent loss of moisture and slowly sugars start to crystallize out and the dextrin grow tougher, then only the biscuit grow tougher and set. Cooling should be gradual and slow (Bloksma, 1990).

Checking is the most common defect which occurs in the biscuits after they are prepared and not noticeable during processing. Checking refers to the hair like structure which might refer to weakening of the structure and breakage might occur after 24 hrs of packaging. Hard, semi-sweet biscuits are prone to checking rather than rotary moulded soft types which is due to its low fat and low sugar content leading to higher gluten development. As gluten has higher affinity towards water, it will extract it from the gelatinized starch present in the hot biscuit and so cause stresses to be set up. This problem is supposed to be further aided by rapid shrinkage of the biscuit due to rapid cooling. Hence, checking can be reduced or eliminated if the baking is slow and the cooling is gradual rather than rapid (Manley, 1998a).

### 2.8.6 Packing and storage

Biscuits are low moisture content food. Their mandatory standards state them to be of low moisture content, mainly below 6%. The relative humidity of freshly baked biscuit is very low so in order to prevent rapid uptake of moisture from the atmosphere, the biscuits must be packed in a water vapour resistant material. Up take of moisture by biscuit make them prone to microbial attack similarly open access to the atmosphere make them prone to oxidative rancidity as fat is a major ingredient used during biscuit making (Paine and Paine, 1983).

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

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

Packaging in case of biscuits must be close up together in order to provide a mutual reinforcing effect which prevents them from breakage. Packaging of biscuits at commercial level is generally done in triple laminates consisting of polyethylene, aluminum foil and paper (Paine and Paine, 1983). The characteristics of the packaging materials are described in Table 2.6.

**Table 2.6** Some characteristics of packaging materials

| Component | Properties |
|---|---|
| LDPE | Moisture and vapour barrier, heat sealing medium |
| Paper | Stiffness, low cost, opacity, printable. |
| Aluminum foil | Opacity, good water vapour and gas barrier. |
| Oriented polyethylene terephthalate | Gas barrier, strength, grease resistant. |
| PVC | Transparency, rigidity, gas barrier |
| Polypropylene | Easy sealing, resistance to oil, grease. |
| HDPE | Stiff, smooth, resistant to chemicals, moisture, gas, harder than LDPE. |

Source: Robertson (2012)

### 2.9 Sensory perception of biscuits

Before launching the biscuit in the market, a panel of experts evaluate the overall acceptability of the final product which is known as sensory perception or evaluation. Many basis of evaluation include appearance, crispiness, crumb color, Flavor and finally the overall acceptability. The evaluation is marked on the score cards which is later on discussed in order to find out the best product. Consumer research, case history research is also some of the factors that need to be included which drawing conclusions from the analysis (Smith, 1972).

### 2.10 Fenugreek plant

#### 2.10.1 Introduction and origin

Fenugreek belongs to family Leguminosae and subfamily papilionacae. It is annual crop mainly grown for use as a spice in many parts of the world (Acharya *et al.*, 2006) . The scientific name of fenugreek is *Trigonella foenum graecum*. It is named Trigonella, from Latin language that means "little triangle" due to its yellowish-white triangular flowers (Flammang *et al.,* 2004). Fenugreek crop is very useful legume crop that can be used in livestock feed, fixation of nitrogen in soil and increasing fertility (Ahari *et al.*, 2009). Fenugreek seeds have been valued as medicinal material from very early times. Fenugreek is chemugric has a wide use for industrial purposes. Its seeds are considered to be of commercial interest as a source of a steroid diosgenin, which is of importance to the pharmaceutical industry (Mehrafarin *et al.*, 2010). Its dried seeds have wide application in food and beverages. The leaves of fenugreek plant are edible and often used as vegetable dish in many parts of India. Fenugreek has been reported to be an important medicinal plant with large number of medicinal properties such as restorative and nutritive properties (appetite stimulant) with hypocholesterilemic, antidiabetic, antileukemic and antimicrobial effects (Acharya *et al.,* 2008).

It is one of the oldest medicinal plants, originally from south Eastern Europe and western Asia, but grown now mainly in India and also in certain parts of Asia, northern Africa, Europe and the United States (Altuntaş *et al.*, 2005). It is also known as methi in Nepal, India and also as Fenugrec (France), Bockshorklee (Germany), Koroha (Japan), Hulba (Arab), Pazhitnik (Russia) and Ku-Tou (China) (Daniel, 2006). In North Africa, it has been grown for fodder in Saharan oases from very early times (Alaoui, 2005). The Greek named the plant “telis”, which means “green” and Romans learned from Greeks that this plant was valuable fodder (Petropoulos, 2002).

The use of Fenugreek dates back as far as six thousand years ago. Through discoveries, archaeologists believe this spice was used as early as 4000 BC, when remains of this herb where discovered in Tell Halal, Iraq. Fenugreek was considered to be a medicinal drug and used by the Ancient Egyptians for this purpose. They believed that Fenugreek could treat burns and induce childbirth. They also used it in mummification. The Greek physician, Hippocrates, used it as a soothing herb. Other ancient Greeks used Fenugreek as a cure for infections. The ancient Romans used it to treat fevers and respiratory and intestinal issues. They also used it to help heal wounds. During the first Jewish-Roman war, Fenugreek was mixed with boiling oil. This mixture was used to keep invaders from entering the city. Fenugreek is often served with food during Rosh Hashanah. It is believed that eating Fenugreek is symbolic for helping one to increase their blessings in the coming year (Anon., 2014).

### 2.10.2 Chemical constituents of fenugreek

The chemical composition of fenugreek (such as seeds, husk and cotyledons) showed that endosperm had the highest (4.63 g/100 g) saponin and (43.8 g/100 g) protein content. As against this, husk contains higher total polyphenols. The extracts of endosperm husk, and fenugreek seed at about 200 µg concentration exhibited antioxidant activity 72%, 64%, and 56% respectively by free-radical scavenging method (Abdouli *et al.*, 2014).

The seeds of fenugreek contain about 0.1–0.9% of diosgenin and are extracted commercially. The plant tissue cultures from seeds of fenugreek when grown under optimal conditions have been found to produce as much as 2% diosgenin with smaller amounts of trigogenin and gitongenin. Seeds also contain the saponin (fenugrin B). Fenugreek seeds have been found to contain several coumarin compounds as well as a number of alkaloids (e.g., trigonelline, gentianine, carpaine). The large amount of trigonelline is degraded to nicotinic acid and related pyridines during roasting. The major bioactive compounds in fenugreek seeds are believed to be polyphenol compounds, such as rhaponticin and isovitexin (Abdouli *et al.*, 2014).

Small amount of volatile oils and fixed oil has been found in fenugreek seeds. Blank *et al.* (1997) have found the odor active compounds based on the fenugreek aroma detection with the help of Gas Chromatograph and these includes the olfactometry diacetyl, 1-Octene-3-one, sotolon, acetic acid; 3-Isobutyl-2-methoxypyrazine, butanoic acid, isovaleric acid, 3-isopropyl-2-methoxypyrazine, caproic acid, eugenol, 3-Amino-4,5-dimethyl-3, linalool, (Z)-1,5-Octadiene-3-one, 4-dihydro-2(5H)-Furanone with characteristic aroma of buttery like, roasty/earthy, metallic, pungent, paprika like, sweaty/rancid, flowery, musty, spicy respectively. Out of all these volatile compounds, sotolon was reported to be found most predominantly in (5s)-enantiomeric form (95%) in fenugreek (Abdouli *et al.*, 2014).

A study was conducted on sweat of human after fenugreek ingestion and it has been concluded that compounds responsible for the strong maple-syrup odor present in sweat after fenugreek ingestion are due to the following components including the following: pinene; 3-octen-2-one, 2,5-dimethylpyrazine, β-; camphor; terpinen-4-ol; 4-isopropyl-benzaldehyde; neryl acetate and β-caryophyllene but it was observed that 2,5-dimethylpyrazine to be a major component responsible for sweat odor contributing compound (Abdouli *et al.*, 2014). A list of chemical constituents is shown in Table 2.7.

**Table 2.7** Chemical constituents of fenugreek

| S. No. | Chemical constituents 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. |
| Other | Coumarin, lipids, vitamins, minerals. 28% mucilage; 22% proteins; 5% of a stronger-swelling, bitter fixed oil. |

Source: Yadav *et al.* (2011)

### 2.10.3 Traditional uses

The medicinal value of fenugreek seeds is mentioned in ayurvedic texts as well as in Greek and Latin pharmacopoeia. The ayurvedic texts praise this herb for its power as an aphrodisiac, but modern vaidyas seem to be using it more for digestive and respiratory problems stemming from an excess of kaph (phlegm) and vat (wind). In ancient Egypt, methi was used to ease childbirth and to increase milk flow, and modern Egyptian women are still using it today to relieve menstrual cramps, as well as making hilba tea out of it to ease other kinds of abdominal pain. The Chinese call it hu lu ba, and also use it for treating abdominal pain. Though this cool season crop is grown in most corners of the world, its uses and people's awareness of its value vary considerably. In India, fresh methi ka saag (the stems and leaves of the plant) is very commonly cooked as a winter vegetable, and the seeds are used year-round as a flavoring agent for various dishes. The seeds are also eaten raw as sprouts and used medicinally. In Egypt and Ethiopia, methi is used in baking bread, and the Swiss use it for flavoring cheese. In the USA, it is mainly used to make spice blends for soups and stews (Singh, 2022).

The herb of fenugreek has been used for centuries as a cooking spice in European countries and it remains a popular ingredient in curry powders, pickles and spice mixtures in India Pakistan, Bangladesh and other Asian countries. Fenugreek has been used in the folk medicines for the treatment of cellulitis, boils, and tuberculosis. Fenugreek remained a key ingredient in a 19th century patent medicine for dysmenorrheal and postmenopausal symptoms. It also has been recommended for the promotion of lactation. The seeds of fenugreek have been used as an orally as insulin substitute for reduction in blood glucose, and the extracts from seed have been reported to lower blood glucose levels (Madar and Stark, 2002).

### 2.10.4 Nutraceutical properties of Fenugreek

Fenugreek has a beneficial effect on cleansing the blood and as a diaphoretic it is able to bring on a sweat and to help detox the body. Due to pungent aroma of fenugreek, that is smelt on the skin and in under-arm perspiration. Fenugreek is also known for its lymphatic cleansing activity though its vital role is to irrigate the cells with nutrients and to remove toxic wastes, dead cells and trapped proteins from the body. Block in the lymphatic system can mean poor circulation of fluid, fluid retention, pain, energy loss and disease, anywhere in the body of a person. Fenugreek maintains mucus conditions of the body, mostly the lungs, by helping to clear congestion. It also acts as a throat cleanser and mucus solvent that also eases the urge to cough. Drinking water in which seeds of fenugreek have soaked helps in softening and dissolving, accumulating and hardening the masses of cellular debris. Fenugreek has been used to relieve colds, bronchial complaints, influenza, asthma, catarrh, constipation, sinusitis, pleurisy, pneumonia, sore throat, laryngitis, hay fever tuberculosis and emphysema (Abdouli *et al.*, 2014).

### 2.10.5 Utilization of fenugreek in various food products

A list of general uses of various parts of fenugreek in different food products is shown in Table 3 below. Due to rich source of natural dietary fiber in fenugreek, it has established itself in the modern food ingredient or functional food. Fenugreek as a hydrocolloid, which is fenugreek gum (soluble fiber of fenugreek), gives textural, appeal, thickening, emulsifying, stabilizing, gelling, and encapsulating properties. So the dietary fiber, more importantly soluble fiber can find their way into nutrition and dairy products, cereal bars, yogurts, and nutritional beverages. The powder of soluble fiber or total dietary fiber can be mixed with juices of fruit, seasonings and other spice mixes. Directly it can be used to formulate tablets or capsules along with the other vitamins and nutrients necessarily needed. It can also be used in milk shakes, dressings, soups, candies and sweets. It has been used to fortify bakery flour for pizza, pizza, cake mix, bread, bagel, muffins, flat bread, tortilla and noodles, fried, baked corn chips. Bakery foods such as bread, pizza, cakes and muffins have been prepared by using flour fortified with eight to ten percent soluble dietary fiber. When fiber fortified flour was used for making oil fried snacks, 8–15% of less oil absorption only takes place which is really appreciable in terms of unwanted fat intake (Kumar and Maliakel, 2008).

