PREPARATION AND QUALITY EVALUATION OF COMPOSITE BREAD FROM WHEAT, OAT AND MAIZE FLOUR

by

Sira Shrestha

Department of Food Technology

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

2019

PREPARATION AND QUALITY EVALUATION OF COMPOSITE BREAD FROM WHEAT, OAT AND MAIZE FLOUR

A dissertation submitted to the Department of Food Technology, Central Campus of Technology, Tribhuvan University, in partial fulfillment of the requirements for the degree of B. Tech. in Food Technology

by

Sira Shrestha

Department of Food Technology

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

2019

Tribhuvan University

Institution of Science and Technology

Department of Food Technology

Central Campus of Technology, Dharan

Approval Letter

This ***dissertation*** entitled ***Preparation and Quality Evaluation of Composite Bread from Wheat, Oat and Maize Flour*** presented by **Sira Shrestha** has been accepted as the partial fulfillment of the requirements for **Bachelor degree in Food Technology.**

Dissertation committee

1. Head of the Department ____________________________

(Mr. Basanta K. Rai, Prof.)

2. External Examiner ____________________________

(Mr. Birendra Yadav, Asst.Prof.)

3. Supervisor ____________________________

(Mr. Bunty Maskey, Asst. Prof.)

4. Internal Examiner ____________________________

(Mrs. Geeta Bhattarai, Assoc.Prof.)

June, 2019

Acknowledgement

I am deeply indebted to my respected supervisor Mr. Bunty Maskey, Assistant Professor of Food Technology, Central Campus of Technology, Tribhuvan University, Dharan for his excellent guidance, frequent inspiration, encouragement and valuable suggestions throughout the endeavor.

I am eternally thankful to Prof. Dr. Dhan Bahadur Karki (Campus chief, Central Campus of Technology), Professor Basanta Kumar Rai (HOD, Department of Food technology) and Associate Professor Geeta Bhattarai (Chairperson, Central Department of Food Technology) for their generosity and co-operation in providing an opportunity and facilities to perform this work successfully.

I cannot stay without thanking all my classmates for their help in preparing the final work. Special thanks must be bestowed to my friends Bishesh Vishwokarma, Kumar Subedi Nabindra Shrestha, Anjana Shrestha, Bandana Pant, Shweta Chaudhary, Sudip Bhattarai and my juniors Stuti Sapkota, Archana Bhatta, Sangita Shah, Pragya Poudel, Barsha Baniya for their co-operation to make this work successful.

I am deeply indebted to all the teachers, all the technical and non-technical staffs of Central Campus of Technology. I salute all those whose perceptions, observation and inputs have helped me directly or indirectly.

Above all, I am indebted to my parents without whose constant encouragements, inspirations and affection, I would never have reached this far, and this work would have never seen the light of the day.

Date of submission: June, 2019

…………………………

(Sira Shrestha)

Abstract

Composite bread is the baked product made from the mixture of flours from tubers rich in starch, protein rich flour or cereals with or without wheat flour to make the bread more nutritious. The aim of this study was to prepare and evaluate composite bread from wheat, oat and maize flour. Design expert was employed for formulating the recipe of bread. The obtained 7 formulations of composite bread from wheat, oat and maize with varying levels of oat to wheat flour including constant amount of maize flour i.e. 5% were prepared in the lab where the range of oat flour used was 0-40 parts with wheat flour. The proximate composition of prepared wheat flour, oats flour and maize flour were carried out in the lab. Bread was prepared by straight dough method and samples were subjected to sensory evaluation for consumer acceptability. The best formulation and control bread were subjected for proximate analysis along with evaluation of mineral content, phenolic content and antioxidant activity.

From sensory evaluation, 10 parts oat flour incorporated bread was found to be significantly best ($p<0.05$). In most of the formulations, color, crumb appearance, texture, taste, flavor and overall acceptability were significantly ($p<0.005$) affected by the variation in oat and wheat flour. The protein, ash, fiber, fat, calcium, iron, polyphenols and antioxidant activity were found to be higher in oat flour incorporated bread comparison to control bread. These findings suggest that oat flour can be successfully incorporated in refined wheat flour up to the concentration of 10 parts along with maize flour of 5 parts without any adverse effect on sensory attributes.

Contents

List of Tables

List of Figures

List of Plates

List of Abbreviation

Abbreviations	Full forms
AACC	American Association of Cereal Chemist
ADD	Activated dough development
ANOVA	Analysis of Variance
CBP	Chorleywood Bread Process
CHD	Coronary heart disease
CVD	Cardiovascular disease
DPPH	2,2-diphenyl-1-picrylhydrazyl
EFSA	European Food Safety Authority
GAE	Gallic Acid Equivalent
GI	Glycemic Index
LI-BIRD	Local Initiatives for Biodiversity Research Development
NARC	Nepal Agriculture Research Council
SDF	Soluble Dietary Fiber
WF	Wheat Flour

Part I

Introduction

1.1 General introduction

Bread is one of the most staple and the oldest ‘processed’ food consumed by humanity. Traditionally bread is based on flour derived from the cereal wheat. Many other types of cereals, pulses and even legumes are milled to give a ‘flour’ but the ability of the proteins present in wheat to transform a gruel of flour and water into a glutinous mass is currently limited to wheat and a few other commonly used cereal seeds (Cauvain and Young, 2007). The aim of all bread making processes is essentially the same, namely to convert wheat flour and other ingredients into a light, aerated and palatable food. (Cauvain and Young, 2006). The first requirement in bread making is formation of gluten network, the second is the aeration of mixture by incorporation of gas, the third is coagulation of material by heating it in the oven so that the gas is retained in and the structure of the material is stabilized. The advantage of having an aerated, finely vesiculated crumb in the baked product is that it is easily masticated (Kent and Evers, 1994). To make good bread, dough made by any process must be extensible enough for it to relax and to expand while it is rising. Good dough is extensible if it will stretch out when pulled. It also must be elastic, that is, have the strength to hold the gases produced while rising, and stable enough to hold its shape and cell structure. Two proteins present in flour (gliadin and glutenin) form gluten when mixed with water. It is gluten that gives dough these special properties. Gluten is essential for bread making and influences the mixing, kneading and baking properties of dough. To produce large and nicely textured loaf of bread the following conditions should be fulfilled.

1. There must be sufficient sugar initially present in the flour and have enough di-static activity in it to produce reserve sugars during fermentation to insure a continuous sufficiency of gas to leaven the dough fully.
2. The protein of the dough must be of satisfactory quality and good enough in quantity to hold sufficient gas to produce.
3. The dough must be approximately ripe at the time of baking and baking should be done under suitable conditions (Kent and Amos, 1967).

Composite bread is a baked product, the primary ingredients of which are composite flour, yeast, salt and water. Technically, composite bread may be different from the whole wheat flour bread in having composite flour, instead of wheat flour alone and other ingredients remaining same. Composite flour is the mixture of flours from tubers rich in starch (e.g. cassava, yam, sweet potato) and/or protein-rich flours (e.g. soy, peanut) and/or cereals (e.g. maize, rice, millet, buckwheat), with or without wheat flour. In other words, flour prepared by replacing wheat flour partly or completely with the flour of other cereals or starchy substances is known as composite flour. The main objective of the composite flour is to make the wheat flour more nutritious enriching its deficient components (e.g. essential amino acids like lysine, methionine and minerals) by adding the flour of other cereals (Ohimain, 2014).

The oats belong to the family of poaceae and is commonly known as *Avena Sativa*. Oats are generally regarded as a minor cereal crop when considered in terms of grain produced annually, or areas sown for production. Traditionally, most of the crop has been used as animal feed. Oats has been recognized as a healthful and nutritious cereal containing high concentration of soluble fiber and dense nutrients. Oats have been linked to the health claims attributed to the use of β-glucans and are valuable sources of β-glucans. As harvested; oats retain their hull, which accounts about 25-30% of the seed. Oats for food use are first dehulled, because hulls are not suitable for humans without processing although readily digested by ruminants. However, properly processed makes it useful fiber ingredient for the food industry. Oat has recently attracted its research and commercial attention mainly due to its high nutritional value. Oats is a good source of antioxidant vitamin E (tocols), phytic acid, phenolic acid and avenantramides. It contains relatively high levels of protein, lipids (unsaturated fatty acids), vitamins, antioxidants, phenolic compounds and minerals. The main part of the physiological effects of oats soluble fiber is due to the elevation of viscosity. Oats are good sources for these functional ingredients like β- glucan, with studies clearly demonstrating their potential health benefits. Irrespective of nutritionally rich cereal, it has physiological benefits like positive effect on reducing hyperglycaemia, hyperinsulinaemia, hypercholesterolemia and several other benefits (Ahmed *et al.*, 2014).

Maize known as *Zea mays* L. is a monoic annual plant belongs to maidas tribe and the grass family of gramineae. Maize is the third important crop after wheat and rice and is grown in more countries than any other crop in the world. This plant is the largest grown cereal in the world with doubled grain yield per unit area compared to wheat and barley. The major chemical component of the maize kernel is starch, which provides up to 72 to 73 percent of the kernel weight. After starch, the next largest chemical component of the kernel is protein. Protein content varies in common varieties from about 8 to 11 percent of the kernel weight. Most of it is found in the endosperm. After carbohydrates, proteins and fats, dietary fibre is the chemical component and vitamins (provitamin A (carotenoids), niacin, vitamin E and vitamin C) found in the greatest amounts (Orhun, 2013).

1.2 Statement of the problem

Bread is a very popular ready food for many people around the world. People are consuming bread made from wheat flour or other sources. In this context, increasing interests in the study of products which are health beneficial as well as nutritious have been in light for many of us. Wheat flour (WF), the vital ingredient for bread making has low protein content as well as lower protein quality due to deficiency in essential amino acid such as lysine and threonine. The use of composite flour for improving protein quality of bread and making functional bread has been increasing (Dooshima *et al.*, 2014).

Oats is a cereal with abundant food uses and nutritive value but study and production on it is very less in context of Nepal and world as a whole. Very few attempts have been made to utilize this food material as an ingredient in other foods. Also oat has been recognized as a healthful and nutritious cereal containing high concentration of soluble fiber, dense nutrient and antioxidant activity (Villarino *et al.*, 2014). Maize is the second most important crop in Nepal and is nutritionally superior to others cereals in many ways, except in protein value. Maize compared with wheat and rice is higher in fat, iron and fiber content. Therefore, preparation of bread from composite flour of wheat, oat and maize flour can improve nutritional and sensory quality of bread (Ndife *et al.*, 2011).

1.3 Objectives

The objectives of this study are as follows:

1.3.1 General objectives

The main objective of this study is the preparation and quality evaluation of composite bread from wheat, oat and maize flour.

1.3.2 Specific objectives

1. To carry out the chemical analysis of raw material i.e. wheat, oats flour and maize flour.
2. To optimize oat flour along with maize flour in bread.
3. To analyze physicochemical and sensory properties of prepared breads.
4. To perform cost evaluation of the product.

1.4 Significance of the study

Oats is one of the most nutritious grain cereals, high in protein and fiber. Oat protein is generally greater than that found in other cereal grains. It contains high amount of vitamins and minerals (Ahmad and Zaffar, 2014).Many positive effects of oats are associated with beta-glucan, due to its beneficial effect on serum cholesterol levels, and recently the European Food Safety Authority approved health claims for beta-glucan (EFSA, 2009). Oats contain many essential amino acids (methionine, cysteine, threonine, isoleucine, tryptophan, valine, leucine, histidine, methionine, phenylalanine, and tyrosine) necessary for human body (Biel *et al.*, 2009) and high antioxidant activity components such as tocopherols, tocotrienols, and flavanoids (Koenig *et al.*, 2014).

Maize is the traditional crops grown for food, feed and fodder and is the second most important crop after rice in terms of production in Nepal. It is the most important cereal grain in the world, providing nutrients for humans and animals and serving as a basic raw material for the production of starch, oil and protein, alcoholic beverages, food sweeteners and, more recently, fuel. Maize nutritionally is superior to others cereals in many ways, except in protein value (Orhun, 2013).

