PREPARATION AND QUALITY EVALUATION OF BUCKWHEAT FLOUR INCORPORATED BISCUIT

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A dissertation submitted to Central Campus of Technology, Food Technology Instruction Committee, in partial fulfilment of the requirements for the degree of B. Tech. in Food Technology

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Approval Letter

This *dissertation* entitled *"Preparation and quality evaluation of Buckwheat flour incorporated biscuit"* presented by *Ananta Raj Poudel* has been accepted as the partial fulfilment of the requirements for Bachelor in Food Technology.

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Ananta Raj Poudel

Abstract

This work was carried out at Pashupati Biscuit Industry (P) Ltd., Duhabi, Sunsari, Nepal. The physicochemical and mineral content of buckwheat flour incorporated biscuit (BFB) and wheat flour biscuit (WB) was studied comparatively. BFB were prepared with the incorporation of buckwheat flour in 10, 15 and 20% concentration with refined wheat flour to assess the quality and acceptability of the biscuit. The sensory analysis of BFB of different concentration was carried out, samples showed a significant difference at 5% level of significance in sensory attributes. From the mean sensory scores sample B (15%) was selected as the best formulation and subjected for further proximate analysis. The moisture, fat, crude fiber, protein, and ash content of BFB and WB were found to be 2.30, 18.0, 0.78, 7.08, 1.7 and 1.89, 17.26, 0.65, 6.92, 1.3 respectively. All the evaluated values showed a higher range in BFB comparatively. Further, BFB and WB were subjected for iron content evaluation. The iron content in BFB and WB was 0.03200mg/10gm and 0.01720mg/10gm respectively. At 5% level of significance the two samples were significantly different from each other. Shelf life of the BFB was studied for 90 days at 30 day interval. The rise pattern of acid value (AV), showed no significant difference between the consecutive studies although initial value of 0.12 and the final 0.23 were different significantly. The rise pattern in moisture content and peroxide value (PV) showed significant difference after 30 days. The initial, 0 day and final, 90 day values of AV, PV and moisture content were found to be 0.23 mg KOH/ gm oil, 1.96 meqv peroxide/kg fat and 4.1% respectively which was below the standard unacceptable level. These findings suggest that buckwheat flour can be successfully incorporated in refined wheat flour up to the concentration of 15% without any adverse effect on sensory attributes. The iron content and the fat content were found to be higher in comparison to normal wheat flour biscuit.

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Part I

Introduction

1.1 General introduction

Biscuit, this word has its origin from the Latin word *Bixcuit* where, Bix- means twice and –cuit means baked (Balfour, 1976). Biscuit is a kind of crispy dry bread, more or less hard, variously flavoured and usually unleavened, prepared usually in small, flat, thin cakes (Smith, 1972).

During ancient days biscuit was one of the best and most suitable foods to be carried along during long voyages especially due to its higher shelf life and nutritive value. Those biscuits were called as ship's biscuit; Walker's dictionary defines: 'Biscuit is a kind of hard, dry bread, made to be carried to sea as a substitute of bread; it is a composition of fine flour, almonds and sugar.'

Based upon these old themes, biscuits have gone through various modifications through new trials till this date. Nowadays various ranges of biscuits with distinct identical qualities and composition are found in the market.

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

Usually the production of biscuit has placed wheat flour as its sole composition, but addition of other valuable flour at a certain extent can be done for nutritional value addition of the produce without adversely affecting the must properties of the biscuit.

Buckwheat is cultivated as a major food crop in the upper and mid hilly regions of Nepal. Besides it's consumption in the form of *dhindo*, *roti*, *khole* and *malpuwa*, no other significant product has been made till date. Besides being nutritionally rich buckwheat also contains a very important antioxidant called rutin. Hence buckwheat can also be used as medically beneficial food for high blood pressure and heart patients. Various products such as noodles, cake and bread are made using whole as well as composite buckwheat flour in Japan, Korea, America and many other countries (NARC, 2010).

Buckwheat's beneficial effects are due in part to its rich supply of flavonoids, particularly rutin. Flavonoids are phytonutrients that protect against disease by extending the action of vitamin C and acting as antioxidants.

Buckwheat's lipid-lowering activity is largely due to rutin and other flavonoid compounds. These compounds help maintain blood flow, keep platelets from clotting excessively. Platelets are compounds in blood that, when triggered, clump together, thus preventing excessive blood loss, and protect LDL from free radical oxidation into potentially harmful cholesterol oxides. All these actions help to protect against heart disease. Buckwheat is a very good source of manganese and a good source of magnesium and dietary fibre. Buckwheat contains two flavonoids with significant health-promoting actions: rutin and quercitin. The protein in buckwheat is a high quality protein, containing all eight essential amino acids, including lysine. Buckwheat lacks gluten.

Natural antioxidants present in buckwheat may inhibit lipid peroxidation in food and improve food quality and safety. Buckwheat seed contains antioxidants such as rutin and can be stored for a long time without apparent chemical changes (Dietrych-Szostak & Oleszek, 1999)

No any former researches in the field of formulation of buckwheat flour mixed biscuits have been undertaken in context of Nepal. Thus the need of this research work has been deemed.

Nutritional contribution of buckwheat flour and products for the seven essential minerals, *i.e.,* iron, zinc, copper, manganese, calcium, magnesium, potassium and phosphorus, to their recommended dietary allowances has been estimated from experimental data (Ikeda, 2001). Recommended dietary allowances (RDA) for these minerals have been established in several countries. The experimental estimation has shown that one hundred grams of buckwheat flour can provide approximately 10 to 100 percent of the RDA for zinc. Copper, manganese, calcium, magnesium, potassium and phosphorus, but can provide only a few percent of RDA for calcium. Therefore, we conclude that buckwheat flour and its resultant products can be a good source of zinc, copper, manganese, magnesium, potassium and phosphorus.

Hence, production of buckwheat biscuits in context of Nepal will be beneficial not only for industries but also for farmers and consumers as well.

1.2 Objectives of the study

1.2.1 General objective

General objective of this work is to incorporate parts of buckwheat flour in the wheat flour biscuit and to evaluate the quality of the biscuit.

1.2.2 Specific objective of the study

The specific objectives of the study are:

- To determine and compare the proximate composition of the wheat flour biscuit and buckwheat flour incorporated biscuit.
- To determine the iron content, shelf life and acceptability of the formulated buckwheat flour biscuit and compare it with wheat flour biscuit.

1.3 Justification of the work

Snacks are the major food bulk consumed by a human every day. Hence, alike lunch, dinner and breakfast, snacks also should be rich in nutritional value. Biscuits have become a famous snack in developing country like ours due to its low price and ready to eat instant characteristics. Hence, such food must be nutritionally as rich as possible.

Many researches have been carried out in context of the nutritional value of normal wheat flour biscuits. Conclusions drawn from those research, point towards a major short coming in such biscuits to be lacking mainly in mineral content. So, the need of this study has been deemed in order to fulfill the shortcoming in wheat biscuits by partial incorporation of buckwheat flour which is richer in mineral content in comparison to normal wheat flour.

Nutritional contribution of buckwheat flour and products for essential minerals, *i.e.*, zinc, iron, copper, manganese, calcium, magnesium, potassium and phosphorus, to their recommended dietary allowances has been estimated from experimental data (Ikeda, 1996).

Recommended dietary allowances (RDA) for these minerals have been established in several countries. The experimental estimation has shown that one hundred grams of buckwheat flour can provide approximately 10 to 100 percent of the RDA for zinc, copper, manganese, calcium, magnesium, potassium and phosphorus, but can provide only a few percent of RDA for calcium. Therefore, we conclude that buckwheat flour and its resultant products can be a good source of iron, zinc, copper, manganese, magnesium, potassium and phosphorus.

Hence, production of buckwheat biscuits in context of Nepal will be beneficial not only for industries but also for farmers and consumers as well.

1.4 Limitations of the study

- The specific variety of buckwheat and wheat used couldn't be determined due to time and technical constraints.
- Only iron content of the product was studied, although other minerals like zinc and magnesium are also present in abundance according to the former literatures.
- The shelf life of the product could only be studied for 3 months due to time constraint.

