STUDY ON THE EFFECT OF INCORPORATION OF NAKED BARLEY FLOUR ON THE PHYTOCHEMICAL, NUTRITIONAL AND SENSORY PROPERTIES OF BISCUITS

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Approval Letter

This *dissertation* entitled *Study on the effect of incorporation of Naked barley flour on the phytochemical, nutritional and sensory properties of biscuits* presented by Samita Limbu has been accepted as the partial fulfilment of the requirement for the B. Tech. degree in Food Technology.

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Abstract

Naked barley was collected from Armala VDC, Kaski, Nepal. Naked barley is an underutilized crop that is suitable for the production of functional food. Refined wheat flour and Naked barley flour blends at varying levels were prepared and subjected for baking to prepare biscuits. Effect of addition of naked barley flour at different proportions (0%, 6.25%, 8%, 12.5%, 17%, 18.25% and 25%) on nutritional and phytochemical properties were studied by proximate analysis, Folin-Coicalteu method for TPC, modified aluminium chloride assay method for TFC and colorimetric DPPH assay for antioxidant activity.

Proximate analysis of the refined wheat flour and naked barley flour showed that naked barley flour is superior in protein, fat, fiber and ash content in comparison to wheat flour. Phytochemical analysis showed a significant increase in TPC, TFC and antioxidant capacity of the biscuits as the percentage of naked barley flour increased in the wheat biscuits. As the properties were also studied for the biscuit dough, baking process resulted in significant decrease in TPC and TFC while increase in antioxidant capacity. The sensory analysis of the all the samples of biscuits were carried out where samples showed significant differences at 5% level of significance in sensory attributes. From the statistical analysis, sample F (18.75% naked barley fraction) was selected as the best formulation and thus subjected for proximate analysis comparing it with control biscuit. The moisture, protein, crude fiber, fat and ash content is significantly higher in the naked barley incorporated biscuits while carbohydrate content is less. Results showed that naked barley flour can be incorporated successfully up to the concentration of 18.75% with better nutritional and phytochemical properties.

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List of Abbreviations

Abbreviations	Full form
EFSA	European Food Safety Authority
ANOVA	Analysis Of Variance
LDL	Low-density lipoprotein
WHO	World Health Organization
CVD	Cardiovascular Disease
FCMP	Full Cream Milk Powder
SMP	Skimmed Milk Powder
LSD	Least Significant Difference
SMS	Sodium metabisulphite
GRAS	Generally Recognized as Safe
FDA	Food and Drug Administration
NBS	Nepal Bureau of Standards
GMS	Glycerol monostearate
BHA	Butylated Hydroxyanisole
ABC	Ammonium bicarbonate
SBC	Sodium bicarbonate
TPC	Total Phenolic Content
FA	Ferulic acid
GBF	Germinated Barley Foodstuff
NARC	Nepal Agricultural Research Council
GAE	Gallic Acid Equivalent
DPPH	2,2-diphenyl-1-picrylhydrazyl
TFC	Total Flavonoid Content

Part I

Introduction

1.1 General introduction

Baking technique is one of the earliest and oldest techniques in the food processing field. In Nepal bakery industry is one of the major food processing industries (Shrestha, 1995). Bakery products have a diverse group of biscuits varieties ranging from high to low fat, high to low sugar and more other combinations. Nowadays, biscuits are the most popular amongst consumers as a better source of carbohydrates, proteins, dietary fibers along with quite good shelf life and serve as ready-to-eat product. Mostly biscuits are made with refined wheat flour which is deficient in some essential amino acids and other nutrients and completely lack of essential fibers. Therefore, to enhance the nutritional value of biscuits they can be fortified with wide varieties of cereals which make them fiber rich and beneficial to health (Kaur *et al.*, 2017).

Hulless barley is a good source of dietary fibre providing soluble and insoluble dietary fibre-fractions. Mixed-linkage (1→3)(1→4)-β-D-glucans(β-glucan) are a major part of the soluble dietary fibre (SDF) in barley. In Europe, the European Food Safety Authority (EFSA) has come to the decision that a claim like “regular consumption of β-glucans contributes to maintenance of normal blood cholesterol concentrations” will be allowed. Investigating the effects of non-processed or minimally processed barley beta-glucans at doses of at least 3g/d have shown a statistically significant decrease in LDL-cholesterol in both normocholesterolaemic and hypercholesterolaemic subjects. Beta-glucans occur naturally in the bran of cereal grasses such as barley (~7%), oats (~5%), rye and wheat (1-2%). The cholesterol-lowering effect of beta- glucan depends on the increased viscosity that reduces the reabsorption of bile acids, increases the synthesis of bile acids from cholesterol, and reduces circulating (LDL) cholesterol concentrations. Until then, the interest of food producers and consumers in using hulless barley for food purposes may increase, although up to now, barley flour is hardly used for human consumption (EFSA, 2009b).

The higher the β-glucan content in barley flours the worse are the baking qualities, due to the high water binding capacity and thus decreasing the water availability for the gluten network (Siebenhandl-Ehn *et al.*, 2011).

1.2 Statement of problem

Today many researches regarding functional foods have been constantly done worldwide. In order to uplift the health status of people around the world the technology of incorporating functional ingredients in our daily diet has been initiated (Roberfroid, 2001). A study of prevalence of cardiovascular diseases risk factors conducted in Kathmandu (Dhungana *et al.*, 2018) shows that diet is the most dominant risk factor causing 98% of the CVD. Thus Nepal should focus on improving the dietary behavior of people.

Considering the benefits of Naked barley it can be incorporated in biscuits which are consumed in large amount in our daily lives. It can be an effective way to supply bioactive compounds and antioxidants in peoples' diet and the occurrence of cardiovascular diseases as well as age-related neurological dysfunctions can be reduced (Youdim and Joseph, 2001). As its hull freely threshes out it does not need much processing and is thus suitable for using in food. Further due to the presence of β-glucan, its baking properties are poor thus demanding a study on the optimization of the percentage of incorporation of naked barley flour in biscuits. Limited studies have been reported on cookie making behavior of whole naked barley flour and effects of baking on antioxidant properties (Siebenhandl-Ehn *et al.*, 2011).

1.3 Objectives of the study

1.3.1 General objective

The general objective of this work is to study the effect of incorporation of Naked Barley flour on the nutritional, phytochemical and antioxidant properties of wheat flour biscuits.

1.3.2 Specific objectives

The specific objectives of the study are:

- To determine and compare the proximate composition of wheat and Naked barley flour.

- To determine and compare the proximate composition of Wheat flour biscuits and Naked barley flour incorporated biscuits.
- To determine and compare the phytochemical and antioxidant properties of the formulated cookie doughs and the biscuits.
- To study the effect of naked barley flour incorporation on the sensory properties of biscuits.

1.4 Significance of the study

Today people are being more aware of health and the concept of functional food is growing. According to the concept, functional foods are the foods in our daily diets supplied with functional ingredients which can prevent nutrition related diseases (Roberfroid, 2001). The advantages of obtaining phenolic compounds from cereals rather than fruits and vegetables are that cereals are dry, easy to store and to process into shelf-stable products for a long duration. The current consumer interest in nutrition and health gives room for research in barley and potential benefits in human diet (Hambira, 2009). Replacing wheat flour with barley flour will definitely dilute the wheat gluten proteins but since high gluten content is not a requirement for biscuits, barley flour could be a potential raw material for cookie making having improved bioactive value (P. Sharma and Gujral, 2014).

Barley features high protein, high fiber, high vitamin, low fat, and low sugar when compared with other crops. It has higher amount of phenolic compounds and antioxidant activity as compared to the more widely consumed cereals wheat and rice. The risk imposed by the consumption of free radicals and oxidation products could be lowered by the intake of dietary phenolics (P. Sharma and Gujral, 2014). Barley outperforms other cereals under various environmental stresses due to its winter-hardy, drought-resistant, and early maturing nature and is thus generally more economical to cultivate (Cook, 2013). The study on hulless variety of barley is very less worldwide. Hulless barley can be more useful in baked products as it doesn't need dehulling for making flour (Siebenhandl-Ehn *et al.*, 2011).

1.5 Limitation of the study

- β-glucan content of naked barley flour couldn't be determined due to infeasibility in the laboratory.

Part II

Literature review

2.1 History and position of biscuits

The word biscuit derives from *panis biscoctus* which is Latin for twice-cooked bread and refers to bread rusks that were made for mariners (ships biscuits) from as long ago as the Middle Ages. The dough pieces were baked and then dried out in another, cooler, oven. They were very unattractive being made more or less from flour and water. It all started in Britain and many biscuit types that were first developed and produced in Britain are still made and enjoyed all round the world. Britain led the industrial revolution which involved the design and construction of machines and can thereby also claim to be a leader in developing the biscuit industry. Biscuits are a very significant part of the food industry in most countries of the world. Their success can be attributed to at least four key factors (Manley, 2011a):

1. their relatively long shelf life
2. their great convenience as food products
3. the human liking and weakness for sugar and chocolate
4. their relatively good value for money.

The word biscuit in the English language is certainly old. Dr Samuel Johnson in his dictionary, published in 1755, gives a primary definition as ‘a kind of hard dry bread, made to be carried to sea’, and a secondary one of ‘a composition of fine flour, almonds and sugar, made by the confectioners’.

According to Manley (2011a), at the end of the eighteenth century there are reports that dough mixing was done initially by hand then was finished off by the mixerman jumping into the trough and treading it with his bare feet! A certain amount of mechanisation was introduced to form a rough sheet of dough but the pieces were then cut out by hand as rectangles which were in turn worked by hand into circles and dockered (holes pierced through the dough piece) before baking. The sheeting machine was known as a brake. The early ovens were fi red by coal but the travelling ovens were firstly heated with superheated steam through tubes running along the length of the oven. Later ovens were fired directly with gas and electrically heated ovens appeared much later. Some of the earliest biscuits took the form of various fermented crackers such as cream crackers and soda crackers. The digestive biscuit was introduced by Alexander Grant in 1892. In 1898, Huntley and Palmer, then the largest biscuit manufacturer in the world, was producing about 400 varieties of biscuit. The surprising point is that many of the most popular biscuits today were being sold over 100 years ago (Muir, 1968).

2.2 Classification of biscuits

It is generally recognized that biscuits are cereal based and baked to a moisture content of less than 5%. The cereal component is variously enriched with two major ingredients, fat and sugar, but thereafter the composition is almost endless. The name ‘cookie’ can be regarded as synonymous with biscuit but the former is more comprehensive in meaning in the USA and the latter in the UK. Groupings have been made in various ways based on (Manley, 2001):

- the name, e.g., biscuits, crackers and biscuits, which are based on the texture and hardness
- the method of forming of the dough and dough piece, e.g., fermented, developed, laminated, cut (simple or embossing, moulded, extruded, deposited, wire cut, coextruded, etc.
- the enrichment of the recipe with fat and sugar (hard and short dough)

2.3 Short dough biscuits

Short dough biscuits are distinguished from others in that they are made from dough that lacks extensibility and elasticity. Wheat flour or some other farinaceous material is the major ingredient but the quantities of fat and sugar present in the dough create a plasticity and cohesiveness of the dough with minimal formation of gluten network and the role of gluten is minimum in this kind of biscuits (Flint *et al.*, 1970). Thus, low gluten flour is used generally less than 8%. They contain higher fat content (25-35%) and sugar (30-45%) and are low in moisture. The higher gluten content should be avoided which can be achieved by:

a) Weak flour
b) Lower moisture content
c) Short mixing time
d) Less aerating agents (Joshi, 2012)

Therefore the challenge is to achieve adequate dispersion of ingredients with minimum kneading. (Flint *et al.*, 1970) has stated that there is no continuous protein matrix (at least in those made from good quality soft wheat flour) and that the fat is present in the form of large globules or of larger interconnected masses between the starch-protein masses. The texture is typically relatively coarse as there is much coalescence of the gas bubbles that form during the baking. The features of the doughs of this group result in biscuits which tend to become larger in width and length as they bake rather than shrink as is the case for crackers and semi-sweet types. Sales of biscuits included in this broad group far exceed all others in the markets of developed countries (Manley *et al.*, 2011).

2.4 Raw materials for biscuit making

Raw materials for biscuit making can be divided into major and minor ingredients. Those which are used in large amounts and are the basic components of biscuit making are called major ingredients i.e., flour, sweetening agents and fat. They are mixed with other minor ingredients (such as baking powder, skimmed milk, emulsifier, and sodium metabisulphite, sodium bicarbonate, ammonium bicarbonate, colouring agents and flavouring agents) to form dough containing a well-developed gluten network. The nature and quantity of ingredients in the dough determine the quality of the biscuit (Mamat and Hill, 2018).

2.4.1 Major Ingredients

2.4.1.1 Wheat flour

Wheat flour is the principal component of nearly all biscuits and major advances in technology of flour milling coincided with the development of biscuit manufacturing. Of all the cultivated cereals, flour from wheat is almost unique in that it forms a dough. The reserve proteins from wheat, i.e., the gluten proteins, are responsible for the dough forming capacity of wheat flour. Gluten permits the retention of gas bubbles during baking of a dough to give open textured and pleasant eating products (Delcour *et al.*, 2011).

Milling wheat can be described as hard, medium or soft, based on the physical characteristics of the wheat grain. Hard types tend to have high protein contents (10–14%). Soft wheats produce a fluffier type of flour with less damaged starch and with lower water absorption. Their protein levels are typically low or very low (8–11%) and their protein gives gluten that is less elastic and resistant to deformation and more extensible before being broken thus mostly used for biscuit. The position of medium wheat is evidently intermediate (Delcour *et al.*, 2011) .

2.4.1.1.1 Particle size of flour

As stated by (Delcour *et al.*, 2011) the finer the particles, the higher will be the specific surface area and therefore the higher water absorption. As biscuits are baked to low moisture levels, it can be assumed that less water in dough will save energy during baking. However, the effects are different for different type of biscuits. Finer flour for hard doughs tends to give biscuits with a higher density and less development during baking, whereas finer flour for short doughs gives biscuits with lower density, more development during baking and less spread on the oven band. The mean particle size for most biscuit flours is around 50 μm with less than 10% more than 130 μm (Delcour *et al.*, 2011).

2.4.1.1.2 Starch

Starch is the most abundant component of wheat flour (*ca.* 70–75%). Starch has an important role for the texture of biscuits and is the major structural element. In dough and finished products, starch acts as filler held in the matrix provided by the other material present. The structural changes that occur during gelatinization of starch is an important phenomenon and include changes of shape and size of the granules (swelling) , water absorption, melting of the crystallites and the progressive solubilisation of the starch (H. Jacobs and Delcour, 1998).

It has been found that in the process of milling, as the endosperm is fractured and then crushed, some of the starch granules are physically damaged, losing crystallinity order. This has a profound effect on the water absorption property of the flour when dough is made as the power of damaged starch to absorb water is about three times that of undamaged granules. In soft wheat flour, damaged starch is typically below 3%. For biscuits, since the finished product must be almost completely dry, the amount of water used to make the dough should be a minimum, so flours of low water absorption and hence low starch damage (i.e. soft flour) are to be favoured (Mamat and Hill, 2018).

2.4.1.1.3 Protein

The gluten proteins (~80–85% of total wheat protein) are the major storage proteins of wheat: Gliadins and Glutenins. They show very low solubilities in water or dilute salt solutions, due to their low levels of amino acids with ionisable (charged) side chains and high hydrophobicity (Lafiandra *et al.,* 2001). Glutenin gives solidity to the product while gliadin is the binding agent imparting the soft sticky character to the gluten (Kent and Evers, 1994).

Specification given by East African Standard for ‘Biscuit flour’ comprising of white wheat flour obtained by milling a blend of hard and soft wheat with a high percentage of soft wheat for biscuit manufacture is given in Table 2.1.

Table 2.1 Flour specification for biscuit manufacture

S.No.	Characteristics	Requirements
1.	Moisture content, max. %m/m	13
2.	Fibre content, max. %m/m	1.0
3.	Total ash content, max. %m/m	0.55
4.	Residue on sieving through 180-micron sieve, max.%	0.50
5.	Protein content, min. %m/m (N×5.7)	8.0

Source: (EAS, 2011)

The specification for different types of flour is given in Table 2.2.

Table 2.2. Typical specifications for different types of flour

Property	Soft flour (%)	Medium flour (%)	Strong flour (%)
Protein	8.0	10.3	13.2
Wet gluten	25.0	26.0	31.0
Fat	0.0	1.0	2.4
Carbohydrate	80.0	76.3	66.9
Ash	0.3	0.5	0.5
Water absorption	53.0	58.0	60.0

Source: (I. Davidson, 2016a)

2.4.1.2 Fat as a shortening agent

Fats and oils are essential ingredients in nearly all bakery products. Fats are esters of fatty acids and glycerol that form triglycerides in which three fatty acids are attached to a glycerol molecule. The common fats used in bakery products are lard, beef fats, and hydrogenated vegetable oils. The variation in the fat properties such as its melting point and solid fat content has a profound effect on its physical form (Mamat and Hill, 2018).