**Table 2.8** General uses of fenugreek.

| S. No. | Component used | Utilization of fenugreek |
|---|---|---|
| 1 | Seeds | Bread |
| 2 | Fenugreek seeds, leaves | Biscuits |
| 3 | Seeds | Extruded product |
| 4 | Fenugreek gum | Extruded products |
| 8 | Seed, leaves | Culinary use (color, flavor, aroma) |
| 9 | Leaves and seeds | Spice and seasoning |
| 11 | Seeds, leaves | Organoleptic character improver |
| 12 | Seed | Maple syrup and artificial flavoring |
| 14 | Seed | (Mixed with flour for bread, yellow dye) food |
| 16 | Seed | Dietary fiber, galactomannan |
| 17 | Seed | Curries, condiments, pickles, chutneys as a flavoring |
| 18 | Seed | Food stabilizer, adhesive and emulsifying agent |

Source: Abdouli *et al.* (2014)

## 2.11 Nutritional composition and anti-oxidant of fenugreek

Fenugreek is an old medicinal plant and has been commonly used as a traditional food and medicine. The seeds of fenugreek contain lysine and L-tryptophan rich proteins, mucilaginous fiber and other rare chemical constituents such as saponins, coumarine, sapogenins and trigonelline, which are thought to account for many of its presumed therapeutic effects, may inhibit cholesterol absorption and thought to help lower sugar levels. Fenugreek seeds have also been reported to exhibit pharmacological properties such as anti-tumor, anti-viral and antioxidant activity (Saini *et al.*, 2016).

Fenugreek seeds are processed by using different processing methods i.e. soaking, germination and roasting. Raw fenugreek seed flour contains higher amount of dietary fiber followed by soaked seed flour, roasted seed flour and germinated seed flour. Processing of fenugreek seeds improves invitro starch digestibility and invitro protein digestibility. Soaking, germination and roasting enhances total phenolic content and the antioxidant activity of fenugreek seed flour. Processing of fenugreek seeds also decreases phytic acid content as compared to raw seeds (Pandey and Awasthi, 2015).

**Table 2.9** Changes in nutritional composition, anti-nutritional, total phenolic content and antioxidant activity of fenugreek seed flour

| Parameters | Raw | Soaked | Germinated | Roasted |
|---|---|---|---|---|
| Moisture % | 6.3±0.34 | 5.2±1.03 | 5.5±0.30 | 4.2±0.14 |
| Crude Protein % | 32.7±0.43 | 35.1±0.70 | 41.2±1.10 | 36.8±1.00 |
| Crude Fat % | 4.8±0.16 | 4.6±0.15 | 3.7±0.12 | 4.5±0.22 |
| Ash % | 3.7±0.25 | 4.0±0.31 | 4.6±0.30 | 3.8±.22 |
| Crude fiber % | 6.0±0.07 | 6.0±0.10 | 8.8±0.6 | 6.2±0.11 |
| Carbohydrates by difference % | 46.1±1.14 | 44.8±1.30 | 35.7±0.56 | 43.9±1.23 |
| Total dietary fiber (TDF) % | 45.4±0.56 | 41.7±0.63 | 31.3±0.74 | 40.9±0.84 |
| Ca mg/100 g | 70.5±0.68 | 68.2±0.86 | 70.7±0.62 | 71.2±0.77 |
| P mg/100 g | 544.5±1.26 | 612.8±0.90 | 632.3±0.92 | 611.9±0.27 |
| Fe mg/100 g | 11.6±0.59 | 10.7±0.19 | 11.5±0.31 | 13.1±0.25 |
| Zn mg/100 g | 5.7±0.34 | 5.2±0.20 | 5.5±0.02 | 6.2±0.12 |
| In vitro starch digestibility (mg maltose released/g meal) | 36.3±0.71 | 39.3±0.51 | 46.1±0.33 | 37.3±0.43 |
| In vitro protein digestibility % | 48.6 ±1.07 | 57.4±0.67 | 63.0±0.73 | 55.8±0.58 |
| Total phenolic content (mg of gallic acid equivalents/g of sample) | 45.4±0.02 | 54.4±0.01 | 80.8±0.02 | 48.5±0.01 |
| Antioxidant activity % | 18.1±0.70 | 60.7±0.57 | 73.9±0.45 | 32.0±0.76 |
| Phytic acid mg/100 g | 552.3 ±2.52 | 504.2±2.52 | 308.7±1.95 | 327.1±2.00 |

Source: Pandey and Awasthi (2015)

**Table 2.10** Proximate composition, mineral content and antioxidant activity of raw and processed fenugreek feeds

| Parameters | Raw | Soaked | Germinated |
|---|---|---|---|
| Moisture% | 6.92$^{b}$±0.83 | 8.81$^{c}$±1.2 | 6.39$^{b}$±0.55 |
| Fat% | 4.23$^{c}$±0.75 | 3.66$^{a}$±0.28 | 3.48$^{a}$±0.35 |
| Crude fibre% | 7.51$^{b}$±0.53 | 7.22$^{b}$±0.52 | 9.26$^{d}$±0.59 |
| Ash% | 3.65$^{b}$±0.46 | 4.17$^{c}$±0.96 | 1.04$^{a}$±0.83 |
| Iron mg/100g | 11.19$^{d}$±0.92 | 9.75$^{c}$±0.62 | 6.53$^{b}$±0.58 |
| Calcium mg/ 100g | 84.17$^{b}$±1.28 | 76.10$^{a}$±1.01 | 90.2$^{c}$±1.57 |
| Phosphorus mg/100g | 515.7$^{a}$±2.17 | 604.27$^{d}$±2.68 | 612.17$^{d}$±2.54 |
| TPC (mg GAE/gm) | 173.38$^{b}$±12.29 | 157.92$^{a}$±5.70 | 184.31$^{c}$±7.02 |
| DPPH % | 13.72$^{a}$±0.88 | 35.85$^{c}$±0.92 | 48.84$^{d}$±1.12 |

Source: Saini *et al.* (2016)

## 2.12 Preparation of Soaked Fenugreek Seed Flour

Fenugreek seeds are cleaned and freed of broken seeds, dust and other foreign materials. The seeds then are soaked in tap water for 12h at 37$^{o}$C with a seeds : water ratio of 1:5 (w/v) is used. The unimpeded water is discarded and the soaked seeds is rinsed twice by distilled water.

The soaked seeds are dried in an oven at 55-60$^{o}$C for 10-12 hr. The dried seeds are ground to particles passing through 20 mesh sieve which is similar to the size of wheat flour (Ibrahium and Hegazy, 2009).

## 2.13 Effect of fenugreek seed flour on physical characteristics of biscuits

Physical properties of biscuit affect consumer acceptance and repeat sales of biscuits (Agrawal and Syed, 2017). Physical characteristics of biscuits such as weight, diameter, thickness and spread ratio are significantly affected with increase in the level of fenugreek seed flour (Hooda and Jood, 2005b). In research done by Hooda and Jood (2005b), average width of control (wheat) biscuit was 3.01 cm whereas that of fenugreek suplemented biscuit varied from 2.66 to 2.36 cm (soaked) and 2.96 to 2.53 cm (germinated) at 5-20% level of substitution. The average thickness of control biscuit was 0.78 cm and for other supplemented levels, it varied from 0.81 to 0.89 for soaked and 0.84 to 0.97 cm for germinated at 5-20% levels. Spread ratio of control biscuit was 3.85, which decreased significantly from 3.28-2.65 in soaked fenugreek at 5-20% levels.

In research done by Agrawal and Syed (2017), the weight of cookies increased linearly with addition of germinated fenugreek seed flour from 13.99 g in control to 14.18 g at 5-15% levels. Diameter of cookies gradually decreased as the level of germinated fenugreek seed replacement. The average diameter of control cookies was 5.35 cm whereas that of supplemented cookies varied from 5.2 to 5 cm at 5-15% levels of fenugreek seed flour.

## 2.14 Effect of incorporation of fenugreek on sensory properties of biscuit

According to the study done by El-Naggar (2019), with increasing the level of raw fenugreek seed flour, soaked fenugreek seed flour or germinated fenugreek seed flour from 5% to 20% in the formulation, the sensory scores for color, texture and flavour of biscuits significantly decreased. Results indicated that there is no significant differences among control wheat biscuit sample and biscuit sample containing 5 or 10% fenugreek seed flour in all sensory properties.

### 2.14.1 Color

According to Hooda and Jood (2005a) on study of wheat bread incorporated with fenugreek flour, as the level of fenugreek flours in the blend was increased, the crust color of the breads changed from creamish white to the dull brown. The 20% blend level of the fenugreek flours produced the darkest color, however, score differed with different fenugreek flours. When compared on mean basis, the color scores for raw, soaked and germinated fenugreek flour blends were 5.66, 5.50 and 5.37 respectively as against 7.15 for the control bread. This suggested that the darker color of bread was observed in germinated fenugreek supplemented flour and comparatively lighter color were observed in case of supplementation with raw and soaked fenugreek flours. Crust color is the result of the maillard reaction between reducing sugars and proteins. The increased protein content of the fenugreek flour blended breads in the present study probably caused the darkest crust color (Hooda and Jood, 2005a).

### 2.14.2 Flavor

According to Hooda and Jood (2005a) on study of wheat bread incorporated with fenugreek flour, the flavor of soaked and germinated fenugreek supplemented breads did not differ significantly at all the substitution levels whereas in case of raw fenugreek supplemented bread it was lowest at 20% level of substitution. On mean basis, flavor score of breads were 5.60, 5.76 and 5.96 for raw, soaked and germinated fenugreek supplemented breads, respectively indicating that germinated fenugreek flour supplemented bread had better flavor while raw fenugreek supplemented bread had poor flavor score. Addition of fenugreek to wheat flour modified the taste and mouth feel and impart fenugreek flavor to the bread at 20% level of substitution, there was a sharp decrease in the scores for odor and taste, probably due to the dominating flavor of fenugreek (Hooda and Jood, 2005a).

### 2.14.3 Taste

Hooda and Jood (2005a) found that the raw, soaked and germinated fenugreek flour supplemented breads had satisfactory taste score upto 15% level of substitution. Thereafter, the taste became bland and bread was considered little bit bitter, probably due to dominating taste of fenugreek flour. However, at 20% level of substitution of all fenugreek flour in wheat flour had poorest taste score which was considered bitter in taste (Hooda and Jood, 2005a).

### 2.14.4 Texture

According to Agrawal and Syed (2017) as the level of fenugreek seed powder (FSP) increased from 5% to 15% in cookies, there was a decrease in hardness, chewiness, springiness and cohesiveness value. At 15% level of FSP, the adverse effect was more pronounced. The hardness was found to be decreased with increase in the fortification level of FSP (Agrawal and Syed, 2017). According to Hooda and Jood (2005a) noticeable changes were observed in the texture of breads. The texture was observed to decrease significantly with the increase in substitution of fenugreek flours with wheat flour. Maximum score of

4.87 was noticed at 20% substitution level of raw fenugreek while in other fenugreek, the score at 20 per cent level were 4.71 for soaked fenugreek and 4.60 for germinated fenugreek (Hooda and Jood, 2005a).

## 2.15 Chemical composition of fenugreek seed flour suplemented biscuit

Nutritional evaluation of wheat biscuits supplemented with untreated and treated fenugreek flour done by Hooda and Jood (2005b) is shown in Table 2.11.

**Table 2.11** Proximate composition of fenugreek supplemented biscuits (g/100 g, on dry matter basis)

| Supplementation level (%) | Moisture | Fat | Protein | Crude fibre | Ash | Carbohydrate |
|---|---|---|---|---|---|---|
| Control (wheat) | 3.15±0.02 | 21.12±0.05 | 9.21±0.13 | 2.05±0.02 | 1.80±0.05 | 62.7±0.30 |
| W:RF | | | | | | |
| 95:5 | 3.18±0.01 | 21.19±0.03 | 9.84±0.11 | 2.24±0.03 | 1.83±0.03 | 61.7±0.47 |
| 90:10 | 3.25±0.02 | 21.28±0.06 | 10.5±0.08 | 2.46±0.01 | 1.88±0.02 | 60.6±0.13 |
| Mean | 3.21±0.02 | 21.23±0.04 | 10.2±0.16 | 2.35±0.05 | 1.85±0.02 | 61.2±0.33 |
| CD (P < 0:05) | NS | NS | 0.39 | 0.09 | NS | 1.07 |
| W:SF | | | | | | |
| 95:5 | 3.17±0.03 | 21.18±0.10 | 9.77±0.09 | 2.30±0.03 | 1.80±0.01 | 61.8±0.26 |
| 90:10 | 3.23±0.02 | 21.21±0.07 | 10.4±0.17 | 2.55±0.01 | 1.83±0.02 | 60.8±0.12 |
| Mean | 3.20±0.02 | 21.19±0.06 | 10.1±0.16 | 2.42±0.06 | 1.81±0.01 | 61.3±0.25 |
| CD (P < 0:05) | NS | NS | 0.53 | 0.10 | NS | 0.80 |
| W:GF | | | | | | |
| 95:5 | 3.19±0.01 | 21.15±0.06 | 10.1±0.14 | 2.37±0.02 | 1.86±0.01 | 61.3±0.46 |
| 90:10 | 3.27±0.02 | 21.06±0.05 | 11.0±0.11 | 2.70±0.03 | 1.91±0.02 | 60.0±0.24 |
| Mean | 3.27±0.02 | 21.10±0.04 | 10.6±0.22 | 2.53±0.07 | 1.88±0.01 | 60.7±0.37 |
| CD (P < 0:05) | 0.07 | NS | 0.49 | 0.10 | NS | 1.25 |
| CD (P < 0:05) of all treatments | 0.06 | NS | 0.58 | 0.20 | NS | 1.03 |

W, wheat flour; RF, raw fenugreek; SF, soaked fenugreek; GF, germinated fenugreek; NS, non-significant. *Values are means SD of three independent determinations.