Therefore, incorporation of wheat flour with oat flour including constant maize flour to make bread provides a good opportunity to improve the nutritional quality. The consumption of product with high dietary fibre, protein, vitamins and mineral helps to rise the nutritional status of the population.

1.5 Limitations of study

1. Size of oats flour and maize flour was not maintained as per the standard.
2. Shelf life of the product could not be studied.
3. Instrumental textural analysis could not be carried out.

Part II

Literature review

2.1 Composite bread

Composite bread is a baked product, the primary ingredients of which are composite flour, yeast, salt and water. As discussed earlier, technically composite bread may be different from the whole wheat flour bread in having composite flour, instead of wheat flour alone and other ingredients remaining same. Composite flours are the mixture of flours from tubers rich in starch (e.g., cassava, yam, sweet potato) and/or protein-rich flours (e.g. soy, peanut) and/or cereals (e.g., maize, rice, millet, buckwheat), with or without wheat flour (Seibel, 2006). The use of composite flours with or without wheat gives rise to technical problems in the production of baked goods, particularly composite bread (Dooshima *et al.*, 2014). From the baker's point of view the most important component of wheat flour is the protein of the gluten that plays a decisive role in dough formation, gas retention and the structure of the crumb. So, in order to produce bread with its characteristic structure and firmness, wheat containing gluten cannot be completely eliminated from bread (Villarino *et al.*, 2014).

According to Kent and Evers (1994), wheat flour can be substituted up to 30% with non-gluten millet flour in preparation of bread. The percentage of non-gluten millet flour that can substitute wheat flour also depends upon the strength of the wheat flour. The substitution can be increased further in case of other baked but unleavened goods like biscuits, cookies, pastry, pasta, etc. Bread has been man's food for at least 6000 years. The purpose of bread making is to present the cereal flours to the consumer in an attractive, palatable and digestive form (Campbell and Martin, 2012). It was probably the first processed food ever produced and remains the most widely acceptable. Bread is one of the few universal staples which is complete in itself and requires no additional preparation. Though it is not perfect nutritional source of protein, it is however, a principal source of both calories and protein for a lot of people because of unique structural properties of hydrated wheat protein (Johnson and Peterson, 1974).

2.1.1 Developments in Composite Flour Program

The Composite Flour Program was established by the Food and Agriculture Organization in 1964 to find new ways of using flours other than wheat, particularly maize, millet and sorghum, in bakery and pasta products, with the objective of stimulating local agricultural production, and saving foreign exchange, in those countries heavily dependent on wheat imports. Since, then several research works and trails have already proven the success of composite baked products (Kent and Evers, 1994).

The ingredients used in composite flours must take account of the raw materials available in the country concerned. The objective is to save as much expensive imported wheat as possible when making bakery products (Bibiana *et al.*, 2014).

In the late 1960s, tests were carried out in Brazil in which 75% wheat flour was mixed with the relevant amounts of potato, maize or cassava flour. The baking tests were conducted on the basis of the Chorleywood bread process. The same flours were used as raw materials for biscuits, but the proportion of wheat flour was reduced to 50%. Most of the trails with composite flours have been carried out in Africa because of its continually growing population. Reports are available from Senegal, Niger and Sudan (Berghofer, 2000). In the bread sector the task here was to produce typical French bread with composite flour. The proportion of wheat flour in the different mixtures varied greatly, the maximum being 70%. Europe and North America produce sufficient quantities of bread cereals, so theoretically they have no need to market and use composite flours at all (Dooshima *et al.*, 2014). But constantly widening ranges of bread and small baked goods and the emergence of certain types of bread as “functional food” have led to an interest in mixtures of wheat flour with other agricultural raw materials. Composite flours are an ideal partner in programs to combat celiac disease (Kim and Ruiter, 1969; Abdel-Kader, 2000). In Asia, traditionally, rice and tapioca have been cultivated as carbohydrate sources. Flour from tapioca (tapioca starch) is used to replace wheat flour in some applications, mainly in pastry (Noorfarahzilah *et al.*, 2014).

2.1.2 Composite flour program in Nepal

Local initiatives for Biodiversity, Research and Development (LI-BIRD) in collaboration with Nepal Agriculture Research Council (NARC) carried out research and development work for 3 years (2002-2004) in finger millet with similar objectives in Kaski and Nuwakot districts of Nepal. The various public awareness activities conducted by the project on nutritional importance of finger millet through FM radio, print materials, food fairs and festivals, workshops, school programs raised awareness among consumers and producers. This resulted increase demand in millet products by conscious groups (intellectuals, diabetics, young generation and foreigners) in Pokhara. According to entrepreneurs, departmental stores and many shops requested for supplying millet bread, cookies and namkin to sale in Pokhara) (LI-BIRD, 2007).

2.2 Development of bread making process

The men learnt to grind the grains into crude form of flour that could be blended with water and baked to improve its character. The Swiss lake dwellers mixed the ground grains, wheat, barley and millet with water, rolled the ground mass into sheets and baked these on hot stones. These early bread were unleavened. The fore runners of modern baking may be the practices started in Egypt about 3500 years ago. The Egyptians observed that if the bread dough was allowed to cure for several hours, it would expand and they baked it, they obtained a spongy light loaf of bread, probably this was the first leavened bread with natural microflora (Pederson, 1971).

For hundreds of years, bread was leavened by the addition of sour dough and in the oldest book of the bread making, written in 240 BC it is mentioned that old dough was made up into yeast cakes and ferment new dough, up to years 1859 it thought that dough fermentation was just something that happened, but then the great French Scientist, Louis Pasteur showed that it was the result of microorganisms that convert sugar to carbon dioxide which is the gas that aerates the dough (Fance, 1972).

The earliest bread making by bulk dough fermentation process includes mixing basic ingredients and fermenting at 80ºF for 3 h. It was slightly changed by sponge dough process in USA. It included kneading 3/4 of total flour, 2/3 of water and all the yeast and allowing fermenting for 4-5 h. The flour was mixed with sponge (Bennion, 1967).

In 1960s "Green dough process" of Holland reduced the fermentation time. In this process dough is given an extended mixing and dividing and then molded into balls immediately.

Here bulk dough fermentation disappeared but used extended fermentation of discrete lumps of dough (Kent and Evers, 1994).

Revolution of bread making process had done by the American workers in 1962 AD. They developed the mechanical modification of dough whereby it could be fermented after pan fermentation. Intense mechanical mixing of dough by a machine modified the structure of dough in such a way that bulk fermentation could be omitted without loss of bread quality In 1952, Dr. Baker went one step further than simple mechanical by combining it with continuous mixing in a two stage process. The first step is to preferment a sugar solution with yeast for a period of 2-4 h. This brew also contains a number of other ingredients including these days large proportion of the total flour in the recipe. The brew is combined with the flour and melted fat in a continuous mixer to form the dough. This process was called Do-maker process (Clarke and Arendt, 2005).

CBP (Chorleywood Bread Process) first developed in 1958 by British baking industries at Chorleywood. Further discoveries with this process were published by baking industry in 1961 AD. Characteristic feature of CBP are:

1. Use of high power batch or continuous dough mixer.
2. Use of 75 ppm ascorbic acid (on the basis of flour weight).
3. Use of 0.7% fat (on the basis of flour weight).
4. Absence of any brew or preferment.
5. Addition of extra water.
6. Raising the yeast level.

Advantages of CBP over tradition British 3-h bulk fermentation process are:

1. Saving of about 60% of time in the conversion of raw materials into bread.
2. Saving of about 75% of space in the dough room previously occupied by fermenting dough.
3. Reduction of 75% of the quantity of dough in the course of processing at time and hence reduced risk of loss in case of plant stoppage.
4. Increase yield of about 4% of bread due to retention of flour solids, extra water and yeast.
5. A lower rate of staling in bread.

About the same time (1960) of the development of CBP, another alteration of bulk fermentation as a means of developing dough was developed in USA. It named as Activated dough development process (ADD) by Chorleywood workers. They used 40 ppm potassium bromate and 50 ppm of Ascorbic acid. Advantages of ADD process over CBP were in terms of time, space and yield though long mixing times (Clarke and Arendt, 2005).

2.3 History of bread making in Nepal

Before 2007 BS (1950 AD), production of bread loafs started by Rana's family. For several years, bread for public was produced in small bakeries. The dough was made by hand and baked in small wood charcoal heated bhatties. Bread is made in this manner still in parts of Nepal. Upgrading this traditional method of bread making means using dough mixer and several accessory machineries added in the unit. The ovens in many places are fired by wood. In Kathmandu and some other big towns, several big scale bakeries with electrical ovens and big scale machinery have come into operation. The quality of breads produced by these bakeries is very standard and can be compared with developed countries. Most of the big hotels in Kathmandu and Pokhara are having their own bakeries and showrooms (Lamsal, 2018).

The first professional bread industry in Nepal was Krishna Pauroti Bhandar, located in Kathmandu is professionally still famous in Kathmandu valley. Many professional bakers are not intended to improve the quality of bread. The concerned department should give simple, hygienic and economic technology to the bakers so that bakery industry can flourish. Bread produced by such technology will be of better quality and cheap to consumer (Khanal, 1997).

2.4 Raw materials for bread making

Four basic ingredients are required for the manufacture of bread, namely, wheat flour, yeast, salt and water. If anyone of these four is omitted, bread as we know it cannot be made. Two other ingredients are often added, fat to improve softness and keeping quality and sugar in many areas to increase sweetness. Nowadays, whole range of additives is employed for various reasons, for example to improve fermentation, keeping properties, moisture retention, volume, crumb structure and to prevent mold growth (Flynn and James, 1981).

Eggs, milk and milk products are also used in bread according to their varieties. Eggs are excellent improver and they improve the handing properties by stabilizing the dough, so that the result of increased volume and boldness are obtained (Bennion, 1967).

2.4.1 Wheat flour

For normal bread making, flour from grist containing a large proportion of strong wheat is required. Good bread making flour is characterized by having protein which is in quantity and of satisfactory quality in respect of elasticity, strength and stability, satisfactory gassing properties and amylase activity, satisfactory moisture content not higher than about 14% to permit safe storage and satisfactory color. Starch is a major component of wheat flour (69%) which is composed of amylase and amylopectin. It is the main factor imparting softness in crumb. Some of the starch granules in flour become damaged during the milling process. It is believed that flour amylases are able to attack only the damaged or available starch to supply sugar during fermentation. Excessive starch damage however, has an adverse effect on the quality of bread, loaf volume is decreased and the bread is less attractive in appearance (Flynn and James, 1981).

2.4.2 Yeast

Yeast or *Saccharoromyces cerevisiae* is group of minute fungi capable of fermenting a sugar solution producing carbon dioxide and alcohol. Baker's yeast is a different strain and it must be fresh and active. The quantity used is related inversely to the time of fermentation and to the temperature of the dough (Kent and Evers, 1994).

Yeast action in fermentation has three main functions are:

1. To produce carbon dioxide, in sufficient quantities and at the right time to inflate the dough and produce a light spongy texture which will result in palatable bread when correctly baked.
2. To produce a complex mixture of chemical compounds of many types, which contribute to the flavor of the bread.
3. To help bring about the essential changes in the gluten structure known ripening or maturing of the dough (Bennion, 1967).

The activity of yeast depends upon its enzymes, coenzymes and activator contents. There is little or no growth during the first 2 h after the yeast is added to the dough, but some growth in 2 to 4 h, if that time is allowed before baking and then a decline in growth in 4 to 6 h. Fermentation by the yeast begins as soon as the dough is mixed and continues until the temperature of the oven inactivates the yeast enzymes (Frazier and Westhoff, 2005).

Yeast available in the market is in two forms, one is the compressed yeast, sold in wax wrapped blocks containing about 70% moisture. Compressed yeast loses about 6.5% of its activity during two weeks storage time at 4.5°C and another is the active dry yeast containing about double amount of active ingredients when compared to compressed yeast. Although compressed yeast is suitable for any yeast leavened product, active dry yeast has certain advantages like stability at room temperature, ease of measuring and better dispersibilty and is therefore preferred and used by most of the bakery plants. It can tolerate drying, high sugar concentration and some inhibitors better than can compressed yeast strains. It is essential that the dry yeast be rehydrated with water between 40.5-43°C before use (Arora, 1980).