Fat gives typical ‘melt in the mouth’, crumbly texture which is characteristic of biscuits. During the mixing of the dough the fat will form a coating around the flour to limit the water in the mix from coming into contact with the flour. This is vitally important in a biscuit because when water comes into contact with flour along with the action of mixing which adds energy, the gluten (protein) in the flour is allowed to ‘develop’, i.e. become more extensible and strengthen the dough (Atkinson, 2011). Fat also influences the dough machinability during processing, the dough spread after cutting-out, and textural and gustatory qualities of the biscuit after baking (Mamat and Hill, 2018).

A secondary function of adding fat to the dough is its ability to coat any small air bubbles which may be present which in turn makes the bubble structure more stable preventing the bubbles rupturing and coalescing into larger bubbles. In this function of coating air bubbles, a fat with a developed crystalline structure is more effective than a liquid oil and to achieve this in highly automated biscuit plants (Atkinson, 2011).

Shortenings are meant to be soft fats that produce a crisp, flaky effect in baked products. Unlike oils, shortenings are plastic and dispense as a film through the batter and prevent the formation of a hard, tough mass. The functions induced by shortenings include: tenderization; mouth feel; structural integrity; lubrication; air incorporation; heat transfer; and shelf life extension (Sarba *et al.*, 2015). Liquid fat is unsuitable for baking products. It is 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 (Smith, 1972). Research by Sarba *et al.* (2015) suggests Solid fat content (SFC) in shortenings in ranges of 15-25% at usage temperature (23°) is recommended for shortening to have excellent baking performance.

Although simple fats are widely used and perform well in many applications, the lack of naturally occurring high melting point fat or fats with particular melting characteristics is a problem. However, a number of techniques have been developed over the years to modify the commonly occurring oils and fats to give the physical properties needed for the specialist applications. The first of these techniques which has been in use for well over 100 years now is known as hydrogenation. This process takes a low melting point oil or fat and increases the melting point by converting some or all of the unsaturated fat into saturated fat and as the degree of unsaturation decreases the melting point increases. If the product is only partially hydrogenated, then this is the scenario that can give rise to the presence of trans fats which is an emerging health threat (Atkinson, 2011).

In order to obtain the best product, the hydrogenated vegetable oil to be used in biscuit making should possess the following properties:

1. It should possess good white to creamy color. After keeping the fat at 50°C for 24 hours and filtering, its color should be comparable with the control sample of oil.
2. It should have a smooth, uniform texture, free from any oil separation and large grains.
3. The fat should have a bland odour and taste.
4. The fat should have a wide plastic range to suit particular production techniques and the product.
5. The crystalline structure of fat should be stable during mixing and after baking.

6. The fat should possess reasonable shelf life on its own without the addition of antioxidants.
7. The fat should be preparing from the blend of oils, which will not cause fat bloom during the storage of biscuits.

Source: (Mukhopadhyay, 1990)

Fat must be refined to remove any physical and chemical impurities along with unwanted colours and flavours. It is mainly done in two steps: degumming and removal of free fatty acids. Bleaching and deodorizing is also done (Atkinson, 2011).

2.4.1.2 Sweetening agents

Sucrose is a disaccharide. It plays an important role in the baking process. However, sucrose is rarely used as the sole sugar in biscuit recipe. Relatively small amounts of reducing sugars, typically 10-20% by weight of the total sugars, are added to most recipes. These reducing sugars are usually added in the form of syrups containing 70- 80% dry solids. Sugar, depending on the level and type, influences the various rheological characteristics of biscuit dough (Mamat and Hill, 2018).

Sugar has many functions in biscuit making and can be listed as below:

1. Sucrose in biscuit doughs dissolves, or partially dissolves depending on the amount of water present, and then recrystallizes or forms an amorphous glass (a supercooled liquid after baking). In this way it strongly affects the texture of the baked biscuit. The size of the sucrose crystals, and therefore their rate of dissolution as the dough piece warms in the oven, affects the spread of short doughs as they bake and affects the appearance and crunchiness of the baked biscuit (Manley, 2011c).
2. Sugar inhibits gluten development during dough mixing by competing with the flour for the recipe water, resulting in less tough and more crumbly biscuits (Mamat and Hill, 2018).
3. As sucrose dissolves during the mixing and baking, it contributes to the liquid phase of the dough to the point where the sucrose solution is saturated, the amount of sucrose depresses the amount of water needed in the dough. Sucrose shifts the starch gelatinisation point to a higher temperature thus allowing the dough to have

more time to rise in the oven (Manley, 2011c). A study by (Spies and Hoseney, 1982) shows that two independent mechanisms were proposed according to the results obtained; 1) the water activity lowering effect (antiplasticiser), and 2) interaction of sugars with starch chains that stabilise the amorphous regions in starch, thus increasing the energy required to gelatinise the starch.

4. Reducing sugars combine with amino acids (from protein) in the Maillard reaction which occurs during baking, and which is the way in which dark and attractive surface colours are formed.
5. Syrups are also used as humectants (materials that prevent the loss of water from food) and, as such, prevent the baked textures being too hard and brittle.
6. Sugars are readily absorbed in the human gut and are valuable sources of energy in our food.
7. In its various crystal forms and sizes sucrose can be used as a surface decoration for biscuits. In certain cases a surface dusting of sucrose will melt during baking, cooling afterwards as a glass thus giving an attractive gloss or glaze (Manley, 2011c).
8. Sucrose contributes to longer shelf life by retarding fat rancidity (Mamat and Hill, 2018).

2.4.2 Minor Ingredients

2.4.2.1 Emulsifiers

Emulsifiers, when added to a foodstuff, make it possible to form or maintain a uniform dispersion of two or more immiscible substances or to aid the wetting (film formation) of a liquid over a solid. Emulsifiers are used to reduce interfacial tension to ensure a better dispersion of the dispersed phase and to achieve greater stability. Emulsifiers are polar lipids; they have two parts: the polar region which will be attracted to the water phase, the hydrophilic part, and the non-polar which is attracted to the fatty or oil phase, the lipophilic part. All these surfactants are effective at very low levels (less than 2% of the recipe weight) so are classed as minor ingredients or food additives.

Fats in biscuits reduce the hardness by interrupting the gluten structure in the dough. By using small amounts of emulsifier the fat phase is spread more uniformly over the hydrophilic ingredients such as flour, sugar, etc., in the dough. The fat phase is thus more effective if it tends to be in films or very small droplets rather than globules. By attention to the amounts and types of emulsifier, it has been possible to reduce the fat content and still have acceptable properties of biscuits. (Manley, 2011b).

Lecithin is a natural food substance which occurs in all living matter but is found in significant quantities in egg yolk (8–10%) and soya beans (2.5%). Commercial lecithin is almost entirely of soya origin because of the cost. The average composition of the main ingredients as given by (Minifie, 1989):

Table 2.3 Chemical composition of commercial lecithin

Ingredients	Composition (%)
Soya bean oil	35
Chemical lecithin (phosphatidyl choline)	18
Cephalin (phosphatidyl ethanolamine)	15
Inositol phosphatides	11
Other phospholipids and polar lipids	9
Carbohydrates (sterol, glucoside)	12

Source: Minifie (1980)

Commercial lecithin is a fluid or a plastic paste which, if used in excess, imparts unpleasant flavours. The usage rates may be expressed relative to the weight of flour (normally between 0.5 and 1.0%) or the weight of fat (upto 2%).

Emulsifiers may also find uses for the following developments in biscuit technology:

- Use of liquid rather than plasticised fats in biscuit dough mixing.
- Control of dough and cream fat plasticity while in storage or when used directly from a chiller/emulsifier, by modifying crystal type.
- Improving tolerance to different levels and qualities of protein in biscuit flours, in respect of dough machinability and oven lift in crackers and semi-sweet types
- Stabilising emulsions or suspensions required as premixes for automated mixing systems (Manley, 2011b)

2.4.2.2 Leavening agents

Leavening agents are a group of predominantly inorganic salts which, when added to dough either singly or in combination, react to produce gases which form the nuclei for the textural development within a biscuit during baking (Manley, 2011a). The leavening action is responsible for good volume, improved eating quality, and a uniform cell structure. Leavening can be achieved by various methods, including yeast fermentation, the mechanical incorporation of air by mixing and creaming, formation of water vapor during baking, and the creation of carbon dioxide and/or ammonia by chemical leaveners. However, creation of the initial air bubbles during the mixing phase is critical before any of the other leavening agents can take effect (Hazelton *et al.*, 2004) .

2.4.2.2.1 Sodium bicarbonate (Soda)

The most widely used source of carbon dioxide in chemically leavened systems is the reaction of sodium bicarbonate or baking soda ($NaHCO_3$) with an acid, usually the acidic salt of a weak mineral acid. The leavening acid promotes a controlled and nearly complete evolution of carbon dioxide from sodium bicarbonate in an aqueous solution. Some examples include monocalcium phosphate monohydrate $(CaH_4PO_4)_2 \cdot H_2O$, sodium acid pyrophosphate ($Na_2H_2P_2O_7$), and potassium acid tartrate ($KHC_4H_4O_6$). When these agents combine with water, they react to form-controlled amounts of carbon dioxide. Sodium bicarbonate also raises dough pH (Hazelton *et al.*, 2004). The reaction takes place as follows:

2.4.2.2.2 Ammonium bicarbonate (Vol)

Ammonium bicarbonate (NH_4HCO_3) generates carbon dioxide, ammonia, and steam when heated. It is readily soluble in water and is very alkaline giving softer doughs which require less water for a given consistency. The dissociation is particularly rapid at about 60°C, that is, well into the oven as the dough pieces are baked (Manley, 2011a). Unlike sodium bicarbonate, which leaves a residue of alkaline sodium carbonate, ammonium bicarbonate leaves no residue when it decomposes by heat. It, therefore, has no effect on the pH of the baked product. If there is more than about 5% moisture in the baked product, however, the ammonia gas will dissolve in this water and impart an ammoniacal flavor to the product.

For this reason, ammonium bicarbonate is used only in low-moisture products (Vetter, 2003).

In many cases it has been found satisfactory and convenient to eliminate all acidulants in biscuit doughs and to use only Vol and soda. The soda is there primarily as a means of controlling the acidity of the biscuits (Manley, 2011a).

2.4.2.3 Milk solids

Milk, butter and cheese have been traditional ingredients for baking due to their flavour and exceptional nutritional values. The protein and reducing sugar (lactose) contents of milk products contribute strongly to the Maillard reaction which gives golden brown surface coloration to biscuits during baking. It is usual to use the dried products, either full cream milk powder (FCMP) or skimmed milk powder (SMP) because of ease of handling, good storage life and low moisture content. The amino acid spectrum of the proteins (casein and albumins) are very valuable for human nutrition and complement the proteins derived from cereals (Manley, 2011a).

SMP has therefore found widespread use as a minor dough ingredient both to give subtle flavour and textural improvements and to aid surface colourings. It is a rather expensive ingredient for these roles and the use of cheaper sources of reducing sugars (whey powder, glucose and invert syrups, and maltodextrin powders) have tended to replace it. If SMP is not well dispersed in the dough the small lumps will appear as dark brown or black specks in the baked biscuits. This problem is normally overcome by dispersing the powder in some cold water before it is added to the mixer (Manley, 2011a) . A typical skimmed milk powder should have a maximum insolubility value of 0.1%, in water, and maximum moisture of 4%. Storage in moisture-proof containers at the optimum temperature of 15°C should maintain the SMP in good condition for at least 12 months (Walstra *et al.*, 2006).

2.4.2.4 Processing aids

2.4.2.4.1 Water

Water is often thought of as a processing aid or catalyst, rather than as an ingredient. It is incorporated at the dough stage but driven off during baking. Water functions in several ways, including hydrating flour proteins and starch, dissolving sugars, salts, and various leavening chemicals, aiding in ingredient distribution and helping control dough temperature.

Dough's consistency is directly related to its water content, or absorption. Many factors affect dough absorption. Approximately 46% of flour's total absorption is associated with the starch, 31% with protein, and 23% with the pentosans. Acceptable consistency can be obtained only after sufficient water is present to hydrate the flour. This is regarded as bound water and controls the dough's consistency. As bound water layers are ''stacked up,'' some of the water is held less and less strongly, resulting in water that can escape (evaporation) and/or migrate as free water (Hazelton *et al.*, 2004).

All the water added to biscuit doughs is subsequently removed in the oven, but the quality of the water used may have an effect on the dough especially traces of copper and other certain metals have been found to have a marked effect on the development of rancidity in fats and oils. Doughs made with very soft water are softer and weaker than those made with hard water (Manley, 2011a).

2.4.2.4.2 Common salt (Sodium chloride NaCl)

Salt is used in almost all recipes for its flavour and flavour-enhancing properties. Moreover, salt has a slight effect on the consistency of hard doughs, because it has a strengthening effect on gluten. Salt also controls fermentation and aids in suppressing undesirable bacteria. Its most effective concentration is around 1–1.5% based on the flour weight, but at a level of more than 2.5% the taste becomes unpleasant (Manley, 2011a). Salt should be free from agnesium and calcium chloride as the minerals may cause rancidity (Joshi, 2012)

2.4.2.4.3 Sodium metabisulphite

Sodium metabisulphite is a processing aid used to modify the quality of gluten and the rheology of dough. It makes the dough flexible for better sheeting, and this is the rationale for sodium metabisulfite's working as reducing agent. It reacts with the cysteine amino acids in dough, creating S-sulfocysteine residues within the protein structure, which inhibit the restoration of disulfide bonds. Essentially, it acts as a cap, covering the reactive thiol group on cysteine, so it is unavailable to reform disulfide bonds. Lack of adequate disulfide bonds means that dough can't form a strong gluten network (Fort, 2016,). The solubility of SMS in water is approximately 39 g/100 ml solution at 20°C. The use of about 0.03 units of this salt per 100 of flour allows at least a 10% reduction of dough water (to give a similar consistency) and a significant reduction of mixing time compared with doughs using no SMS (Manley, 2011a). Sodium metabisulfite is GRAS as a chemical preservative, regulated by the (FDA, 2017)

2.4.2.5 Flavoring and coloring agents

2.4.2.5.1 Flavoring agents

The flavour of a food is the combined effects of taste, smell and mouthfeel. The introduction of aromatic ingredients as a contribution to flavour can be made to biscuits and other cooked products in three principal ways (Manley, 2011a):

1. by including the flavour in the dough or batter before baking
2. by dusting or spraying the flavour after baking
3. by flavouring a non-baked portion, such as cream filling, icing, jam or mallow, which is applied later

To obtain good distribution in a dough, the flavor should be creamed with the sugar and shortening at the beginning of mixing. Except, from the added flavours, it can be obtained from various ingredients like nuts, fruits etc. Most commonly used favouring agents are common salt, yeast extracts, spices and essences (Joshi, 2012).

2.4.2.5.2 Coloring agents

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

2.4.2.6. Antioxidants

Antioxidants 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, flavour or odour 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 metabisulphite and potassium metabisulphite are used as conditioning agents. Special fortifying agents like protein, vitamins, fruits, nuts, chocolates etc. can also be mixed with biscuits (Smith, 1972).

2.4.2.7. Other ingredients

Dried fruits such as currants, sultanas, raisins, figs, dates; glace cherries, are used in biscuits. Nuts such as coconut, walnut, almonds, groundnuts, hazel nuts, cashew nuts, etc are also used. Spices such as ginger, cloves, cinnamon, pepper, coriander, cardamom, celery seeds etc are used as flavourings (Whiteley, 1971). Monosodium glutamate, yeast extracts, etc are used as flavor enhancers. Cocoa and chocolate, colorings, artificial sweeteners are also used (Manley, 2011b).

2.5 General and mandatory requirements of biscuits as published by NBS

Biscuit should be properly baked, crisp and uniform in texture and appearance. They should not possess rancid flavor, fungal infection, off odour and any insect infestation. The mandatory standards as described by Nepal Bureau of Standards (NBS) is given in Table 2.4.

Table 2.4 The mandatory standard for biscuits

S. No	Characteristics	Requirements
1	Moisture	6.00% max
2	Acid insoluble ash (on dry basis)	0.1% max
3	Acidity of extracted fat (as oleic acid)	2.00% max

Source: (NBS, 2040)

For filled biscuits any of the fillers like jam, jellies, marshmallow, cream, caramel, figs, raisins etc. can be used. The biscuits may be coated with caramel, cocoa or chocolates. Use of antioxidant as well as permitted preservative can be done not exceeding the maximum dosages.

2.6 Nutritive value of biscuit

Biscuit is a ready to eat good source of nutrient as it contains carbohydrates, fats, proteins, minerals and vitamins. Proteins are nutrients for growth and repair of tissues while carbohydrate and fat provide heat and energy. Similarly, minerals provide nutrient for bone growth. Vitamins are responsible for normal metabolic activities and maintaining normal vitality of the body. Nutritive value of biscuit is given in Table 2.5.

Table 2.5 Nutritional composition of wheat biscuit

Constituents	Weight per serving in 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)

2.7 Technology involved during biscuit making

Technology is the factor which enables easy doing of something by significantly reducing the labour, time, expenditure and increasing the quality. Technology is always beneficial until it is under control. Hence the skill to handle the available technology is the fore most need during an operation. Not only the technology controller or his department is responsible with the machinery control but with the total control from ingredient purchase to sales (Rao, 1985). The technology of biscuit production is shown in Fig. 2.1.