Source: Hooda and Jood (2005b)

Chemical composition of cookies supplemented with germinated fenugreek seed flour found by Agrawal and Syed (2017) is presented in Table 2.12.

**Table 2.12** Chemical composition of cookies

| Formulation | Proximate composition (g/100 g) | | | | | |
|---|---|---|---|---|---|---|
| | Moisture | Crude fat | Crude Protein | Crude fiber | Ash | Carbohydrate |
| Control | 3.12 | 25.60 | 9.62 | 1.22 | 1.20 | 58.37 |
| $FC_1$ | 3.28 | 25.49 | 10.48 | 1.49 | 1.24 | 57.49 |
| $FC_2$ | 3.35 | 25.34 | 11.35 | 1.81 | 1.35 | 55.87 |
| $FC_3$ | 3.57 | 25.17 | 12.25 | 2.10 | 1.39 | 54.73 |
| SE ± | 0.013 | 0.010 | 0.011 | 0.011 | 0.018 | 0.015 |
| CD at 5% | 0.040 | 0.032 | 0.036 | 0.034 | 0.055 | 0.046 |

*Where $FC_1$ : 5% GFSP, $FC_2$ : 10% GFSP, $FC_3$ : 15% GFSP; Values are means of three determinations.

Source: Agrawal and Syed (2017)

Antioxidant activity estimation of fenugreek supplemented bread done by Afzal *et al.* (2016) is shown in table 2.13.

**Table 2.13** Antioxidants in fenugreek seed flour supplemented bread

| Treatments | TPC (mg GAE/ 100g) | Flavonoids (mg CE/g ) | DPPH (%) | β-carotene& Linoleic acid assay (%) |
|---|---|---|---|---|
| $T_0$ | $99.00^{d}\pm4.45$ | $2.13^{c}\pm0.09$ | $31.00^{c}\pm1.27$ | $29.00^{c}\pm1.18$ |
| $T_1$ | $238.00^{c}\pm10.94$ | $2.50^{b}\pm0.10$ | $45.00^{b}\pm1.93$ | $37.00^{b}\pm1.70$ |
| $T_2$ | $341.00^{b}\pm16.02$ | $2.68^{ab}\pm0.10$ | $49.00^{ab}\pm2.20$ | $40.00^{ab}\pm1.88$ |
| $T_3$ | $413.00^{a}\pm19.82$ | $2.91^{a}\pm0.12$ | $51.00^{a}\pm2.39$ | $43.00^{a}\pm2.10$ |

Values expressed are means ± standard deviation; $T_0$:Wheat Flour (Control); $T_1$: 5% fenugreek seeds powder supplemented wheat flour; $T_2$:10% fenugreek seeds powder supplemented wheat flour; $T_3$: 15% fenugreek seeds powder supplemented wheat flour.

Source: Afzal *et al.* (2016)

Evaluation of mineral and phytic acid of biscuit supplemented by fenugreek seed flour done by Ibrahium and Hegazy (2009) is shown in table 2.14.

**Table 2.14** Mineral and phytic acid content of biscuits supplemented by soaked and germinated fenugreek seed flours

| Mineral content (mg/100g) | Control | Substitution level (%) | | | |
|---|---|---|---|---|---|
| | | 5%(SF) | 10%(SF) | 5%(GF) | 10%(GF) |
| Iron | $4.65^{b}$ | $7.15^{a}$ | $7.44^{a}$ | $7.42^{a}$ | $7.81^{a}$ |
| Calcium | $36.23^{c}$ | $41.37^{b}$ | $43.96^{b}$ | $44.24^{b}$ | $47.89^{a}$ |
| Zinc | $2.24^{c}$ | $2.67^{b}$ | $2.93^{b}$ | $2.81^{b}$ | $3.19^{a}$ |
| Phytic acid | $175^{a}$ | $140^{b}$ | $128^{b}$ | $112^{c}$ | $98^{c}$ |

*a, b and c means in the same raw with different superscripts are different significantly

Source: Ibrahium and Hegazy (2009)

# Part III

## Materials and methods

### 3.1 Materials

#### 3.1.1 Wheat flour

Wheat flour named ‘Fortune maida’ produced by Nutri Food Pvt. Ltd., Sonapur, Sunsari, Nepal was used for biscuit making. The maida was purchased from local market of Dharan.

#### 3.1.2 Fenugreek seed

Fenugreek seed was collected from local market of Itahari, Sunsari.

#### 3.1.3 Butter

Butter manufactured by DDC, Biratnagar was used as shortening agent.

#### 3.1.4 Sugar and Salt

Sugar in pulverized form and iodized common salt were used. There were bought from local market of Dharan.

#### 3.1.5 Skim milk powder

Skim milk powder named ‘Safal Skim Milk Powder’ manufactured by Sujal Dairy Pvt. Ltd., Pokhara was used.

#### 3.1.6 Baking powder

Baking powder named ‘Weikfield baking powder double action’ manufactured and packed by Weikfield food Pvt. Ltd., Pune, India was used.

#### 3.1.7 Flavor and Invert syrup

Flavor of vanilla named “Bhanbhori vanilla” manufactured by Bhanbori Pvt. Ltd. and Honey named “Dabur honey” manufactured by Dabur India Limited as invert syrup bought from local market of Dharan were used.

### 3.1.8 Packaging material

Low density polyethylene (50 µ as mentioned in the label) bought from local market of Dharan was used for packaging of the product.

## 3.2 Chemicals required

The required chemicals were obtained from Central Campus of Technology lab which are shown in Appendix B.

## 3.3 Apparatus required

The required chemicals were obtained from Central Campus of Technology lab which are shown in Appendix C.

## 3.4 Method of experiment

### 3.4.1 Experimental Design

Design Expert v7.1.5 software was used to create the recipe. D-optimal Design was used to formulate the recipe. The independent variable for the experiment is concentration of fenugreek seed powder used to make biscuit.

### 3.4.2 Formulation of recipe

The recipe formulation for soaked fenugreek seed flour incorporated biscuit was carried out as given in Table 3.1.

**Table 3.1** Recipe formulation of biscuit

| Ingredients | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| Wheat flour | 100 | 99.874 | 99.748 | 99.50 | 99.252 | 99.126 | 99 |
| Fenugreek powder | 0 | 0.126 | 0.252 | 0.5 | 0.748 | 0.874 | 1 |
| Sugar | 35 | 35 | 35 | 35 | 35 | 35 | 35 |
| Fat | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
| SMP | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Baking powder | 3.25 | 3.25 | 3.25 | 3.25 | 3.25 | 3.25 | 3.25 |
| Salt | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 |
| Flavor | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Invert syrup | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Water | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

The biscuit was made as per the recipe formulation done and coded name A, B, C, D, E, F and G were given to each recipe. Composite biscuit were of soft dough type method as in Fig. 3.2.

## 3.5 Preparation of soaked fenugreek seed flour

Fenugreek seeds were washed with tap water to remove dirt particles. They were freed from broken seeds and other foreign materials. Then seeds were soaked in tap water for 12 h at 37°C. A seed to water ratio of 1:5 (w/v) was used. The unabsorbed water was discarded. The soaked seeds were rinsed twice in distilled water and then dried in cabinet dryer at 55°C for 12 h. The dried samples were ground to fine powder in an electric grinder to particles passing through 595 micron sieve which is similar to the size of wheat flour and then packed in plastic container, sealed and stored in ambient condition (Ibrahium and Hegazy, 2009). The process for preparing fenugreek seed powder is shown in Fig. 3.1.

Fenugreek seeds

↓

Washing

↓

Freed from foreign materials

↓

Soaking in tap water (seed to water ratio of 1:5) for 12 h at room temperature

↓

Cabinet drying at 55°C for 12 h to get 11% moisture content

↓

Pulverizing

↓

Sieving (595 μ)

↓

Fenugreek seed flour

**Fig 3.1** Process of making soaked fenugreek seed flour

Source: Ibrahium and Hegazy (2009)

### 3.6 Determination of threshold of soaked fenugreek seed flour

The independent variable of the experiment was fenugreek seed flour. The determination of threshold for fenugreek seed flour was carried out by preliminary sensory analysis. Substitution of fenugreek seed flour in wheat flour were 0.25%, 0.5%, 1% and 1.25% for preliminary sensory analysis. The preliminary sensory analysis was carried out which concluded that above 1% soaked fenugreek seed flour, the biscuit was unacceptable for panelists due to its high bitterness . Therefore, the threshold for soaked fenugreek seed flour was set between 0 and 1%.

### 3.7 Preparation of soaked fenugreek seed flour incorporated biscuit

Fat and sugar were firstly creamed. Salt was dissolved in water and added to the prepared cream mixture. As creaming process was continued, composite flour and baking powder were added and stirred well together. The full prepared dough was sheeted 5-6 mm thick and was cut to form required circular shape. The formed biscuit were baked at 200°C for 15 min. After cooling to about 35°C, the biscuits were packed in LDPE packages. The process for preparing fenugreek seed flour incorporated biscuit is shown in Fig. 3.2.

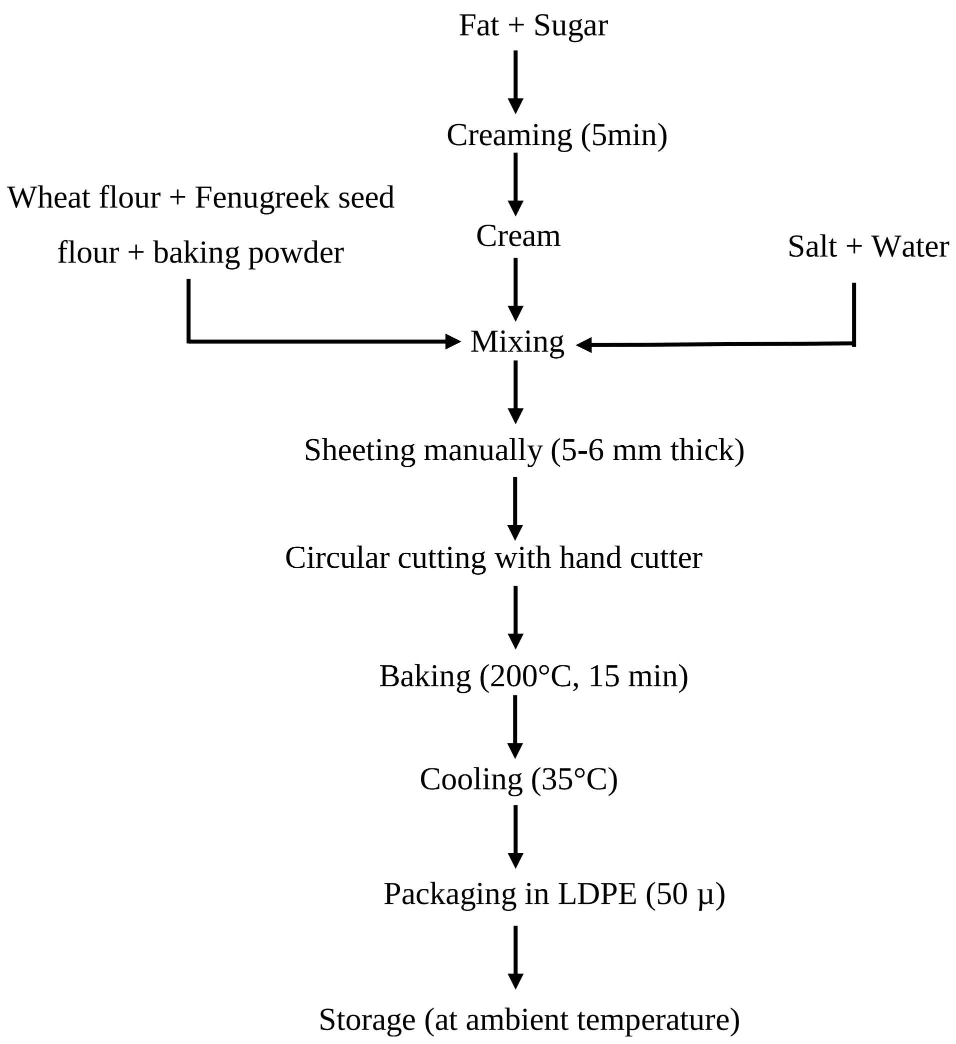


**Fig. 3.2** Flow chart for the preparation of soaked fenugreek seed flour incorporated biscuit

Source: Uchenna and Omolayo (2017)

## 3.8 Analysis of raw materials and product

### 3.8.1 Physical parameter analysis

#### 3.8.1.1 Spread ratio

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

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

Where diameter was measured in mm by Vernier caliper and thickness was measured in mm by screw gauge.