The packaging of the biscuit was done in simple polypropylene bags rather than laminates due to technical constraints.

Part II

Literature Review

2.1 Classification of biscuits

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

2.1.1 Soft dough biscuits

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

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

Soft dough biscuits are less versatile because of the inherent nature of the dough.

2.1.2 Hard dough biscuits

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

2.1.2.1 Semi sweet biscuits

The flour used in this type of biscuits should be as weak as possible. Its higher water content and relatively low amount of sugar and fat produces an extensive gluten system and structure. Many flour formulations contain cornstarch or arrowroot to an extent of 10% of the flour weight in order to weaken the flour strength. Further to prevent excessive gluten development, a long mixing time followed by addition of sodium metabisulphite is carried out. Rapid cooling should be avoided as these types of biscuits are highly susceptible to checking (George, 1981).

2.1.2.2 Fermented dough biscuits

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

2.1.2.3 Puff dough biscuit

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

2.2 Chemical composition of biscuits

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

Table 2.1 Chemical composition of biscuit

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

(Source: Smith, 1972)

2.3 Raw materials for biscuit making

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

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

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

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

2.3.1 Major ingredients

2.3.1.1 Flour

Flour is the basic raw material for biscuit making responsible for the major bulk of biscuit (Whitely, 1971). The flour used in biscuit and cracker vary in strength and baking characteristics (Bohn, 1956)

Wheat grain is the only grain naturally capable of producing flour capable of being made into a low density baked product. Wheat is botanically named as *Triticum vulgare.* Wheat flour for biscuit making is obtained from the endosperm in the form of particle size enough to pass through a flour sieve usually 100 mesh per linear inch (Kent, 1983)

Wheat flour is unique among all the cereal flours in that it forms an elastic mass when mixed with correct proportion of water. This unique property is due to the presence of insoluble proteins, collectively called gluten. The gluten forming proteins (Glutenin and gliadin) constitute about 75-80% of the total flour proteins (Mukhopadhyay, 1990). Glutenin gives solidity to the product whilst gliadin is the binding agent imparting the soft sticky character to the gluten. Gliadin is soluble in 70% alcohol and may be extracted from flour whereas glutenin is soluble in alcohol and water (Gorinstein *et al*., 2002).

Gluten is elastic, cohesive and rubbery and holds together and holds together the various ingredients of the dough. It has the property of holding the gases given off during fermentation and during baking. It sets in oven to form the firm, porous, open texture during baking which are necessary in the production of biscuits and crackers. Thus gluten is the necessary framework, forming the sustaining wall of the whole structure of baked products (Bohn, 1956).

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

The strong flour protein has long links with few bonds while weak flour protein has short links with many bonds. During biscuit making weak and easy to stretch, soft wheat flour is found to be better (K.C., 1999).

Beside the natural quality of flour, the modifications in the flour strength can be done by various treatments. Treatment of the flour with sulphur dioxide reduces the flour strength. Heat treated flour added to untreated flour is claimed to strengthen the flour. According to Kent (1983), improvers have some effect upon the nature and character of the gluten and cause it to behave, during fermentation, like the gluten of the stronger flour.

The flour should be free flowing, dry to touch, should be creamy in colour and free from any visible bran particles. It should also have a characteristic taste and should be free from musty flavour and rancid taste.

As suggested by Biscuit Bakers Institute book in Biscuit, Crackers and Cookies vol.1, the protein specifications for cookie flour is mentioned in Table 2.2.

Table 2.2 Protein specification for various flours

Type	Specifications	Protein (%)
Soft cookie flour	General sweet goods	7-8
Medium cookie flour	Cracker dough - up, rich cookies	8-9
Strong cookie flour	Cracker sponge	8.5-10

(Source: Arora, 1980)

The characteristics as required in flour is given in Table 2.3.

Table 2.3 Requirements for flour characteristics

S. No.	Characteristics	Requirements
1.	Moisture content	13.0% max.
2.	Gluten content on dry basis	7.5% min.
3.	Total ash on dry basis	0.5% max.
4.	Acid insoluble ash on dry basis	0.05% max.
5.	Protein (N×5.7) on dry basis	9.0% min.
6.	Alcohol acidity as H_2SO_4 in 90% alcohol	0.1% max
7.	Water absorption	55% min.
8.	Sedimentation value	22% min.
9.	Uric acid (mg/100 gm)	10 max
10.	Granularity	To satisfy the taste

(Source: Arora, 1980)

2.3.1.1.1 Corn flour

Corn flour and maize starch are prepared from the cereal *Zea mays*. Maize is Indian corn. The two chief varieties are known respectively as flint maize and dent maize (Sanchez, 2002). Corn flour is milled from the endosperm of maize and is very nearly pure starch, because of its high starch content; it can be used to weaken flour which is too strong.

2.3.1.1.2 Rice flour

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

2.3.1.1.3 Oat flour and oat meal

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

2.3.1.1.3 Soya flour

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

2.3.1.1.4 Arrowroot flour

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

2.3.1.1.5 Buckwheat flour

Buckwheat (*Fagopyrum esculentum*) is botanically classified as fruit and also referred to as pseudo-cereal. Buckwheat flour is highly rich in mineral content mainly iron and zinc. Buckwheat flour is rich in vitamin content mainly the vitamin ‘B’ group, as well as the antioxidant content in buckwheat flour is very high. Buckwheat flour has higher oil absorption capacity which gives better mouth feel. Buckwheat flour is gluten free and hence partial incorporation of buckwheat flour seem to be beneficial nutritionally (NARC, 2010).

2.3.1.2 Fat or shortening

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

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

During mixing fat also helps in entrapment and retention of the air, which is highly necessary for a product for its good texture. Fat also lubricates the formed gluten molecules to distribute it to various sites during sheeting and hence preventing the agglomeration of the gluten molecules. Fat also plays a vital role in the softness, texture palatability and keeping quality of the product (Manley, 1985).

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

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

1. It should possess good white to creamy color.

2. After keeping the fat at 50°c for 24 hrs and filtering, its color should be comparable with the control sample of oil.
3. The fat should have a smooth, uniform texture, free from any oil separation and large grains.
4. The fat should have a bland 'clear' odour and taste.
5. The fat should have a wide plastic range to suit particular production techniques and the product.
6. The crystalline structure of fat should be stable during mixing and after baking.
7. The fat should possess reasonable shelf life on its own without the addition of antioxidants. The acid value and peroxide value of the extracted fat should not exceed 0.3 mg KOH/ gm oil and 3 meqv peroxide/kg fat respectively.
8. The fat should be prepared from the blend of oils, which will not cause fat bloom during the storage of biscuit.

(Mukhopadhyay, 1990)

2.3.1.3 Sweetening agents

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

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

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

2.3.2 The minor ingredients

2.3.2.1 Emulsifying agents

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

Various recipes in biscuit making include those from high fat recipe to low fat recipe, with low water and high water respectively. In the low fat recipes, process problems are associated with gluten development and dough machinability but in high fat recipes, there is more concern for the fat, to give maximum textural effects, dough stickiness and control of spread while baking (Manley, 1983).