2.4.3 Salt

Functions of salt in bread making are:

1. Primarily to flavor to the bread.
2. To confer bloom or wholesome appearance on the finished loaf.
3. To tighten up and give stability to the gluten of the flour and enable a bold loaf to be produced with firm cutting crumb.
4. To prevent yeast working too fast in process dough and to control the action of acid producing bacteria in dough.
5. To help to keep the loaf moist after with drawl from the oven (Bennion, 1967).

Salt is largely responsible for crust color in bread made from normal flour because of its controlling influence on fermentation. If the speed of fermentation is retarded by the use of increased amount of salt there will be less sugar used by the yeast to produce gas. In consequence, there will be more sugar caramelized on the crust producing a high crust color. If there is too little salt used, the opposite happens and there is little crust color (Fance, 1972).

2.4.4 Water

Water is an essential part of bread formulation and helps in the following manner.

1. The most important function of water is the formation of bread gluten from flour which makes the dough flexible.
2. Helps in controlling the viscosity or toughness of dough.
3. Helps in making the starch digestible.
4. Helps in controlling the temperature of dough and also contributes towards proper mixing of minor ingredients in flour.
5. Helps in the fermentation process.

The water to be used in for bread formulation should be fresh, clean, soft water and free from any microorganism and limited mineral content. Dissolved mineral and organic matter present in the water can affect the flavor, color and physical attributes of the finished baked goods (Arora, 1980).

Dough should have a pH value of 5-6, that is acidic. If sufficient alkaline water were mixed in dough so as to give an alkaline condition, the activities of the yeast, diastase and lactic acid bacteria would be restrained so that the production of gas and acidity would be slow and the time necessary for ripening the dough greatly increased (Fance, 1972).

When flour is mixed with water at dough making both the gluten and starch absorb water within the range of dough temperature which may be stated as 70-90ºF. There is no doubt that proteins of the flour take up the water much more readily then the starch. Determination of the moisture percentage in a piece of wet gluten washed out at 70ºF from an average flour show that the dry gluten holds nearly twice its own weight of water, whereas somewhat similar experiments with starch would indicate that at the same temperature the dry starch does not hold more than 40% of its own weight of water (Bennion, 1967).

The flour from strong wheat (with higher protein content) and flour from hard wheat (with a higher damaged starch grain) require more water than is needed by flour from weak (lower protein) or soft (less damaged starch) wheat to make a dough of standard consistency (Kent and Evers, 1994).

2.4.5 Sugar

Although sugar is not an essential ingredient of the bread formulation, yet it is added to improve the texture, taste and flavor of the bread. In very small and cottage scale unit it is added as crystallized sugar while mechanized units incorporate it as corn syrups, sucrose or invert syrup (Arora, 1980).

Ordinary cane sugar is used not so much to increase gas production as to improve the color and bloom of the loaf, for there is naturally present in a normal flour sufficient sugar for gas production. Cane sugar can be used at the rate 0.5 kg per sack to supplement any deficiency in the natural product as in those flours obtained from some of the white wheat. With dough lying for a long period especially in overnight doughs added sugar may prove a danger, for it is readily broken down by lactic acid bacteria, thus increasing the acidity. Too much sucrose however will slow down fermentation. If very sweet dough is prepared adding 10% or more of sucrose at once, the growth of the yeast and the formation of carbon dioxide may be slow (Das and Das, 2002).

Glucose can also be used. This will be fermented by the yeast directly; it can used in quantities up to 0.6 kg per sack to improve the bloom and color of the bread. Invert sugar at the rate of 1.3 kg per sack is a very effective bread improver, bringing about the physical modification of the gluten so that well-conditioned dough is produced and bread with a more mature moist crumb and good crust color results (Bennion, 1967).

2.4.6 Fats

Shortenings are used in bread for increased calorific value longer preservation period, better finish and taste and to improve its gas retaining characteristics. Generally, hydrogenated oils are used. Research over many years has shown that fats are better improvers than vegetable oils. Fats have power of preventing the toughness of gluten, according to the methods and amount used. All fats are therefore shortening agents. Fats confer flavor according to the type used (Arora, 1980).

2.4.7 Milk and milk products

The advantages derived from the use of milk products are as follows:

1. They confer a delicate flavor on the crumb of the loaf.
2. They improve the bloom and color of the bread.
3. They assist in the production of a thin, biscuit like crust.
4. They improve the texture and sheen of the crumb.
5. Skimmed milk powder enables the flour to take up slightly more water and the softer dough obtained can be worked more easily.
6. They increase the mineral content of the loaf and hence its value as a food especially for children.

When any type milk product is used other than fresh whole milk, it should always be used in conjunction with fat generally in the proportion half the weight. Skim milk powder (SMP) alone will always tend to produce drier eating bread due to influence of the casein. The milk sugar is not fermentable by yeast so that milk is essentially an enriching agent and improver. When higher proportion of milk are used, attention must be paid to baking temperature because of the amount of sugar in dough which readily caramelizes and can cause excessive crust color (Bennion, 1967).

The addition of milk to the dough raises the pH because of the presence of butter salts in the milk. Milk consequently retards amylase activity. However, in presence of acid salts such as calcium hydrogen phosphate or the acetic acid of vinegar this retardation may be eliminated and gas formation may even be increased by the milk through the improved nutrition of yeast (Meyer, 1987).

Raw or pasteurized milk decreases the baking qualities of flour unless the milk is first heated. It is believed that milk contains some substance which increase the activity of proteolytic enzyme and consequently during fermentation period faster the formation of gluten which is too sticky (Shukla *et al.*, 2016).

2.4.8 Malt products

Malt products are available to the baker in three forms malt flour, malt extract (which is thick, viscous and amber colored syrup) and dehydrated malt extracts which in the dry crystal form (Fance, 1972).

Some patents flours are low in amylase activity and this is rectified by the addition of malted wheat flour or malted barley flour with the diastatic value of the malt extract and malt extract greater proportion than the dried product (Shukla *et al.*, 2016).

Malt flour is manufactured by passing the malted grain through fluted rollers, similar to the break rollers used in the milling of wheat. It is then sieved to remove the coarse particles. Malt being very dry and brittle the outer coating breaks up into fine particles so that the resultant flour is reddish brown in color (Fance, 1972).

2.4.9 Malt extract

The malt is disintegrated and mixed with an equal volume of water and macerated for 6 h. Four times the amount of water is then added and the mixture is digested for 1 h at a room temperature not exceeding ~54.5ºC so that the maximum conversion of starch to sugar is obtained. The sweet liquors are separated and transferred to vacuum pans where concentration is carried out at such a temperature that the diastase is not destroyed when the correct consistency is obtained the syrup is transferred to drum. Ordinary malt extract may be converted into a dry crystalline powder by removing the remaining water in travelling band vacuum oven (Bennion, 1967).

2.4.10 Other improvers

Soya flour, lecithin, eggs, gelatinized starch or scalded flour are generally used as improvers (Bennion, 1967).

A rapid acting reducing agent, L-cysteine and a slow acting oxidizing agent potassium bromate or a mixture of potassium bromate and ascorbic acid are added at the dough mixing stage using convectional slow speed mixing equipment. The reducing agent accelerates the uncoiling and reorientation of the protein molecules and the oxidizing agent follows up by stimulating the formation of cross links stabilize the desired elastic three dimensional gluten network (Kent and Evers, 1994).

Rao and Rao (1991) studied on the effect of potassium bromate or ascorbic acid on rheological characteristics and bread making quality of commercial wheat flours. Ascorbic acid brought about greater changes in the baking qualities as compared to the potassium bromate. Soft wheat flour responded more than medium or hard wheat flours to improvers.

The effect of potassium bromate on rheological characteristics was more pronounced, when the pH of the dough was lowered to less than 5.0, potassium bromate and ascorbic acid brought about greater improvement in the milk bread as compared to other varieties such as plain sugar and fruit bread (Lamsal, 2018).

2.5 Oats

2.5.1 Scientific classification of oats

Oat belongs to the Poaceae family and the genus: *Avena*, which comprises about 70 species, a few of which are cultivated. *Avena stativa* (common oat) is the most important among cultivated oats (*A. stativa*, *A. byzantina*, *A. fatua*, *A. diffusa* and *A. orientalis*) among others because of its multifunctional characteristics and nutrition. *A. sativa* and *A. byzantina* K. Koch known as the white oat and red oat, respectively, are the primary oats grown commercially (Liangli *et al.*, 2012).

Systematic position of *Avena stativa*

Kingdom: Plantae

Division: Spermatophyta

Class: Monocotyledons

Order: Cyperales

Family: Poaceae / Gramineae

Genus: *Avena* L.

Source: USDA (1983)

2.5.2 Cultivation and production of oats plant

Oats are best grown in temperate regions. They have a lower summer heat requirement and greater tolerance of rain than other cereals, such as wheat, rye or barley, so are particularly important in areas with cool, wet summers, such as Northwest Europe and even Iceland. Oats are an annual plants and can be planted either in Autumn (for late summer harvest) or in the spring (for early autumn harvest) (Beloshapka *et al.*, 2016).

In 2016, global production of oats was 23 million tons, led by Russia with 21% of the world total, followed by Canada with 13% of the total. Other substantial producers were Poland, Australia, and Finland, each with over one million tons (Bhaduri, 2013). The production of oats in world is shown in Table 2.1.

Table 2.1 Oats production in 2016

Country	Tons
Russia	4,761,365
Canada	3,018,100
Poland	1,358,079
Australia	1,299,680
Finland	1,037,400
United states	940,130
World	22,991,780

Source: Chan (2017)

2.5.3 Oats in Nepal

Winter feed deficit is one of the critical problems related to ruminant production in Nepal whereas quality feeding management considering leguminous as well as non-leguminous fodder cultivation is grossly lacking (Upreti and Upreti, 2013). Oats are considered most important cereal fodder crop grown in Nepal during the winter season. Oats are quick growing, palatable, succulent and nutritious (Suttie *et al.*, 2005).

In Nepal, fodder oats have been under testing since 1970s, but the two cultivars Kent and Swan were distributed to relatively large numbers of farmers, especially after 1980s. Usually oat is an erect annual with a fairly good tillering habit. It attains a height of 1-2 m. The panicles are lax and effuse. The inflorescence may be equilateral or unilateral. The main axis and lateral branches end in a single apical spikelet. The grain is long and slender or spindle shaped and usually covered with fine hair at the upper end. The leaves may have a length of 25 cm and more. Roots are fibrous (Devkota *et al.*, 2015).

In spite of its advantages to grow as winter fodder- farmers have not been cultivating oats widely due to several factors associated with it. This necessitate establishing demonstration block of promising winter fodder cultivation at the farmers' field so that demonstration effect of cultivation practices and dry matter yield potential of fodder, such as oats would help farmers in convincing towards opt of these practices. Accordingly a field trial was conducted in three research sites of Gorkha, Tanahu and Chitwan with the objective to establish demonstration unit at farmers' field by using promising fodder oats and legumes, and to demonstrate cultivation practices and fodder yield potentials in terms of dry matter and chemical composition (Upreti and Upreti, 2013).

2.5.4 Grain structure of oats

Grain structure is an expression of grain composition as it reflects properties from the standpoint of plant physiology. The plant does not synthesize or incorporate component into structures unless they have a specific function in preservation or propagation of the species. Cereal chemist and technologist, on the other hand are interested in another set of properties the function the grain or its fractions can perform in the production of nutritious foods, that have good shelf life, and are acceptable to the consumer (Pomeranz, 2005).

Thus, in a way, grain structure forms the link between composition that is the source of our basic knowledge of biological systems and utilization of those components in food production. The grain structure of oats is shown in Fig. 2.1.