Preparation of ingredients

↓

Creaming, mixing, Kneading

↓

Laying of dough

↓

Forming (Moulding, sheeting, cutting, etc)

↓

(Moulding for soft dough biscuits)

↓

Baking (temp. 140℃ to 240℃, time 3 to 12 min)

↓

Cooling (6 to 7 min)

↓

Secondary processing (coatings, etc)

↓

Packaging

↓

Storing (RH 40% to 59%, temperature: 23 to 27 ℃)

Fig. 2.1 Flow sheet of manufacturing process of biscuit

Source: (Smith, 1972)

2.7.1 Preparation of ingredients

2.7.1.1 Wheat flour

Flour is taken from the bottom of a silo, or after a sieving system, via rotary seal into a stream of air and is blown to a hopper which is mounted on a weighing system, probably above a mixer.

2.7.1.2 Fat soluble ingredients

GMS, Lecithin, BHA are sugar are to be mixed with melted semisolid/liquid shortening.

2.7.1.3 Water soluble ingredients

Glucose syrup, salt and SMP are to be mixed with some water together and color (if used) and SMBS both separately in some water. ABC and SBC are separately mixed with water and then mixed with flavor.

2.7.1.4 Metering of ingredients

Metering is probably the most important aspect of process control. Errors in metering may have an effect throughout the rest of the manufacturing process. In most plants insufficient is known about the precision of metering and deviations from standard are not recorded systematically so comparison with biscuit size and quality is difficult or impossible. So, it is important that metering is done properly. In most factories ingredients are metered to mixers by a combination of automatic (for bulk-handled materials) and manual (for small ingredients) methods (Manley, 2001)

2.7.2 Creaming, mixing, kneading

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

c. the solution of a solid in a liquid

d. the kneading of the mass to impart development of gluten from flour proteins which have been hydrated at an 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 formation of dough for the very many types of products that are called biscuits (Manley, 2011b).

2.7.2.1 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 ingredient 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 cream and mixed at slow speed in the mixing machine for around 10 min. 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 a wide spread attack on the flour to form any significantly higher amount of gluten network. Mostly short cake rotary and wire cut doughs are mixed by this method, in order to control flow and volume during baking. A significant factor in such mixings is the amount of water used (Whiteley, 1971).

2.7.2.2 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 doughs. Due to the relatively higher water content in these doughs, it results in very satisfactory gluten production and formation (Manley *et al.*, 2011).

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).

2.7.3 Laying of dough

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

2.7.4 Forming

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

2.7.4.1 Sheeting, gauging and cutting

Of the various means of forming pieces for baking from a mass of dough, sheeting, gauging and cutting is the most versatile and commonly used method. The integrated set of machines that form dough pieces from a mass of dough are commonly referred to as a "cutting machine". A cutting machine represents a straightforward mechanization of the old manual method whereby a mass of dough was rolled out and then dough pieces were cut with a cutter of the desired shape and size (Caballero *et al.*, 2016).

Laminating is done for several reasons: (Caballero *et al.*) it helps to repair the sheet that was formed using a simple pair of rolls; (2) uniform stress distribution can be achieved by turning the folded dough through 90˚; (3) consecutive and repetitive cycles of rolling and folding cause more working of dough and develop a delicate structure in baked products; and (4) flaky structure can be obtained in products by spreading fat between two layers (Caballero *et al.*, 2016).

Within the sheeter the dough is compressed and worked to remove air and it is inevitable that some stresses are built up in the gluten structure. There is also a small increase in dough bulk density. Flanges at the ends of the rolls prevent the dough from being extruded from the ends of the rolls and ensure that the emerging sheet is always of the desired width. The new sheet of dough then passes to one or more sets of gauge roll pairs which reduce the thickness to that required for cutting. Like the sheeter, there are flanges on one of the gauging rolls to prevent the dough extruding sideways and to maintain a full width of dough sheet. Sometimes, having been reduced in thickness, the sheet is folded or cut and piled up to form many laminations before being further gauged to a final desired thickness (Manley, 2001).

Once the dough has been relaxed, it passes on to the cutting operation. Two different types of cutting methods exist: reciprocating cutters and rotary cutters. Reciprocating cutters consist of heavy block cutters that stamp out one or more pieces at a time (Caballero *et al.*, 2003). For maintaining a perfect size and shape, it is necessary that the dough sheet travels at constant speed under the cutter, which drops over the dough sheet, moves along with the dough, and comes to the original position before (Caballero *et al.*, 2016). The cutter head may have a dual action, whereby the cutter drops first, followed by a docking head or an embossing plate (Caballero *et al.*, 2005). Reciprocating cutters are rarely used nowadays (Manley, 2001).

The Rotary cutters are of two types, those that employ two rolls, one immediately after the other, and those with only one roll. In case of two roll types the dough sheet, on a cutting web, is pinched between engraved rolls (mounted in series) and a rubber-coated anvil roll(s). The first roll dockers the dough, prints any surface pattern or type and thereby pins the dough onto the cutting web. The second roll is engraved with only the outline of the biscuit and cuts out the piece leaving a network of scrap. A single-roll rotary cutter achieves both dockering, pinning and outline cutting with only one roll. In many cases this works well and there is a saving in capital equipment, but there is a strong tendency to lift the dough piece from the cutting web because the pinning down facility is not independent of the cutting pressure (Manley, 2001). Scrap can be reused either by mixing it with a fresh batch of dough in the sheeter with the help of the 'scrap return,' or it is added in the horizontal mixer while kneading a new batch (Caballero *et al.*, 2003).

2.7.4.2 Rotary moulding

Principally, the dough is forced into moulds with patterns, name, type and docker holes. The excess dough is scraped off with a knife bearing upon the mould and thereafter the piece is extracted from the moulds onto a web of cotton canvas or other fabric. Short dough may be sheeted, gauged and cut with an embossing type cutter in a similar way as for extensible dough, but the advantages of a moulder are:

a. It is not necessary to form and support a dough sheet

b. Difficulties of gauging are eliminated

c. There is no cutter scrap dough which must be recycled.

Until recently only short dough could be formed with a rotary moulder. That is because, as is explained below, the scraper knife drags back cohesive dough so the mould is not filled and the resulting dough piece is incomplete (Manley, 2001).

2.7.4.3 Extruding and depositing (Wire cutting)

These basically consist of a hopper over a system of two or three rolls which force the dough into a pressure/balancing chamber underneath. The rolls may run continuously or intermittently and may be capable of a short period of reverse motion to relieve the pressure and cause a suck back at the dies or nozzles at the base of the pressure chamber. This means that dough can be forced continuously or intermittently out of the pressure chamber. The machine spans the width of the plant and is usually situated over the oven band. In the case of certain drier, wire cut dough and rout types (continuously extruded) which are subsequently cut into lengths before baking, the machine is over a normal canvas conveyor and not the oven band. Dough pieces formed on a conveyor may be spaced out as they are transferred onto the oven band. Wire cutting gives attractive product appearance but production efficiency is not as good as with other methods of dough piece forming (Manley, 2001).

2.7.5 Baking

The baking and drying of dough is the essence of biscuit making. Baking involves heating the dough. The physics of heat transfer, heat flux, includes convection, conduction and radiation which are difficult concepts to appreciate and evaluate where only the temperature at selected places can be measured in an oven (Manley, 2001). 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). Baking time varies from 3 to 12 min and depends on the product. Temperature also varies from 140 to 240°C, varies from zone to zone, and is product specific (Caballero *et al.*, 2016).

2.7.5.1. Oven

A traveling or band oven is extensively used for industrial baking processes, whereas small bakeries rely on simple ovens or static ovens, which usually have a heated box with a door and different trays and can be heated by means of electricity, gas, or wood. The traveling or band oven is a tunnel that is enclosed, is insulated, and bears different sections/zones. Oven length ranges from 30 to 150 m, with an average length of about 60 m and a band width of 1–2 m. The oven consists usually of 3–7 zones with different temperature and air profiles, which are controlled separately as for the baking profile of the product. In a continuous oven, temperature and heat transfer conditions can be controlled throughout the oven during the baking process. Industrial ovens usually run on fuels such as petroleum gas, oil, or electricity, which heat the atmosphere around the product either directly or indirectly via heat exchangers (Caballero *et al.*, 2003).

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 (Hazelton *et al.*, 2004). 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 (Smith, 1972).

2.7.5.2 Changes during baking

2.7.5.2.1. Development of structure

Development of the internal structure of a biscuit takes place mainly in the first quarter or third of the baking period. The changes are all temperature related and involve several aspects of the recipe and the form of the dough piece. Bubbles of gas and water vapour are formed which expand and result in a large reduction in the density of the dough. It is the open porous structure that gives a biscuit a pleasant eating texture. The development of the structure is often known as oven spring. The conditions for giving maximum spring, which are sustained through the remainder of the bake, are imperfectly understood but the changes to the dough piece that are involved include:

a) heating the starch and proteins to levels where swelling, gelatinisation, denaturation and setting occur.

b) liberation of gases from leavening chemicals

c) expansion of these bubbles of gases as a result of increasing temperature which also increases the water vapour pressure within them as well as rupture and coalescence of some of these bubbles

d) loss of moisture from the product surface by evaporation followed by migration of moisture to the surface and continued loss to the oven atmosphere

e) increase of sugar solution concentration as the temperature rises

f) reduction in consistency of sugar solutions and fat with temperature rise.

Starch gelatinisation occurs in the temperature range 52–99°C and the change is somewhat time dependent. Proteins are denatured and coagulated above about 70°C. Gas is liberated from chemical leavening agents at a significant rate from about 65°C. It can also be seen that the volume increase due to water vapour accelerates very rapidly above 70°C. Fats used in baking melt completely well below any of these temperatures. It will be seen that as the different parts of the dough piece reach about 65°C expansion and loss of flexibility are converging forces. Too much expansion and the structure ruptures, too little expansion and a dense close structure forms (Manley, 2001).

2.7.5.2.2. Reduction in moisture

Ideally, moisture loss should occur after the structure has set but obviously this is impossible to achieve throughout the dough piece. Moisture can only be lost from the dough piece surface so migration to the surface by capillary action and diffusion must occur. Both phenomena are accelerated by temperature gradients so a rapid heating of the whole product to 100°C is required during this stage of baking. If the surface is heated too much, and it dries too rapidly (as will occur if there is much air movement in the oven), colour changes occur prematurely and it is thereafter difficult to dry the biscuit enough without excessive surface coloration (Manley, 2011a) .

The moisture gradient across a dough piece increases during drying and as the biscuit structure dries the starch/protein structure shrinks. While hot the biscuit is flexible enough to withstand these shrinkage stresses, but a phenomenon known as ‘checking’ may occur if a large moisture gradient remains after the biscuit leaves the oven. As the biscuit cools moisture equilibrates (moving from the wetter areas to drier) and the shrinkage stresses so developed may cause cracks to form. This is called checking. The best way to prevent checking is to ensure that the total moisture content of the biscuit is low so that any gradients will be small (Manley, 2011a).

2.7.5.2.3. Color changes

Although there is a change to a yellow-brownish hue during baking, the term colour here is used to imply merely a darkening, reduction in reflectance, of the biscuit surface (Manley, 2011a). After the moisture has been mainly evaporated from the dough pieces, the temperature of the surface rises quickly and the colour will change from around 150°C. There are three processes which contribute to the browning of the biscuits. Caramelisation is a non-enzymatic browning reaction, which is caused by the breakdown of sugars at high temperatures. The caramelisation of different sugars occurs at different temperatures: fructose at 110°C, glucose 160°C and sucrose at 160°C. Caramelisation results in both colour and flavour development. A second browning process, dextrinisation, is the breaking down of starch molecules by heating. This produces pyrodextrins which are brown in colour and have a distinctive flavour. Dextrinisation of the starch occurs at temperatures of 100–200°C (I. Davidson, 2016b).

The third browning process is known as the Maillard reaction. This is a complex chemistry in which many compounds are formed at high temperatures by the reaction of reducing sugars and amino acids (I. Davidson, 2016b). The Maillard reaction, non-enzymic browning, involves the interaction of reducing sugars with proteins to produce attractive reddish-brown hues. This occurs around 150–160°C and will happen only in a moist situation. It is not possible to reheat baked biscuits to increase significantly the surface colour due to the Maillard reaction (Manley, 2011a).

As drying continues, the colouration due to the changes already described will develop in the thinner or more exposed areas of the biscuit. This change is accompanied by the development of a bitterness of flavour. A condition known as ‘perishing’ will occur if this continues throughout the biscuit structure. Perished biscuits are bitter and unpleasant (Manley, 2001).

Table 2.6 Temperature related changes in biscuit during baking

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

Source: (Mukhopadhyay, 1990)

2.7.6. Cooling

According to Manley *et al.* (2011) cooling is one of the most important parts of biscuit production. As biscuits emerge from the oven, they are very hot nearly at a range of 210-214˚F, 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 crystallise out and the dextrin grow tougher, then only the biscuit grows tougher and set. Cooling should be gradual and slow (Smith, 1972).

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 gelatinised 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, 2001).

2.7.7 Secondary processing

In many cases biscuits which have been baked and cooled are subjected to further treatment in the form of coatings or sandwiching with flavored materials. These treatments are known as secondary processes. The additions include chocolate, fat-based creams, water icing, marshmallow, caramel toffee and jam or jelly. There may be a single addition such as chocolate or a combination of two or more materials. In the latter case each addition is usually made separately, and a cooling or drying period allowed between each (Manley *et al.*, 2011).

Secondary processing allows a much greater variety of flavors, textures and appearance to be achieved than by baking alone. The additions may result in a biscuit becoming a confectionery product and the materials used are more akin to the sugar confectionery industry than flour confectionery. It may be that chocolate enhances a biscuit or a biscuit fills out and enhances a chocolate product. In-line secondary processing is not always the case and in many factories baked and cooled biscuits are collected in tins or trays to be coated or sandwiched, etc., at a later time (Manley, 2011a)

2.7.8 Packaging

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 vapor resistant packaging material. 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. Packaging in case of biscuits must be close 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 primary pack is the moisture proof unit which is to be offered for sale to the consumer. Secondary packaging into groups of 10, 20 or more in boxes or cases is for ease of storage and transportation, but as this packaging may have a significant effect on the mechanical protection afforded to the primary packs, it should be designed carefully (Manley, 2001).

2.7.9 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 bases 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 while drawing conclusions from the analysis (Dawadi and Prakash, 2008).

2.8 Barley (Hordeum vulgare)

2.8.1 Historical Background

Barley was one of the first cereal crops to be domesticated. There is substantial evidence that portrays the role of barley as a sustaining source of food in the evolution of humans. Archaeologists have found evidence suggesting the existence of six-row barley in Egypt along the River Nile around 17,000 years ago (Badr *et al.*, 2000) and in the Middle East that it was a staple food as far back as 5,000 BC (Hambira, 2009).

Hordeum vulgare subsp. *spontaneum* (wild barley) is said to be the ancestor of today's barley. Greek and Roman scholars such as Hippocrates or Pliney the Elder, respectively, considered barley as a healthy and nourishing food and barley gained recognition for medical treatments. Probably due to the nourishing properties and the ruggedness of the crop, barley became a major food especially for poor people throughout history (Siebenhandl-Ehn *et al.*, 2011). Barley played important role in ancient Greek and Roman culture as a staple bread-making grain as well as important food for athletes (Acharya, 2008).

2.8.2 Scientific classification of barley

Scientific classification of commercial barley is as follows

Kingdom: Plantae - Plants

Subkingdom: Tracheobionta - Vascular plants

Superdivision: Spermatophyta - Seed plants

Division: Magnoliophyta - Flowering plants

Class: Liliopsida - Monocotyledons

Subclass: Commelinidae

Order: Cyperales

Family: Poaceae - Grass family

Genus: *Hordeum* – barley

Species: *vulgare*   (Gina, 2008)

2.8.3 Production and utilization in the world

Barley is an important crop for direct human consumption, animal feed and industrial applications. It also ranks fourth among the cereals in worldwide production (Wei-wei *et al.*, 2016). Barley outperforms other cereals under various environmental stresses due to its winter-hardy, drought-resistant, and early maturing nature and is thus generally more economical to cultivate (Cook, 2013). These features are mainly linked to the presence of genetic factors that allow synchronizing the vegetative cycle of the plant with the environment. This allowed to have early spring varieties suitable for environments with a prolonged cold weather and short spring-summer seasons and tardive winter varieties able to fully exploit all the productive potential of temperate climates. Depending on climate conditions, soil characteristics, agricultural practices (i.e., irrigation), but also a variety of cultivated and technological innovations, barley's yield has changed during the time starting from 1.39 (in 1960) to 2.99 t/ha (in 2017) (Tricase *et al.*, 2018).

The genus *Hordeum* has center of diversity in central and southwestern Asia, West North America, southern South America and in the Mediterranean (Bothmer., 1992). Cultivated barley grown in a range of diverse environment that vary from sun-Arctic to sun-Tropical, with greater concentration in temperate areas and high altitudes of tropics and subtropics. Other than cool highlands, barley is grown in the tropic as it is no suited to warm humid climates (Nevo and Shewry, 1992). Most production of barley takes place in Europe (62%), followed by Asia (15%) and America (13%). From the statistical data the world production of barley is around 144 million tonnes with Russia , France and Germany being the highest producing countries (Sen-nag, 2017).