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

2.3.2.2 Leavening agents

Leavening are the gassing agents which causes the dough to spring off or puff up to give a porous open texture to the final product. Ammonium and sodium bicarbonate are the major chemical leaveners, while yeasts are the biological leaveners. Similarly, mechanical leavening can be done by incorporating the air within the dough matrix by mechanical agitation. Reaction of two or more chemicals also leads to production and incorporation of gas, mainly the reaction takes place between bicarbonates of ammonia as well as sodium with acidulants. To discuss about the major and most common leavening agent the baking powder, it should possess the following properties (Smith, 1972):

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

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

$$\underset{\text{Ammonium bicarbonate}}{NH_4HCO_3} \xrightarrow{\text{heat}} \underset{\text{Ammonia}}{NH_3} + \underset{\text{Carbon dioxide}}{CO_2} + \underset{\text{Water}}{H_2O}$$

$$\underset{\text{Ammonium carbonate}}{(NH_4)_2CO_3} \xrightarrow{\text{heat}} \underset{\text{Ammonia}}{2NH_3} + \underset{\text{Carbon dioxide}}{CO_2} + \underset{\text{Water}}{H_2O}$$

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

$$\underset{\text{Sodium bicarbonate}}{NaHCO_3} + \underset{\text{Acid}}{HX} \longrightarrow NaX + \underset{\text{Carbon dioxide}}{CO_2} + \underset{\text{Water}}{H_2O}$$

$$NaHCO_3 + \underset{\text{Cream of tartar}}{C_4H_5O_6K} \longrightarrow \underset{\text{Sod. Pot. Tartarate}}{C_4H_4O_6NaK} + CO_2 + H_2O$$

Both sodium and ammonium bicarbonate react with acidic ingredients if any, in the dough. Use of an excess ammonium bicarbonate makes the dough more alkaline and thereby may affect the protein structure of the dough. Use of an excess sodium bicarbonate will affect the crumb and crust colour often with an accompanying unpleasant or soapy taste, unless any acidic material is used to neutralise the residual sodium carbonate (Bohn, 1956).

2.3.2.3 Milk solids

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

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

2.3.2.4 Salt (Sodium chloride)

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

2.3.2.5 Flavoring and coloring agents

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

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

2.3.2.6 Water

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

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

2.3.2.7 Anti-oxidants

Anti-oxidants act as a retarding or inhibiting agent in the onset of oxidation rancidity. As biscuit is rich in nutrients and fat content, it is highly prone to oxidative rancidity, so role of antioxidant is essential for prolonging the shelf life of the product. There are a number of naturally occurring substances as well as many man-made chemicals which possess anti-oxidant 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 biscuit (Smith, 1972).

2.4 General specifications of biscuits as published by NBS

Biscuit should be properly baked, crisp and uniform in texture and appearance. They should not possess rancid flavour, fungal infection, off odour and any insect infestation. For filled biscuits any of the fillers like jam, jellies, marshmallow, cream, caramel, figs, raisins etc can be used. The biscuits may be coated with caramel, cocoa or chocolates. Use of antioxidant as well as permitted preservative can be done not exceeding the maximum dosages. The general specifications of biscuits as described by Nepal Bureau of Standards (NBS) is given in Table 2.4.

Table 2.4 General specification for biscuits

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

(Source: NBS, 2040)

Processed edible oil used in biscuit making means any edible vegetable oil neutralized with alkali, refined, deodorized and bleached.

2.5 Nutritive value of biscuits

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 Nutritive value of biscuit (values per 100 gm)

Weight per serving in gram	100 gm
Calories (kcal)	480
Protein (gm)	5.2
Fat (gm)	20.2
Carbohydrate (gm)	71.0
Calcium (gm)	0.04
Phosphorous (gm)	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)

2.6 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

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

Creaming and mixing

↓ (Performing is done for hard dough and fermented dough biscuits.)

Laying the dough

↓

Dough forming

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

Baking

↓ (Temp. 176˚C to 204˚C for 4 to 6 minutes)

Cooling

↓ (For 6 to 7 minutes)

Wrapping and packaging

↓

Labelling and storage (RH 40 to 59% at room temp.)

(Source: Smith, 1972)

Fig. 2.1 Flow sheet of manufacturing process of biscuit

2.6.1 Dough mixing

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

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

a. Creaming up method

This method of mixing up of dough mixing includes two steps, during first step the sugar and fat are blended together to fine dissolution after that other ingredients like milk powder, water, invert syrup, lecithin, color, essence, salt are mixed up for around 3-5 mins. 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 mins. 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 (Whitely, 1971).

b. 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.

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).

2.6.2 Laying the 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).

2.6.3 Forming and performing

The shaping or forming of the dough into various shapes and to the required thickness before baking is one of the major steps in biscuit making. In case of hard dough and fermented dough forming is done by using sheeters and laminators, which reduces the thickness of the dough to convert 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).

2.6.4 Baking

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

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

Every oven used till date consists of four basic parts:

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

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

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

Chemical changes include:

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

Temperature in the baking oven has different effect on the raw dough which is shown in Table 2.6.

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)

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

2.6.5 Cooling

Cooling is one of the most important part of biscuit production. As biscuits emerge from the oven they are very hot nearly at a range of 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 grow tougher and set. Cooling should be gradual and slow (Bloksma, 1990).

Checking is the most common defect which occurs in the biscuits after they are prepared and not noticeable during processing. Checking refers to the hair like structure which might refer to weakening of the structure and breakage might occur after 24 hrs of packaging. Hard, semi-sweet biscuits are prone to checking rather than rotary moulded soft types which is due to its low fat and low sugar content leading to higher gluten development. As gluten has higher affinity towards water, it will extract it from the 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, 1984).

2.6.5 Packaging and storage

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

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

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

Packaging in case of biscuits must be close up together in order to provide a mutual reinforcing effect which prevents them from breakage. Packaging of biscuits at commercial level is generally done in triple laminates consisting of polyethylene, aluminium foil and paper (Paine and Paine, 1983). The characteristics of the packaging materials are described in Table 2.7.

Table2.7 Some characteristics of packaging materials

Component	Properties
Low density polyethylene	Moisture and vapour barrier, heat sealing medium.
Paper	Stiffness, low cost, opacity, printable.
Aluminium foil	Opacity, good water vapour and gas barrier.
Oriented polyethylene terepthalate	Gas barrier, strength, grease resistant.
PVC	Transparency, rigidity, gas barrier.
Polypropylene	Easy sealing, resistance to oil, grease.

(Source: Briston and Katan, 1974)

2.7 Sensory perception of biscuits

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

2.8 Buckwheat (*Fagopyrum esculentum*)

Buckwheat (*Fagopyrum esculentum*) a dicotyledonous plant belonging to *Polygonaceae* family, is a moisture loving, cool climate, annual grain with much potential for use in sustainable tropical cropping systems. . Buckwheat has been grown as a grain crop in China for over 1000 years. Two buckwheat species are commonly cultivated namely, Common buckwheat (*F. esculentum*) and Tartary buckwheat (*F. tartaricum*), the former one is known as *mithe phapar* while the next one is known as *tite phapar* in local language. It is one of the quickest growing green manure crops, taking only 4–5 weeks from seeding to flowering. Buckwheat is a broadleaf, annual crop that reaches 2–5 ft (60–150 cm) in height. It has a single, succulent stem with several branches. Its flowers vary from white or light green to pink or red. It forms a dense, fibrous root system with a deep taproot. Most of its roots are concentrated in the top 10 inches of the soil. Cultivation of buckwheat is not only confined to a particular type of soil or fertilization requirement and furthermore is grown at higher altitudes above 3,000 meters in Nepal and Bhutan (Valenzuela, 2002).

Buckwheat is primarily a human food crop, used similarly to cereal grains such as wheat or oats. Even though buckwheat is not a true cereal, it is sometimes called a “pseudo cereal”. Buckwheat seeds are dehulled and the remaining seed material, called a groats, is ground into flour. The flour is often mixed with flour from other cereal grains to make breads, breakfast cereals or other multi-grain products. In Japan, buckwheat and wheat flour are used to make the popular “soba” noodles. In Russia, where buckwheat is native, it is used in a variety of food products, including roasting the whole groats to make “kasha.” Buckwheat is high in lysine, which wheat and corn are low in. The protein content of dehulled buckwheat is about 12%, with only 2% fat (Myers, 2002).

2.8.1 Nutritional value of buckwheat

Buckwheat seed contains various kinds of nutrients such as protein and starch. Thus products made from buckwheat seed serves as valuable sources of these nutrients. Buckwheat seed also contains some kinds of minerals at relatively high levels. Therefore, buckwheat products appear to be a key source of minerals for people who consume them. Despite of the importance of buckwheat as a food, minerals in buckwheat and its products are still not fully characterized in view of nutrition and functionality (Ikeda, 2001).