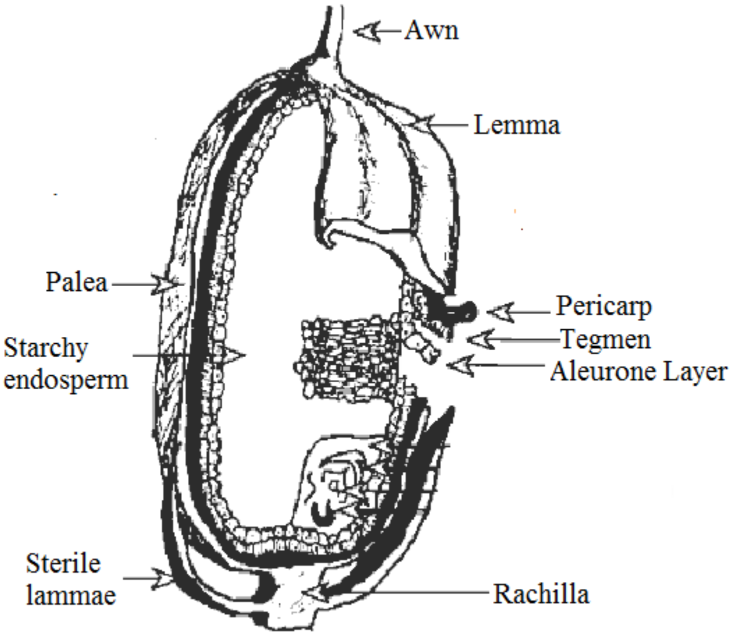

Fig. 2.1 Oats grain

Source: Kent and Amos (1983)

The structure and adherence of the hull may contribute to protection of grain during germination or malting and protection against insect infestations. Germ retention during threshing and separation during processing depend on the germ structure and location in the kernel. The sub aleurone and central endosperm layers differ in cell size, shape, and structure and in composition, especially with regard to protein contents and quality (Gambuś *et al.*, 2011). The main factor in grain hardness of the main components, the strength of interaction within the cell, and the interaction of individual cells to produce overall grain structure (Pomeranz, 2005).

Endosperm structure and hardness is related to oats conditioning, to breakage in milling, and to the structure and composition of the milled flour particles. Milling quality is governed by morphological characteristics of oats kernel and it mechano-physical properties and by the methods of grinding and separation. Reducing changes in texture and structure during drying of maize and rice are important in minimizing breakage during handling, storage, and transportation, dust formation, and infestation. Difference in grain structure are expressed in difference in composition, gradient of components in grain tissues, and the end-use properties. Those differences have important nutritional implications. New microscopic methods to determine grain structure, composition, and end-use properties have the potential of contributing to improved nutritional quality and utilization of cereals by modifying restructuring grain morphology through classical plant breeding and genetic engineering (Pomeranz, 2005).

2.5.5 Chemical composition and nutritive value of oats

The biochemical composition of oats grain is shown in Table 2.2 and mineral and vitamin content of oat grain is shown in Table 2.3.

Table 2.2 Biochemical composition of oats grain.

Parameters	Husked oats	Naked oats
Protein content, %	10.58±0.67	15.71±1.10
Fat content, %	5.15±0.19	9.66±1.87
Starch, %	48.08±0.29	31.55±3.72
Total dietary fiber, %	17.63±1.52	22.97±1.89
β-glucan, %	3.15±0.19	3.29±0.26

Source: Zhao *et al.* (2014)

Table 2.3 Mineral and vitamin content of oat grain (per 100 g of edible portion).

Parameters	Value per 100 g
Calcium (mg)	54
Iron (mg)	4.72
Magnesium (mg)	177
Potassium (mg)	429
Zinc (mg)	3.97
Manganese (mg)	177
Thiamin (mg)	0.763
Riboflavin (mg)	0.139
Niacin (mg)	0.961
Pantothenic acid (mg)	1.349
Vitamin B-6	0.119

Source: Liangli *et al.* (2012)

2.5.6 Nutritional component of oats

2.5.6.1 Oats starch

Starch constitutes about 60% of oat grain. It is mainly a constituent of endosperm. There is considerable difference observed between the physicochemical properties of oats starch and other cereal starches. Differences in physicochemical properties are also observed in different cultivars of oats. These differences are probably due to differences in the magnitude of interaction between and among starch chains within the amorphous and crystalline regions of the native granules and by the chain length of amylose and amylopectin fractions of oats starch. Oats starch offers untypical properties such as small size of granules, well developed granule surface and high lipid content (Berski and Ptaszek, 2011).

2.5.6.2 Oats protein

Oats is consider as potential source of low cost protein with good nutritional value. Oats has a unique protein composition along with high protein content of 11–15%. Cereal proteins have been classified into four types according to their solubility as follows: albumins (water-soluble), globulins (salt water-soluble), prolamins (soluble in dilute alcohol solution) and glutelins (soluble in acids or bases). Oat protein not only differs in the structural properties but also differs in distribution of protein fraction in comparison to other cereal grains (Gambuś *et al.*, 2011). Other cereals such as wheat and barley have characteristic protein matrix which lacks in oat. In wheat and some other cereals, the storage protein is insoluble in salt solutions, while in oats, a large portion of salt water soluble globulins also belong to the storage proteins of the endosperm (Klose *et al.*, 2009).

Oats contains lower quantity of prolamins (15%) relative to the high amount of globulins (80%) of the total oats protein. Prolamins (avenins) are low molecular weight fractions of oats proteins. These prolamins are soluble in 50–70% ethyl alcohol or 40% 2-propyl alcohol. Prolamins have high percentage of glutamine and proline and are low in lysine as compared to the other protein fractions (Robert and Nozzolillo, 1985). Avenins, a type of prolamins, have storage function similar to that of other cereal prolamins. Glutelin values are reported to be varying from 5 to 66% of the total protein as they are difficult to be completely solubilised and are dependent on the extraction solvent and solvent concentration. Of the total metabolically active proteins of oats, water soluble albumin accounts for most of the fraction. Albumins account for about 1–12% of the total oats protein. In general, albumin and globulin have higher lysine content. Thus oats are rich in lysine content compared to other cereals while they have rather lower content of glutamic acid and prolamin (Lapvetelainen, 1994).

Celiac disease is triggered by the ingestion of gluten in gluten intolerant persons. Gluten is an alcohol soluble complex protein present mostly in wheat and other related cereals such as barley and rye. In individuals who are genetically susceptible, the ingestion of gluten causes an inappropriate small intestinal immune response characterized by villous atrophy and crypt hyperplasia (Fasano and Catassi, 2001), resulting in malabsorption of protein, fats, carbohydrates, soluble vitamins, folate and minerals especially, iron and calcium. The only therapy available at present is to completely exclude gluten from the diet of the individual. Oat contains comparatively more favourable and nutritionally more valuable composition of protein fractions (Capouchova, 2004). However, it has long been debated, whether oats can be considered safe for celiac patients. Rasane *et al.* (1953) and Baker and Ream (1976) recommended complete elimination of oats; while, Ripsin *et al.* (1992) and Janatinen *et al.* (2006) advocated the use of oats in celiac diet. The use of oats in gluten free diet depends on the composition of the protein fractions; albumins, globulins, prolamins (avenins) and glutelins.

Prolamins together with glutelins forms the reserve protein located in the grain endosperm, which forms about 60–70% of the grain proteins of cereals. The prolamin fractions are less susceptible to hydrolysis and hence are also difficult to digest. The prolamins content in oats (10–15% of the total protein) is rather low as compared to wheat (40–50%), rye (30–50%) and barley (35–45%). Kumar and Farthing (1995) stated that avenins (oats prolamins) could be responsible for toxicity in the celiac patients only if oats is consumed in high amounts, as compared to rye and barley. Amount of prolamins in oats varies with species, variety and time of cultivation. However, recently European commission regulation No. 41/2009 has included oats amongst permitted ingredients, if the gluten content does not exceed 20 ppm (mg/kg) (Henkey, 2009).

2.5.6.3 Dietary fibers

Dietary fibers are an essential part of the human diet. They consist of many substances of plant origin that are not digested in the human upper gastrointestinal tract. They include polysaccharides such as cereal *β*-glucan, arabinoxylans and cellulose. Dietary fibers are located in the cell walls of the grain (Dawkins *et al.*, 1999). The outer layers, the seed coat and the pericarp contribute significantly to the insoluble dietary fiber content of the grain. According to American Association of Cereal Chemists (AACC), a dietary fiber is defined as “the edible part of plant or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. It includes polysaccharides, oligosaccharides, lignin and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation and/or blood cholesterol attenuation and/or blood glucose attenuation (AACC, 2016).

2.5.6.4 Lipids

Lipid content and composition are important for nutritional quality, energy content, flavor/off-flavor attributes and pasting properties. Oats contain the highest lipid or oil concentration of the cereals but it does not compete as a source of edible oil when compared to the concentrations in oil seed crops. Oat lipids are highly unsaturated. Lipid content varies from 2 to 11% and is dependent on variety. The fatty acid composition of oat lipids is significant to the nutritional and physical qualities of oats (Salehifar and Shahedi, 2010). The three primary fatty acids in oat varieties are linoleic, oleic, and palmitic, comprising about 95% of the total fatty acids. The two major fatty acids, linoleic and oleic acid, both unsaturated, are important for human and animal nutrition. Palmitic acid, the major saturated fatty acid, increases oil stability against peroxidation (Zwer, 2010).

2.5.6.5 Antioxidant

Oats are rich in antioxidants including vitamin E, avenanthramides, which are unique to oats, phenolic acids, flavonoids, sterols, and phytic acid. Antioxidants protect the body from membrane damage, cancers, heart disease and age-related deterioration (Sandhu *et al.*, 2017). Vitamin E is a generic term for compounds that show biological activity linked to a-tocopherol. Among the cereals, oats and barley have the highest concentrations. The primary oat tocol is a-tocotrienol with smaller amounts of a-tocopherol. Tocopherols are located in the embryo and the source for tocotrienols is the starchy endosperm. The total concentration of tocols in 12 varieties grown in the USA at three locations was 19.0–30.3 mg kg^{-1} (Peterson *et al.*, 2002).

2.5.7 Oats: as a functional food

Oats has been recognized as a healthful and nutritious cereal containing high concentration of soluble fiber and dense nutrients. Irrespective of nutritionally rich cereal, it has physiological benefits like positive effects on reducing hyperglycaemia, hyperinsulinaemia, hypercholesterolemia and several other benefits are discussed in this review (Chauhan *et al.*, 2018). The main part of the physiological effects of oats soluble fiber is due to the elevation of viscosity and that is due to soluble fiber such as (1→3, 1→4) - β -D-glucan or β-glucan. β-glucan has been shown to have effects on the glycaemic, insulin, and cholesterol responses to foods. Oats are good sources for these functional ingredients like β-glucan, with studies clearly demonstrating their potential health benefits (Ahmed *et al.*, 2014).

Oats are source of different dietary fiber components of mixed-linkage (1→3), (1→4)-β-D-glucan arabinoxylans and cellulose. The neutral cell wall of polysaccharide β-glucan has outstanding functional and nutritional properties. It achieves high viscosities at relatively low concentrations and is of particular importance in human nutrition. Soluble fiber of oats has been reported to reduce elevated blood cholesterol, triglyceride, and glucose levels. Oats also good sources of insoluble fiber functions as a water-holding-capacity agent and can reduce intestinal transit time when present in adequate amounts in food (Paton *et al.*, 1995).

2.5.8 Oats flour

Oats flour may be milled directly from grinding rolled oats. The fines streams from groat cutting and flaking typically end up in the oat flour stream. Grinding is usually accomplished by hammer mills, but pin mills and other types of size reduction equipment are also used alone or in sequence. Corrugated rolls, commonly used for wheat milling, are unsuitable for oats milling because the grooves tend to plug due to the high fat content of oat groats (Menon and Watson, 2016). Youssef *et al.* (2016) reported proximate values of moisture content, crude protein, crude fat, crude fiber, total ash and carbohydrate 12, 13.1, 9.2, 1, 1.8 and 62.8% respectively.