Recently more than 70% of barley crop has been used for feed; about 21% has been intended to malting, brewing, and distilling industries; lesser than 6% has been consumed as human food (Tricase *et al.*, 2018). The popularity of barley to be used in foods have surely increased since few years as epidemiological studies have associated the regular consumption of barley with its potential to reduce the risk of certain diseases, such as chronic heart disease , colonic cancer , high blood pressure , and gallstones (Idehen *et al.*, 2017).

In context of Nepal, Barley is grown from the terai, up to an elevation of 4000 m in Nepal, but is a staple food crop mainly in the hills and mountains in the west of the country. According to the statistical reports, the total barley production has increased over the years and has reached 31,000 metric tons in 2019 which is a decreased value compared to 37,000 metric tonnes in 2014 (US department of agriculture, 2019).

2.8.4 Types of barley

Barley can be classified based on physical properties, processing and grain composition. Some of the classes include spring or winter types, two-rowed or six-rowed, hull or hulless, malting or feed use, normal, waxy or high amylose starch, high lysine, high β-glucan, and proanthocyanidin - free. Barley may also be divided by the number of kernel rows in the head. There are two types: two-row barley and six-row barley. Two-row barley has lower protein content than six-row barley but higher enzyme content. High protein barley is best suited for animal feed or malt that has a large adjunct content. Two-row barley is best suited for pure malts (Hambira, 2009). All the three spikelets attached at each end of the node are all fertile while only the central spikelet is fertile in the two-rowed. There are naked and hulled barleys, the hulled barleys being the older forms (Newman, 2008).

2.8.5 Structure of Barley

There are several distinct species of barley, although the most commonly cultivated is designated as two-rowed or two-eared barley. In general structure, the barley grain resembles wheat and oats as shown in Fig 2.2. The caryopsis is composed of the pericarp, germ and endosperm while the hull contains the lemma and palea. The barley grains are harvested with the hull intact; however, some cultivars are hulless. The hull makes up 10% of the kernel and the average barley kernel weighs approximately 35 mg (Sidhu et al 2007). The aleurone layer can be blue or white depending on the cultivar. The germ makes up 3% of the kernel and is rich in lipids thereby making the grain susceptible to rancidity.

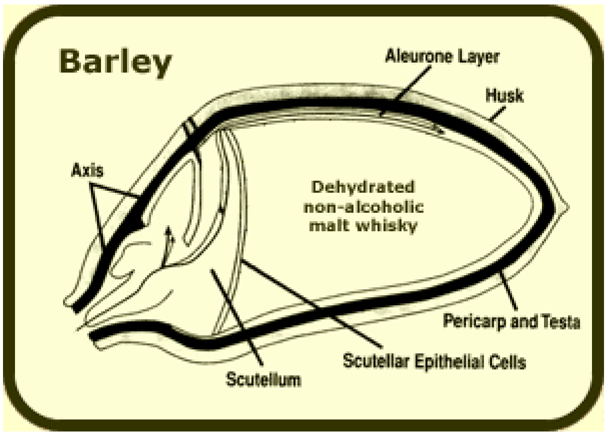

Fig 2.2 Structure of barley (Hambira, 2009)

2.8.6 Naked Barley

In hulless barley the expression of the recessive naked caryopsis gene (*nud*, nudum) prevents the intergrowth of husks and caryopsis. Consequently, the kernels thresh free and lemma and palea do not adhere to the caryopsis at maturity. This is why hulless barley requires no further dehulling for the production of food. Cultivation is as old as that of hulled barley but is less common worldwide due to lower yields and only minor breeding activities (Atanassov *et al.*, 2001); Its domestication is believed to have occurred after the hulled type around 6500 BC (Pandey *et al.*, 2006).

Cultivation of naked barley is less common worldwide than that of hulled barley. Its distribution is skewed towards East Asia, namely to the Himalaya range (Nepal, Bhutan and Tibet), China, Korea and Japan where it accounts to as much as 95% of the domesticated barley in some areas (Sun and Wang, 1999). The cultivation is rare in the Western World (Europe, North America) and in Australia where hulled types are prevalent. Hulless barley landraces from the highlands of Nepal turns out to be distinct from the Chinese, Korean and Japanese types. Furthermore, it was shown that these landraces are different from western hulled barley (German cultivars) and Canadian naked barley cultivars (Pandey *et al.*, 2006).

Taxonomically naked barley belongs to:

Family: - Graminae

Sub-family: -Frestucoideae

Tribe: - Hordeae

Genus: - *Hordeum*

Species*:-vulgare* (Harris, 1962)

2.8.6.1 Composition of Naked barley

Compared to hulled barley, the free-threshing character of hulless barley proportionally increases contents of protein and the limiting amino acids lysine and thereonine, respectively and as well levels of β-glucan but lowers contents of insoluble dietary fibre components (Xue *et al.*, 1997). In barley growing naked forms are of growing importance, also as grains are characterized by a low fibre content, and a higher content of starch in comparison with husked cultivars. Changing the chemical composition involves the improvement of digestibility and energy for monogastric animals. It also has high straw yield, moderate lodging resistance, as well as an early time of maturing (Tobiasz R. *et al.*, 2012)

Table 2.7 Proximate composition of naked barley grain

Component	Percentage
Moisture	11.57
Protein	11.77
Crude fat	2.90
Crude fiber	4.20
Ash	2.64
Carbohydrates	78.49

2.8.6.1.1 Major constituents

Starch

Most of the carbohydrate in naked barley is starch amounting 60 – 70% of the dry matter which serves as energy source during germination (Duffus and Cochrane, 1993). Mono- and di-saccharides (sucrose, glucose, fructose and maltose) are present in lesser amounts, but their concentration is twice as high as in other cereals. Starch is the major component in barley kernels. Starch itself is composed of two types of glucose polymers namely the highly branched amylopectin and the linear amylose. According to the proportions of amylose and amylopectin barley can further be classified: normal naked barley contains

25 – 30% amylose, waxy varieties less than 15% whereas high-amylose cultivars more than 35% amylose (Siebenhandl-Ehn *et al.*, 2011).

Non- Starch Polysaccharides

Barley is a good source of both soluble and insoluble dietary fibre. The dietary fibre helps in maintaining the health of the digestive system. It is the soluble fibre in the cell wall consisting of (1→3), (1→4) β -glucans and pentosans (arabinoxylans) that is responsible for the lowering of the serum cholesterol, postprandial blood glucose and insulin levels in human which can impact both heart disease and type II diabetes (Ullrich, 2014). Arabinoxylans are found in the husk but are abundant in the walls of the aleurone cells and starchy endosperm. This major group of noncellulosic polysaccharides is made up of the pentoses, arabinose and xylose (Hambira, 2009). The dietary fibre contents in cereals will vary depending on component of the grain, cultivar and processing conditions. The total dietary fibre in barley was 10% dry basis (Sidhu *et al.*, 2007). High-amylose and waxy hulless barley contains approximately 7 or 8% β-glucans, whereas regular hulless barley comprises significantly less (4.6%). Study conducted by (Kinner *et al.*, 2011) shows that the β-glucan content of naked barley is sufficiently high to meet the requirements of the EFSA health claim (3 g/day) (EFSA, 2009a).

The higher the β-glucan content in barley flours the worse are the baking qualities, due to the high water binding capacity and thus decreasing the water availability for the gluten network (Gill *et al.*, 2002).

Proteins

Cereal grains have relatively low protein content (10-12%) compared to legume seeds. In barley, the protein content varies between 7 to 14.6% (Guerrieri2004). Wheat, barley and rye have similar amino acid composition (Guerrieri, 2004). The storage proteins, prolamins and glutelins are deficient of tryptophan, lysine and methionine. Threonine, after lysine is one of the limiting amino acid in barley. While the hull contains low protein concentration, its proteins are high in lysine. The germ proteins are high in lysine while the endosperm has lower lysine content but higher than in other cereals. Prolamins in barley are referred to as hordein accounting for 40% of total proteins. However, prolamins are low in lysine. High contents of glutamic acid and proline have been found in the endosperm of barley . Protein synthesis occurs throughout the fruiting season of the plant (Hoseney, 1992).

Table 2.8 Amino acid composition of naked barley (g amino acid/100 g crude protein)

Amino acid	Range
Alanine	3.14 – 4.39
Arginine	4.13 – 5.21
Aspartic acid	4.17 – 6.23
Cystine	1.69 – 2.60
Glutamic acid	16.7 – 25.5
Glycine	3.29 – 4.85
Histidine	2.15 – 2.96
Isoleucine	3.06 – 4.12
Leucine	5.85 – 7.57
Lysine	2.79 – 4.13
Methionine	1.39 – 1.92
Phenylalanine	4.29 – 5.57
Proline	8.25 – 12.2
Serine	3.53 – 4.24
Threonine	2.76 – 3.72
Tyrosine	1.10 – 2.33
Valine	4.32 – 5.94

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

Lipids

Barley lipids make up approximately 3% of the kernel. Most of the lipids are found in the germ. Other lipids are contained in the starch granules in quantities relative to amylose. Barley contains non-polar lipids (72%), glycolipids (10%) and phospholipids (21%) (Hoseney, 1992). Barley fatty acids contain higher levels of linolenic acid and are more saturated than those found in wheat. A distinct characteristic of barley is that it contains all eight naturally occurring tocopherols (Govind *et al.*, 2018) . The germ tissues contain tocols while the starchy endosperm and aleurone layer contain the tocotrienols. Studies with non-starch lipids have shown that only the polar lipids have a positive effect on baking performance, whereas the nonpolar lipids have the opposite effect. If the term “specific baking activity” would be defined, polar lipids would be found to affect the baking performance of wheat flour to a considerably greater extent than proteins (Koehler and Wieser, 2013).

2.8.6.1.2 Minor Constituents

Micronutrients

A study done by (Ragaee *et al.*, 2006) shows that barley had the highest levels of phosphorous, calcium, potassium, magnesium, sodium, copper and zinc compared to millet, wheat, rye and sorghum. The aleurone layer contains the majority of the minerals (P, K, Mg, Ca, Zn and S) in cereals while the vitamins (Vitamin E, B1, B2 and B3) are mainly found in the aleurone or scutellum (Hambira, 2009). The average mineral content varies significantly, and this appears to be due to a number of factors, including the variety in question, the growing and soil conditions and fertilizer application. A high portion of phosphorus in barley grain is bound to the phytate complex (51-66%) making much of the phosphorous unavailable to monogastric animals. Yet naked barley contains more phosphorous than common cereal grains and the phosphorous bioavailability of naked barley is higher than that in other grains (Harrold, 1999). The amounts of copper, iron, manganese and zinc present in naked barley grain may vary to a large extent due to growing conditions (Duffus and Cochrane, 1993).

Phytochemicals

Naturally occurring chemical substances in plants are referred to as phytochemicals (Zielinski and Kozlowska, 2000b). Though a large percentage remains unknown, about 5000 of the phytochemicals present in plants have been identified (K. K. Adom and R.H., 2002).When consumed together, grains contribute phytochemicals that complement those found in fruits and vegetables (Hambira, 2009). It is hypothesized that phytochemicals that are located in the fibre matrix, in addition to or instead of the fibre itself, are responsible for the reduced risk of various diseases associated with oxidative stress, such as cancer, cardiovascular and neurodegenerative diseases (D. R. Jacobs and Steffen, 2003).The most common phytochemicals are phenolic compounds. They belong to various groups of compounds and these include simple phenols, hydroxybenzoic and cinnamic acid derivatives, flavonoids and coumarines, among others (Naczk and Shahidi, 2004). Genetics, environment and processing are the factors that influence the amount of phytochemicals in cereals (H. Zhao *et al.*, 2006).

Total Phenolic content

Several phenolic compounds are present in whole grains; however, bound phytochemicals are predominant. These phenolic compounds in plant-based foods exist as free or covalently bound to non-starch polysaccharides and account for the sensory characteristics such as the appearance, taste, smell and oxidative stability (Naczk and Shahidi, 2004). The role of whole grain in the prevention of colon cancer is partly due to the fact that bound polyphenols may survive digestion and may reach the colon possessing anti-cancer effects. When bound phenolics reach the colon they are acted upon by microbial flora; consequently, the freed phenolics can now be absorbed by the colon endothelial cells (Sidhu *et al.*, 2007). The total dietary intake of phenolic compounds is estimated to be 1 g/day. It is 10 times higher than that of vitamin C and 100 times higher than vitamin E (Scalbert, 1992). Barley has higher amount of phenolic compounds (0.1–0.4%) and antioxidant activity as compared to the more widely consumed cereals wheat and rice (P. Sharma and Gujral, 2014) , corn and oats (K. K. Adom and R.H., 2002). The thermal processing of food may have either increasing or lowering effect on phenolic compounds and antioxidant activity (Randhir *et al.*, 2008).

Free phenolic contents ranged from 167.9 ± 12.1 to 282.0 ± 5.5 mg GAE/100 g grain (DW) showing 61.2% contribution to the total phenolics. The bound phenolics are linked with cell wall polysaccharides and proteins, and the content varied from 166.0 ± 5.0 to 199.0 ± 4.1 mg GAE/100 g grain (DW), showing 46.7% to 49.7% contribution to the total phenolics (Zhu *et al.*, 2015). For barley, the variety is the most significant factor influencing TPC not the type – hull-less or hulled (Kruma *et al.*, 2016). The prominent phenolic compounds in the blue hulless barley grains were gallic acid, benzoic acid, syringic acid, 4-coumaric acid, naringenin, hesperidin, rutin, (+)-catechin and quercetin. Among these, protocatechuic acid, chlorogenic acid and (+)-catechin were the major phenolic compounds in the free phenolics extract. The most abundant bound phenolics were gallic acid, benzoic acid, syringic acid, 4-coumaric acid, benzoic acid, dimethoxybenzoic acid, naringenin, hesperidin, quercetin and rutin (Yang *et al.*, 2018).

(Holtekjolen *et al.*, 2008) reported that the baking of bread containing barley flour exhibited decrease in TPC. The decrease in TPC may be attributed to alteration in the chemical structure of the phenolic compounds, possible polymerization leading to reduced extractability and oxidation (P. Sharma and Gujral, 2014).

Phenolic acids

Phenolic acids consist of two classes namely hydroxybenzoic acids and hydroxycinnamic acids. The former includes gallic, ρ-hydroxybenzoic, vannilic, syringic and protocatechuic acids. The hydroxycinnamic acids include *p*-coumaric, caffeic, ferulic and sinapic acids. (Holtekjolen *et al.*, 2008) reported that antioxidant activity of grains depends on the structure of phenolic acids. The CH=CH-COOH group in the cinnamic acid derivatives gives higher antioxidant capacity compared to the COOH group of the benzoic acid derivatives. Ferulic acid (4-hydroxy-3-methoxycinnamic acid; FA) is the most abundant phenolic acid in cereals making approximately 90% of the total polyphenols (Nordkvist *et al.*, 1984). A supplement containing 500 mg of ferulic acid twice a day over a short-term period had no effects on body fat and body weight and also no adverse effects on kidney and liver function (Bumrungpert *et al.*, 2018).Phenolic acids are distributed unevenly in the layers of the bran; consequently, different grain fractions have varying antioxidant activities. The outer layers contain the highest contents of phenolic acids while the starchy endosperm contains low levels (Holtekjolen *et al.*, 2008).

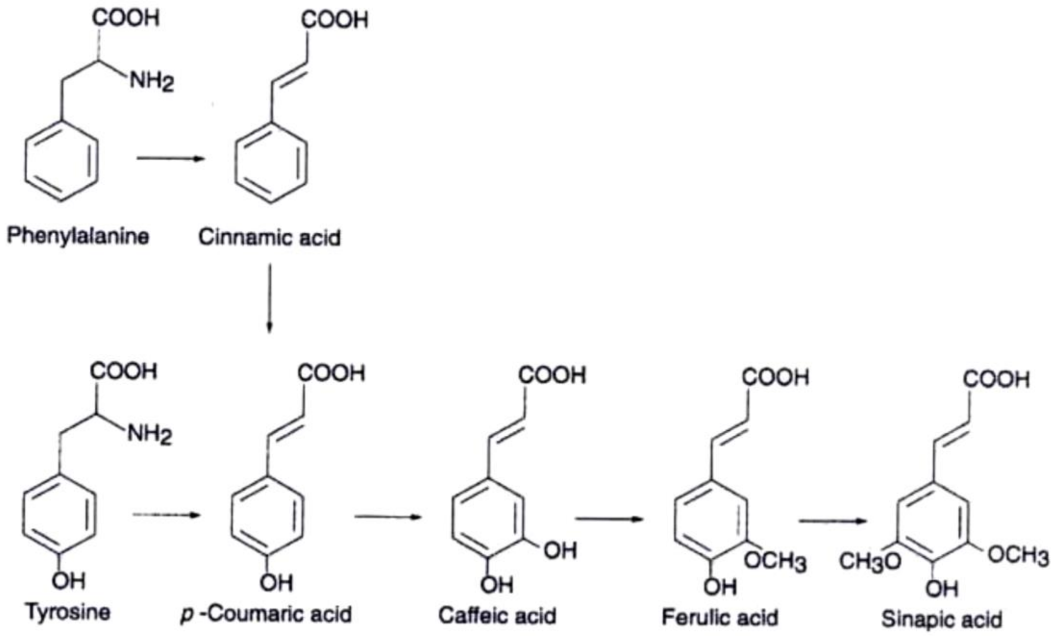

Fig 2.3 Intermediates in the phenylpropanoid biosynthesis (Rice-Evans *et al.*, 1997).