#### 3.8.1.2 Volume

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

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

Where, t = Average thickness of biscuit (mm)

d = Diameter of biscuit (mm)

#### 3.8.1.3 Density

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

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

### 3.8.2 Physicochemical analysis

#### 3.8.2.1 Moisture content

Moisture content of the sample was determined by heating in an oven at 103 ± 2°C to get constant weight (Ranganna, 1986).

#### 3.8.2.2 Crude fat

Crude fat content of the samples was determined by solvent extraction method using Soxhlet apparatus using petroleum ether as solvent as discribed by Ranganna (1986).

#### 3.8.2.3 Crude protein

Crude protein content of the samples was determined indirectly by measuring total nitrogen content by micro Kjeldahl method. Factor 6.25 was used to convert the nitrogen content to crude protein (KC and Rai, 2007).

#### 3.8.2.4 Crude fiber

Crude fiber content of the samples was determined by the method given by Ranganna (1986).

#### 3.8.2.5 Total ash

Total ash content of the samples was determined by following the method given by Ranganna (1986) using muffle furnace.

#### 3.8.2.6 Carbohydrate

The carbohydrate content of the sample was determined by difference method.
Carbohydrate (%) = 100-(protein + fat + ash + crude fiber)

## 3.9 Determination of iron

The iron content of the biscuit was determined by colorimetric method as described by KC and Rai (2007).

## 3.10 Calcium content

The calcium content of the sample was determined by using volumetric method as per KC and Rai (2007).

## 3.11 Total polyphenol content

Determination of total phenol was carried out with Folin-Ciocalteau reagent as mentioned by Sadasivam and Manickam (1996). Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium producing a blue colored complex (molybdenum blue). 0.5 ml of the extract and 1ml of Folin-Ciocalteu reagent was mixed and incubated at room temperature for 15 min. Then 2.5 ml of saturated sodium carbonate was added and further incubated for 30 min at room temperature and absorbance was measured at 760 nm.

### 3.12 Antioxidant Activity

Antioxidant activity of the extracts of samples and products were determined by method described by Vignoli *et al.* (2011) with slight variation. Different concentration of samples was made with 80% methanol. 1 ml of prepared extract was mixed with 2 ml of 0.1 mM DPPH solution and left in dark for 30 min and absorbance was measured at 517 nm. Blank samples were prepared with methanol and DPPH.

% inhibition was expressed in percentage using the equation:

$$\% \text{ inhibition} = \frac{A_b - A_s}{A_b} \times 100$$

Where, $A_b$ is the absorbance of control, $A_s$ is the absorbance of test sample.

### 3.13 Sensory analysis

The sensory analysis for overall quality was carried out with twelve semi-trained panelists which consisted of teachers and students of Central Campus of Technology. The parameters for sensory evaluation were texture, crispiness, color, taste, flavor and overall acceptability. Sensory evaluation was performed according to the 9-point Hedonic Scale as in appendix A.

### 3.14 Statistical analysis

The analyses were carried out in triplicate. Statistical calculations were performed in Microsoft office Excel 2013. All the data obtained in this experiment were analyzed for significance by Analysis of Variance (ANOVA) using the statistical program known as Genstat Discovery Edition 3. From this, means were compared using Fisher's protected LSD (Least Significance Difference) at 5% level of significance.

### 3.15 Packaging and storage of the biscuit

Low Density Polyethylene (LDPE) bag with zip was used for the packaging of the biscuits and was stored at ambient temperature.

# Part IV

## Results and discussion

The soaked fenugreek seed flour incorporated biscuit was prepared by supplementing soaked fenugreek seed flour in various levels. The quality of biscuits depends upon the quality of ingredients used mainly wheat flour. Therefore, wheat flour and soaked fenugreek seed flour were analyzed to find their proximate composition.

The wheat flour and soaked fenugreek seed flour were collected and mixed with other ingredients to formulate soaked fenugreek seed flour incorporated biscuit of 0, 0.126, 0.252, 0.5, 0.748, 0.874 and 1 part. The best among the seven variations was determined by carrying out sensory evaluation. Total polyphenol content, antioxidant activity and detail nutritional value of the best product was determined.

### 4.1 Proximate composition

The proximate composition of wheat flour, raw fenugreek seed flour as well as soaked fenugreek seed flour was obtained as given in Table 4.1 and T-test for chemical parameters of raw and soaked fenugreek seed flour is given in Appendix I.

**Table 4.1** Chemical composition of wheat flour, raw fenugreek seed flour and soaked fenugreek seed flour.

| Parameters (db) | Wheat flour* | Raw FSF* | Soaked FSF* |
|---|---|---|---|
| Moisture content (%) | 12.68±0.35 | $10.5^{a}$±0.37 | $11.37^{a}$±0.54 |
| Crude protein (%) | 10.24±0.26 | $26.96^{a}$±0.38 | $28.26^{a}$±0.13 |
| Crude fat (%) | 1.22±0.63 | $8.19^{a}$±0.16 | $7.58^{a}$±0.21 |
| Crude fiber (%) | 0.42±0.53 | $6.7^{a}$±0.18 | $6.89^{a}$±0.30 |
| Total ash (%) | 0.53±0.34 | $2.97^{a}$±0.05 | $3.09^{a}$±0.13 |
| Carbohydrate (%) | 87.59±0.59 | $55.18^{a}$±0.21 | $54.17^{b}$±0.31 |
| Gluten content (%) | 8.1±0.23 | - | - |

*Values are the means of three determinations ± standard deviations.

The moisture content of the wheat flour was 12.68% which is in normal range as described by Arora (1980). The protein content of wheat flour is very important parameter in estimating the baking quality of the flour. The protein content of the wheat flour was found to be 10.24% which lie within the standard range as describe by Arora (1980). The fat content of wheat flour was found to be 1.22% while the ash content was found to be 0.53% which lie in the standard range as described by Arora (1980).

Moisture content of unprocessed (raw) fenugreek flour increased from 10.5 to 11.37% on dry basis. The protein content increased from 26.96 to 28.26% in raw and soaked respectively. Pandey and Awasthi (2015) also found the lower level of protein content in raw than soaked fenugreek seed powder. This might be due to reduction of seed nitrates into plant protein or ammonium compounds during soaking (Hooda and Jood, 2007). Slight increase in crude fiber was noticed from 6.7 to 6.9% in raw and soaked fenugreek seed flour. Hooda and Jood (2007) found that soaking fenugreek seeds for 12 h reduced total dietary fiber but not total carbohydrates and crude fiber contents. Soaking of fenugreek seeds decreased fat content to 7.58%. A similar trend in decrease in fat content in fenugreek seeds after soaking has been observed by Pandey and Awasthi (2015). Total carbohydrates significantly decreased on soaking. As soluble carbohydrates got dissolve in water, the decrement in carbohydrates was seen (Zakrzewska *et al.*, 2010). In general, soaking did not show any significant changes in total ash (3.09%) (Pandey and Awasthi, 2015).

## 4.2 Mineral content of raw material

Mineral content of raw and soaked fenugreek seed flour is shown in the Table 4.2.

**Table 4.2** Mineral content of raw and soaked fenugreek seed flour

| Minerals | Raw FSF* | Soaked FSF* |
|---|---|---|
| Iron content (mg/100 g) | $10.97^{a}\pm0.89$ | $9.21^{b}\pm0.65$ |
| Calcium content (mg/100 g) | $81.4^{a}\pm0.58$ | $73.93^{b}\pm0.78$ |

*Values are the means of three determinations ± standard deviations.

Raw fenugreek seed flour contained higher total mineral content such as 81.4 mg/100 g calcium and 10.97 mg/100 g iron. On soaking, significant decrease was observed in iron content (9.21 mg/100 g) and calcium content (73.93 mg/100g). These data are similar to the data reported by Pandey and Awasthi (2015). Comparatively lower contents of mineral when soaked in water might be due to leaching of some amount into soaking water (Pandey and Awasthi, 2015).

### 4.3 Phytochemicals composition of fenugreek seed flour

Fenugreek seeds are rich source of bioactive phytochemicals including polyphenols and antioxidants.

Phytochemicals composition of fenugreek seed flour is shown in Table 4.3.

**Table 4.3** Phytochemicals composition of raw and soaked fenugreek seed flour

| Parameters | Raw FSF* | Soaked FSF* |
|---|---|---|
| TPC (mg GAE/g) | $136^{a}\pm3$ | $155^{b}\pm2$ |
| DPPH % | $15.28^{a}\pm0.89$ | $40.34^{b}\pm0.93$ |

*Values are the means of three determinations ± standard deviations.

In the case of fenugreek seed, total phenolic content significantly ($P<0.05$) increased on soaking from 136 to 155 mg GAE/g. The antioxidant capacities of the fenugreek seeds were analyzed by using the free radical scavenging capacity (DPPH). DPPH % of raw fenugreek seed flour was found to be 15.28%. The DPPH % increased significantly to 40.34% on soaking fenugreek seeds. Pandey and Awasthi (2015) also found that the total phenolic content and DPPH radical scavanging ability of raw fenugreek seed sample was significantly lower than processed fenugreek seed sample. Saini *et al.* (2016) also found that, in response to phenolic content, antioxidant activity increased significantly after soaking. Increase in total phemolic content was seen since soaking modifies the quantitative and qualitative phenolic compounds of legumes and the changes depend on the type of legume and soaking conditions. From the result, it may be concluded that soaked fenugreek posseses more health potential compared to non-germinated fenugreek seeds (Ojha *et al.*, 2018).

### 4.4 Effects of fenugreek seed flour on physical parameters of biscuits

Physical characteristics of biscuits such as thickness, diameter, spread ratio, weight, volume and density were affected by the substitution increment of level of fenugreek seed flour which is presented in Appendix J.

#### 4.4.1 Spread ratio

Mean scores for spread ratio of biscuit sample of different formulation is shown in Figure 4.1.

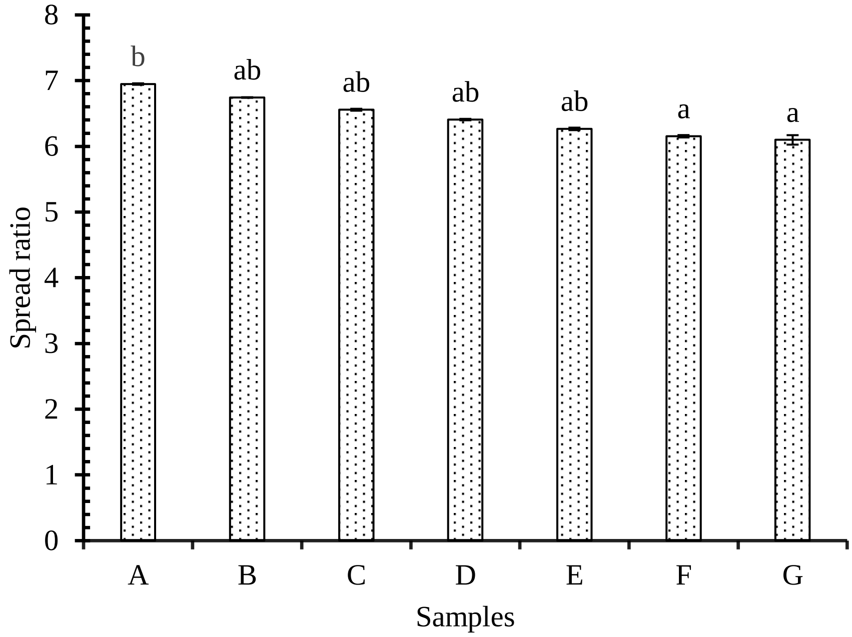


**Fig. 4.1** Mean scores for spread ratio of biscuit sample of different formulation

*Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance.

The changes in diameter and thickness are reflected in spread ratio which was calculated by dividing the diameter (D) by thickness (T) of biscuits. Spread ratio of control biscuits was found to be 6.94, which was decreased consistently from 6.74 to 6.09 with increase in fenugreek seed flour from 0.12-1%. Reduced spread ratio of fenugreek seed flour incorporated biscuits were attributed to the fact that composite flours apparently form aggregates with increased numbers of hydrophilic sites available for competing for limited free water in biscuit dough. Rapid partitioning of free water of these hydrophilic sites occurs during dough mixing and increase dough viscosity, thereby limiting biscuit spread and top grain formation during baking (Hooda and Jood, 2005b).