In 2004 the AACCI (American Association of Cereal Chemists International) provided a definition for whole oats flour as: “whole oats flour is produced from clean, 100% groats, or from products derived without material loss from whole groats, by stabilizing and size reduction” (Bhaduri, 2013).

2.5.8.1 Benefits of oats flour

a. Reduces heart disease risk

An epidemiologic study published in the *Archives of Internal Medicine* looked at the relationship between dietary fiber intake and the risk of coronary heart disease (CHD) and cardiovascular disease (CVD) in 9,776 adults. The researchers found that subjects consuming the most fiber, 20.7 g per day, had 12% less CHD and 11% less CVD compared to those eating the least amount (5 g per day) of fiber (Khanal, 1997).

b. Lowers cholesterol

Another reason that oats flour is so great for the heart is that it has been shown to lower LDL ("bad") cholesterol. Specifically, it's the beta-glucan (β-glucan) found mainly in the endosperm cell wall of oats that's believed to be responsible for decreasing total serum cholesterol and LDL cholesterol. Well β-glucan is a highly glutinous soluble fiber so as it travels through the small intestine, it actually limits the absorption of dietary cholesterol (Mickee, 2015).

c. Helps diabetics

Research shows that, in moderation, oats can be a healthy and helpful food for diabetics and others struggling with blood sugar issues. The aim of a 2015 scientific review was to figure out if oats intake is beneficial for diabetic patients. The researchers looked at 14 controlled trials and two uncontrolled observational studies, and the findings are quite impressive (Lamsal, 2018).

Compared with the controls, "oats intake significantly reduced" the concentrations of A1c and fasting blood glucose as well as total cholesterol and LDL cholesterol. The conclusion of the review is that oats intake can benefit both blood sugar control and lipid profiles in type 2 diabetics, making it a great addition to any diabetic diet plan (Prasad *et al.*, 2015).

d. Satiety star

If you have ever eaten a bowl of oatmeal for breakfast, you are familiar with how oats can hold you over really well until your next meal. Since oats flour is really just ground-up whole oats, adding it to your meals and recipes can also help you feel more satisfied after consuming it (Dawkins *et al.*, 1999).

Scientific research published in the *European Journal of Clinical Nutrition* aimed to produce a validated satiety index of common foods. Many different foods were tested, and oatmeal ended up being rated No. 1 among breakfast foods and No. 3 overall. A scientific review published in 2016 suggests that it's likely the beta-glucan content of oats that has such a positive effect on perceptions of satiety (Barro and Real, 2017).

2.6 Maize

Maize is the third important food grain after wheat and rice, and its demand is increasing because of its increased use for biofuel production. Starch is the main component of maize, which is produced by wet milling process. Maize starch functionality varies with the starch structure and composition, which vary with genotypes and cultural practices (Habbal and Samaan, 2012). Sugary maize starch has lower crystallinity, while waxy maize starch has greater crystallinity as compared to normal maize starches. The sugary maize starch has lower gelatinization temperature and enthalpy. The maize starch with long-branch chain length amylopectin and higher crystallinity has higher gelatinization temperature and enthalpy. The maize products (canned, frozen, and boiled sweet maize) have lower glycemic index (GI) than white rice and wheat flour bread. Waxy maize starch is more rapidly digested and have high GI than high-amylose starches. Thermal treatments such as autoclaving, baking, steam cooking, and parboiling processes affect starch digestibility and consequently the GI of maize-based products. Maize also contains various bioactive constituents, such as carotenoids, anthocyanins, and phenolic compounds, which vary with maize type. Maize has a higher antioxidant capacity compared to wheat, oat and rice (Sharma *et al.*, 2012).

2.6.1 Classification of maize

Systematic position of *Zea mays*

Kingdom: Plantae

Division: Spermatophyta

Class: Monocotyledons

Order: Cyperales

Family: Poaceae

Genus: *Zea* L.

Source: USDA (1983)

2.6.2 Chemical and nutritive value of maize

Maize (*Zea mays*), known as "corn" in the U.S.A., is used for animal feeding, for human consumption, and for manufacture of starch, syrup and sugar, industrial spirit and whisky. The products of milling include maize grits, meal, flour (and its hydrolysis products), protein (gluten feed) and corn steep liquor (Kent and Evers, 1994).

Maize has a lower nutritive value than that of wheat, in particular being deficient in the vitamin niacin (nicotinic acid), and having a relatively low content of protein, which is deficient in lysine (as is wheat protein) and in tryptophan (Fast and Caldwell, 2000).

Maize contains dietary fiber which has several beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation (Habbal and Samaan, 2012). Table 2.4 shows the soluble, insoluble and total dietary fiber in maize. Such dietary fiber from other sources can also be incorporated into the corn flakes so as to increase its efficiency. Similarly Table 2.5 shows the chemical composition of corn grits.

Table 2.4 Soluble and insoluble dietary fiber in common and quality protein maize (%).

Maize type	Dietary fiber		
	Insoluble	Soluble	Total
Highland	10.94 ± 1.26	1.25 ± 0.41	12.19 ± 1.30
Lowland	11.15 ± 1.08	1.64 ± 0.73	12.80 ± 1.47
QPM Nutricta	13.77	1.14	14.91

Source: Beressani and Breuner (1989)

Table 2.5 Chemical composition of corn grits

Parameters	% composition
Moisture content	11.7
Protein	7.0
Fat	0.6
Ash	0.2
Carbohydrate	80.3
Crude fiber	0.2

Source: FAO (1992)

2.6.3 Uses of maize grits

Maize grits (groats, hominy grits) are used in the following ways (Kent and Evers, 1994)

1) Domestically, to make a porridge, by boiling. In Italy, maize porridge, often made with cheese for flavoring, is called "polenta"
2) In the food processing industry, to make the ready-to-eat breakfast cereal "corn flakes"
3) As a brewing adjunct, providing up to 40% of the mash
4) For the manufacture of wallpaper paste
5) For the manufacture of glucose by "direct hydrolysis"

2.6.4 Health benefits of maize

a) Healthy eyes

Macular degeneration and cataracts are among the world's most common visual impairments and major causes of blindness Lutein and zeaxanthin are the predominant carotenoids in corn, accounting for approximately 70% of the total carotenoid content. Commonly known as macular pigents, these compounds exist in your retina, the light-sensitive inner surface of your eye, where they protect against oxidative damage caused by blue light. Observational studies likewise suggest that high dietary intake of lutein and zeaxanthin may be protective, but not all studies support this (Kent and Evers, 1994).

b) Prevention of diverticular disease

Diverticular disease (diverticulosis) is a condition characterized by pouches in the walls of your colon. The main symptoms are cramps, flatulence, bloating and less often bleeding and infection. Popcorn and other high-fiber foods were once believed to trigger this condition. Men who ate the most popcorn were 28% less likely to develop diverticular disease than those with the lowest intake (Orhun, 2013).

c) Prevention of anemia

Anemia is caused by the deficiency of vitamin and minerals such as iron in the body. The body needs iron folate to form new blood cells. Maize is very good source of iron, per 100 g of serving, it provides an impressive of 2.7 mg of iron (Kent and Evers, 1994).

d) Prevention of cancer

An antioxidant rich diet will prevent cancer. Maize contains carotenoid antioxidant that are proven to protect the eyes and skin from oxidative damage. Popped corn can be a good way to get those antioxidants in (Begum *et al.*, 2013).

e) Good source of fiber

Fiber helps in several ways by reducing cholesterol and making the digestive system more efficient. Maize provides a healthy and impressive amount of fiber. Fiber helps to prevent constipation and also lowers cholesterol levels.

f) Gluten free

People suffering from gluten intolerance often suffer intense reaction to the enzyme, causing different types of health complications. Maize and maize flour is a good option as it is naturally gluten free and a better option than other grains (Liangli *et al.*, 2012).

2.7 Bread making process

There are three stages in the manufacture of bread, mixing and development of the dough, aeration of the dough and oven baking of the dough (Bennion, 1967).

2.7.1 Dough mixing

Main ingredients of bread are wheat flour, water, yeast and salt. Other ingredients may be malt flour, soya flour, yeast food, milk and milk products, fats, fruits and gluten. When these ingredients are mixed in correct proportions, the following phenomena take place:

1. The proteins in the flour begins to hydrate i.e., to combine with some of the water to form a cohesive material called gluten which has peculiar extensible properties, it can be stretched like elastic and posses a certain degree of recoil or spring. The elastic properties which are developed during mixing appear to involve sulfhydril groups possibly their oxidation to disulphide bonds, possibly the formation of new bonds.
2. Evolution of the carbon dioxide gas by action of the enzymes produced by the yeast upon the sugars.

These are mixed using water at temperature that will bring the mixture to about 27ºC (80ºF). The yeast is dispersed in some of the water and the salt dissolved in another portion, yeast suspension, the salt solution and the rest of the water are then blended with the flour. Thorough mixing and correct dough development demand correct absorption of water to produce ideal clear dough. Such dough will produce a loaf with qualities superior to any loaf made from dough which is badly mixed. Dough processed correctly gives even texture and uniform, soft and moist crumb (Kent and Evers, 1994).

2.7.2 Dough fermentation

Enzymes for panary fermentation are diastase (α and β amylase) in flour, maltase, invertase and the zymase complex in the yeast. Starch in the flour is broken down into maltose by amylase enzymes. Maltose is splitted to glucose by maltase. Cane sugar added is splitted to glucose and fructose by invertase enzyme and these products are converted into carbon dioxide and alcohol by zymase complex. Most of alcohol thus produced is driven off during the baking process. Secondary product e.g., acids, carbonyls and esters may affect the gluten or import flavor to the bread (Bennion, 1967).

During the fermentation, conditioning of the dough takes place when the flour proteins (gluten) mature i.e., become elastic and springy and therefore capable of retaining a maximum amount of carbon dioxide gas produced by the yeasts. The conditioning results from action on the gluten by:

(1) proteolytic enzymes from the yeast, from the malt or added otherwise and
(2) the reduction in pH by acids added and formed (Shukla *et al.*, 2016).

Adequate gas should be produced during fermentation process, otherwise the loaf will not be inflated sufficiently. Gas production depends upon quantity of soluble sugar present in flour, its diastatic power and granulation (Kent and Evers, 1994).

2.7.3 Straight dough process

In this process, dough is made in one stage. Fermentation of may vary (2-12 h) and many variable factors come into operations which may affect the development of dough over such a range of time. To make good quality bread, it requires a fermentation time range of 2.5-5 h and flour containing about 11% of good quality gluten forming protein. The gluten formed should be of better stability and moderate elasticity (Bennion, 1967).

2.7.4 Sponge process

In sponge system, only a part of flour is mixed at first with all the yeast and sufficient water to make dough which is allowed to ferment for some hours. This first dough made is called sponge is then broken down and the remainder of flour, water and salt added to make a dough of required consistency which is given for short fermentation time only before proofing and baking (Flynn and James, 1981).

As a result of enzymatic changes which take place in the gluten of the sponge, the dough ripens very much quicker and the bread produced has softness of crumb which is unique. Such bread breaks down very readily in mouth dissolves without effort. This process is the longer process and requires less yeast than is used for straight dough process (Kent and Evers, 1994).

2.7.5 Knock back

As the fermentation is going on, volume of fermenting dough increases continuously. As the dough rises, the interior contains intimate cell like structure in the form of very network. Each cell is filled with carbon dioxide, knocking the dough removes carbon dioxide and develops gluten, rendering it more elastic and capable of producing a better and more even textured loaf, knock back should be carried out at the early stages of fermentation, otherwise, it has little effect on the desired texture of finished product (Bennion, 1967).

2.7.6 Dividing

The next step in bread making is the division of the dough into the sizes required for the finished bread, either by hand or machine. Hand division is coupled with weighing of each piece. Machine division is by volume and results in greater accuracy and hence uniformity in size of product. The pieces of divided by unshaped dough are next rolled into a ball. This has two fold objectives. Firstly, it expels the spent gas which has accumulated during the fermentation stage and secondly it allows a regular shaped piece of dough to be presented to the final shaper or molding machine (Flynn and James, 1981).