Zinc is one of essential minerals for humans. Zinc may be profoundly important for human health. Focusing to zinc, it has been estimated that buckwheat contains a relatively high level of zinc as compared to other cereals (Ikeda, 1991). Furthermore, studies have shown that a relatively-high level of zinc in buckwheat flour is released after pepsin pancreatic digestion. Recent studies clarify changes in various minerals, besides zinc, in buckwheat flour after enzymatic digestion (Ikeda, 2001). Analysis has shown that high levels of zinc, copper and potassium in buckwheat flour are released on enzymatic digestion; moderate levels of magnesium and phosphorus are released; and low levels of calcium and manganese are released. Studies have also shown that 100 gm of buckwheat flour can fulfill 10 to 100% of RDA of minerals namely, Zinc, Copper, Iron and Potassium (Suzuki, 2005).

The incorporation of buckwheat can be justified in composite flour based biscuits as it has beneficial neutraceutical properties and its gluten-free nature can play important role in preventing celiac problem. Buckwheat flour addition into noodle formulation has been observed to show considerable effects on cooking quality, chemical and sensory properties and color values of noodles (Bilgicli, 2008). Cereal grains, including soft wheat are low in protein (7 to 14%) and deficient in some amino acids such as lysine. Buckwheat on the other hand, is higher in protein quality than other cereal grains and could be used to support certain amino acids such as lysine, histidine, valine and leucine. Keeping in view of the neutraceutical and other functional properties of buckwheat, the present study was undertaken with the objectives to compare the functional properties of buckwheat flour with wheat flour and incorporate it in biscuits to assess the quality and acceptability of biscuits (Baljeet, 2010). The amino acid composition in buckwheat flour is richer than the wheat flour which is as described in Table 2.8.

Table 2.8 Amino acid composition (g/100g of protein) of groats of buckwheat samples

Amino acids	Sample
Aspartic acid	10.38
Threonine	3.93
Serine	5.07
Glutamic acid	18.73
Proline	3.01
Glycine	6.33
Alanine	4.72
Cystine	2.67
Valine	5.7
Methionine	2.15
Isoleucine	4.09
Leucine	7.01
Tyrosine	2.98
Phenylalanine	4.79
Lysine	6.07
Histidine	2.56
Arginine	9.84

(Source: Bonafaccia, 2003)

According to Herbert (1987), the iron content in wheat flour is 1.7 mg/100 gm. Due to higher level of gluten content, mainly in case of celiac patients; the real absorption of iron in body is very negligible. The higher gluten content also affects the absorption of other minerals including calcium and magnesium. Hence, due to the technological and nutritional property of buckwheat it has been suggested that its use could improve the intake of iron, calcium and fiber in case of celiac patients.

The iron content in buckwheat flour is 4.1 mg/cup serving (160gm), which fulfills 23% of the recommended daily intake (RDI) of iron. Iron is important to replace the hemoglobin loss mainly in pregnant women, lactating mother and children. The daily intake suggested for normal male is 7 mg/day, while that for lactating mother, pregnant women is 12-16 mg plus the normal RDI of women that is 10-20 mg/day. Although legumes are also the good source of iron, absorption of iron from legume source is very low because the bran binds the iron tightly and makes it unavailable for absorption.

The lethal dose of iron is 3gm/day in case of children, while in case of adult it is 200-250 mg/ kg of body weight. Hence from all these facts and buckwheat flour can be considered as safe and abundant source of iron (Saturani, 2010).

2.4.2 Shelf- life of biscuits

Moisture and oxidative rancidity of the biscuit are the main factors responsible for the shelf-life reduction of the biscuit in terms of palatability. Sunlight causes loss of crust color. Although moisture proof and opaque packaging are used the deterioration of crust color and onset of rancidity is likely to occur at any time which might be due to the atmospheric oxygen present inside the packet. Normally packed and stored biscuits are prone to rancidity and color fading in about 60 days while biscuits stored in sealed jars and dark cupboards at 70-75˚F were safe till 12 months (Smith, 1972).

Mainly increase in moisture content, onset of fat deterioration or increase in peroxide value and sometimes maillard reaction is considered responsible for off-flavor. Natural antioxidants if present in the raw materials might help in preventing oxidation. Buckwheat is a good source of natural anti-oxidants like rutin and quercitin. Incorporation of buckwheat flour in the biscuit might help in preventing oxidative rancidity of the final product (Lin *et al*., 2008).

Part III

Materials and methods

3.1 Raw material

Wheat flour in the form of *maida* used for biscuit making was obtained from Mahalaxmi Flour Mills, Duhabi, Sunsari. Common type Buckwheat grains were obtained from Prakashpur, Sunsari and the flour was milled and obtained from local flour mill to a 90 mesh size. Vegetable ghee of OKI brand, Thailand was used as shortening agent. Sugar in the form of pulverized sugar was used. Skimmed milk powdered (SMP) of Britannia India Limited, India was used. Other raw materials like common salt, invert syrup, sodium and ammonium bi-carbonate, lecithin and flavour were used from the regular store of Pashupati Biscuits Industry (P) Ltd., Duhabi, Sunsari, Nepal.

The recipe formulation for the buckwheat flour incorporated biscuit (25 kg lot) was carried out as given in Table 3.1.

Table 3.1 Recipe Formulation for biscuit

Ingredients	Quantity (Parts)	10% BF (Kg)	15% BF (Kg)	20% BF (Kg)
Wheat flour	90, 85, 80	22.5	21.25	20.00
Fat	35	8.75	8.75	8.75
Pulverised sugar	40	10	10	10
Salt	0.3	0.075	0.075	0.075
Sodium bicarbonate	1	2.5	2.5	2.5
Ammoniuim bicarbonate	1	2.5	2.5	2.5
Soya lecithin	2	5	5	5
Invert syrup	5	12.5	12.5	12.5
Flavor (Butter DRS)	1.5	3.75	3.75	3.75
Milk powder (Full cream)	2	5	5	5
Buckwheat flour (BF)	10, 15, 20	2.5	3.75	5
Water	6	1.5	1.5	1.5

3.2 Method of preparation

Raw materials were weighed as per the calculated recipe as given in Table 3.1. Then after creaming up of shortening, pulverized sugar, syrups, milk powder, part of water, lecithin and half of buckwheat flour was done till the mixture appeared as a creamy mass. The mixing was done in mixing machine at top speed for 3-5 minutes as per requirement. Addition of buckwheat flour early in the mixture was to enhance its naturally borne emulsifying capacity as described by Smith (1972).

Now to the creamy mass remaining buckwheat flour, wheat flour and chemical leaveners dissolved in water was added. Now the remaining water was also added and the mixing was carried out on two speed mixer on low speed for 10 minutes. The adequacy of mixing was checked by studying the adhering nature of the dough in the dye.

Now, thus prepared dough was left for 15 minutes for laying, in order to achieve maturity of dough for easy machinability and good surface gloss as described by Smith, (1972). The matured dough was then fed in the embossing dye or moulders directly.

The dough was then conveyed to continuous baking oven where it was baked for 5 minutes with the last damper open. Baked biscuits were now cooled for 20 minutes and then packed in poly propylene bags and stored in a cool and dry place for further analysis. The standard baking temperature profile set for the study was as given in Table 3.2.

Table 3.2 Baking temperature profile of the oven

	1st Zone	2nd Zone	3rd Zone
Top	176˚C	232˚C	221˚C
Bottom	204˚C	237˚C	204˚C

The method of preparation of biscuit of 25 kg batch was carried out as given in Fig 3.1.