### 4.4.2 Density

Mean scores for density of biscuit sample of different formulation is shown in Figure 4.2.

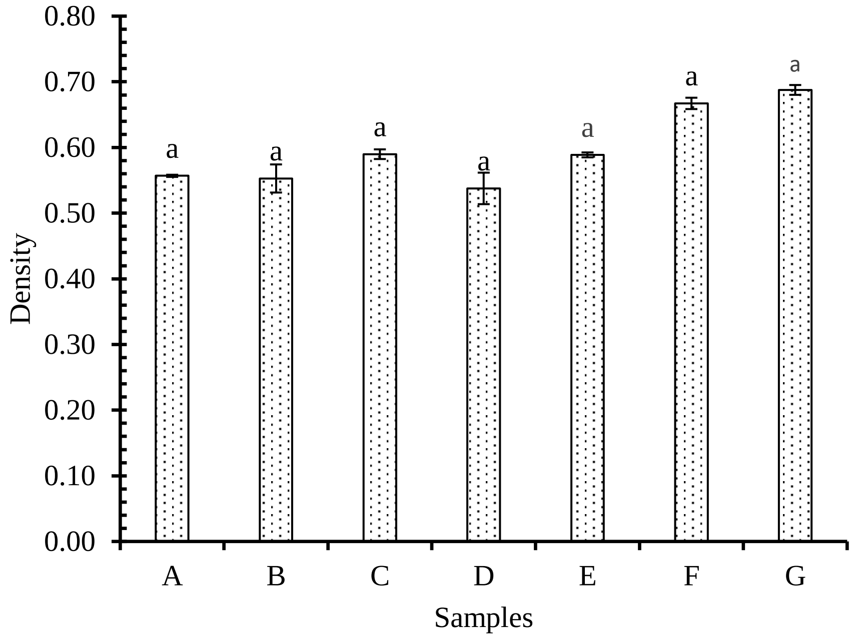


**Fig. 4.2** Mean scores for density of biscuit sample of different formulation

*Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance.

There is no significant difference in density with increasing the level of soaked fenugreek seed flour.

## 4.5 Sensory analysis of fenugreek seed flour incorporated biscuit

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

### 4.5.1 Color

The mean sensory score for color were found to be 7.62, 7.62, 7.3, 7.38, 7.84, 7.15 and 6.38 for the biscuit formulation A, B, C, D, E, F and G respectavely. The obtained mean values of treatments are presented in Fig. 4.3.

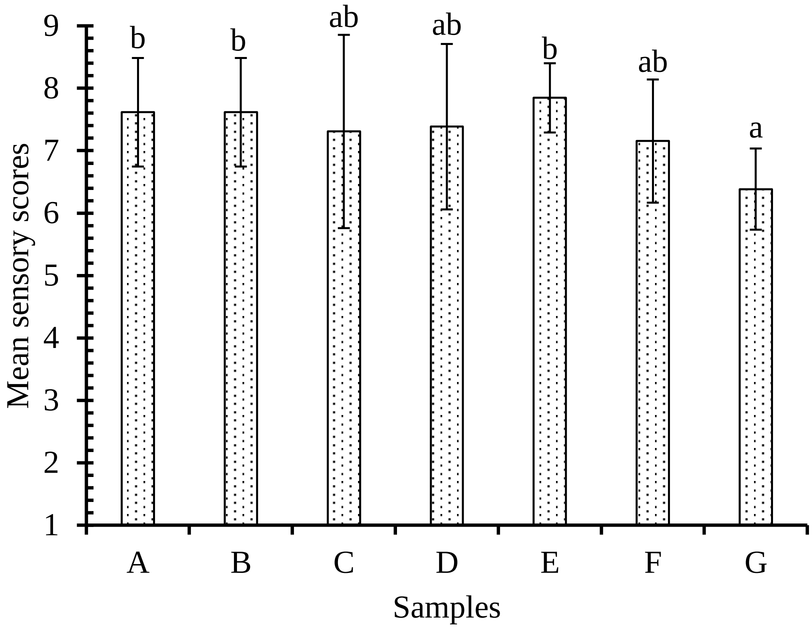


**Fig. 4.3** Mean sensory scores for color of biscuit sample of different formulation

*Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by 12 panelists.

The analysis of variance showed that in case of color, sample A, B, C, D, E, and F and showed significant difference ($P<0.05$) with sample G. The highest mean score was for sample E followed by B, which are not significantly different with control sample A. The lowest mean score was for sample G (1% fenugreek seed flour). According to El-Naggar (2019) with increasing level of fenugreek seed flour in the formulation of biscuit, the sensory scores for color gradually decreases. Also, Agrawal and Syed (2017) found that the color of the cookies changed from creamy to dark brown as substitution level increased due to dull color of fenugreek seeds.

### 4.5.2 Texture

The mean sensory score for texture were found to be 7.46, 7.38, 7.46, 7.77, 7.31, 7.15 and 7.08 for biscuit formulation A, B, C, D, E, F and G respectively. The obtained mean values of treatments are presented in Fig. 4.4.

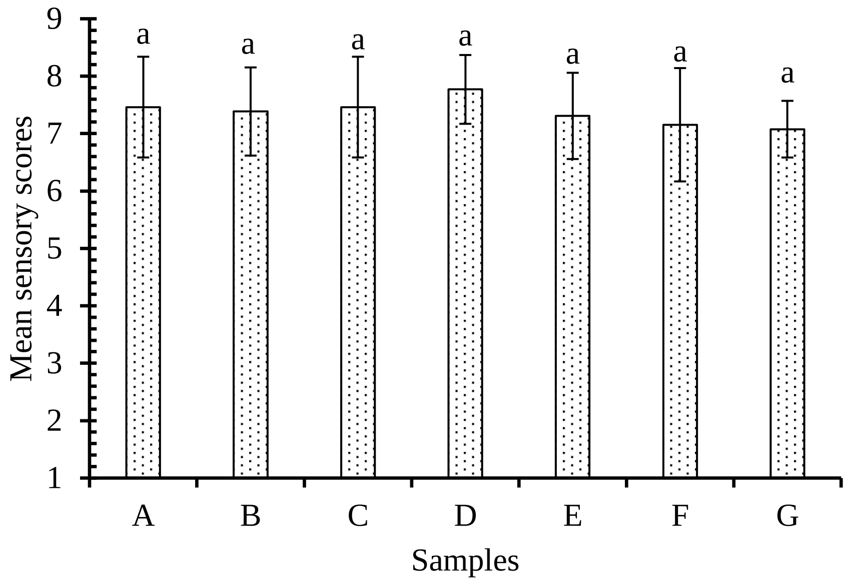


**Fig. 4.4** Mean sensory scores for texture of biscuit sample of different formulation

*Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by 12 panelists.

The mean sensory score for texture of sample D was found to be 7.77 which was the highest score of all the biscuit formulations. The texture of all samples was not significantly different. Sample D showed firm texture and no cracks. Sample F and G had slightly poor texture. Hooda and Jood (2005b) also reported the decrease in texture with increase in fenugreek seed flour. The decrease in texture of biscuit with increase in fenugreek seed flour might be due to loss of crispiness. According to El-Naggar (2019), with increasing level of soaked fenugreek seed flour in the formulation, the sensory scores for texture and of biscuits significantly decreased.

### 4.5.3 Crispiness

The mean sensory score for crispiness were found to be 6.38, 7.08, 7.54, 7.62, 7.85, 7.85 and 7.31for biscuit formulation A, B, C, D, E, F and G respectively. The obtained mean values of treatments are presented in Fig. 4.5.

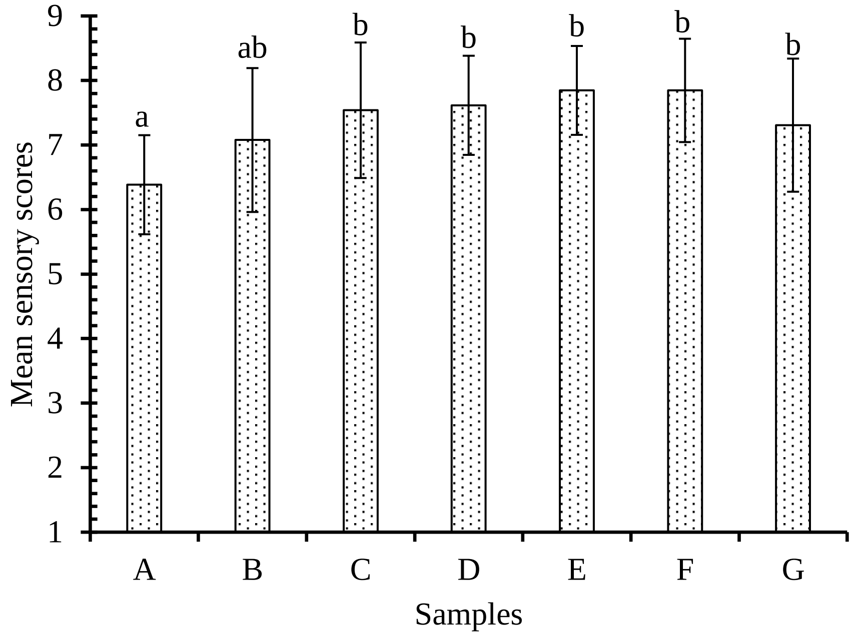


**Fig. 4.5** Mean sensory scores for crispiness of biscuit sample of different formulation

*Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by 12 panelists.

The mean sensory score was found to be highest for sample F and E followed by sample D. Samples C, D, E, F and G were not significantly different in terms of crispiness. Sample A got least score and was significantly different with all other samples. Use of excessive non glutinous flour reduces the textural strength of biscuit and leads to increase in crispiness, which causes greater acceptability due to slight cohesive nature rather than being too elastic (Schober *et al.*, 2003).

### 4.5.4 Flavor

The mean sensory score for Flavor were found to be 7.07, 7.53, 7.23, 7.76, 7.46, 6.76 and 6.3 for biscuit formulation A, B, C, D, E, F and G respectively. The obtained mean values of treatments are presented in Fig. 4.6.

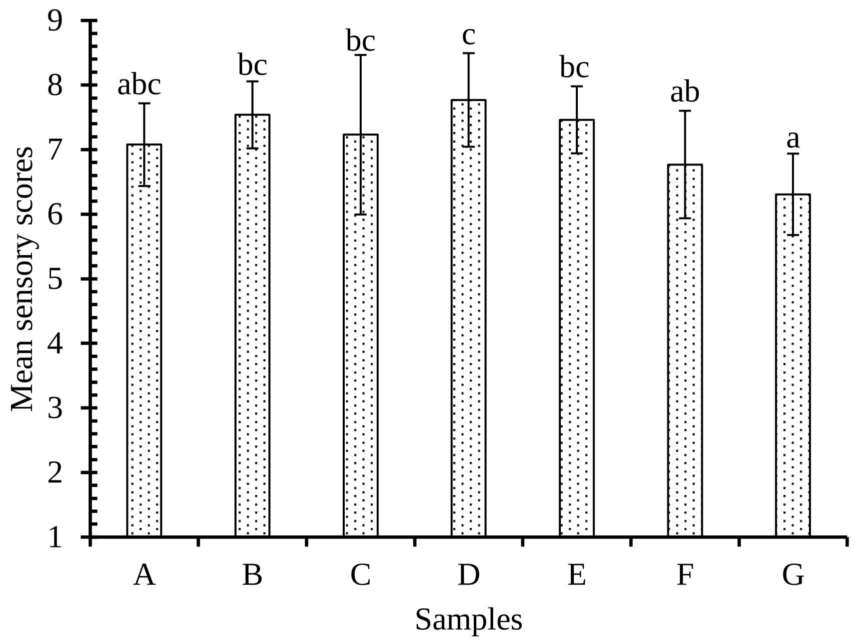


**Fig. 4.6** Mean sensory scores for flavor of biscuit sample of different formulation

*Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by 12 panelists.

The mean sensory score for flavor of sample D was found to be superior and significantly different ($p<0.05$) among the different formulations. The sample D got a high sensory score due to the correct proportion of wheat and fenugreek seed flour (99.5:0.5). The lowest mean sensory score was of sample G (1% fenugreek seed flour). Addition of fenugreek seed flour to wheat flour modified the taste and mouth feel and impart fenugreek flavor to the biscuit at 1% level of substitution. There was a sharp decrease in score, probably due to the dominating flavor of fenugreek (Hooda and Jood, 2005b). Hooda and Jood (2005b) also reported the decrease in flavor with increase in fenugreek seed flour. Also, El-Naggar (2019) found that with increasing level of soaked fenugreek seed flour in the formulation, the sensory scores for flavor and of biscuits significantly decreased.