2.7.7 Proofing

The ball of dough is given an intermediate proofing, a resting period of about ten minutes before final shaping to allow it to recover its extensibility and elasticity. The ball of dough is then shaped as required. After shaping, there is final proofing period which is again a continuation of fermentation, allowing the shaped dough piece to double its size prior to baking. This period lasts from 45 min to 60 min (Kent and Evers, 1994).

2.7.8 Baking of dough

When the dough is in fully expanded state (called "full proof") baking is started. Once the loaf is in oven, physical, chemical and biological changes become rapid (Fance, 1972).

As the temperature of loaf rises in oven, baking the yeast works faster and produces large quantities of gases. This condition in oven is termed as oven spring. After attaining of temperature ~42ºC, the yeast cells are inactivated and they are killed when loaf centre reaches ~54ºC. Gelatinization of starch and its degradation takes place as temperature rises gradually to ~77ºC. Diastase enzyme becomes inactivated after the temperature ~77ºC has reached. At a temperature of 50ºC the process of denaturation and coagulation of protein starts and proceed rapidly up to 80ºC. Steam and alcohol escapes from the centre of the loaf, while its surface loses a large proportion of its moisture and the crust begins to form. As baking proceeds, evaporation of water takes place and at 110-120ºC, yellow dextrins are produced and these change into brown dextrins and caramel to form the red brown color at

~71ºC. The dark brown color is produced at temperature beyond 200ºC. It is also interesting to note that yeast activity ceases after 20 min and diastatic activity after 26-30 min according to temperature of the oven (Bennion, 1967).

Humidity of the oven is also of importance for the expansion of loaf to good shape. If the humidity is too great, the bread has tough leathery crust and an excessive shine which is unattractive. Insufficient humidity in oven causes rapid evaporation of moisture from skin of the loaf.

2.7.9 Bread cooling

After taking out bread loaves from oven it should be cooled rapidly so that it can be packed for distribution. During cooling moisture moves from interior outward towards the crust and to atmosphere, if the moisture content of the crust rises considerably during cooling, the texture of the crust becomes leathery and tough and attractive crispness of freshly baked bread is lost. Excessive drying during cooling results in weight loss and poor crumb characteristics. The aim of cooling is to lower the temperature without much loss of moisture. Bread loaf can be cooled by counter flow of air at ~21ºC and 80% relative humidity within 2-3 h. If bread is packed before cooling, steam coming from loaf condensates on the crust surface called sweating (Kent and Evers, 1994).

2.8 The technology involved in dough formation

Wheat gluten consists mainly of the storage protein of wheat endosperm, i.e., gliadin and glutenin. Upon hydration and during processing, gliadin and glutenin interact to a unique viscoelastic gluten network, envisaged as being necessary for holding the gases and for producing light porous crumb textured bread. Recent work has confirmed that the elastic properties of gluten are due to the glutenin fraction, whilst the viscous properties come from the gliadin fraction. An appropriate balance in the amount of these two major protein components of wheat gluten is required for achieving the desired bread quality (Khatkar and Schofield, 1997). The glutenin polypeptides are joined head-to-tail via S-S (disulphide) bonds in a linear chain. The glutenin polymerise into a linear chain by intermolecular S-S bonds between the cysteine residues located in the α-helical regions near N- and C terminal ends of high molecular glutenin subunits. The central domain is thought to be rich in repetitive β-turns which form stable β-spiral structure. Under stress conditions, the β- spiral structures undergo deformation and on release of stress, the β-spirals resume the energetically more favorable original conformation. The presence of cysteine residues at either end of glutenin molecules allows deformation/reformation to occur in the central spiral region (Schofield and Booth, 1983; Shewry *et al.*, 1992).

Wheat proteins favor hydrophobic interactions due to their low solubility; on the other hand, soy proteins are more water soluble and they exhibit hydrophilic characteristics in a soy-wheat composite dough system (Arendt and Zannini, 2013). An initial step towards improving the dough and baking quality of soy-wheat composites has been reported; it involves the use of a heat treatment that increases the size distribution of the soy protein and its hydrophobicity, thereby increasing the contribution to the ‘‘unextractable polymeric protein’’ (UPP) of the soy in the composite dough. A higher % UPP is reported to contribute to dough strength in wheat dough (Maforimbo *et al.*, 2008).

2.9 Factors affecting bread quality

Good bread is made from good ingredient. Therefore, the selection of raw materials in making of bread is very important to do to achieve expected quality of the final product. Some of the factors that affect the quality of bread are as follows:

2.9.1 Flour

The main ingredient in making of bread is flour. The most suitable flour in making of good bread is the refined flour. The most suitable flour in making of good bread is the flour that has high protein content (> 12.5%). Eighty-five percent of proteins in the flour are glutenin and gliadin, and the rest are globulin, albumin and protease. When flour is mixed with water, it will make a gluten form which has a cohesive and extensive characteristic. This gluten will have an influence in holding the forming of carbon dioxide gas in the dough during the fermentation by yeast (Zarh, 2010).

2.9.2 Water

Water when mixed with flour will form gluten base. Besides that, the function of water is to be a dissolve agent and distributes the other materials in dough to be well blended and also controlling the structure of dough (Shewry *et al.*, 1992).

2.9.3 Leavening agent

Leaven is used in bread making to produce carbon dioxide and ethyl alcohol through sugar fermentation. Bread leaven is a kind of yeast (*Saccharomyces cerevisiae*). Leaven could be classified into two types of yeast. The first is wet yeast which contain 60-70% of water and the second is dry yeast which contain 7-8% of water (Zarh, 2010).

2.9.4 Salt

Salt is required in the manufacture of bread to give a taste. It helps controlling the rate of fermentation and strengthens the gluten and improves dough extensibility and the ability of holding the gas. Dough that does not have enough salt will be soft, the rate of fermentation will be too fast, will produce bland bread and also will make a rough texture of bread (Kent and Evers, 1994).

2.9.5 Sugar

There are several types of sugar that can be used, which are sucrose, dextrose, fructose, and maltose. Each has a different degree of sweetness. Sugar in bread making is used as a food for yeast. The remaining sugar after being used by yeast is to provide sweetness and an influence factor in the process of caramelization during roasting and also contribute in the forming of brown color in the bread (Pederson, 1971).

2.9.6 Fat

The use of fat in bread making will give an influence in gluten lubrication, increasing the volume of the dough and in an easy way during cutting. Fat in the dough also could increase the extensibility and elasticity of the dough. So the dough becomes more adaptive to the machine and easy to handle. Fats also influence in good flavor and aroma and also help to control water evaporation, so it can maintain the tenderness of bread during storage (Zarh, 2010).

2.10 Nutritional value of bread

Bread is one of the complete foods available for human consumption. Most lacking factor in bread is fat which is generally compensated by the addition of butter, margarine. Typical composition of bread is shown in the Table 2.6.

Table 2.6 Typical composition of bread

Constituents	White bread	Whole bread
Water (%)	40	45
Protein (%)	6.5	6.3
Fat (%)	1.0	1.2
Starch, sugar, etc (%)	51.2	44.8
Cellulose (%)	0.3	1.5
Mineral matters (%)	1.0	1.2

Source: Bennion (1967)

Normal bread contains all the amino acids but lysine is deficient in it. Enriched bread e.g., composite bread, egg bread, milk bread, etc., supplement the deficiency (Fance, 1972).

The most important vitamins in bread are those of vitamin B1 and B2. Vitamin C is absent in bread. Vitamin D exists in two major forms D2 and D3. Three main minerals in flour are calcium, phosphorus and iron and in bread sodium is added in the form of sodium chloride. Calcium content of whole meal bread is greater than white bread but is unavailable to the body. All cereals are poor source of calcium so that chalk is added to all white flour by statute (14 oz per sac), whole meal also has more iron content than wheat flour. Again less of it is absorbed in the body so that iron is added in white flour by statuate (1.65 mg/100 g flour). Whole meal bread contains 287 mg of phosphorus per 100 g of meal as compared with mg/100 g of white flour. Phosphorus in cereals antagonizes the absorption of calcium from other sources e.g., cheese, milk and fish. In higher extraction flours, some of the phosphorus is contained in phytic acid which combines with calcium and produces phytates which are not utilized by digestive system. Bread provides about 26% of our total calcium and 30% of total intake of iron. Phytic acid is hydrolyzed to phosphoric acid and inositol by the enzyme phytase, optimum activity occurring at 55ºC. Probably 60% of the phytic acid in flour is hydrolyzed during bread making (Kent and Evers, 1994).

2.11 Wheat flour and bread standards in Nepal

According to Nepal Rajpatra Standards (2057 B.S.), wheat flour and white bread should possess the following criteria as shown in Table 2.7.

Table 2.7 Wheat flour and bread standards in Nepal

Parameters	Wheat flour	Bread
Moisture	≤ 14% (130-133ºC/2 h)	-
Total ash	≤ 0.70% (dry wt. basis)	-
Acid insoluble ash (in HCl)	≤0.1% (dry wt. basis)	0.1% [a] , ≤ 0.2% [b]
Alcoholic acidity (as H_2SO_4)	≤ 0.12% (dry wt. basis)	≤ eq. of 7.5 ml N NaOH/100
Gluten	≥ 8.0% (dry wt. basis)	-
Guar gum	-	≤ 0.5%
Total soluble solids	-	≥ 60%
Improvers (e.g., $CaPO_4$)	-	≤ 0.25%
Anti-mold agent (e.g., Ca-propionate)	-	≤ 0.5%

a= simple bread, b= spice/fruit bread and c= 90% alcohol

Source: Nepal Rajpatra Standards (2057)

Part III

Materials and methods

3.1 Raw materials

3.1.1 Wheat Flour

Wheat flour named 'fortune maida' produced by Nutri Food Pvt. Ltd., Sonapur, Sunsari, Nepal was used for muffin making. The maida was purchased from the local market of Dharan.

3.1.2 Oats

Rolled oats named 'D lite' was brought from the local store of Dharan at the rate of Rs. 289 per kg.

3.1.3 Maize

Corn grit was purchased from local market of Dharan.

3.1.4 Butter

Butter named 'Delicious fat spread' manufactured by Kaira District Co-operative milk producer's Union Ltd., Anand, India was used as shortening agent which was also brought from local market of Dharan.

3.1.5 Baker's Yeast

Yeast named Angel Yeast manufactured by Angel Yeast (Dehong) Co., Ltd., Hubei, P. R. China was used ad was obtained from the lab of Central Campus of Technology.

3.1.6 Sugar and salt

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

3.2 Chemicals and equipment

All chemicals, glasswares and equipment required were used from the laboratory of Central Campus of Technology, Dharan.

3.3 Method of experiment

3.3.1 Methodology

Design Expert v 7.1.5 software is used to create the recipe. Rotatable central composite design is used to formulate the recipe.

3.3.2 Formulation of recipe

The recipe formulation for the oats and maize flour incorporated bread was carried out as given in Table 3.1.

Table 3.1 Recipe formulation for bread

	A	B	C	D	E	F	G
Wheat flour (parts)	95	85	82	75	68	65	55
Oats flour (parts)	0	10	13	20	27	30	40
Maize flour (parts)	5	5	5	5	5	5	5
Fat	4	4	4	4	4	4	4
Sugar	20	20	20	20	20	20	20
Yeast	2	2	2	2	2	2	2
Salt	1	1	1	1	1	1	1
Water (parts)	65	65	65	65	65	65	65

3.4 Preparation of oats flour and maize flour

Oats flakes and maize grits were grinded separately with the help of grinder. The oats and maize were powdered in the laboratory pulverized to a fineness that 90% of the powder passed through 400 µ sieve. The flour obtained was shield in a plastic container and stored at ambient condition for further processing.

3.5 Preparation of composite bread

Composite bread was prepared by mixing wheat, oat and maize flour along with salt, sugar and water for 20 min and fermentation to about 45 min. This was followed by knock-back, scaling and weighing and first proofing for about 30 min. After first proofing, moulding, panning and final proofing was carried out to about 1 h. Then baking in oven at 220°C for 30 min, depanning, cooling, slicing and packaging was done. Detail is shown in the Fig. 3.1.