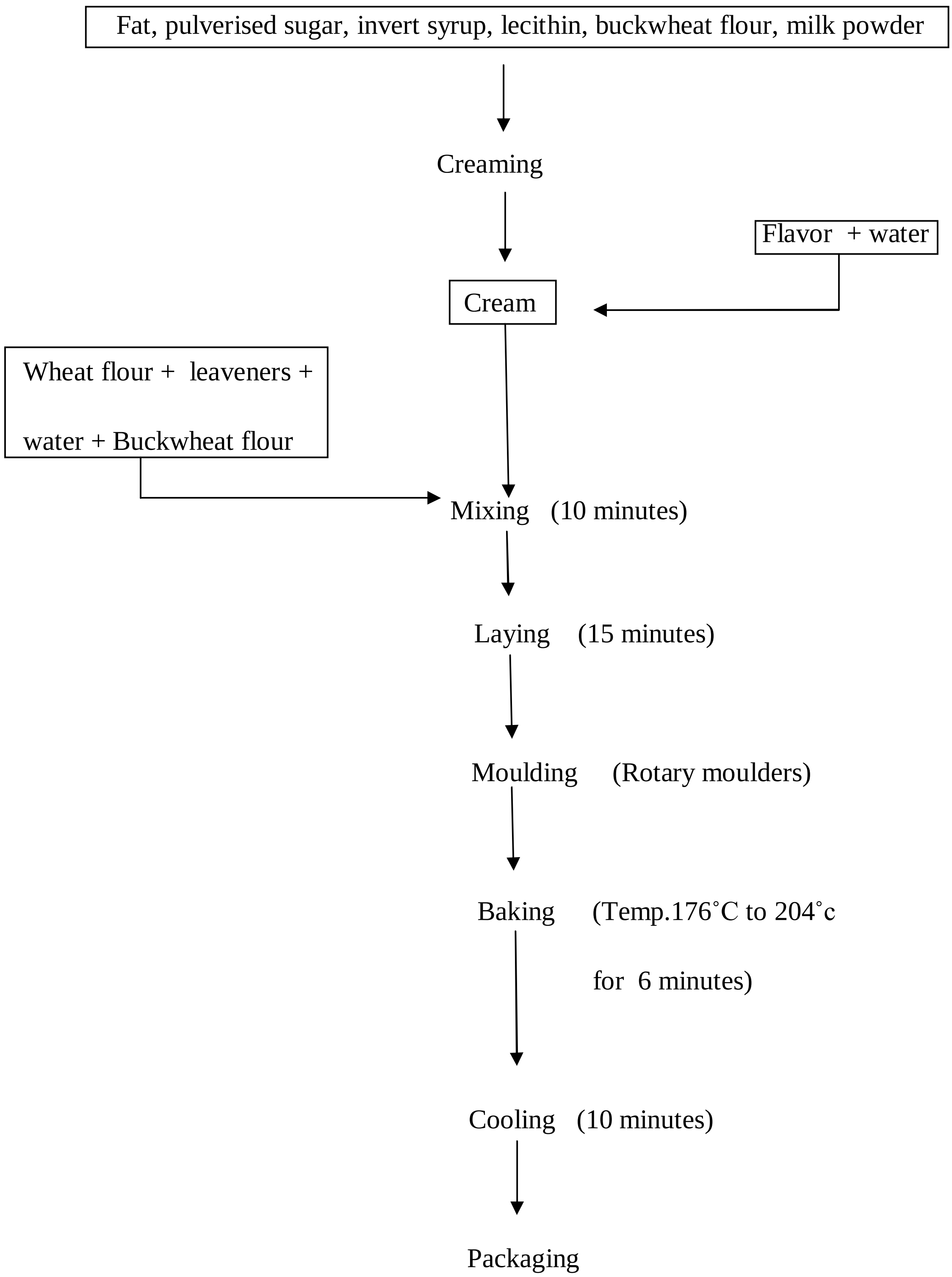


Fig 3.1 Flow chart for the production of biscuit

3.3 Analytical Procedures

3.3.1 Determination of moisture content

Moisture content of the sample was determined by weight loss during heating in a thermostatically controlled oven at 105^0C by hot air oven method as described in Ranganna (2002).

3.3.2 Determination of ash content

Ash content of the biscuit samples and the flour was determined by hot air oven as described in AOAC (1991).

3.3.3 Determination of crude fat

The crude fat content of the sample was determined by solvent extraction method as described by Ranganna (2000).

3.3.4 Determination of Gluten content

The gluten content of the flour was determined by hand washing method as described by Pearson (1981).

3.3.5 Determination of Crude fibre

The crude fibre content of the flour and biscuits was determined as described by FAO (1980).

3.3.6 Determination of Crude protein

The crude protein content of the flour and the biscuit sample was calculated as described by Ranganna (2000).

3.3.7 Determination of Iron

The iron content of the biscuit was determined by colorimetric method as described by Ranganna (2000).

3.3.8 Determination of Carbohydrate

Carbohydrate content was determined by difference method.

3.3.9 Sensory analysis

The sensory evaluation for overall quality was carried out with 10 semi- trained panelist. The parameters for sensory evaluation were color, flavor, crispness, texture and overall acceptability. Sensory evaluation was performed by hedonic rating test.

3.3.10 Statistical analysis

All the data obtained in this research work were analyzed by the statistical program known as Genstat which was developed by Lawes Agricultural Trust (1995). From this mean ANOVA (No blocking at 5% Level of significance), LSD and interaction effects were obtained to determine whether the sample were significantly different from each other and also to determine which one is superior among them. The specimen evaluation card used for the sensory test appears in the appendix.

3.3.11 Determination of shelf life

Shelf life of the product was determined by studying the acid value (AV), Peroxide value (PV) of the extracted fat and the moisture content of the biscuit. The analysis was carried out once in a month basis.

3.3.12 Packaging and storage of the biscuit

Polypropylene bags were used for the packaging of the biscuits. Used polypropylene was of 20 micron, biaxially oriented and having a density of 0.901 gm/cm^3. It was clear and it possessed good water vapour resistance than LDPE.

Part IV

Results and discussion

The wheat flour and the buckwheat flour were collected and mixed with other ingredients to formulate buckwheat flour incorporated biscuit of 10%, 15% and 20% of buckwheat flour incorporation. Proximate composition of the flour as well as biscuit was carried out. The best product among the three variations was determined by carrying out sensory evaluation and the detailed nutritional value of the best product was analysed.

The proximate composition of buckwheat flour as well as wheat flour was obtained as given in Table 4.1.

Table 4.1 Proximate composition of wheat flour and buckwheat flour

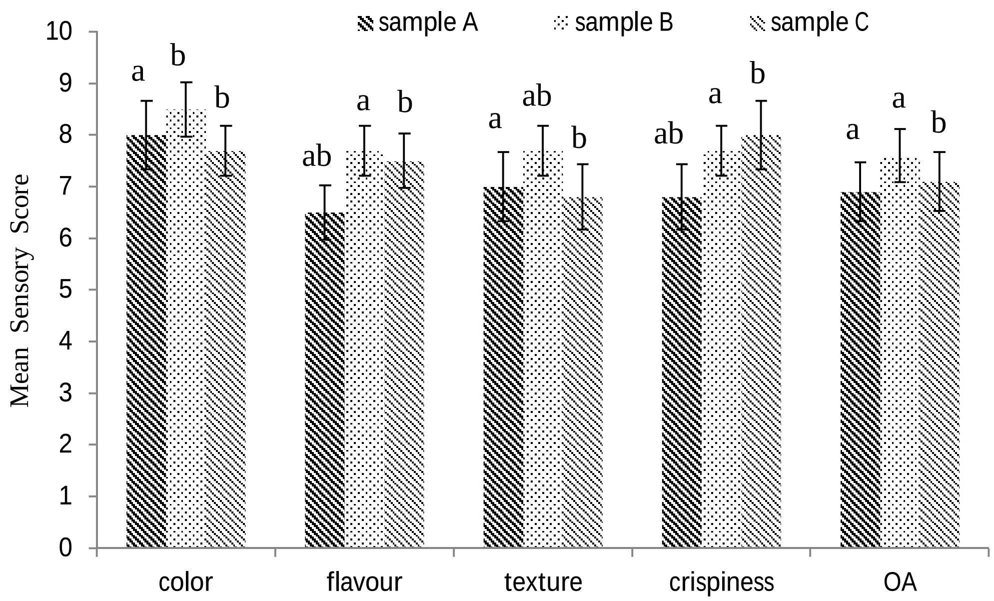
Parameters in %	Wheat flour*	Buckwheat flour*
Moisture	12.30 (0.35)	13.29 (0.45)
Crude protein	9.90 (0.25)	10.69 (0.67)
Crude fat	1.50 (0.73)	2.22 (0.43)
Crude fibre	0.45 (0.46)	0.70 (0.67)
Total ash	0.56 (1.23)	1.71 (0.69)
Carbohydrate	75.29 (0.45)	71.39 (1.1)
Gluten content	8.10 (0.89)	0

* The values are the means of triplicate samples and the values in the parenthesis are standard deviation.