### 4.5.5 Taste

The mean sensory score for taste were found to be 6.92, 7, 7.07, 7.69, 7, 6.61 and 6.53 for biscuit formulation A, B, C, D, E, F and G respectively. The obtained mean values of treatments are presented in Fig. 4.7.

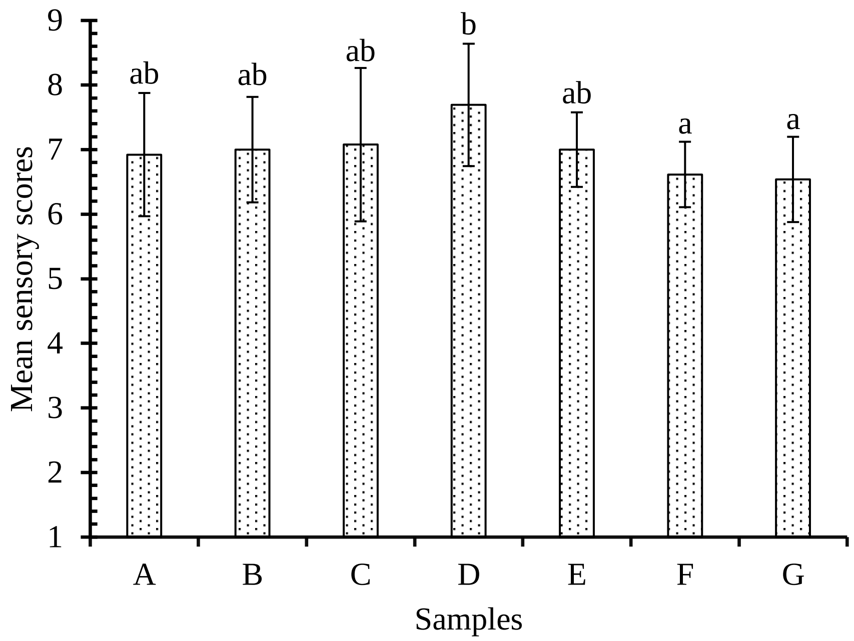


**Fig. 4.7** Mean sensory scores for taste of biscuit sample of different formulation

*Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by 12 panelists.

The mean sensory score for taste of sample D was found to be superior and significantly different ($p<0.05$) scored among the different formulations. Sample G (1% fenugreek seed flour) had poorest taste score. Fenugreek seed flour supplemented biscuits had satisfactory taste up to 0.5% level of substitution. This may be due to the incorporation of fenugreek seed flour, as the taste became bland and biscuit was considered little bit bitter due to dominating taste of fenugreek flour (Hooda and Jood, 2005a). Similarly, study of Agrawal and Syed (2017) revealed that taste of cookies decreases with increasing level of fenugreek seed flour. This was might be due to unacceptable bitter flavor imparted by fenugreek seed flour.

### 4.5.6 Overall acceptability

The mean sensory score for overall acceptability were found to be 7.3, 7.46, 7.3, 7.69, 7.3, 6.84 and 6.61for biscuit formulation A, B, C, D, E, F and G respectively. The obtained mean values of treatments are presented in Fig. 4.8.

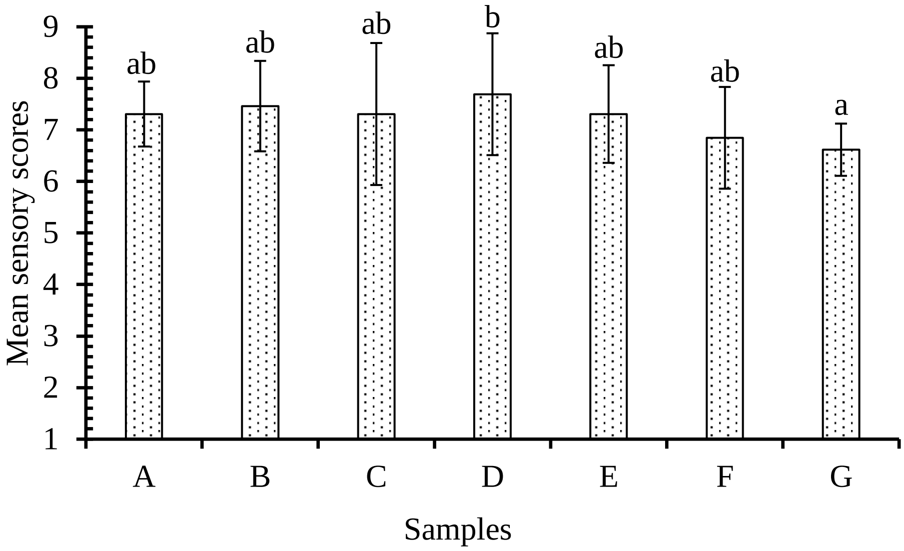


**Fig. 4.8** Mean sensory scores for overall acceptability of biscuit sample of different formulation

*Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by 12 panelists.

Sample D scored highest ($p<0.05$) in overall acceptability, which might be due to good texture as adequate amount of gluten was developed in sample D. Similarly in sample D, not too much amount of fenugreek seed flour, but adequate amount of fenugreek seed flour gave biscuit a good flavor. Sample G showed lowest score in overall acceptability which could be as a result of highest amount of fenugreek seed flour incorporated in it. In case of color only the control biscuit got higher score than sample D.

From the overall acceptability rating, it was concluded that soaked fenugreek flour could be incorporated upto 0.5% level in the formulation of biscuits without affecting their sensory quality.

Hence, from the statistical analysis, sample D with 0.5% soaked fenugreek seed flour incorporation was found to be best (optimized) product.

### 4.6 Chemical composition of optimized product

The composition of the optimized and control biscuit from chemical analysis was carried out. The result of the analysis is given in the Table 4.4.

**Table 4.4** Chemical composition of soaked fenugreek seed flour incorporated biscuit and wheat biscuit.

| Chemical composition (db) | Product A (Control) | Product D (Optimized) |
|---|---|---|
| Moisture content (%) | $2.42^{a}\pm0.15$ | $2.53^{b}\pm0.19$ |
| Crude protein (%) | $9.54^{a}\pm0.17$ | $10.74^{b}\pm0.20$ |
| Crude fat (%) | $20.15^{a}\pm0.23$ | $20.93^{b}\pm0.19$ |
| Crude fiber (%) | $0.545^{a}\pm0.13$ | $0.59^{b}\pm0.11$ |
| Total ash (%) | $1.38^{a}\pm0.09$ | $1.6^{b}\pm0.07$ |
| Carbohydrate (%) | $68.39^{a}\pm0.11$ | $66.14^{b}\pm0.13$ |
| Calcium (mg/100 g) | $23.21^{a}\pm0.13$ | $25.58^{b}\pm0.21$ |
| Iron (mg/100 g) | $0.42^{a}\pm0.30$ | $0.75^{b}\pm0.35$ |
| DPPH % | $22.21^{a}\pm1.13$ | $26.67^{b}\pm1.58$ |
| TPC (mg GAE/100 g) | $80^{a}\pm2.34$ | $87^{b}\pm1.79$ |

*Values are the means of three determinations ± standard deviations.

It is seen that moisture, protein, crude fiber, fat and ash increased significantly whereas total carbohydrate was found to be decreased significantly in prepared biscuits with substituting 0.5% of wheat flour by fenugreek seed flour. Increase in moisture of biscuits was due to increased level of soaked fenugreek seed flour that has tendency to absorb water because of hydrophilic nature (Agrawal and Syed, 2017). The ash content of biscuit increased in fenugreek seed flour incorporated biscuit from 1.38% (control) to 1.6%. The increase in ash content may be due to the high mineral content of fenugreek seed flour i.e. iron, calcium, magnesium and zinc (Hooda and Jood, 2005b). Control biscuits had 9.54% protein content. In case of supplemented biscuits, it was found to be 10.74%. The increase in protein content of fenugreek supplemented biscuits might be the result of appreciably higher protein content of fenugreek. Hooda and Jood (2005b) also reported higher protein of biscuit prepared from blends of wheat-fenugreek flours. The moisture content ranged from 2.42% in wheat biscuit to 2.53% in fenugreek seed flour incorporated biscuit. The increase in moisture content may also be due to increase in protein content (Mustafa *et al.*, 1986). Fat content increased from 20.15% to 20.93% in superior product. This is due to high fat content of fenugreek seed flour than wheat flour. Crude fiber of superior biscuit significantly increased from 0.545% to 0.59%. This may be due to the higher contents of crude fiber constituents in fenugreek (Hooda and Jood, 2005b).

Control biscuits had low total calcium (23.21 mg/100g) and iron (0.42 mg/100g) contents. Mineral contents increased on substitution of soaked fenugreek seed flour in wheat flour. Calcium and Iron content of fenugreek seed flour incorporated biscuit significantly increased to 25.58 mg/100g and 0.75 mg/100g respectively. This might be due to higher mineral content of fenugreek seed flour (Hooda and Jood, 2005b). Ibrahium and Hegazy (2009) also reported the similar result where Iron, Calcium and Zinc content increased significantly with substitution of wheat flour by soaked and germinated fenugreek seed flour. Total phenolic content increased from 80 mg/100g of control biscuit to 87 mg/100g of optimized biscuit. Total phenolic content in soked fenugreek seed flour supplemented biscuit was found higher when compared with control biscuit because fenugreek seed flour is better source of polyphenols than wheat flour (Afzal *et al.*, 2016). The free radical scavenging activity (DPPH) for biscuit prepared from wheat flour was found lower than fenugreek seed flour supplemented biscuit. DPPH % of control biscuit was found to be 22.21% and increment of antioxidant activity was seen in fenugreek seed flour supplemented biscuit where DPPH% was found to be 26.67%. The reason for increased in DPPH is due to higher amount of phenolic content in the Fenugreek seeds (Márcio and Isabel, 2013). Afzal *et al.* (2016) also reported increase in % DPPH in fenugreek supplemented bread which is due to the higher activity of fenugreek.

## 4.5 Cost of the fenugreek seed flour incorporated biscuit

The total cost associated with best product was calculated and the cost of fenugreek seed flour incorporated biscuit per 100 g was NRs. 15.34 including overhead cost and profit of 10% (Calculation is given in Appendix L).

# Part V

## Conclusions and recommendations

### 5.1 Conclusions

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

1. The iron content and calcium content of fenugreek seed decreased after soaking.
2. The TPC and % of DPPH was increased after soaking of fenugreek seed.
3. The soaked fenugreek seed flour can be incorporated upto 0.5 part with wheat flour denoted by sample D, with no adverse effect on sensory quality of biscuits.
4. The nutritional quality of the biscuit seemed to be enhanced in the case of fiber, protein, fat, iron, calcium, DPPH and total phenolic content.

### 5.2 Recommendations

The experiment can be further continued with the following recommendations:

1. Study of germinated fenugreek seed flour incorporated biscuit can be carried out.
2. Study of shelf life of the product can be carried out.
3. Entrepreneur can utilize soaked fenegreek seed flour upto 0.5 parts to enrich nutritional value of general biscuits without hampering consumer acceptance.
4. Other products like cookies, bread, muffins can be prepared by using soaked fenugreek seed flour.
5. Production cost of the prepared soaked fenugreek seed flour incorporated biscuit was reasonable, within the reach of general population. So, its commercialization could be done.

# Part VI

## Summary

Biscuit is a popular ready-to-eat baked product made of primary ingredients wheat, sugar and fat. Although, its popularity is in the form of important diet as snack or breakfast, it has plenty of rooms for further improvement in its nutritional composition and health beneficiary aspects. Fenugreek seed is pleasantly bitter in taste but it is a good source of many nutrients and has medicinal properties. The overall objective of this research was to utilize soaked fenugreek seed flour in the development of a nutritious low cost biscuits for nutritional benefit of poor people of Nepal and other developing countries.

For the formulation of recipe, Design Expert v7.1.5 software was used. Seven different biscuit formulations, namely A (100 parts wheat flour), B (0.126 part soaked fenugreek seed flour), C (0.252 part soaked fenugreek seed flour), D (0.5 part soaked fenugreek seed flour), E (0.748 part soaked fenugreek seed flour), F (0.874 part soaked fenugreek seed flour), and G (1 part soaked fenugreek seed flour) were prepared by soft dough process. The other ingredients fat 33 parts, pulverized sugar 35 parts, salt 0.75 part, SMP 4 parts and baking powder 3.25 parts, Flavor 0.5 parts and invert syrup 2 parts were taken constant. The biscuit formulated was of soft dough type. The seven different biscuit samples were prepared and subjected to sensory evaluation. The sensory analysis was carried out based on texture, crispiness, color, taste, flavor and overall acceptance. The data obtained were statistically analyzed using two way ANOVA (no blocking) at 5% level of significance. Sample D (SFSF:WF :: 0.5:99.5) got the highest mean sensory score. The proximate analysis for moisture, crude protein, crude fat, crude fiber and total ash and carbohydrate of raw and soaked fenugreek seed flour were found to be 10.5%, 26.96%, 8.19%, 6.7%, 2.97%, 55.18% and 11.37%, 28.26%, 7.58%, 6.89%, 3.09%, 54.17% respectively. Similarly proximate analysis for moisture, crude protein, crude fat, crude fiber, total ash and carbohydrate of control biscuit and sample C were found to be 2.42%, 9.54%, 20.15%, 0.545%, 1.38%, 68.39% and 2.53%, 10.74%, 20.93%, 0.59%, 1.6%, 66.14% respectively.