Wheat flour + oats flour + maize flour

↓

Addition of water, salt, sugar

↓

Dough mixing (20 min)

↓

Fermentation (45 min)

↓

Knock-back

↓

Scaling and weighing by dividing

↓

First proofing (30 min)

↓

Moulding

↓

Panning

↓

Final proofing (1 h)

↓

Baking in oven (220°C for 30 min)

↓

Depanning

↓

Cooling

↓

Slicing and packing

Fig. 3.1 Flowchart for bread making process

Source: Kent and Evers (1994)

3.6 Analysis of raw materials and products

3.6.1 Determination of physical properties of product

3.6.1.1 Weight

The weight of bread was determined by weighing balance.

3.6.1.2 Volume

Volume of bread was determined by rapeseed displacement method as mentioned in white bread (AACC, 2016).

3.6.1.3 Specific loaf volume of the bread

Specific loaf volume of bread is defined as the ratio of the volume of bread to the weight of bread. The specific loaf volume of bread was determined as illustrated in Al-Saleh and Brennan (2012).

$$\text{Specific loaf volume} = \frac{\text{volume of bread}}{\text{weight of bread}}$$

3.6.2 Proximate analysis

3.6.2.1 Moisture content

Moisture content of the sample was determined by heating in an oven at 100 ± 5°C to get constant weight (Ranganna, 1986).

3.6.2.2 Crude fat

Crude fat contentof the samples was determined by solvent extraction method using Soxhlet apparatus and solvent petroleum ether (Ranganna, 1986).

3.6.2.3 Crude protein content

Crude protein content of the samples was determined indirectly by measuring total nitrogen content by micro Kjeldahl method. Factor 6.25 was used to convert the nitrogen content to crude protein (Ranganna, 1986).

3.6.2.4 Crude fiber content

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

3.6.2.5 Total ash

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

3.6.2.6 Carbohydrate

The carbohydrate content of the sample was determined by difference method (AACC, 2016).

Carbohydrate = 100 – (% protein + % fat + % ash + % crude fiber)

3.7 Iron content

The iron content of the sample was determined as per Ranganna (1986). The iron in foods was determined by converting the iron to ferric form using oxidizing agents like potassium persulphate or hydrogen peroxide and then treating with potassium thiocyanate to form the red ferric thiocyanate which was measured colorimetrically at 480 nm.

3.8 Calcium content

The calcium content of the sample was detemined as per Ranganna (1986). Calcium was precipitated as calcium oxalate. The precipitate was dissolved in hot dilute H_2SO_4 and titrated with standard $KMnO_4$. 1 ml of 0.01N $KMnO_4$ is equivalent to 0.2 mg calcium.

3.9 Determination of total phenolic content

Total phenol content was determined as described by Waterhouse (2002). 0.5 ml of the extract was taken in which 2.5 ml of 10 % Folin-Ciocalteu reagent was added and then 2.5 ml of 10 % sodium carbonate was added. Then the prepared solution was incubated for 30 minutes at 45ºC and then the absorbance was taken at 765 nm against a reagent blank. Gallic acid was taken as standard and the result was expressed as mg of gallic acid equivalents (GAEs) per g of the dried extracts.

3.10 Free radical scavenging capacity

DPPH free radical scavenging activities (antioxidant activities) of extracts were determined by method described by (Vignoli *et al.*, 2011) with slight variation. Different dilutions of the extracts were made using 80% methanol. Then 1 ml of the extract was mixed with 2 ml of 0.1 mM DPPH solution. The absorbance was read at 517 nm after 30 min incubation in the dark. Finally, percentage scavenging activity was determined using following equation

$$\%\text{scavenging activity} = 100-[(A_c-A_s) \times 100 / Ac]$$

Where A_c is the absorbance of control and A_s is the absorbance of test sample.

3.11 Sensory analysis

Statistical analysis of the sensory scores was obtained from 12 semi-trained panelists using 9-point hedonic rating scale (9=like extremely, 1= dislike extremely) for composite bread formulations as in appendix A. The parameters for sensory evaluation were crumb appearance, color, texture, flavor and overall acceptability.

3.12 Statistical Analysis

The obtained data was analyzed statistically by Genstat Discovery Edition 3, for Analysis of Variance (ANOVA) at 5% level of significance. The data obtained from proximate analysis and sensory evaluations were subjected to one and two way ANOVA.

Part IV

Results and discussion

This work was carried out for the preparation of different bread formulation with varying proportion of oats flour and wheat flour with constant proportion of maize flour i.e. 5%. Seven formulations incorporating different proportion of oat flour with respect to wheat flour along with 5% maize in every samples namely A (0% oat flour), B (10% oat flour), C (13% oat flour), D (20% oat flour), E (27% oat flour), F (30% oat flour) and G (40% oat flour) were prepared. As bread is the product widely consumed by general population, oats flour and maize flour incorporated bread adds value to the nutritional profile along with price compatibility. At first, the major raw materials were subjected to chemical analysis.

4.1 Proximate composition of wheat flour, oats flour and maize flour

The proximate composition of wheat flour, oats flour and maize flour were determined. Determined results are presented in Table 4.1.

Table 4.1 Proximate composition of wheat flour, oats flour and maize flour

Parameters	Wheat flour (%)	Oats flour (%)	Maize flour (%)
Moisture (g/100 g)	13.07±0.116	14.07±0.120	12.08±0.126
Crude protein (% db)	9.17±0.152	11.94±0.050	8.32±0.136
Crude fat (% db)	1.07±0.060	8.3±0.100	0.75±0.098
Crude fiber (% db)	0.45±0.012	1.04±0.006	0.54±0.046
Total ash (% db)	0.44±0.036	1.6±0.006	0.44±0.015
Carbohydrate (% db)	88.87±0.079	77.12±0.274	89.95± 0.334

Gluten content (%)	8.1±0.25	-	-
Iron (mg/100 g)	3.42±0.008	13.76±0.75	1.04±0.1
Calcium (mg/100 g)	34±0.15	54.70±0.36	8.77±1.0
Polyphenols (mg GAE/g)	0.87±0.01	1.71±0.40	52.46±0.25
Antioxidant activity (DPPH% inhibition)	4.56±0.15	17.36±0.75	62.64±0.63

*Values are the means of triplicates ± standard deviation.

The moisture content, protein, fat, crude fiber, ash and carbohydrate of wheat flour were determined. The moisture content of the wheat flour was in normal range as described by Arora (1980). The protein content of wheat flour was within the range of 8-13.8% reported by Kent and Evers (1994). Protein content of wheat is highly influenced by the environmental conditions, grain yield, available nitrogen and the variety genotype. The fat content of wheat flour was found to be 1.55%.

The moisture content, protein, fat, crude fiber, ash and carbohydrate of oats flakes were determined. Crude protein was within range as reported by Arendt and Zannini (2013) which was 7.7-14.8 %. The crude fat content was however higher that that obtained by Beloshapka *et al.* (2016) and Zhao *et al.* (2014). The difference in proximate composition may be due to the factors like varieties, climatic conditions, soil type, maturity, fertility geographical conditions and others.

The proximate composition of the corn grits according to which the moisture content, protein, fat, ash, crude fiber and carbohydrate of the corn grits were determined on dry weight basis. Comparing these results with the results reported by Fast and Caldwell (2000), we see that the moisture content is slightly higher. The protein, fat, ash and crude fiber content are also slightly higher than the values reported by Fast and Caldwell (2000) but the carbohydrate content is slightly lower.

It is reported that oat has high phenolic content and antioxidant activity. The phenolic content and antioxidant activity was found to be higher than it was reported by Chauhan *et al.* (2018), this may be due to the use of instant oat flour. As the instant oat has gone through thermal processing which might release more bound phenolic acids from the breakdown of cellular constituents. Also several authors claims that higher properties of thermally processed food could be due to the formation of Millard products, which render high antioxidant activity (Sandhu *et al.,* 2017).

4.2 Physical parameters of composite bread

Physical parameters of bread such as loaf volume, weight and specific loaf volume were affected by the substitution increment of the level of oats flour as maize flour was constant which is presented in the Table 4.2. The data shows changes in physical parameters such as weight and loaf volume of bread with incorporation of oat in bread. Weight was found to be highest in sample G (161.32 g) and lowest in sample A (control) (145.27 g). It might be due to high water absorption capacity of increased fiber content.

There was decrease in loaf volume of bread from 330.67 to 305.23 cm^3 and specific loaf volume from 2.27 to 1.89 cm^3/g with increase in level of oat flour with constant maize flour. The decrease in loaf volume is may be due to the dilution effect of non-wheat flour on gluten content of wheat flour and has been expressed as decrease in loaf volume when using composite flours for bread preparation. This finding loaf volume and specific loaf volume is slightly less than previous work of Chauhan *et al.* (2018) and greater than Bibiana *et al.* (2014) and Dooshima *et al.* (2014).

Table 4.2 Physical parameters of composite bread

Samples	Loaf volume (cm^3)	Weight (g)	Specific loaf volume (cm^3/g)
A	330.67±2.08	145.27±1.12	2.28±0.009
B	321.33±1.53	148.60±1.85	2.16±0.014
C	319.0±2.00	1150.17±0.76	2.13±0.001
D	314.26±1.73	153.26±1.23	2.05±0.004
E	3.10.35±1.89	156.31±0.32	1.99±0.007
F	308.63±2.01	158.17±1.70	1.95±0.006
G	305.23±1.29	161.32±1.34	1.89±0.006

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

4.3 Sensory properties of bread

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

4.3.1 Effect of formulation on color

The mean sensory score for color of bread samples of different formulation are shown in Fig. 4.1. Statistical analysis showed that the partial substitution of wheat flour with oats flour along with constant 5% maize flour had significant effect ($p<0.05$) on the color of the different bread formulation. Product B got highest score may be due to the appropriate amount of oats flour (10%). There is no significant difference in the color of product B and product A. reduced sensory acceptability of composite flour bread for color with increase in percentage of other flour has been reported.

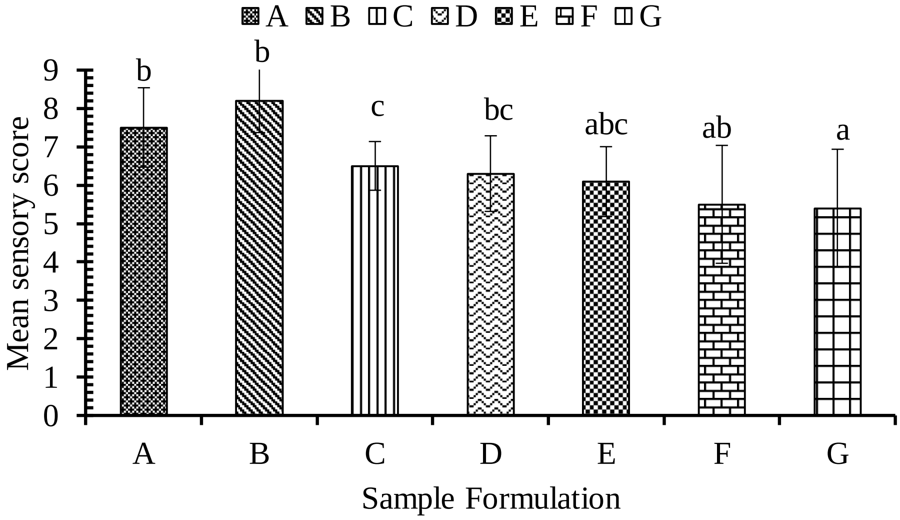

Fig. 4.1 Mean sensory scores for color of bread of different formulations. Bars with similar alphabets at the top are not significantly different.

4.3.2 Effect of formulation on crumb appearance

The average mean scores of crumb appearance are shown in Fig. 4.2. The product B got highest score while F and G ranked lowest score because incorporating high levels of oats flour protein depresses loaf volume, gives poor crumb characteristics and decreases acceptability.