Flavonoids

A group of phytochemicals found in cereals are flavonoids which are located in the pericarp. The main classes of dietary flavonoids include flavanols, flavones, flavanones, anthocyanidins, isoflavanoids and flavonols. Major flavonoids in hulless barley are catechin, naringin, hesperidin, myricetin, quercetin, naringenin, kaempferol and rutin (Yang *et al.*, 2018). Flavonoids are formed in plants from the aromatic amino acids phenylalanine and tyrosine and malonate (Neish, 1986). Flavonoids have shown activity as scavengers of various oxidizing species including superoxide anion, hydroxyl radical and peroxyl radicals (Harborne *et al.*, 2000). Flavonoids compounds are accumulated in the vacuoles of plant cells. Flavonoids give the plant a rich taste. The role of flavonoids in flowers is to provide colors which is due to the presence of anthocyanin which is attractive to plant pollinators and in leaves, these compounds are increasingly believed to promote physiological survival of the plant, protecting it from, for example, fungal pathogens and UV-radiation.

Flavonoids have been reported to exert multiple biological property including antimicrobial, cytotoxicity, anti-inflammatory as well as antitumor activities but the best-described property of almost every group of flavonoids is their capacity to act as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species (Saxena *et al.*, 2012).

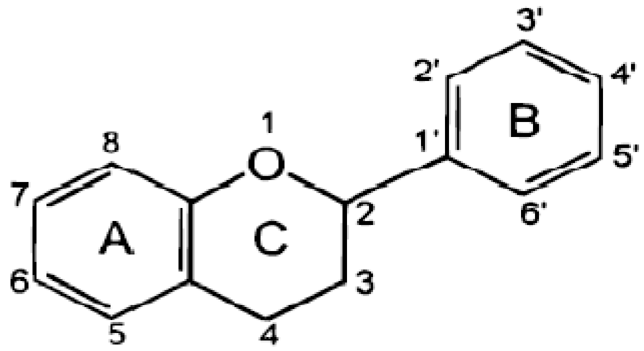

Figure 2.4: Basic flavonoid structure (Pietta, 2000)

Flavonoids have health-promoting properties due to their high antioxidant capacity in both in vivo and in vitro systems (Cook, 2013). Considerable attention has been directed towards flavonoids due to their beneficial effects as antioxidants in the prevention of human diseases such as cancer and vascular diseases and some pathological disorders of gastric and duodenal ulcers, allergies, vascular fragility and viral and bacterial infections (Zand *et al.*, 2002). The mean intake of flavonoids worldwide ranges between 150 and 600 mg/day expressed as aglycones without thearubigins (Cévoli et al., 2016). . Based on the average daily intake of flavonols (68 mg) and isoflavones (20– 240 mg) in Asian populations, dietary exposures at these doses are not likely to cause adverse health effects. To date, no human data on the long-term effects of high dose supplementation are available (Skibola and Smith, 2000)

Upon baking, significant decrease in total flavonoid content has been reported by (Holtekjolen *et al.*, 2008) and upon incorporation of hulled barley flour and of corn flour by (Li *et al.*, 2011) in bakery products. This is due to the thermal sensitivity of flavonoid compounds as reported by (P. Sharma and Gujral, 2014).

2.8.6.1.3 Anti- nutrients and allergen compounds in naked barley

The content of common anti-nutrients in cereals, including naked barley, is considered to be low when compared with legumes such as faba beans, peas and lupines. Protease inhibitors, especially trypsin inhibitors, decrease the digestibility and biological value of ingested protein and retard growth when sufficient amounts are present in the diet. Amylase inhibitors may affect the digestibility of starch. Both protease and amylase inhibitors have been identified in naked barley (Palmer, 1989). However they do not appear to be responsible for any serious anti-nutritional activity in humans as both inhibitor types tend to be heat labile (Klopfenstein, 2000).

Amylase inhibitor accumulates in naked barley grain during grain development (Duffus and Cochrane, 1993). Chymotrypsin inhibitors are present in the starchy endosperm and the aleurone layer (Kreis and Shewry, 1992). Lectins, sometimes called phytohemagglutinins, are glycoproteins that bind to certain carbohydrate groups on cell surfaces, such as intestinal epithelial cells, where they cause lesions and severe disruption and abnormal development of the microvilli. Although more commonly associated with legumes, cereal grains including barley are also known to contain lectins (Liener, 1989); (Palmer, 1989). As lectins are usually inactivated by heat treatment, they are really only of interest when raw or inadequately cooked food or feed is consumed (Klopfenstein, 2000).

Phytic acid (myo-inositol hexaphosphate) chelates minerals such as iron, zinc, phosphorus, calcium, potassium and magnesium. The bioavailability of these minerals can thus be reduced by the presence of phytic acid in monogastric animals, although in humans, phytic acid does not seem to have a major effect on potassium, phosphorus or magnesium assimilation. Ruminants, on the other hand, are more readily able to utilize phytate-complexed phosphorus because they have abundant amounts of microbial phytase which degrades phytate in the rumen (Harland, 1993). Phytic acid level in barley varities ranges from 0.70 to 0.76% (Bull and Bradshaw, 1995).

Naked barley, along with other gluten-containing cereals such as wheat and rye, is also associated with a condition known as gluten-sensitive enteropathy (also called coeliac disease), which affects genetically predisposed individuals. Gluten is a complex of two major storage proteins in cereals namely prolamin and glutelin. The sensitivity response is triggered by the prolamin fraction of the cereal storage proteins that are hordeins in barley (Anonymous, 2004).

2.8.6.2 Health benefits from Naked Barley

2.8.6.2.1 Cardiovascular disease (CVD)

It is generally recognized that high blood cholesterol level is a significant risk factor in the occurrence of CVDs. In addition, there is evidence indicating that free radicals and other oxidants largely cause most neurodegenerative diseases linked with CVDs, such as Alzheimer's disease and stroke (Mahmoudi T. *et al.*, 2015). Consumption of natural antioxidants, such as polyphenols, in daily diets can produce an effective protective action against oxidative processes generated by these compounds, thereby improving cardiovascular health (Ghafoor, 2015). Recent strategies for reducing the risk of CVDs utilize dietary restrictions to limit cholesterol intake and/or the use of a class of medication, statin, to lower serum cholesterol. Consumption of barley grains rich in phytonutrients may help reduce the dependence on drug use or dietary restrictions for the moderation of CVD. Barley phytosterols can compete with cholesterol for micelle formation in the lumen of the intestine, thus inhibiting cholesterol absorption and increasing secretion and regulation of cholesterol levels. Studies show that the levels of total phytosterols in barley oils are sufficient (0.18e1.44 g/15 g oil) to significantly lower low-density lipoprotein (LDL) cholesterol at reasonable dosages of 15 mL/d (1 tablespoon/d) (Moreau *et al.*, 2007). Lignans, also present in barley, have been found to act as strong antioxidants comparable to FA and better than vitamin E. They have also been reported to lower the risk of CVD (Prasad, 2000).

Among the most economically important cereals (wheat, barley, rice, rye, and oat) , barley has been reported to have the highest amounts of tocols and phytosterols which are strong antioxidants (Andersson *et al.*, 2008). Folate has been associated with cardiovascular health. It is currently one of the most studied vitamins due to its essential role in amino acid metabolism and DNA methylation. Barley grains enriched with folate are, therefore, a good and natural means of obtaining folate from diets and in turn preventing and reducing the risk of cardiovascular ailments (Santilli *et al.*, 2016).

2.8.6.2.2 Diabetes and obesity

Notwithstanding scarce in vivo studies on effects of barley phytochemicals on diabetes and obesity, it is well known that one of the primary pathogenic factors leading to insulin resistance, β-cell dysfunction, impaired glucose tolerance, and ultimately diabetes occurrence is oxidative stress. Oxidative stress, which plays an important role in the pathology of diabetes and obesity, can be moderated by phytochemicals present in barley. An increase in the formation of reactive oxygen species and a decrease in antioxidant defense efficiency are characteristics of both diseases (Evans *et al.*, 2003). Therefore, phytochemicals may prevent the development and progression of obesity and diabetes by reducing oxidative stress (Okarter and Liu, 2010). Barley phytochemicals that may have a role in protecting against diabetes and obesity include various phenolic acids, flavonoids, phytosterols, and tocols (K.K. Adom and Liu, 2002). The antioxidative activity investigated for 80% methanolic extracts originating from different whole grains were in the hierarchy: barley > oat > wheat > rye (Zielinski and Kozlowska, 2000a) . Furthermore, systemic, low-grade inflammation, especially in adipose tissue, is a trademark of obesity and diabetes. In addition to barley phytochemicals' antioxidant properties, barley phytochemical compounds have potent anti-inflammatory actions and could thereby moderate diabetes and obesity risk by this mechanism (Salas-Salvad *et al.*, 2011).

2.8.6.2.3 Cancer

Barley and its products have bioactive compounds with antioxidative and immunomodulatory activities that are associated with cancer moderation. Most studies regarding the chemoprevention of carcinogenesis by barley have been in vitro and have mainly involved the effect of barley fiber, especially β-glucan, and the moderation of this disease. (Kanauchi *et al.*, 2008) investigated anti-carcinogenic benefits of germinated barley foodstuff (GBF), a prebiotic heterogeneous mixture of approximately 80% hemicellulose and insoluble glutamine-rich protein fiber. GBF also contains phytochemicals, especially phenolic acids, present in free or bound forms which has health benefits. (Kanauchi *et al.*, 2008) reported that GBF affects the early stages of the pathogenesis of colon cancer and helps impede transforming hyperproliferative epithelia. In another report by (McIntosh *et al.*, 1993) on the influence of different fiber-rich sources from wheat and barley in diets on tumor incidence, tumor burden, tumor mass index, and dimethyl induced tumor, these were reported to be more effectively prevented by dietary insoluble fiber from barley than from other soluble fiber-rich commercial bran from barley and oat (McIntosh *et al.*, 1993). Spent barley grain, a by-product of the brewing industry, produced the lowest tumor incidence. The abundant content of phenolic compounds in barley suggests that it may also serve as an excellent dietary source of natural antioxidants with antiradical and antiproliferative potentials (Z. Zhao and Moghadasian, 2008). Several studies have shown that FA has tumor inhibition properties (Kumar and Pruthi, 2014). There have also been reports of its ability to inhibit large bowel carcinogenesis (Kawabata *et al.*, 2000). Fermentation of barley (lactic acid) was also reported to produce a novel purple pigment called hordeumin, a type of anthocyanidinetannin pigment whose scavenging activity increases with the length of fermentation. (Deguchi *et al.*, 1999) suggested that the free radical activity of hordeumin results from barley bran polyphenols. Phytochemicals present in barley may be beneficial in moderating the risk factors associated with cancer and therefore should be a subject of further studies.

Part III

Materials and methods

3.1 Materials

3.1.1 Naked Barley

Naked barley was collected from Armala VDC, Kaski, Nepal. It was confirmed that the naked barley belongs to:

Genus: *Hordeum*

Species: *vulgare*

Variety: *nudum* (NARC)

3.1.2 Raw materials for biscuit making

Important raw materials for biscuit making were provided by Asian Biscuits and Confectionery (p) Ltd which included:

- Wheat flour (*Maida*)
- Sodium and Ammonium bicarbonate
- Soya lecithin
- Sodium Metabisulphite

Sugar in the form of pulverized sugar and iodized common salt were used. Skimmed milk powder (SMP) named 'Everyday' of Nestle was used. Vegetable ghee named 'Shakti' was used. Invert syrup was prepared in the laboratory of CCT.

3.2 Methods

3.2.1 Experimental procedure

Design Expert 10 software was used to create the recipe. Rotatable central composite design was used to formulate the recipe. The independent variable for the experiment is percentage incorporation of Naked Barley flour was used to make biscuit ranging from 0 to 25%.

3.2.2 Formulation of recipe

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. Biscuits were of soft dough type method. The recipe formulation for the Naked barley incorporated biscuits was as per the direction of Asian Biscuits and Confectionery (p) Ltd and was carried out as given in Table 3.1.

Table 3.1 Recipe formulation for biscuits

Ingredients	A	B	C	D	E	F	G
Wheat flour	100	93.75	92	87.5	83	81.25	75
Naked Barley flour	0	6.25	8	12.5	17	18.75	25
Fat	33	33	33	33	33	33	33
Pulverised sugar	35	35	35	35	35	35	35
Salt	0.75	0.75	0.75	0.75	0.75	0.75	0.75
SBC	0.75	0.75	0.75	0.75	0.75	0.75	0.75
ABC	2.5	2.5	2.5	2.5	2.5	2.5	2.5
SMBS	1	1	1	1	1	1	1
Soya lecithin	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Invert syrup	2	2	2	2	2	2	2
SMP	4	4	4	4	4	4	4
Water	10	10	10	10	10	10	10

3.2.3 Preparation of Naked barley flour

The naked barley sample was first cleaned by screening to remove impurities such as stones, strings, weed seeds, broken corn etc. and then by winnowing with *nanglo* (flat round woven bamboo tray) to remove dusts, husk, immature grains and other light particles. After cleaning it was ground in a grinder and sieved through a mesh size of 100µ.

3.2.4 Manufacturing process of biscuits

The process for preparing Naked barley flour incorporated biscuits is shown in Fig. 3.2. Slight changes were made in the preparation of biscuit as that given by (Smith, 1972).

Fat, lecithin, pulverized sugar, invert syrup, SMP

Creaming

Salt + ABC + SBC + water

Cream

Flour mixture +

SMBS in 2ml water

Mixing

Forming (5mm thick)

Cutting and Stumping (65mm diameter)

Baking (200^{o}C, 15 min)

Cooling (35^{o}C)

Packaging and storage at ambient temperature

Fig. 3.2 Flow sheet of manufacturing process of biscuits

3.2.5 Analytical procedures

3.2.5.1 Proximate analysis

3.2.5.1.1 Moisture content

Moisture content of the sample was determined by weight loss during heating in a thermostatically controlled oven at 105℃ by hot air oven method as given by AOAC (2005)

3.2.5.1.2 Ash content

Ash content of the biscuits samples and the flours was determined using muffle furnace as described by AOAC (2005) at the temperature of 600-700℃.

3.2.5.1.3 Crude fat

Crude fat content of the samples was determined by solvent extraction method using Soxhlet apparatus and solvent petroleum ether as per AOAC (2005).

3.2.5.1.4 Crude protein

Crude protein content of the samples was determined indirectly by measuring total nitrogen content by micro Kjeldahl method. Factor 5.7 was used to convert the nitrogen content to crude protein as per AOAC (2005).

3.2.5.1.5 Crude fiber

Crude fiber content of the samples was determined by the method given by AOAC (2005).

3.2.5.1.6 Carbohydrate

The carbohydrate content of the sample was determined by difference method as by AOAC (2005).

$$\text{Carbohydrate (\%)} = 100 - (\text{protein} + \text{fat} + \text{ash} + \text{crude fiber}).$$

3.2.5.2 Phytochemical analysis

3.2.5.2.1 Extraction and sample preparation

Ten grams of each cookie dough sample and biscuits were taken and 30 ml of 80 % methanol was added to sample. The mixture was swirled and filtered. This process was repeated for 2 times and then final filtered sample was made to 100 ml for analysis (Amorim *et al.*, 2008)**.**

3.2.5.2.2 Determination of total phenolic content

Total polyphenol content was determined by using Folin-Ciocalteu method (Singleton and and Rossi, 1965). Briefly 1 ml of extract or standard solution of gallic acid (20, 40, 60, 80 and 100 mg/l) was taken in 25 ml volumetric flask which containing 9 ml of distilled water. 1 ml of Folin-Ciocalteu reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added, and the solution was diluted to volume with distilled H_2O and mixed. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank (distilled H_2O) was measured at wavelength of 750 nm. Total polyphenol contents of cookie dough samples and biscuits were reported as mg Gallic Acid Equivalent /100 gm.

3.2.5.2.3 Determination of flavonoid content

Total flavonoid content was determined using a modified aluminium chloride assay method as described by (Ale, 2017). 2 ml of solution was pipette out in a test tube in which 0.2 ml of 5% Sodium Nitrate ($NaNO_3$) was mixed and stand for 5 minutes. 0.2 ml Aluminium Chloride ($AlCl_3$) was pipetted out, mixed in the tube and allowed to stand for 5 minutes. This followed addition of 2 ml of 1N Sodium Hydroxide (NaOH) in the tube and finally volume was made up to 5ml. The absorbance was measured after 15 minutes at 510nm against a reagent blank. The flavonoid content in the extract was determined from the standard curve and expressed in mg quercetin/100gm sample.