The moisture content of both the wheat and buckwheat flour was in normal range as described by Arora (1980). The protein content of the wheat flour taken for biscuit making seemed to be slightly higher than the standard range for soft dough biscuits as described by Arora (1980). The gluten content of the wheat flour was found to be 8.10%, while in case of buckwheat flour gluten was not observed which resembles the gluten less explanation of buckwheat flour as explained by Dietrych-Szostak and Oleszek (1999). The fat content of wheat flour was found to be 1.50% while it was observed to be 2.22% in buckwheat flour.

4.1 Result of incorporation of buckwheat flour in the biscuit

The biscuits prepared from different level of buckwheat flour incorporation were subjected to sensory evaluation. The samples were provided to 10 semi trained panelist. The semi trained panelists expressed their evaluations in the context of various parameters of biscuit namely, color, flavour (taste and smell), texture, crispiness and overall acceptability. The panelists were suggested to provide scores in the score sheets as per their perception. All the scores were summed up and the best product was found out by statistical analysis as shown in Fig. 4.1.



* Bars containing the same alphabets are significantly different

Fig. 4.1 Mean sensory score of different samples of BFB

The ANOVA at 95% level of confidence ($p > 0.05$) showed that the biscuits made for different level of buckwheat flour incorporation, 10%(A), 15%(B) and 20%(C) were significantly different from each other in sensory attributes.

4.1.1 Color

The analysis of variance showed that in case of color, only sample B and C showed significant difference, while sample A and B and sample C and A were not significantly different. Sample C showed too dark reddish color with lower acceptance, which might be due to the excess of buckwheat flour.

4.1.2 Flavor

In case of flavour sample A and B and sample A and C showed significant difference with each other while sample B and C showed no significant difference which might be due to the least flavour development in sample A due to least use of buckwheat flour, as buckwheat flour has higher oil absorption capacity which leads to good flavour development and better mouth feel as stated by Taira (1974).

4.1.3 Texture

The texture of sample B was the best among all the samples. Sample C showed cracks on the upper crust which might be the due to the least gluten development as higher amount of buckwheat flour is used which is gluten less. Although little cracks were seen in Sample A, the texture was tougher then desired which might be due to excessive gluten development as the amount of wheat flour replacement is only 10%. Sample B showed firm texture and no cracks, which might be due to adequate amount of gluten development.

4.1.4 Crispiness

Use of excessive non glutinous flour reduces the textural strength of the biscuit and leads to increase in crispiness, which causes greater acceptability due to slight cohesive nature rather than being too elastic as described by Schober (2003). The sensory scores for the crispiness showed greater acceptability of sample C, which possessed higher amount of buckwheat flour.

4.1.5 Overall acceptability

The overall acceptability mean showed the product B with 15% incorporation of buckwheat flour to be superior, which might be due to good texture as adequate amount of gluten formation was possible in such composition. Similarly, not too much amount of buckwheat flour, but adequate enough to provide good flavour and better oil absorption capacity might have provided good mouth feel in sample B. Higher amount of oil absorption capacity of buckwheat flour might have led to good surface gloss as described by Mukhopadhyay (1990). Hence, from the statistical analysis the overall acceptability of sample B with 15% buckwheat flour incorporation was found to be superior.

4.2 Proximate analysis of buckwheat flour biscuit (15%) and normal wheat biscuit.

The proximate analysis of the best product (Sample B) from sensory and the normal wheat flour was carried out. The result is tabulated in Table 4.2.

Table 4.2 Proximate analysis of buckwheat flour biscuit (BFB) and wheat biscuit.

Parameters in %	BFB*	Wheat biscuit*
Moisture	2.307 (0.89)	1.89 (0.56)
Fat	18.00 (0.78)	17.26 (0.46)
Crude fibre	0.78 (0.34)	0.65 (0.56)
Protein	7.08 (1.67)	6.92 (0.89)
Ash	1.7 (0.45)	1.3 (0.67)

* The values are the means of triplicate samples and the values in the parenthesis are standard deviation.

The proximate composition of biscuits is shown in Table 4.2. The ash content of biscuits increased in BFB. The increase in ash content may be due to the high mineral content in the BFB i.e. iron, copper and magnesium (Francischi *et al.*, 1994). The moisture content ranged from 1.89% in wheat biscuit to 2.30% in BFB. The increase in moisture content may be due to the increase in protein content. Mustafa *et al.*, (1986) reported an increase in moisture content of bakery products with increase in protein content. The fat content of BFB was higher than wheat flour biscuits. This was probably due to the oil retention ability of buckwheat flour during baking process (Rufeng *et al.*, 1995). Higher oil retention improves the mouth feel and retains the flavor of the biscuits. No definite trend in increase or decrease in crude fibre contents was observed. The protein content of biscuits ranged from 7.08 in BFB to 6.92 in wheat biscuit. The biscuits showed an increase in protein content when BWF concentration was increased which might be due to the use of low protein soft wheat flour.

4.3 Effect of incorporation of buckwheat flour on nutritional quality of biscuit on the basis of iron content

Biscuits prepared from the incorporation of buckwheat flour at different level were analysed for the best acceptability and the best sample was further analysed for the iron content and was compared with the control sample, wheat flour biscuit. The amount of iron content determined is given in Table 4.3.

Table 4.3 Iron content in wheat biscuit and buckwheat flour incorporated biscuit.

Sample	Iron Content (%)	
	BFB	WFB
1	0.034	0.017
2	0.031	0.019
3	0.03	0.016
4	0.036	0.016
5	0.029	0.018
Average	0.032	0.0172

The results obtained were subjected for further statistical analysis. From ANOVA the grand mean of iron content in buckwheat flour incorporated biscuit was observed to be 0.03200mg/10gm and the grand mean of iron in wheat flour biscuit was observed to be 0.01720mg/10gm. At 5% level of significance the two samples were significantly different from each other. The comparison of mean between the two samples showed that the iron content in the buckwheat flour incorporated biscuit was higher than the normal wheat flour biscuit, which is as described by (Saturni, 2010).

4.4 Shelf life evaluation of the buckwheat flour incorporated biscuit (BFB)

The shelf life of the BFB was studied for 3 month with triplicate samples. The product was packed in polypropylene bags and stored in cool and dry place. The acid value, peroxide value of extracted fat and the moisture content of the product was evaluated from the date of manufacture up to 3 months. The result of analysis was obtained as shown in Fig. 4.2.

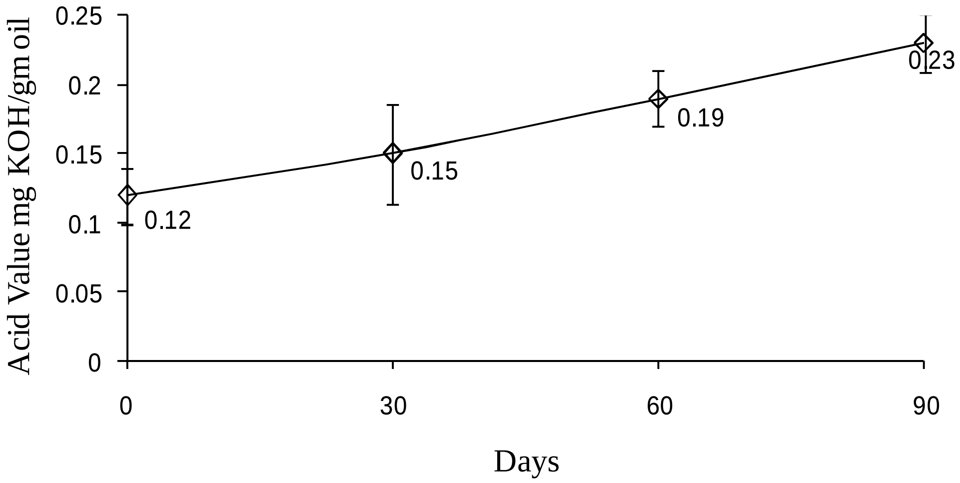


Fig. 4.2 Acid value of the product

The acid value (AV) of the product was observed to be 0.12 at initial which reached 0.23 within 90 days. There was no significant difference between the analysis within 1 month while a significant difference in the acid value of the extracted fat was observed in case of 0 day, 60 day and 90 day analysis, but the acid value was below the unacceptability level of maximum 0.3 mg KOH/ gm oil as described by Mukhopadhyay (1990) till the last date of analysis. The rate of increase in AV shows a gradual increase which suggests that it will be self-preserved till 6 months as described by Smith, 1972.