Further, RFSF, SFSF, control biscuit and sample D were subjected for Iron, Calcium, DPPH and TPC. The Iron, Calcium, DPPH and TPC in RFSF and SFSF were 10.97 mg/100g, 81.4 mg/100g, 15.28%, 136 mg GAE/g and 9.21 mg/100 g, 73.93 mg/100 g, 40.34%, 155 mg GAE/g respectively. Similarly Iron, Calcium, DPPH and TPC in control biscuit and

sample D were 0.42 mg/100g, 23.21 mg/100g, 22.21%, 80 mg GAE/100g and 25.58 mg/100 g, 26.76%, 87 mg GAE/g respectively. At 5% level of significance the two samples were significantly different from each other. Thus prepared biscuits were packed in LDPE and stored at ambient temperature. These findings suggested that soaked fenugreek seed flour can be successfully incorporated in refined wheat flour up to the concentration of 0.5 part without any adverse effect on sensory attributes giving product especially enriched in fiber, protein, fat, Iron, Calcium and Total Phenolic Content with best acceptability. The cost of the final SFSFIB was found to be NRs. 15.34 per 100 g including overhead cost and profit each of 10%.

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# Appendices

## Appendix A

Sensory evaluation score sheet for biscuit

Date:

Name of panelist:

**Name of the products: Soaked fenugreek seed flour incorporated biscuit**

Dear panelist, you are provided with 7 samples of soaked fenugreek seed flour incorporated biscuit with one control and rest are of varying concentration of soaked fenugreek seed flour content. Please test the following sample of biscuits and check how much you prefer for each of the samples. Give the points for your degree of preferences for each samples as shown below:

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

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

| Sample | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| Color | | | | | | | |
| Crispness | | | | | | | |
| Taste | | | | | | | |
| Texture | | | | | | | |
| Flavor | | | | | | | |
| Overall | | | | | | | |

Any Comments:

Signature:

# Appendix B

**B.1** Chemicals required for this research

- Silver nitrate
- NaOH
- Sulphuric acid
- Boric acid
- Petroleum ether
- Phenolphthalein indicator
- Methylorange indicator
- Hydrochloric acid
- Methylred indicator
- Sodium carbonate
- Potassium permanganate
- Potassium metabisulfite
- Sulfuric acid
- Ethanol
- Acetic acid
- Ammonia
- Ammonium oxalate
- Catalyst mixture
- $FeSO_4(NH_4)SO_4.6H_2O$
- Folin-Ciocalteau reagent
- Bromocresol green
- Methanol
- Oxalic acid
- Potassium persulfate
- Potassium thiocyanide

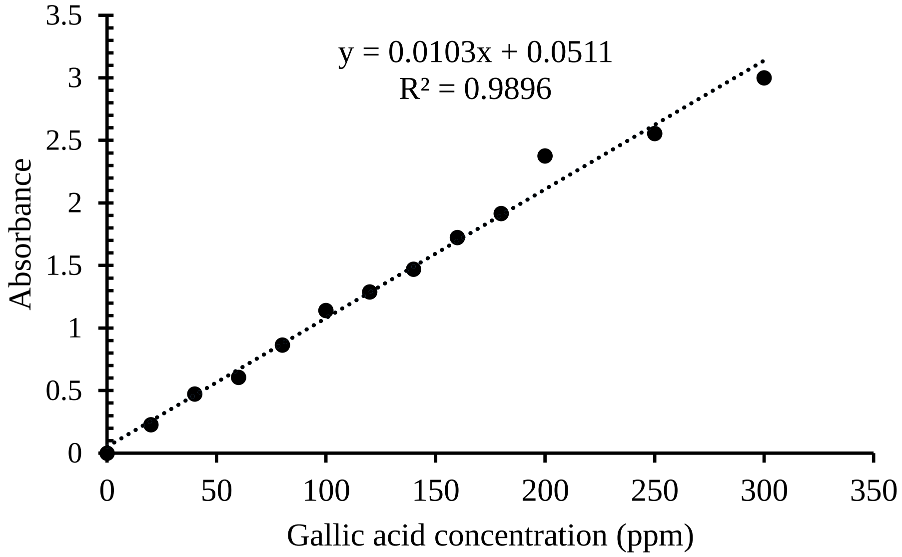
## Appendix C

**C.1** Apparatus required for this research

- Burette
- Conical flask
- Measuring cylinder
- Beaker
- Spectrophotometer
- Pipette
- Cabinet dryer
- Thermometer
- Weighing balance
- Vernier calliper
- Screw gauge
- Hot air oven
- Soxhlet assembly
- Desiccator
- Heating arrangement
- Buchner filter assembly
- Suction pump
- Crucible
- Whatman filter paper
- Muffle furnance
- Silica crucible
- Petridish
- Kjeldahl digestion and distillation set

# Appendix D

**D.1** Standard curve of gallic acid



# Appendix E

## E.1 ANOVA for physical parameters of developed biscuits

**E.1.1** Two way ANOVA (No blocking) for spread ratio

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Sample | 6 | 1.78300 | 0.29717 | 4.29 | <.001 |
| Residual | 14 | 0.96927 | 0.06923 | | |
| Total | 20 | 2.75227 | | | |

**E.1.2** Two way ANOVA (No blocking) for density

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Sample | 6 | 0.06170 | 0.01028 | 0.29 | 0.930 |
| Residual | 14 | 0.48980 | 0.03499 | | |
| Total | 20 | 0.55151 | | | |

# Appendix F

## F.1 ANOVA for sensory characteristics of developed biscuit samples

**F.1.1** Two way ANOVA (No blocking) for texture

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Sample | 6 | 4.0659 | 0.6777 | 1.44 | 0.212 |
| Panelist | 12 | 17.2967 | 1.4414 | 3.06 | 0.002 |
| Residual | 72 | 33.9341 | 0.4713 | | |
| Total | 90 | 55.2967 | | | |

**F.1.2** Two way ANOVA (No blocking) for crispiness

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Sample | 6 | 20.8352 | 3.4725 | 7.00 | <.001 |
| Panelist | 12 | 32.7253 | 2.7271 | 5.49 | <.001 |
| Residual | 72 | 35.7363 | 0.4963 | | |
| Total | 90 | 89.2967 | | | |

**F.1.3** Two way ANOVA (No blocking) for color

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Sample | 6 | 17.6484 | 2.9414 | 2.95 | 0.012 |
| Panelist | 12 | 16.6813 | 1.3901 | 1.39 | 0.189 |
| Residual | 72 | 71.7802 | 0.9969 | | |
| Total | 90 | 106.1099 | | | |

**F.1.4** Two way ANOVA (No blocking) for taste

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Sample | 6 | 11.0330 | 1.8388 | 2.91 | 0.014 |
| Panelist | 12 | 13.3846 | 1.1154 | 1.76 | 0.071 |
| Residual | 72 | 45.5385 | 0.6325 | | |
| Total | 90 | 69.9560 | | | |

**F.1.5** Two way ANOVA (No blocking) for Flavor

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Sample | 6 | 19.4505 | 3.2418 | 6.34 | <.001 |
| Panelist | 12 | 12.2418 | 1.0201 | 1.99 | 0.037 |
| Residual | 72 | 36.8352 | 0.5116 | | |
| Total | 90 | 68.5275 | | | |

**F.1.6** Two way ANOVA (No blocking) for overall acceptability

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Sample | 6 | 10.5275 | 1.7546 | 2.63 | 0.023 |
| Panelist | 12 | 31.0330 | 2.5861 | 3.88 | <.001 |
| Residual | 72 | 48.0440 | 0.6673 | | |
| Total | 90 | 89.6044 | | | |

## Appendix G

**G.1** T-test for chemical parameters of control and superior biscuit samples

**G.1.1** T-test for moisture content

| | Variable 1 | Variable 2 |
|---|---|---|
| Mean | 2.42 | 2.53 |
| Variance | 0.0225 | 0.0361 |
| Observations | 3 | 3 |
| Pearson Correlation | 1 | |
| Hypothesized Mean Difference | 0 | |
| df | 2 | |
| t Stat | -4.763139721 | |
| P(T<=t) one-tail | 0.020680863 | |
| t Critical one-tail | 2.91998558 | |
| P(T<=t) two-tail | 0.041361725 | |
| t Critical two-tail | 4.30265273 | |

**G.1.2** T-test for crude protein

| | Variable 1 | Variable 2 |
|---|---|---|
| Mean | 9.54 | 10.74 |
| Variance | 0.0289 | 0.04 |
| Observations | 3 | 3 |
| Pearson Correlation | 1 | |
| Hypothesized Mean Difference | 0 | |
| Df | 2 | |
| t Stat | -69.2820323 | |
| P(T<=t) one-tail | 0.000104134 | |
| t Critical one-tail | 2.91998558 | |
| P(T<=t) two-tail | 0.000208268 | |
| t Critical two-tail | 4.30265273 | |

**G.1.3** T-test for crude fat

| | Variable 1 | Variable 2 |
|---|---|---|
| Mean | 20.15 | 20.93 |
| Variance | 0.0529 | 0.0361 |
| Observations | 3 | 3 |
| Pearson Correlation | 1 | |
| Hypothesized Mean Difference | 0 | |
| Df | 2 | |
| t Stat | -33.77499075 | |
| P(T<=t) one-tail | 0.000437733 | |
| t Critical one-tail | 2.91998558 | |
| P(T<=t) two-tail | 0.000875465 | |
| t Critical two-tail | 4.30265273 | |

**G.1.4** T-test for crude fiber

| | Variable 1 | Variable 2 |
|---|---|---|
| Mean | 0.545 | 0.59 |
| Variance | 0.0169 | 0.0121 |
| Observations | 3 | 3 |
| Pearson Correlation | 1 | |
| Hypothesized Mean Difference | 0 | |
| df | 2 | |
| t Stat | -3.897114317 | |
| P(T<=t) one-tail | 0.029990329 | |
| t Critical one-tail | 2.91998558 | |
| P(T<=t) two-tail | 0.059980658 | |
| t Critical two-tail | 4.30265273 | |

**G.1.5** T-test for total ash

| | Variable 1 | Variable 2 |
|---|---|---|
| Mean | 1.38 | 1.6 |
| Variance | 0.0081 | 0.0049 |
| Observations | 3 | 3 |
| Pearson Correlation | 1 | |
| Hypothesized Mean Difference | 0 | |
| df | 2 | |
| t Stat | -19.05255888 | |
| P(T<=t) one-tail | 0.001371745 | |
| t Critical one-tail | 2.91998558 | |
| P(T<=t) two-tail | 0.002743489 | |
| t Critical two-tail | 4.30265273 | |

**G.1.6** T-test for carbohydrate

| | Variable 1 | Variable 2 |
|---|---|---|
| Mean | 68.39 | 66.14 |
| Variance | 0.0121 | 0.0169 |
| Observations | 3 | 3 |
| Pearson Correlation | 1 | |
| Hypothesized Mean Difference | 0 | |
| df | 2 | |
| t Stat | 194.8557159 | |
| P(T<=t) one-tail | 1.31682E-05 | |
| t Critical one-tail | 2.91998558 | |
| P(T<=t) two-tail | 2.63364E-05 | |
| t Critical two-tail | 4.30265273 | |

**G.1.7** T-test for calcium

| | Variable 1 | Variable 2 |
|---|---|---|
| Mean | 23.21 | 25.58 |
| Variance | 0.0169 | 0.0441 |
| Observations | 3 | 3 |
| Pearson Correlation | 1 | |
| Hypothesized Mean Difference | 0 | |
| df | 2 | |
| t Stat | -51.31200517 | |
| P(T<=t) one-tail | 0.000189795 | |
| t Critical one-tail | 2.91998558 | |
| P(T<=t) two-tail | 0.00037959 | |
| t Critical two-tail | 4.30265273 | |

**G.1.8** T-test for iron

| | Variable 1 | Variable 2 |
|---|---|---|
| Mean | 0.42 | 0.75 |
| Variance | 0.09 | 0.1225 |
| Observations | 3 | 3 |
| Pearson Correlation | 1 | |
| Hypothesized Mean Difference | 0 | |
| df | 2 | |
| t Stat | -11.43153533 | |
| P(T<=t) one-tail | 0.003782775 | |
| t Critical one-tail | 2.91998558 | |
| P(T<=t) two-tail | 0.00756555 | |
| t Critical two-tail | 4.30265273 | |