3.2.5.2.4 Free radical scavanging capacity

The antioxidant activity of the samples extract was determined using the colorimetric DPPH assay, as described by (Blois, 1958). To determine the radical scavenging activity, 0.1mM solution of DPPH in ethanol was prepared. This solution (1 ml) was added to 3 ml of different extracts in ethanol. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then, absorbance was measured at 517 nm by using spectrophotometer.

The antioxidant activity was calculated from the following equations:

$$\text{Antioxidant Activity (\%)} = \frac{A_0\text{-}A_1}{A_0} \times 100$$

Where, A_0 is the absorbance of the control and A_1 is the absorbance of sample.

3.2.6 Sensory analysis

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

3.2.7 Statistical analysis

The obtained data was analyzed statistically by Genstat 12.1 Edition, for Analysis of Variance (ANOVA) at 5% level of significance. The data obtained from proximate and phytochemical analysis and sensory evaluations were subjected to one-way and two-way Analysis of Variance respectively. All the t-tests were carried out on Ms Excel 2019.

Part IV

Results and discussions

This work was carried out with a goal to study the effect of incorporation of Naked barley flour on the phytochemical properties in wheat flour biscuits.

4.1 Proximate composition of wheat and Naked barley four

The proximate composition of wheat flour and naked barley flour were determined. Determined results are presented in Table 4.1.

Table 4.1 Proximate composition of wheat flour and naked barley flour

Parameters	Wheat flour (%)	Naked barley flour (%)
Moisture	12.57±0.143	10.78±0.120
Crude protein	9.77±0.162	14.18±0.050
Crude fat	1.32±0.098	2.34±0.100
Crude fiber	0.54±0.202	1.96±0.006
Total ash	0.79±0.177	1.46±0.045
Carbohydrate	74.83±0.173	69.28±0.274
Gluten content	8.85±0.213	0

The crude protein content in wheat flour 9.77 was lower than that obtained by Oppong D. *et al.* (2015) and Khanal (1997). The lower value of protein can be attributed with the soil since nitrogen levels can influence protein content however the protein content falls (Oppong D. *et al.*, 2015). Other values have been found close with the values obtained in the above-mentioned studies. Gluten content on Asian wheat flours also found similar value of gluten content as in this work (Barak *et al.*, 2014). Some small variations in values of proximate compositions may result from climate, soil, varieties, maturity, geographical conditions and milling conditions (Oppong D. *et al.*, 2015)

The moisture content, protein, fat, crude fiber, ash and carbohydrate of naked barley flour were found to be similar to the results of a research done by Tobiasz R. *et al.* (2012). The slight differences in proximate composition may be due to the factors like varieties, climatic conditions, soil type, maturity, fertility, geographical conditions and others (Oppong D. *et al.*, 2015). According to Oppong D. *et al.* (2015) Wheat prolamin is known as gliadin and barley prolamin as hordein. As reported by (Dostalek *et al.*, 2006) using sandwich ELISA test, gluten content in barley was found to be 2.0 to 3.0%. However, it was not detected by washing method.

4.2 Phytochemical properties of Cookie dough and biscuits

4.2.1 Total phenolic content

The Total phenolic content (TPC) of both the cookie dough and biscuits according to percentage incorporation of naked barley flour in the refined wheat flour biscuits were analyzed and shown in the Fig 4.1 below. The TPC increased significantly ($p < 0.05$) as the proportion of barley flour in the blends increased. It has been reported that wheat has less phenolic content as compared to barley (Stratil *et al.*, 2007). Whole naked barley flour was used in this study and phenolic compounds are known to be more concentrated in the outer fractions as compared to inner parts of the grain (P. Sharma and Gujral, 2014). (Kaur *et al.*, 2017) reported TPC of refined wheat flour as 11mg GAE/100g and (Brindzova *et al.*, 2009) reported a phenolic content of 11.25 mg GAE/100g in refined wheat flour. (P. Sharma and Gujral, 2014) reported that replacement of wheat flour with barley flour led to a significant increase in total phenolic content. According to (Kruma *et al.*, 2016) the TPC for barley ranged from 351 to 460 mg GAE 100 g^{-1} DW, hull-less (naked) standard varieties being superior in content of total phenolic compounds in grain compared to hulled varieties.

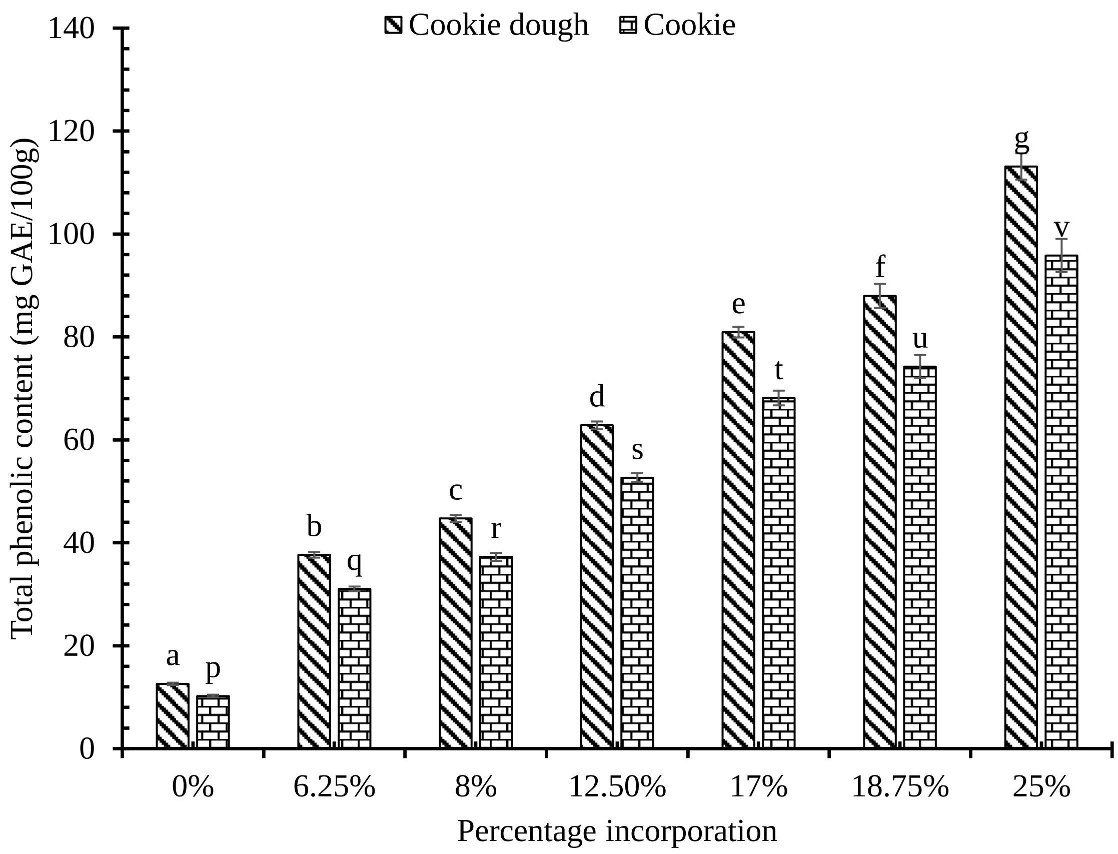

Fig 4.1 Effect of incorporation of Naked barley flour in the TPC of refined wheat flour cookie doughs and biscuits

Baking led to a significant ($p < 0.05$) decrease of upto 18.7% in total phenolic content. (Holtekjolen *et al.*, 2008) reported that the baking of bread containing barley flour exhibited 19.2% decrease in TPC. The decrease in TPC may be attributed to alteration in the chemical structure of the phenolic compounds, possible polymerization leading to reduced extractability and oxidation (P. Sharma and Gujral, 2014).

4.2.2 Antioxidant activity (DPPH free radical scavenging activity)

Increasing the level of Naked barley flour in the wheat barley blends progressively increased the antioxidant activity of the cookie dough from 9.76 to 20.93% (Fig 4.2) and this is attributed to the higher antioxidant activity of the whole naked barley flour as compared to wheat flour. Similar results have been found by (P. Sharma and Gujral, 2014) while incorporating hulled barley flour in refined wheat flour in cookie making. Hulless variety from Nepal was observed to be superior in antioxidant capacity than others by (Hambira, 2009)

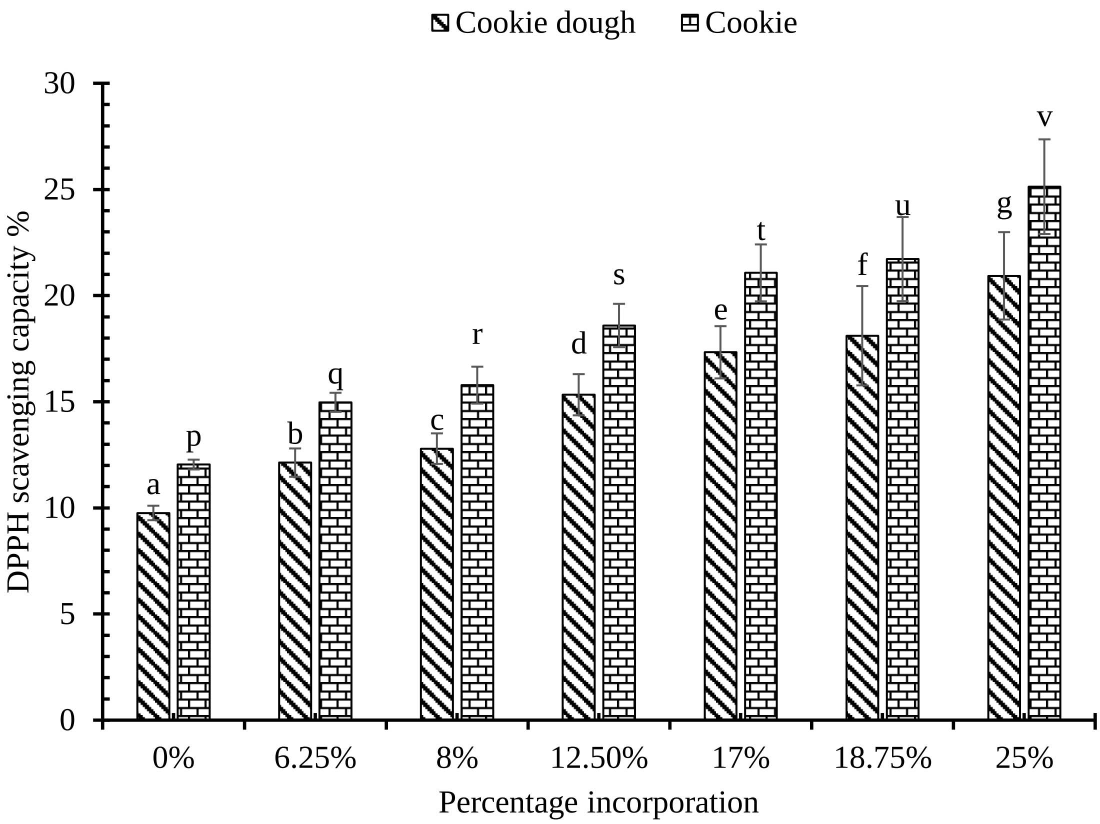

Fig 4.2 Effect of incorporation of Naked barley flour on the antioxidant (DPPH scavenging) capacity of refined wheat flour cookie doughs and biscuits.

Baking further increased the antioxidant activity upto 22.3%. (Holtekjolen *et al.*, 2008) also reported similar results upon baking for bread prepared by incorporating barley flour. (P. Sharma and Gujral, 2014) observed that the baking of fiber incorporated biscuits led to a significant ($p \leq 0.05$) increase in antioxidant activity. The increase in antioxidant activity may be attributed to formation of dark color pigments (brown color) during the baking process due to the Maillard browning and these pigments have been reported to have antioxidant activity (Nicoli *et al.*, 1999)

4.2.3 Total flavonoid content

The total flavonoid content (TFC) of only wheat cookie dough was 28.92 mg Quercetin equivalents/100 g (Fig 4.3). A similar result was reported by (Kaur *et al.*, 2017). A significant ($p < 0.05$) increase in TFC was observed as naked barley flour was incorporated at higher levels in wheat flour. The increase in TFC upon incorporating barley flour in the blends may be attributed to the higher TFC as compared to wheat flour. (Oomah and

Mazza, 1996) reported a total flavonoid content in wheat samples of 407.5 μg rutin equivalents/g whereas barley flour contained TFC upto 527 μg/g.

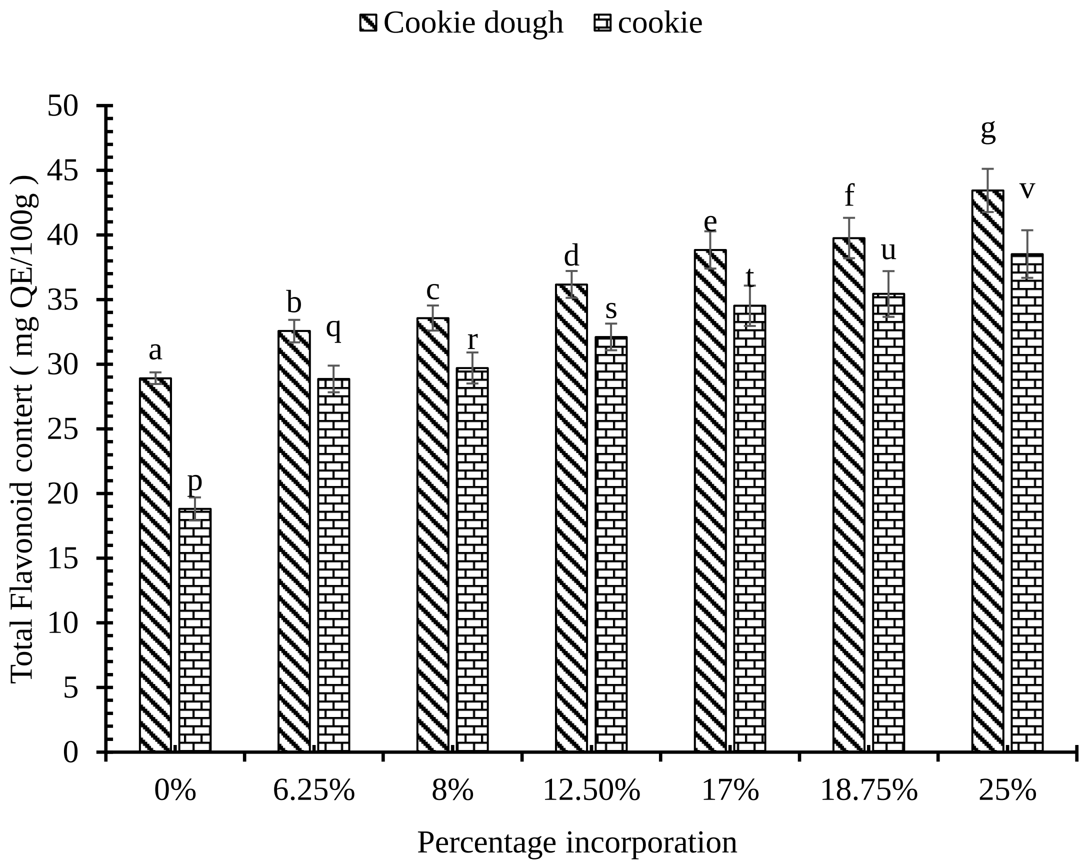

Fig 4.3 Effect of incorporation of Naked barley flour on the Total Flavonoid content of refined wheat flour cookie doughs and biscuits.

Similar increase in total flavonoid content after incorporation of barley flour in refined wheat flour was observed in biscuits by (P. Sharma and Gujral, 2014). Baking of biscuits led to a significant decrease ($p<0.05$) in TFC and it was 11.2% for 25% naked barley incorporated biscuits and 34.8% for only wheat biscuits. Similar decrease in flavonoid content has been reported during baking of biscuits by (Li *et al.*, 2011) prepared from corn flour and (Kaur *et al.*, 2017) from flaxseed flour incorporation. Flavonoids are a group of phenolic compounds which are considered to be more thermal sensitive and they are destroyed during thermal processing (P. Sharma and Gujral, 2014).

4.3 Sensory properties

Statistical analysis of sensory scores obtained from 11 semi-trained panelist using 9-point hedonic rating scale (9= like extremely, 1= dislike extremely) were carried out for the formulated biscuits. The semi trained panelists expressed their evaluations in the context of parameters namely, color, flavor texture and overall acceptability. All the scores were analyzed by ANOVA. The statistical results for sensory evaluation are presented in the Appendix B.

Table 4.2 Sample codes for different Naked barley formulated biscuits

Sample code	Percentage incorporation (%)
A	6.25
B	8
C	12.5
D	17
E	18.75
F	25

4.3.1 Texture

The mean sensory scores for texture were found to be 6.818, 6.545, 6.636, 6.273, 8.000, 8.273 and 5.545 respectively for cookie samples A, B, C, D, E, F and G. Statistical analysis shows that there was significant difference ($p<0.05$) among the samples as shown in Fig 4.4 but no significant difference ($p>0.05$) among the panelists. Sample E and F had the superior values than other samples. Sample G has the lowest value similar with sample D and significantly different than the remaining ones.