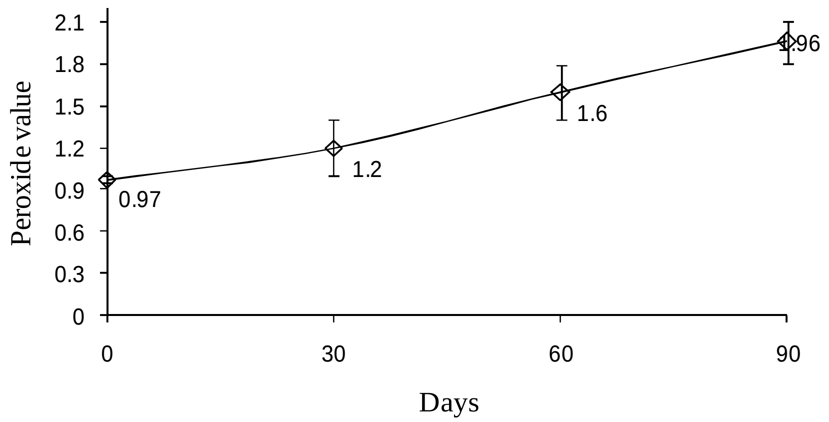


Fig. 4.3 PV of the product

The peroxide value (PV) of the product was observed to be 0.97 at initial which reached 1.96 within 90 days. There was no significant difference between 0 day and 30 day analysis while there was a significant increase in the PV after that till 90 days, but also the value of PV obtained was far below the unacceptable level of maximum 3 meqv peroxide/kg fat as described by Mukhopadhyay (1990). The peroxide value of the product is shown in Fig. 4.3.

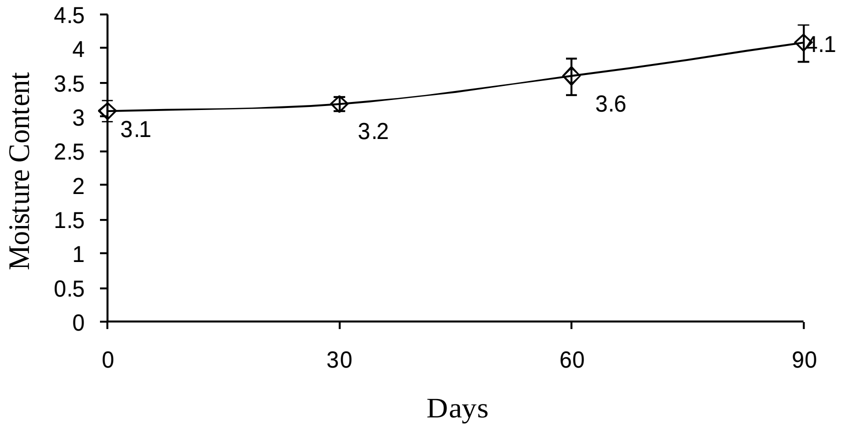


Fig. 4.4 Moisture content of the product.

The moisture content of the product was observed to be 3.1 at initial which reached 4.1 within 90 days as given in Fig. 4.4. There was no significant difference between 0 day and 30 day analysis while there was a significant increase in the moisture content after that till 90 days, but the value was far below the unacceptable level of maximum 6%. Hence, the biscuit could be considered safe for consumption till date.

Hence, the shelf life of the product was evaluated and all the parameters determining the shelf stability of biscuit were found to be within the standard limit. The rate of increase in AV, PV, and moisture content signified that the product would be safe for consumption till standard best before time of 6 months. Packaging in laminate packets would have further increased the stability of the biscuit.

4.4.1 Rate of increase in the AV, PV, moisture content and the projected shelf life

The probable shelf life of the biscuit was projected by studying the observed increase pattern of the values. The rate of increase in the AV was calculated to be 0.0012 mg KOH/ gm oil per day. This rate of increase in the AV will take 250 days to cross the unacceptable value of AV which is 0.3 mg KOH/ gm oil per day.

Similarly, the rate of increase in the PV was calculated to be 0.011 meqv peroxide/kg fat per day. This rate of increase in the PV will take 272 days to cross the unacceptable value of AV which is 3 meqv peroxide/kg fat.

The moisture content was observed to be increasing with a rate of 0.011% per day. This rate of increase in the moisture content will take 545 days to cross the unacceptable value of moisture content which is 6%.

The shelf life of the biscuit was calculated to be higher than the normal 6 months shelf life of biscuit as observed by (Smith, 1972). Although this is just a calculated value, a slight increase in the shelf life of the biscuit might be because of the natural antioxidants like rutin and quercitin present in the buckwheat flour as described by Fabjan *et al*. (2003).

Part V

Conclusions and Recommendations

5.1 Conclusion

On the basis of the study carried out, the following conclusions were drawn.

- The buckwheat flour can be successfully incorporated up to 15%, with no adverse effect on the acceptable quality.
- The nutritional quality of the biscuit seemed to be enhanced in the case of mineral content mainly iron.
- This formulation could help in reducing the amount of gluten in the biscuit which would be beneficial for celiac diseased people.
- Incorporation of buckwheat flour above 15% showed an adverse effect on the textural quality and good body formation.
- The shelf life of the buckwheat flour incorporated biscuit was found to be satisfactory. Further studies on formulation of biscuits with higher shelf-life using buckwheat flour can be done.

5.2 Recommendations

The following recommendations for further research work can be drawn from the work:

- Further analysis on the total mineral content can be done.
- Study about the effect of naturally present antioxidants of buckwheat on the shelf life of biscuits can be carried out.
- Detailed study about formulation of low gluten product can be done.
- Development of neutraceutical food mainly targeted to iron deficient patients can be done.
- Proper production and marketing of this product will surely promote the production of this neglected but very beneficial crop.

Summary

Biscuits are the low cost, processed food which offers good taste along with nutritional values at affordable price with convenient availability. Biscuits have in general, a good shelf life in comparison to most of the other snack items. It is no more only viewed as a luxury tea item snack, but as an essential daily food composition of an average Nepalese household. Since biscuit is a kind of dry food having a long shelf life, the problem of deterioration is very low in comparison to other bakery products. Biscuits owing to their shelf- life can be beneficial for feeding programmes and other immediate catastrophic conditions. Further value of biscuit can be added by incorporating buckwheat flour. Buckwheat (*Fagopyrum esculentum*) is cultivated as a major food crop in the upper and mid hilly regions of Nepal. Besides it's consumption in the form of *dhindo*, *roti*, *khole* and *malpuwa*, no other significant product has been made till date. Besides being nutritionally rich buckwheat also contains a very important antioxidant called rutin. The mineral content of buckwheat is higher in comparison to wheat flour.

This study was mainly focused on the nutritional value addition of normal wheat biscuit by incorporating buckwheat flour at various levels of incorporation. Thus, formulated biscuits were subjected to proximate analysis, iron content and shelf life estimation. The proximate analysis of the flour used was also carried out. The incorporation was carried out as 10%, 15% and 20% and the best product was found out. The wheat flour and buckwheat flour was analysed for moisture, protein, fat, crude fibre, total ash and carbohydrates. The values were found to be 12.30, 8.90, 1.50, 0.45, 0.56 and 68.19% for wheat flour and 13.29, 10.69, 2.22, 0.70, 1.71 and 71.39% for buckwheat flour respectively.