**G.1.9** T-test for DPPH

| | Variable 1 | Variable 2 |
|---|---|---|
| Mean | 22.21 | 26.67 |
| Variance | 1.2769 | 2.4964 |
| Observations | 3 | 3 |
| Pearson Correlation | 1 | |
| Hypothesized Mean Difference | 0 | |
| df | 2 | |
| t Stat | -17.166548 | |
| P(T<=t) one-tail | 0.001688108 | |
| t Critical one-tail | 2.91998558 | |
| P(T<=t) two-tail | 0.003376217 | |
| t Critical two-tail | 4.30265273 | |

**G.1.10** T-test for TPC

| | Variable 1 | Variable 2 |
|---|---|---|
| Mean | 87 | 80 |
| Variance | 5.4756 | 3.2041 |
| Observations | 3 | 3 |
| Pearson Correlation | 1 | |
| Hypothesized Mean Difference | 0 | |
| Df | 2 | |
| t Stat | 22.04428301 | |
| P(T<=t) one-tail | 0.001025746 | |
| t Critical one-tail | 2.91998558 | |
| P(T<=t) two-tail | 0.002051493 | |
| t Critical two-tail | 4.30265273 | |

# Appendix H

**H.1** T-test for mineral content and antioxidant activity of raw and soaked fenugreek seed flour

**H.1.1** T-test for iron

| | Variable 1 | Variable 2 |
|---|---|---|
| Mean | 10.97 | 9.21 |
| Variance | 0.7921 | 0.4225 |
| Observations | 3 | 3 |
| Pearson Correlation | 1 | |
| Hypothesized Mean Difference | 0 | |
| Df | 2 | |
| t Stat | 12.70170592 | |
| P(T<=t) one-tail | 0.003070653 | |
| t Critical one-tail | 2.91998558 | |
| P(T<=t) two-tail | 0.006141307 | |
| t Critical two-tail | 4.30265273 | |

**H.1.2** T-test for calcium

| | Variable 1 | Variable 2 |
|---|---|---|
| Mean | 81.4 | 73.93 |
| Variance | 0.3364 | 0.6084 |
| Observations | 3 | 3 |
| Pearson Correlation | 1 | |
| Hypothesized Mean Difference | 0 | |
| Df | 2 | |
| t Stat | 64.69209766 | |
| P(T<=t) one-tail | 0.00011943 | |
| t Critical one-tail | 2.91998558 | |
| P(T<=t) two-tail | 0.000238859 | |
| t Critical two-tail | 4.30265273 | |

**H.1.3** T-test for DPPH

| | Variable 1 | Variable 2 |
|---|---|---|
| Mean | 15.28 | 40.34 |
| Variance | 0.7921 | 0.8649 |
| Observations | 3 | 3 |
| Pearson Correlation | 1 | |
| Hypothesized Mean Difference | 0 | |
| Df | 2 | |
| t Stat | -1085.129831 | |
| P(T<=t) one-tail | 4.24625E-07 | |
| t Critical one-tail | 2.91998558 | |
| P(T<=t) two-tail | 8.49251E-07 | |
| t Critical two-tail | 4.30265273 | |

**H.1.4** T-test for TPC

| | Variable 1 | Variable 2 |
|---|---|---|
| Mean | 136 | 155 |
| Variance | 8 | 18 |
| Observations | 2 | 2 |
| Pearson Correlation | 1 | |
| Hypothesized Mean Difference | 0 | |
| df | 1 | |
| t Stat | -19 | |
| P(T<=t) one-tail | 0.016738 | |
| t Critical one-tail | 6.313752 | |
| P(T<=t) two-tail | 0.033475 | |
| t Critical two-tail | 12.7062 | |

# Appendix I

**I.1** T-test for chemical composition of raw and soaked fenugreek seed flour

**I.1.1** T-test for moisture content

| | Variable 1 | Variable 2 |
|---|---|---|
| Mean | 10.5 | 11.37 |
| Variance | 0.2738 | 0.5832 |
| Observations | 2 | 2 |
| Pearson Correlation | 1 | |
| Hypothesized Mean Difference | 0 | |
| Df | 1 | |
| t Stat | -5.11765 | |
| P(T<=t) one-tail | 0.061425 | |
| t Critical one-tail | 6.313752 | |
| P(T<=t) two-tail | 0.122849 | |
| t Critical two-tail | 12.706204 | |

**I.1.2** T-test for crude protein

| | Variable 1 | Variable 2 |
|---|---|---|
| Mean | 26.96 | 28.26 |
| Variance | 0.2888 | 0.0338 |
| Observations | 2 | 2 |
| Pearson Correlation | 1 | |
| Hypothesized Mean Difference | 0 | |
| Df | 1 | |
| t Stat | -5.2 | |
| P(T<=t) one-tail | 0.060475 | |
| t Critical one-tail | 6.313751 | |
| P(T<=t) two-tail | 0.1209503 | |
| t Critical two-tail | 12.706204 | |

**I.1.3** T-test for crude fat

| | Variable 1 | Variable 2 |
|---|---|---|
| Mean | 8.19 | 7.58 |
| Variance | 0.0512 | 0.0882 |
| Observations | 2 | 2 |
| Pearson Correlation | 1 | |
| Hypothesized Mean Difference | 0 | |
| Df | 1 | |
| t Stat | 12.2 | |
| P(T<=t) one-tail | 0.026032 | |
| t Critical one-tail | 6.313752 | |
| P(T<=t) two-tail | 0.05206555 | |
| t Critical two-tail | 12.7062 | |

**I.1.4** T-test for crude fiber

| | Variable 1 | Variable 2 |
|---|---|---|
| Mean | 6.7 | 6.89 |
| Variance | 0.0647 | 0.18 |
| Observations | 2 | 2 |
| Pearson Correlation | 1 | |
| Hypothesized Mean Difference | 0 | |
| Df | 1 | |
| t Stat | -1.58333 | |
| P(T<=t) one-tail | 0.179309 | |
| t Critical one-tail | 6.313751 | |
| P(T<=t) two-tail | 0.35861 | |
| t Critical two-tail | 12.7062 | |

**I.1.5** T-test for ash

| | Variable 1 | Variable 2 |
|---|---|---|
| Mean | 2.97 | 3.09 |
| Variance | 0.005 | 0.0338 |
| Observations | 2 | 2 |
| Pearson Correlation | 1 | |
| Hypothesized Mean Difference | 0 | |
| Df | 1 | |
| t Stat | -1.5 | |
| P(T<=t) one-tail | 0.187167 | |
| t Critical one-tail | 6.313751 | |
| P(T<=t) two-tail | 0.374334 | |
| t Critical two-tail | 12.7062 | |

**I.1.6** T-test for carbohydrate

| | Variable 1 | Variable 2 |
|---|---|---|
| Mean | 55.18 | 54.15 |
| Variance | 0.5929 | 0.5776 |
| Observations | 3 | 3 |
| Pearson Correlation | 0.9997 | |
| Hypothesized Mean Difference | 0 | |
| Df | 2 | |
| t Stat | 154 | |
| P(T<=t) one-tail | 2.1E-05 | |
| t Critical one-tail | 2.9199 | |
| P(T<=t) two-tail | 4.2E-05 | |
| t Critical two-tail | 4.30265 | |

## Appendix J

**Table J.1** Physical parameters of developed biscuits

| Samples | Thickness (mm) | Diameter (mm) | Spread ratio | Weight (g) | Volume ($cm^3$) | Density (g/ $cm^{3)}$ |
|---|---|---|---|---|---|---|
| F | $8.7^{de}$±0.02 | $53.29^{b}$±0.3 | $6.15^{a}$±0.02 | $12.88^{d}$±0.1 | $19.31^{a}$±0.4 | $0.67^{a}$±0.05 |
| E | $8.56^{d}$±0.03 | $53.63^{c}$±0.2 | $6.2^{ab}$±0.02 | $11.32^{c}$±0.2 | $19.33^{a}$±0.1 | $0.59^{a}$±0.04 |
| D | $8.42^{c}$±0.01 | $53.95^{d}$±0.4 | $6.4^{ab}$±0.01 | $10.34^{a}$±0.3 | $19.24^{a}$±0.3 | $0.54^{a}$±0.03 |
| G | $8.70^{e}$±0.01 | $53.02^{a}$±0.3 | $6.09^{a}$±0.07 | $13.20^{d}$±0.2 | $19.20^{a}$±0.5 | $0.69^{a}$±0.06 |
| B | $8.13^{a}$±0.01 | $54.82^{f}$±0.3 | $6.7^{ab}$±0.09 | $10.6^{ab}$±0.3 | $19.18^{a}$±0.2 | $0.55^{a}$±0.01 |
| C | $8.28^{b}$±0.02 | $54.29^{b}$±0.1 | $6.5^{ab}$±0.01 | $11.3^{c}$±0.2 | $19.16^{a}$±0.1 | $0.59^{a}$±0.02 |
| A | $8.04^{a}$±0.01 | $55.86^{g}$±0.2 | $6.94^{b}$±0.01 | $10.9^{bc}$±0.2 | $19.70^{a}$±0.3 | $0.56^{a}$±0.00 |

*Values are the means of three determinations ± standard deviations.

## Appendix K

**Table K.1** Summary of ANOVA of sensory evaluation of soaked fenugreek seed flour incorporated biscuit

| Sample | Color | Crispiness | Flavor | Taste | Texture | Overall |
|---|---|---|---|---|---|---|
| F | $7.15^{ab}\pm0.1$ | $7.85^{b}\pm0.8$ | $6.77^{ab}\pm0.8$ | $6.62^{a}\pm0.5$ | $7.15^{a}\pm1$ | $6.85^{ab}\pm1$ |
| E | $7.85^{b}\pm0.6$ | $7.85^{b}\pm0.7$ | $7.46^{bc}\pm0.5$ | $7^{ab}\pm0.6$ | $7.31^{a}\pm0.8$ | $7.31^{ab}\pm0.9$ |
| D | $7.38^{ab}\pm1.3$ | $7.62^{b}\pm0.8$ | $7.77^{c}\pm0.7$ | $7.69^{b}\pm0.9$ | $7.77^{a}\pm0.6$ | $7.69^{b}\pm1.2$ |
| G | $6.38^{a}\pm0.7$ | $7.31^{b}\pm1$ | $6.31^{a}\pm0.6$ | $6.54^{a}\pm0.7$ | $7.08^{a}\pm0.5$ | $6.62^{a}\pm0.5$ |
| B | $7.62^{b}\pm0.9$ | $7.08^{ab}\pm1.1$ | $7.54^{bc}\pm0.5$ | $7^{ab}\pm0.8$ | $7.38^{a}\pm0.8$ | $7.46^{ab}\pm0.9$ |
| C | $7.31^{ab}\pm1.5$ | $7.54^{b}\pm1$ | $7.23^{bc}\pm1.2$ | $7.08^{ab}\pm1.2$ | $7.46^{a}\pm0.9$ | $7.31^{ab}\pm1.4$ |
| A | $7.62^{b}\pm0.9$ | $6.38^{a}\pm0.8$ | $7.08^{abc}\pm0.6$ | $6.92^{ab}\pm1$ | $7.46^{a}\pm0.9$ | $7.31^{ab}\pm0.6$ |

# Appendix L

**J.1** Cost calculation of product (SFSFIB)

| Particulars | Cost (NRs/kg) | Weight in a lot (g) | Cost (NRs) |
|---|---|---|---|
| Wheat flour | 50 | 99.5 | 4.975 |
| Fenugreek seed flour | 400 | 0.5 | 0.2 |
| Sugar | 80 | 35 | 2.8 |
| Fat | 310 | 33 | 10.23 |
| SMP | 480 | 4 | 1.92 |
| Salt | 20 | 0.75 | 0.015 |
| Baking powder | 125 | 3.25 | 0.40625 |
| Flavor | 1790 | 0.5 | 0.895 |
| Invert syrup | 600 | 2 | 1.2 |
| Raw material cost | | | 22.64125 |
| Processing and labor cost (10% of raw material cost) | | | 2.264125 |
| Profit (10%) | | | 2.4905375 |
| Grand total Cost | | | 27.395913 |
| Average weight of SFSFIB (g) | | 10.34 | |
| Total no. of SFSFIB formed | | 17 | |
| Total weight of SFSFIB (g) | | 178.5 | |
| Total cost of SFSFIB (NRs/100g) | | | 15.34 |

**Photo gallery**

**Plate 1**: Soaked and dried fenugreek seed

**Plate 2**: Sheeting and cutting of biscuit

**Plate 3**:  Preparation for baking of biscuit

Plate 4: Baking of biscuit

# Photo gallery

**Plate 5**: Sensory evaluation of soaked fenugreek flour incorporated biscuit