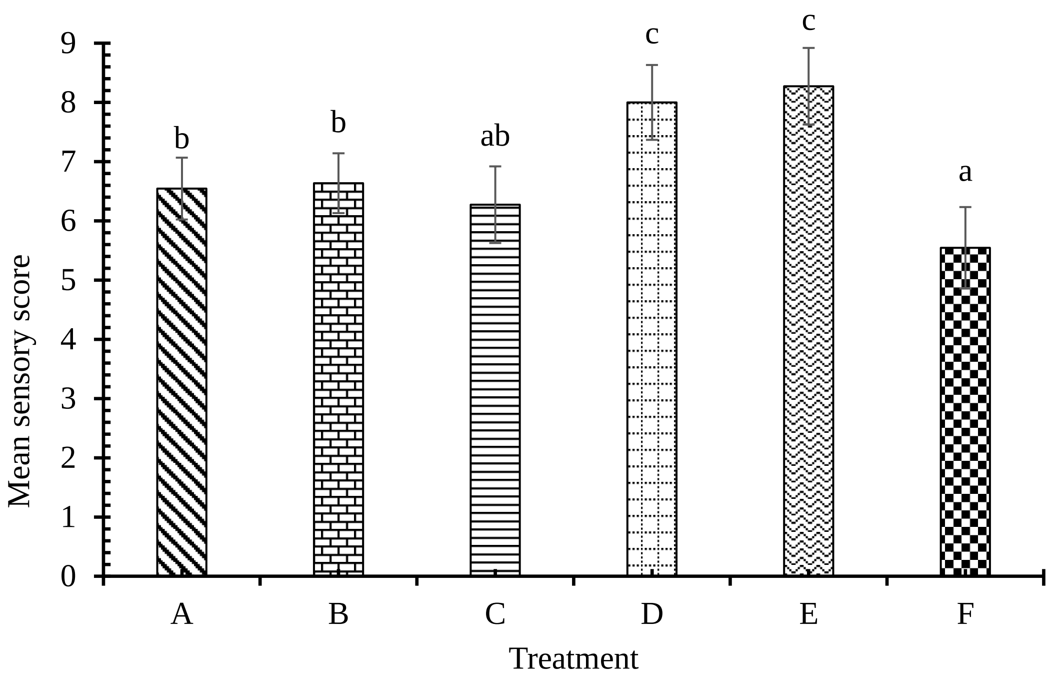

Fig 4.4 Effect of incorporating naked barley flour on the texture of cookie

The score for G was lowest due to the maximum percentage of naked barley flour used. It has been shown in various experiments that the texture is tougher as there is increment of glutenless portions in bakery products like incorporating peanut flour by (Joshi, 2012) and hulless barley in bread by (Siebenhandl-Ehn *et al.*, 2011). Also the high concentration of β-glucan decreases the water availability for the gluten network and thus impairs the baking properties which decreases spreading factor making the texture tougher (P. Sharma and Gujral, 2014). Texture of biscuits become harder since barley contains water absorbing components such as fiber and protein which can contribute in sticky nature of dough which reduces the extensibility of dough and increases fracturability (Kaur *et al.*, 2017).

4.3.2 Colour

The mean sensory scores for texture were found to be 6.636, 5.909, 6.727, 6.000, 7.545, 8.182 and 5.545 respectively for cookie samples A, B, C, D, E, F and G. Again significant differences were observed among the samples but not among the panelists. Sample A, E and F were significantly different ($p<0.05$) from each other while samples B, C, D and G were not significantly different from each other.

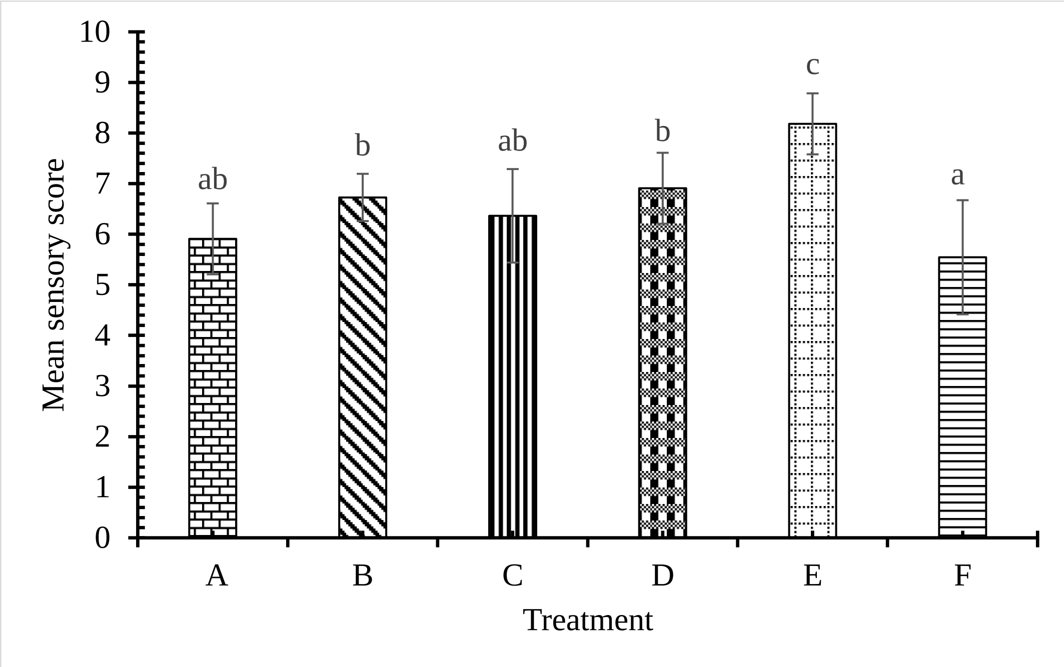

Fig 4.5 Effect of incorporating naked barley flour on the color of biscuits

Decrease in browning of the color in biscuits was observed considering the same time and temperature of baking for all samples which may be attributed by the increase in percentage of whole naked barley flour. Control A had a darker brown colour and sample G had a light brown colour. Besides Maillard reactions browning may also be contributed by caramelization of sugar as cookie formulation is high in sugar. Similar decrease in browning of biscuits were observed by (P. Sharma and Gujral, 2014) as the non-enzymatic browning index of the biscuits decreased as the levels of whole flour increased in the blends which may be attributed to possible dilution of the sugars and proteins of the wheat flour upon incorporation of whole barley flour. From the scores sample F had the most desirable color.

4.3.3 Flavor

The mean sensory scores for texture were found to be respectively 7.000, 6.000, 5.909, 6.182, 7.545, 8.545, 7.273 for cookie samples A, B, C, D, E, F and G respectively. Sample F had the highest score with significantly different ($p<0.05$) value while sample C had the lowest score but not significantly different than B and D. Samples A, E, G had medium values with no significant differences. No significant difference was observed for panelists ($p>0.05$).

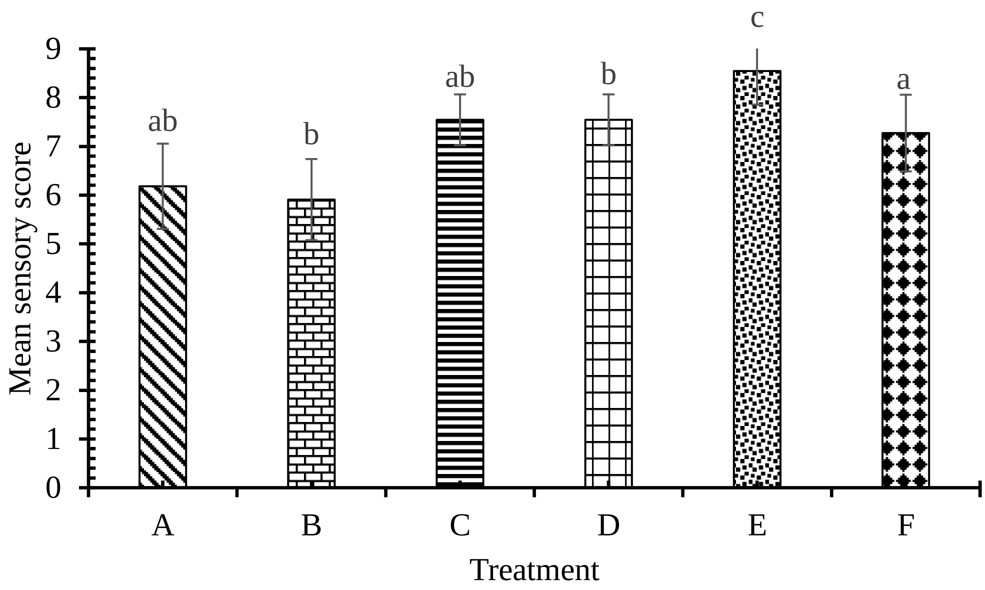

Fig 4.6 Effect of incorporating naked barley flour on the flavour of biscuits

The control A was perceived as good in terms of flavor. As the percentage of naked barley flour has increased the score is seen to decrease and then increase due to the slight nutty flavor of naked barley which is perceived as most optimum for sample F. It may also be linked to the fact that barley flour has high oil absorption capacity which leads to good flavor development and better mouth feel as stated by (P. Sharma and Gujral, 2014).

4.3.4 Overall acceptability

The mean sensory scores for texture were found to be respectively 7.273, 6.091, 6.182, 7.273, 7.636, 8.545 and 5.545, for cookie samples A, B, C, D, E, F and G respectively. Though significant differences among samples have been observed, no significant difference among the panelists were observed from statistical analysis. Sample F had the highest sensory score for overall acceptability making it the best sample. The lowest score was of G which is due to the presence of cracks as the percentage of barley flour is maximum. This might be attributed to increased levels of soluble fiber that lead to increased number of hydrophilic sites available that compete for the limited free water in cookie dough and dilution of gluten protein (P. Sharma and Gujral, 2014). The lowest values are for sample B and C due to the bland flavor and texture comparative to other samples. Sample A, D and E have medium scores as they are acceptable.

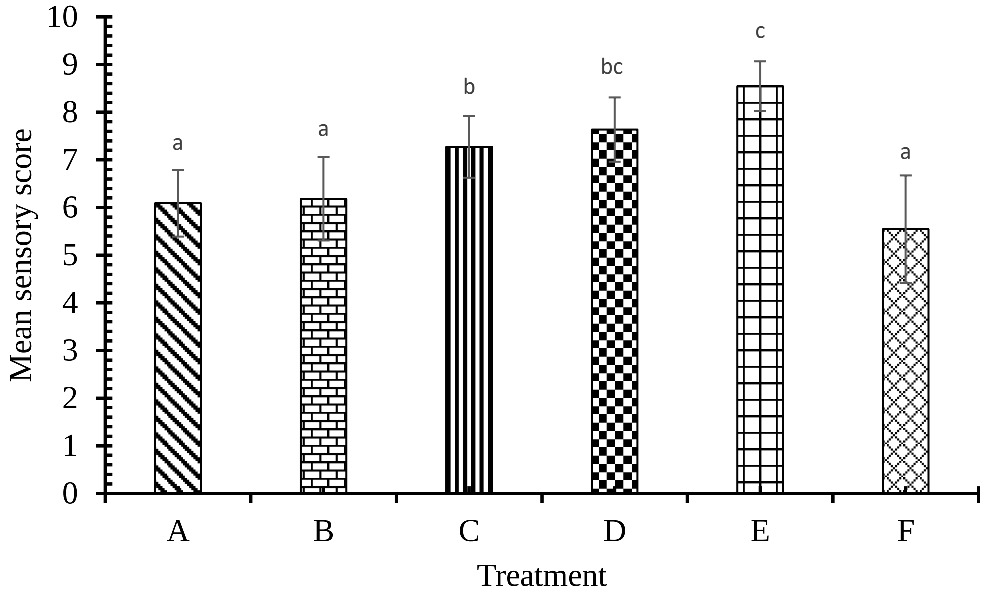

Fig 4.5 Effect of incorporating naked barley flour on the overall acceptability of biscuits

Sample F had the optimum brown colour, texture and overall appearance. It also had the best flavor of barley and also due to high oil absorption capacity. Considering all of the factors, panelists have given it the highest mean sensory score of 8.545. Thus, it is the optimized best product of this experiment which had 18.75% of naked barley flour incorporated into the product.

4.4 Proximate analysis of Optimized naked barley biscuits and Normal wheat biscuits

The proximate analysis of the best product (sample F) from the sensory analysis and normal wheat biscuits (control sample A) was carried out. The result is tabulated in table 4.3.

Table 4.3 Proximate analysis of Optimized naked barley biscuits and Normal wheat biscuits

Parameters in %	Wheat biscuits	Naked barley biscuits
Moisture	2.11[a]	3.42[b]
Fat	26.23[a]	28.02[b]
Crude fibre	0.47[a]	1.44[b]
Protein	8.37[a]	9.45[b]
Ash	0.72[a]	0.91[b]
Carbohydrates	62.10[a]	55.73[b]

From the t-test, significant difference ($p<0.05$) was observed for all the parameters between the two samples. The moisture content significantly increased from 2.11% in control to 3.42% in 18.75% level of substitution. The increase in moisture may be due to more water retaining capacity of the fibers as well as more amount of water required to prepare the biscuit dough having more amount of fiber and β-glucan (P. Sharma and Gujral, 2014). There is increase in protein content from 8.37% to 9.45%. This is because of higher protein (P. Sharma and Gujral, 2014) content of naked barley than wheat flour (Wei-wei *et al.*, 2016) . Similarly, there is increase in crude fiber from 0.47 to 1.44% because of higher fiber content of Naked barley (C. Sharma *et al.*, 2013). Crude fiber is known to aid the digestive system of human.

The fat content has slightly increased from 26.23% to 28.02%. This is due to higher fat content of naked barley flour than wheat flour and also due to high fat absorption by the naked barley flour (P. Sharma and Gujral, 2014) . The increase in total ash content has been observed from 0.72% to 0.91% is due to the higher amount of minerals in naked barley than in wheat flour (Wei-wei *et al.*, 2016). Carbohydrate content reduced from 61.36% to 54.50%. The reduction in carbohydrates might be due to high protein, ash, fat and fiber content of the biscuit. The reduction in carbohydrate could be of help in addressing the risk of sugar intake. The result obtained by (C. Sharma *et al.*, 2013) presented similar pattern in carbohydrate, fat, protein, fiber and ash content. The slight differences in values may have occurred due to different varieties of flour used, cultivation conditions, milling conditions and recipe formulations.

Part V

Conclusions and recommendations

5.1 Conclusions

On the basis of the research, following conclusions can be drawn. Since the work was done under controlled condition on a small scale, its generalization may warrant some reservations.

1. Naked barley flour is superior to wheat flour in terms of crude protein, crude fat, crude fiber and mineral content.
2. Phytochemical analysis showed a significant increase in Total phenolic content, Total flavonoid content and Antioxidant capacity of the biscuits as the percentage of naked barley flour increased in them.
3. The nutritional quality of the Naked barley incorporated biscuits seemed to be enhanced in the case of fiber and protein content.
4. Baking process resulted in significant decrease in TPC and TFC but increase in overall antioxidant capacity.
5. The sensory analysis showed that naked barley flour can be successfully incorporated upto 18.75% without negatively affecting the sensory properties of the wheat biscuits.

5.2 Recommendations

1. Naked barley biscuits can be commercialized by substituting 18.75% of the wheat flour with naked barley flour with improved nutritional and phytochemical qualities and unique sensory attributes.
2. Shelf life of the biscuits and texture analysis can be carried out.

Part VI

Summary

Barley lowers cholesterol, prevents CVD, cancer, diabetes, obesity, etc. It reduces the dependence on drug use or dietary restrictions for the moderation of CVD. There have also been reports of its ability to inhibit large bowel carcinogenesis.

Biscuits with different formulations of refined wheat and naked barley flour were prepared in the lab and dough samples and the biscuits were subjected to phytochemical analysis where total phenolic content, total flavonoid content and antioxidant activity were found out. It showed a significant increase in all the three properties of the biscuits as the percentage of naked barley flour increased in the wheat biscuits. Baking process resulted in significant decrease in TPC and TFC while increase in antioxidant capacity.

The biscuits were also subjected to sensory analysis which was based on colour, flavour, texture and overall acceptability. From the mean sensory scores, sample F (18.75%) was selected as the best formulation and thus subjected for proximate analysis comparing it with control cookie. The colour, texture, flavour was altered due to increase in fiber and beta glucan content on addition of naked barley flour which changed the sensory attributes.

Finally, from the proximate analysis, increase in moisture, protein, fiber, fat and ash content were found in the latter ones. The increase in moisture may be due to more water retaining capacity of the fibers as well as more amount of water required to prepare the biscuit dough having more amount of fiber and β-glucan. Increase in fat content is due to higher fat content of naked barley flour than wheat flour and also due to high fat absorption by the naked barley flour.

Thus, naked barley incorporated biscuits a tasty snack with loads of health benefits so these need to be further researched and commercialized in Nepal which has a great potential for growing naked barley.