The biscuit formulated was of soft dough type. The product was subjected to sensory analysis. The sensory analysis was carried out based on crust color, flavour, crispiness, texture and overall acceptance. The data obtained were subjected to statistical analysis at 95% confidence level. The best product among the three varieties was found out. The biscuit with 15% buckwheat flour was found to be best among the three varieties. At 95% level of confidence it was observed that, the biscuits made for different level of buckwheat flour incorporation, 10%(A), 15%(B) and 20%(C) were significantly different from each other in sensory attributes. In case of color, only sample B and C showed significant difference, while sample A and B and sample C and A were not significantly different. Sample C showed too dark reddish color with lower acceptance, which might be due to the

excess of buckwheat flour. In case of flavour sample A and B and sample A and C showed significant difference with each other while sample B and C showed no significant difference which might be due to the least flavour development in sample A due to least use of buckwheat flour.

The texture of sample B was the best among all the samples. Sample C showed cracks on the upper crust which might be the due to the least gluten development as higher amount of gluten less buckwheat flour is used. Although little cracks were seen in Sample A, the texture was tougher then desired which might be due to excessive gluten development as the amount of wheat flour replacement is only 10%. Sample B showed firm texture and no cracks, which might be due to adequate amount of gluten development.

Use of excessive non glutinous flour reduces the textural strength of the biscuit and leads to increase in crispiness, which causes greater acceptability due to slight cohesive nature rather than being too elastic. The sensory scores for the crispiness showed greater acceptability of sample C, which possessed higher amount of buckwheat flour.

The overall acceptability mean showed the product B with 15% incorporation of buckwheat flour to be superior. This showed that buckwheat flour can be incorporated in biscuits up to 15% with good acceptability. Product B and wheat flour biscuit was further analysed for iron content. The comparison of mean between the two samples showed that the iron content in the buckwheat flour incorporated biscuit was higher than the normal wheat flour biscuit. The result obtained showed that intake of 100 gm of buckwheat flour incorporated biscuit supplemented 22% of the RDI of iron, while the iron content in wheat flour biscuit was significantly low.

Sample B was further analysed for shelf life. The AV, PV, and moisture content of the triplicate samples were analysed on monthly basis for 3 months. The values were all within the acceptable limits. The overall study showed that buckwheat flour incorporated biscuit at 15% incorporation would give a nutritionally enriched product with best acceptability.

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Appendices

Appendix A

1. Sensory score card

Hedonic rating test

Name ………………………… **Date :**

Product: Buckwheat Biscuit

Observe the product by tasting and check how you feel about the product by analyzing different parameter. Give your appropriate point that best describes your feeling about the product. An honest expression of your personnel feeling will help to choose right product.

Score the point according to following remarks

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

Attributes	Sample A	Sample B	Sample C
Crispiness			
Texture			
Color			
Flavor			
Overall acceptability			

Comments:

………………………………………………………………………………………………

……………………………………………………………………

Signature

Appendix B

1. Sensory evaluation of the product

Table B.1 Two way ANOVA (no blocking) for color

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	2	3.2667	1.6333	5.13	0.013
Residual	27	8.6	0.3185		
Total	29	11.8667			

Since F pr. < 0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.2 LSD for Color

Sample code	Mean score	LSD at 0.05	Mean difference	Remarks
A	8	0.518	B-A = 0.5	<LSD
B	8.5		B-C = 0.8	>LSD*
C	7.7		A-C = 0.3	<LSD

(* = Significantly different)

Table B.3 Two way ANOVA (no blocking) for flavor

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	2	8.2667	4.1333	15.72	<.001
Residual	27	7.1	0.263		
Total	29	15.3667			

Since F pr. < 0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.4 LSD for Flavor

Sample code	Mean score	LSD at 0.05	Mean difference	Remarks
A	6.5	0.4705	B-A = 1.2	>LSD*
B	7.7		B-C = 0.2	<LSD
C	7.5		C-A = 1	>LSD*

(* = Significantly different)

Table B.5 Two way ANOVA (no blocking) for Texture

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	2	4.4667	2.2333	6.22	0.006
Residual	27	9.7	0.3593		
Total	29	14.1667			

Since F pr. < 0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.6 LSD for Texture

Sample code	Mean score	LSD at 0.05	Mean difference	Remarks
A	7	0.550	B-A = 0.7	>LSD*
B	7.7		B-C = 0.9	>LSD*
C	6.8		A-C = 0.2	<LSD

(* = Significantly different)

Table B.7 Two way ANOVA (no blocking) for Crispiness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	2	7.8	3.9	10.86	<.001
Residual	27	9.7	0.3593		
Total	29	17.5			

Since F pr. < 0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.8 LSD for Crispiness

Sample code	Mean score	LSD at 0.05	Mean difference	Remarks
A	6.8	0.550	B-A = 0.9	>LSD*
B	7.7		C-B = 0.3	<LSD
C	8.0		C-A = 1.2	>LSD*

(* = Significantly different)

Table B.9 Two way ANOVA (no blocking) for Overall Acceptability

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	2	2.6	1.3	4.28	0.024
Residual	27	8.2	0.3037		
Total	29	10.8			

Since F pr. < 0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.10 LSD for Overall Acceptability

Sample code	Mean score	LSD at 0.05	Mean difference	Remarks
A	6.9	0.506	B-A = 0.7	>LSD*
B	7.6		B-C = 0.5	<LSD
C	7.1		C-A = 0.2	<LSD

(* = Significantly different)

Appendix C

1. Shelf life of the product

Table C.1 One way ANOVA (no blocking) for AV

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	3	0.0218	0.007267	11.47	0.003
Residual	8	0.005067	0.000633		
Total	11	0.026867			

Since F pr. < 0.05, there is significant difference between the samples so LSD testing is necessary.

Table C.2 LSD for AV

Days	Mean score	LSD at 0.05	Mean difference
(A)0	0.12	0.04738	B-A = 0.03<LSD, D-B=0.08>LSD*
(B)30	0.15		C-B = 0.04<LSD, D-C<LSD
(C)60	0.19		C-A = 0.07>LSD*
(D)90	0.23		D-A = 0.11>LSD*

(* = Significantly different)

Table C.3 One way ANOVA (no blocking) for PV

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	3	1.72556	0.57519	22.13	<.001
Residual	8	0.20793	0.02599		
Total	11	1.93349			

Since F pr. < 0.05, there is significant difference between the samples so LSD testing is necessary.

Table C.4 LSD for PV

Days	Mean score	LSD at 0.05	Mean difference
(A)0	0.97	0.3036	B-A < LSD, D-B > LSD*
(B)30	1.2		C-B > LSD*, D-C > LSD*
(C)60	1.6		C-A > LSD*
(D)90	1.96		D-A > LSD*

(* = Significantly different)

Table C.5 One way ANOVA (no blocking) for Moisture content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	3	1.7825	0.59417	13.71	0.002
Residual	8	0.34667	0.04333		
Total	11	2.12917			

Since F pr. < 0.05, there is significant difference between the samples so LSD testing is necessary.

Table C.6 LSD for Moisture Content

Days	Mean score	LSD at 0.05	Mean difference
(A)0	3.13	0.3919	B-A < LSD, D-B > LSD*
(B)30	3.2		C-B > LSD*, D-C > LSD*
(C)60	3.6		C-A > LSD*
(D)90	4.1		D-A > LSD*

(* = Significantly different)

Appendix D

1. Iron content of product

Table D.1 One way ANOVA (no blocking) for iron

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	1	$5.48x10^{-04}$	$5.48x10^{-04}$	107.37	<.001
Residual	8	$4.08x10^{-05}$	$5.10x10^{-06}$		
Total	9	$5.88x10^{-04}$			

Since F pr. < 0.05, there is significant difference between the samples so LSD testing is necessary.

Table D.2 LSD for Iron content

Sample code	Mean score	LSD at 0.05	Mean difference	Remarks
A(Buckwheat flour)	0.032	0.003294	A-B = 0.015	>LSD*
B (Wheat flour)	0.017			

(* = Significantly different)

BIO-DATA

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