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Appendices

Appendix A

Sensory evaluation score sheet Naked barley incorporated biscuits.

Date:

Name of Panelist:

Name of the product: Naked barley incorporated biscuits

Dear panelist, you are provided 7 samples of biscuits containing different proportions of Naked barley and wheat flour. Please taste the following samples and check how much you prefer for each of the samples. Give the points for your degree of preferences for each parameter for each sample as shown below:

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

Like extremely – 9
Like 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

Parameters	Sample Code						
	A	B	C	D	E	F	G
Color							
Flavor							
Texture							
Overall acceptability							

Any Comments:

Signature:

Appendix B

Results of TPC analysis

Table B.1 One way ANOVA of TPC content for cookie dough

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	6	2.093E+04	3.488E+03	77364.45	<.001
Residual	14	6.312E-01	4.509E-02		
Total	20	2.093E+04			

Table B.2 One way ANOVA of TPC content for biscuits

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	6	1.522E+04	2.536E+03	85062.88	<.001
Residual	14	4.174E-01	2.981E-02		
Total	20	1.522E+04			

T-test (two-samples assuming unequal variance) for comparison of TPC between cookie dough and cookie of each formulation

Table B.3 0% incorporation of Naked barley

	Cookie dough	Cookie
Mean	12.58	10.23
Variance	0.1058	0.0288
Observations	2	2
Hypothesized Mean Difference	0	
df	2	
t Stat	9.058584298	
P(T<=t) one-tail	0.005984087	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.011968173	
t Critical two-tail	4.30265273	

Table B.4 6.25% incorporation of Naked barley

	Cookie dough	Cookie
Mean	37.65	31.06
Variance	0.0121	0.0064
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	83.91897637	
P(T<=t) one-tail	6.04324E-08	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	1.20865E-07	
t Critical two-tail	2.776445105	

Table B.5 8% incorporation of Naked barley

	Cookie dough	Cookie
Mean	44.73	37.26
Variance	0.0256	0.0169
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	62.76055337	
P(T<=t) one-tail	1.93037E-07	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	3.86073E-07	
t Critical two-tail	2.776445105	

Table B.6 12.5% incorporation of Naked barley

	Cookie dough	Cookie
Mean	62.81	52.63
Variance	0.1089	0.0729
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	41.3534232	
P(T<=t) one-tail	1.02184E-06	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	2.04369E-06	
t Critical two-tail	2.776445105	

Table B.7 17% incorporation of Naked barley

	Cookie dough	Cookie
Mean	80.92	68.13
Variance	0.0676	0.0196
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	75.01928651	
P(T<=t) one-tail	2.61002E-06	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	5.22004E-06	
t Critical two-tail	3.182446305	

Table B.8 18.75% incorporation of Naked barley

	Cookie dough	Cookie
Mean	87.97	74.25
Variance	0.0196	0.0256
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	111.7752169	
P(T<=t) one-tail	1.92091E-08	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	3.84182E-08	
t Critical two-tail	2.776445105	

Table B.9 25% incorporation of Naked barley

	Cookie dough	Cookie
Mean	113.13	95.82
Variance	0.0289	0.0529
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	104.8289036	
P(T<=t) one-tail	2.48275E-08	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	4.96551E-08	
t Critical two-tail	2.776445105	

Results of Antioxidant capacity analysis

Table B.10 One way ANOVA of antioxidant capacity for cookie dough

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	6	271.987457	45.331243	7146.82	<.001
Residual	14	0.088800	0.006343		
Total	20	272.076257			

Table B.10 One way ANOVA of antioxidant capacity for cookie

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	6	367.57046	61.26174	5111.23	<.001
Residual	14	0.16780	0.01199		
Total	20	367.73826			

T-test (two-samples assuming unequal variance) for comparison of Antioxidant capacity between cookie dough and cookie of each formulations

Table B.11 0% incorporation of Naked barley

	Cookie dough	Cookie
Mean	9.76	12.04
Variance	0.0049	0.0064
Observations	3	3
Hypothesized Mean		
Difference	0	
df	4	
t Stat	-37.14978055	
P(T<=t) one-tail	1.56748E-06	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	3.13495E-06	
t Critical two-tail	2.776445105	

Table B.12 6.25% incorporation of Naked barley

	Cookie dough	Cookie
Mean	12.13	14.97
Variance	0.0064	0.0121
Observations	3	3
Hypothesized Mean		
Difference	0	
df	4	
t Stat	-36.1654	
P(T<=t) one-tail	1.74E-06	
t Critical one-tail	2.131847	
P(T<=t) two-tail	3.49E-06	
t Critical two-tail	2.776445	

Table B.13 8% incorporation of Naked barley

	Cookie dough	Cookie
Mean	12.79	15.78
Variance	0.0016	0.0144
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	-40.9423	
P(T<=t) one-tail	0.000298	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.000596	
t Critical two-tail	4.302653	

Table B.14 12.5% incorporation of Naked barley

	Cookie dough	Cookie
Mean	15.22	18.59
Variance	0.0121	0.0169
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	-34.2761	
P(T<=t) one-tail	2.16E-06	
t Critical one-tail	2.131847	
P(T<=t) two-tail	4.32E-06	
t Critical two-tail	2.776445	

Table B.15 17% incorporation of Naked barley

	Cookie dough	Cookie
Mean	17.33	21.07
Variance	0.0064	0.0081
Observations	3	3
Hypothesized Mean		
Difference	0	
df	4	
t Stat	-53.796	
P(T<=t) one-tail	3.6E-07	
t Critical one-tail	2.13185	
P(T<=t) two-tail	7.1E-07	
t Critical two-tail	2.77645	

Table B.16 18.75% incorporation of Naked barley

	Cookie dough	Cookie
Mean	18.11	21.72
Variance	0.0049	0.0196
Observations	3	3
Hypothesized Mean		
Difference	0	
df	3	
t Stat	-39.947	
P(T<=t) one-tail	1.7E-05	
t Critical one-tail	2.35336	
P(T<=t) two-tail	3.5E-05	
t Critical two-tail	3.18245	

Table B.17 25% incorporation of Naked barley

	Cookie dough	Cookie
Mean	20.93	25.13
Variance	0.0081	0.0064
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	-60.412	
P(T<=t) one-tail	2.2E-07	
t Critical one-tail	2.13185	
P(T<=t) two-tail	4.5E-07	
t Critical two-tail	2.77645	

ANOVA results of TFC analysis

Table B.18 One way ANOVA of TFC for cookie dough

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	6	271.987457	45.331243	7146.82	<.001
Residual	14	0.088800	0.006343		
Total	20	272.076257			

Table B.19 One way ANOVA of TFC for cookie

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	6	733.51783	122.25297	5348.57	<.001
Residual	14	0.32000	0.02286		
Total	20	733.83783			

T-test (two-samples assuming unequal variance) for comparison of TFC between cookie dough and cookie of each formulations

Table B.20 0% incorporation of Naked barley

	Cookie dough	Cookie
Mean	28.92	18.81
Variance	0.0121	0.0081
Observations	3	3
Hypothesized Mean Difference	0	
Df	4	
t Stat	123.207	
P(T<=t) one-tail	1.3E-08	
t Critical one-tail	2.13185	
P(T<=t) two-tail	2.6E-08	
t Critical two-tail	2.77645	

Table B.21 6.25% incorporation of Naked barley

	Cookie dough	Cookie
Mean	32.56	28.86
Variance	0.0196	0.0196
Observations	3	3
Hypothesized Mean Difference	0	
Df	4	
t Stat	32.3683	
P(T<=t) one-tail	2.7E-06	
t Critical one-tail	2.13185	
P(T<=t) two-tail	5.4E-06	
t Critical two-tail	2.77645	

Table B.22 8% incorporation of Naked barley

	Cookie dough	Cookie
Mean	33.57	29.71
Variance	0.0169	0.0225
Observations	3	3
Hypothesized Mean Difference	0	
Df	4	
t Stat	33.6822	
P(T<=t) one-tail	2.3E-06	
t Critical one-tail	2.13185	
P(T<=t) two-tail	4.6E-06	
t Critical two-tail	2.77645	

Table B.23 12.5% incorporation of Naked barley

	Cookie dough	Cookie
Mean	36.17	32.11
Variance	0.0144	0.0256
Observations	3	3
Hypothesized Mean Difference	0	
Df	4	
t Stat	35.1606	
P(T<=t) one-tail	2E-06	
t Critical one-tail	2.13185	
P(T<=t) two-tail	3.9E-06	
t Critical two-tail	2.77645	

Table B.24 17% incorporation of Naked barley

	Cookie dough	Cookie
Mean	38.84	34.52
Variance	0.0289	0.0169
Observations	3	3
Hypothesized Mean Difference	0	
Df	4	
t Stat	34.9632	
P(T<=t) one-tail	2E-06	
t Critical one-tail	2.13185	
P(T<=t) two-tail	4E-06	
t Critical two-tail	2.77645	

Table B.25 18.75% incorporation of Naked barley

	Cookie dough	Cookie
Mean	39.76	35.43
Variance	0.0256	0.0144
Observations	3	3
Hypothesized Mean Difference	0	
Df	4	
t Stat	37.4989	
P(T<=t) one-tail	1.5E-06	
t Critical one-tail	2.13185	
P(T<=t) two-tail	3E-06	
t Critical two-tail	2.77645	

Table B.26 25% incorporation of Naked barley

	Cookie dough	Cookie
Mean	43.44	38.52
Variance	0.0441	0.0529
Observations	3	3
Hypothesized Mean Difference	0	
Df	4	
t Stat	27.3615	
P(T<=t) one-tail	5.3E-06	
t Critical one-tail	2.13185	
P(T<=t) two-tail	1.1E-05	
t Critical two-tail	2.77645	

Appendix C

Standard curves of Gallic acid and Quercetin

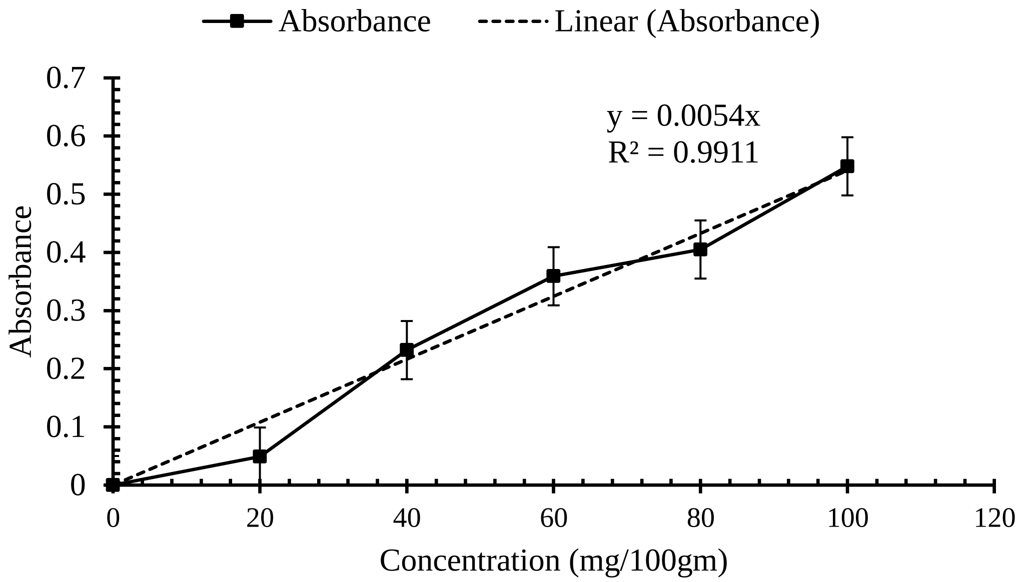

Fig B.1 Standard curve for Gallic acid

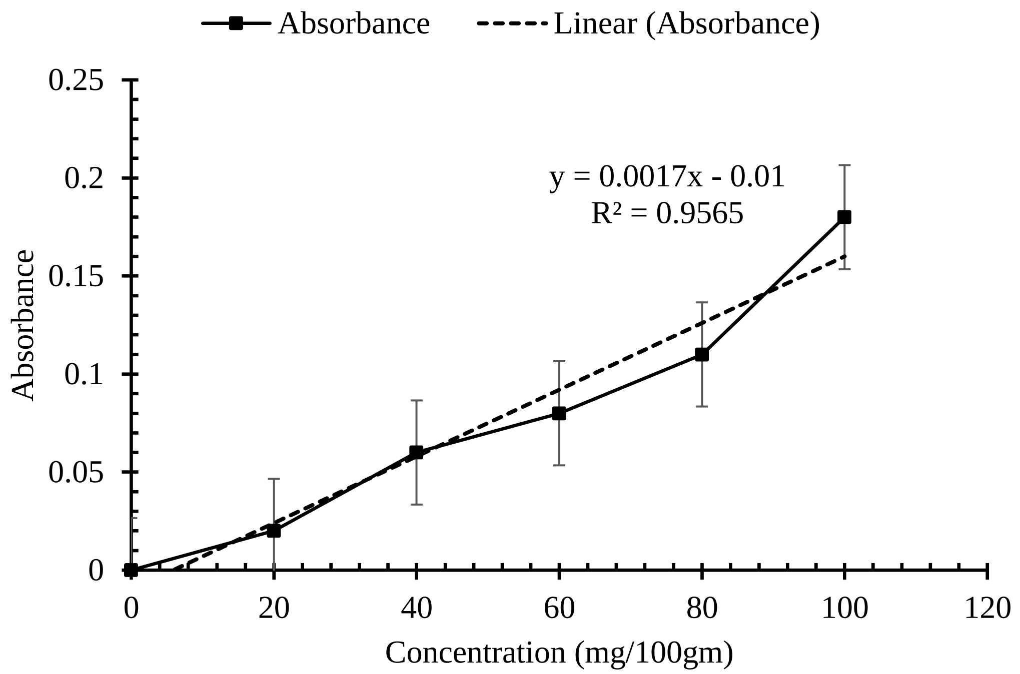

Fig B.2 Standard curve of quercetin

Appendix D

ANOVA results of sensory analysis

Table C.1 ANOVA (no blocking) for color of Naked barley incorporated biscuits

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	6	61.4026	10.2338	15.91	<.001
Panelist	10	2.8571	0.2857	0.44	0.918
Residual	60	38.5974	0.6433		
Total	76	102.8571			

Table C.2 ANOVA (no blocking) for texture of Naked barley incorporated biscuits

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	6	60.7013	10.1169	21.56	<.001
Panelist	10	3.8442	0.3844	0.82	0.612
Residual	60	28.1558	0.4693		
Total	76	92.7013			

Table C.3 ANOVA (no blocking) for flavor of Naked barley incorporated biscuits

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	6	52.8831	8.8139	16.84	<.001
Panelist	10	4.4156	0.4416	0.84	0.589
Residual	60	31.4026	0.5234		
Total	76	88.7013			

Table C.4 ANOVA (no blocking) for overall acceptability of Naked barley incorporated biscuits

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	6	71.7662	11.9610	18.63	<.001
Panelist	10	6.3896	0.6390	1.00	0.458
Residual	60	38.5195	0.6420		
Total	76	116.6753			

Appendix E

Comparison between best sample of Naked barley formulated biscuits and control wheat biscuits

T-test (two-sample assuming unequal variance) for comparison of proximate composition between best sample and the control wheat biscuits.

Table D.1 Moisture content

	Wheat biscuits	Naked barley biscuits
Mean	2.11	3.42
Variance	0.0081	0.0121
Observations	3	3
Hypothesized Mean Difference	0	
Df	4	
t Stat	-15.96453371	
P(T<=t) one-tail	4.50009E-05	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	9.00017E-05	
t Critical two-tail	2.776445105	

Table D.2 Fat content

	Wheat biscuits	Naked barley biscuits
Mean	26.23	28.00666667
Variance	0.0169	0.014533333
Observations	3	3
Hypothesized Mean Difference	0	
Df	4	
t Stat	-17.35686808	
P(T<=t) one-tail	3.2336E-05	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	6.46719E-05	
t Critical two-tail	2.776445105	

Table D.3 Crude Fibre content

	Wheat biscuits	Naked barley biscuits
Mean	0.47	1.44
Variance	0.01	0.0144
Observations	3	3
Hypothesized Mean Difference	0	
Df	4	
t Stat	-10.75566949	
P(T<=t) one-tail	0.000211811	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.000423623	
t Critical two-tail	2.776445105	

Table D.4 Protein content

	Wheat biscuits	Naked barley biscuits
Mean	8.37	10.48
Variance	0.0256	0.0256
Observations	3	3
Hypothesized Mean Difference	0	
Df	4	
t Stat	-16.15132299	
P(T<=t) one-tail	4.29804E-05	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	8.59609E-05	
t Critical two-tail	2.776445105	

Table D.5 Ash content

	Wheat biscuits	Naked barley biscuits
Mean	1.46	2.14
Variance	0.0064	0.0081
Observations	3	3
Hypothesized Mean Difference	0	
Df	4	
t Stat	-9.78105135	
P(T<=t) one-tail	0.00030613	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.00061226	
t Critical two-tail	2.776445105	