Upon hydration and during processing, gliadin and glutenin interact to a unique viscoelastic gluten network, for holding the gases and for producing light porous crumb textured bread. An appropriate balance in the amount of these two major protein components of wheat gluten is required for achieving the desired bread quality. Fat coats the proteins to interfere with gluten development, which creates a tender product. Sugar also interferes with gluten development, but too much fat or sugar will retard the development (Bennion, 1967). Oats flour weakened wheat flour dough by increasing SH concentration. Substitution of gluten proteins by non-gluten-forming proteins causes a dilution effect and consequently weakens the dough. Oats flour proteins interfere with gluten formation in both a direct and an indirect way, the direct effect is related to an interaction between oats proteins and gluten proteins and the indirect effect is related to water availability of wheat proteins (Maforimbo *et al.,* 2008).

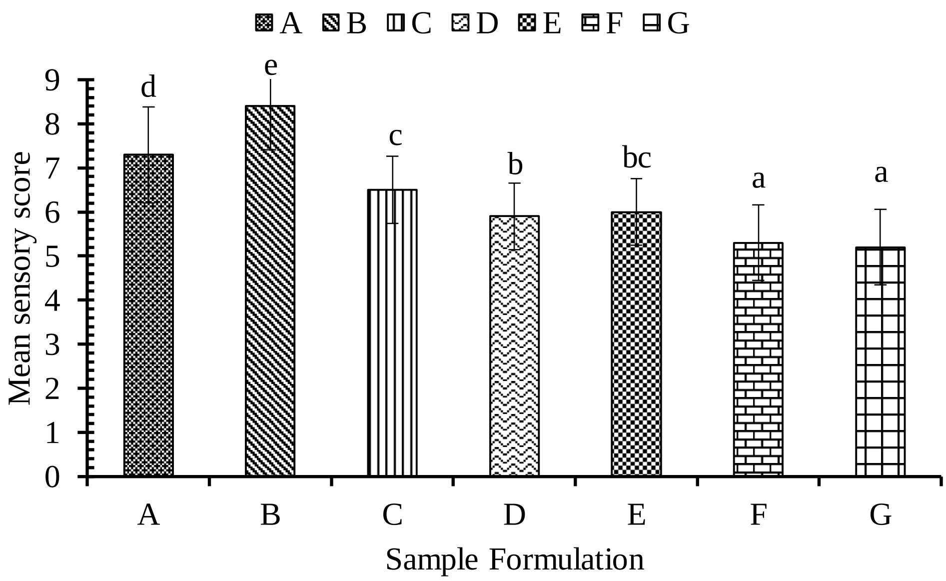

Fig. 4.2 Mean sensory scores for crumb appearance of bread of different formulations. Bars with similar alphabets at the top are not significantly different.

4.3.3 Effect of formulation on texture

The mean sensory score for texture of bread samples of different formulations are shown in Fig. 4.3. The mean score was found to be highest for product B which was significantly different with other formulation.

As proportion of oats flour increases texture score decreases which may be due to increase in firmness of bread. Fresh oats bread was more firmer (stiffer) than fresh wheat bread. This may be attributed to the lack of gluten network formation and smaller air cell structure of bread after oats flour addition (Fance, 1972) as confirmed by the higher density of the oats bread. A perfect texture should be free from lump and hardness and should present a smooth silky surface.

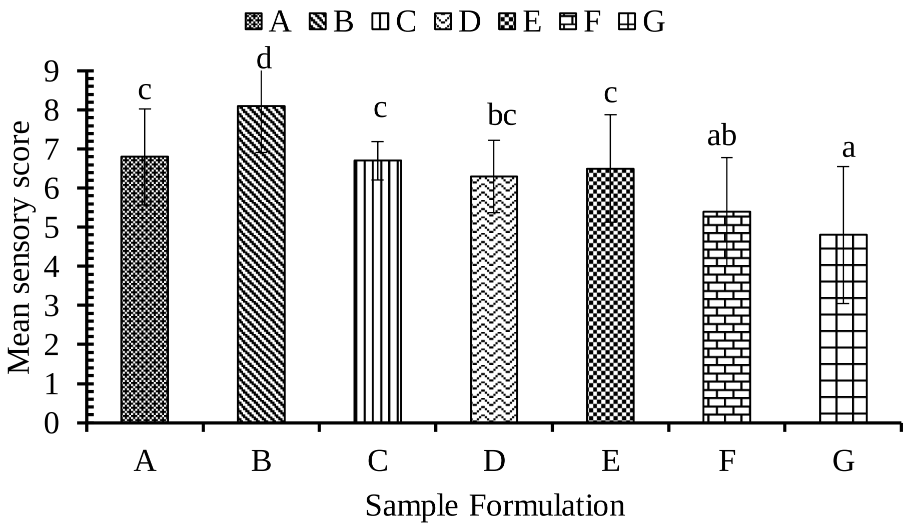

Fig. 4.3 Mean sensory scores for texture of bread of different formulations. Bars with similar alphabets at the top are not significantly different.

4.3.4 Effect of formulation on taste

The mean sensory scores for taste of bread samples of different formulations are shown in Fig. 4.4. The mean sensory score of product B was highest but was not significantly different from product A and C. Product G is the lowest scoring formulations of all which indicates that higher amount of oats flour in the formulations could lower the score and acceptability of the product.

Formulation containing 10% oats flour got high score which may be due to optimum amount of oats flour. Oats flour may gives crumb tenderness and forms structure building of bread. The taste of the 10% oats flour incorporated bread was found to be significantly superior as judged by panelist.

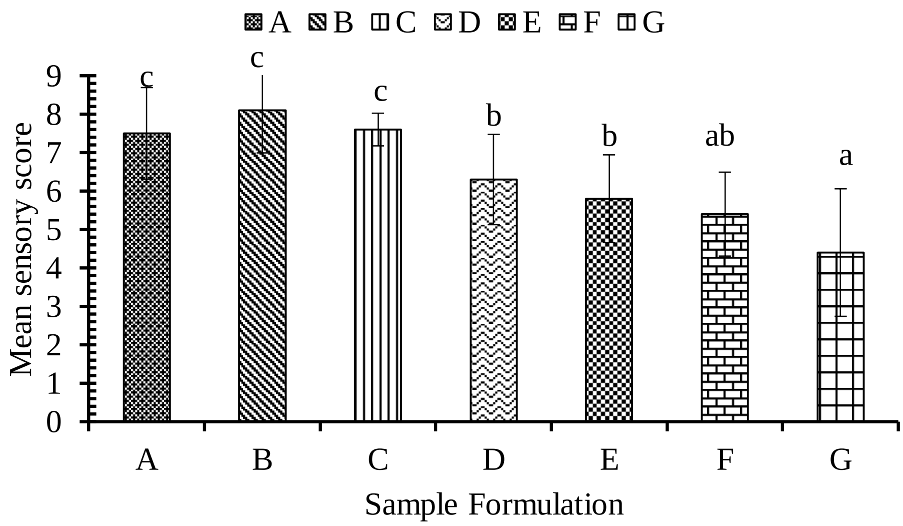

Fig. 4.4 Mean sensory scores for taste of bread of different formulations. Bars with similar alphabets at the top are not significantly different.

4.3.5 Effect of formulation on flavor

The mean sensory score for flavor of bread samples of different formulations are shown in Fig.4.5. The statistical analysis showed that the partial substitution of wheat flour with oats flour had significant effect ($p < 0.05$) on the flavor of the different bread formulation. Product B got highest score which was significantly different from other formulation. Product G got lowest score. From this we can say that as the amount of oats flour increases the acceptability on flavor of product goes on decreasing. The flavor of the 10% oats flour incorporated bread was found to be significantly superior.

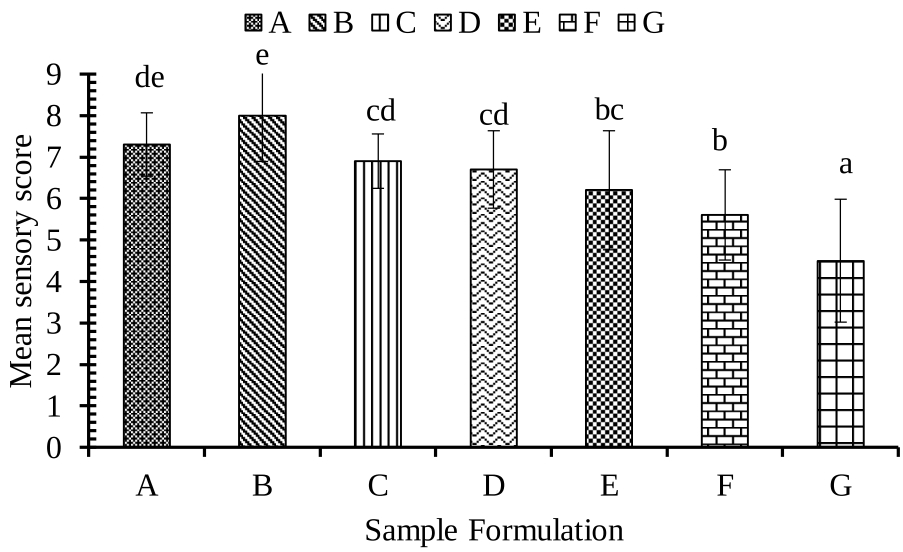

Fig. 4.5 Mean sensory scores for flavor of bread of different formulations. Bars with similar alphabets at the top are not significantly different.

4.3.6 Effect of formulation on overall acceptability

The mean scores of overall acceptability of breads of different formulations are shown in Fig. 4.6 which shows the significant effect ($p<0.005$) on overall acceptability of the different bread formulations with partial substitution of wheat flour with oat flour.

Product B got higher score which was significant different to all bread formulation. Color, crumb appearance, texture, taste and flavor of product B was very much liked. Therefore, product B got high score in terms of overall acceptability. The overall acceptability of the 10% oats flour along with constant 5% maize flour incorporated composite bread was found to be significantly superior.

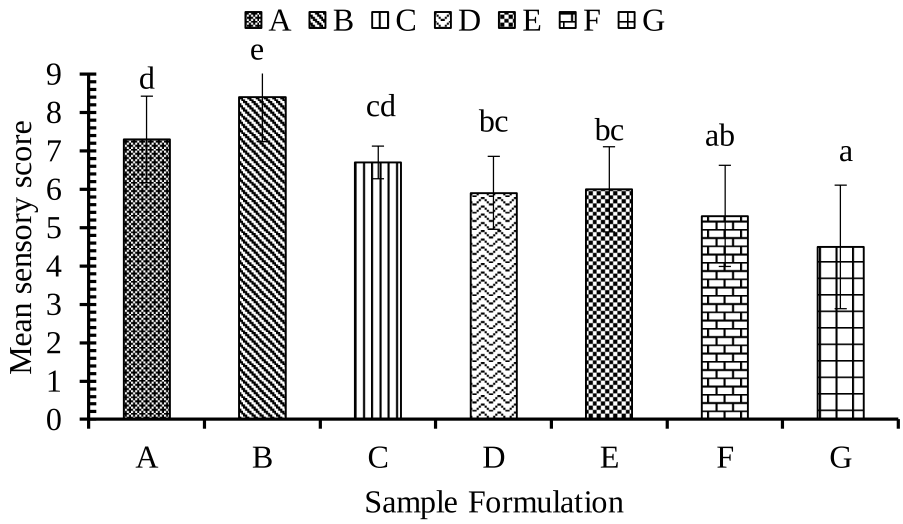

Fig. 4.6 Mean sensory scores for overall acceptability of bread of different formulations. Bars with similar alphabets at the top are not significantly different.

4.4 Chemical composition

Thus from statistical sensory analysis, the best product was found to be composite bread containing 10% oats flour along with constant 5% maize flour. The chemical composition of best formulation bread and control bread were presented in Table 4.3.

Table 4.3 Composition of control bread and best composite flour bread

Chemical composition	Control bread	Best formulation
Moisture (g/100 g)	35.10±0.025[a]	38.98±0.078[b]
Crude protein (% db)	17.60±0.015[a]	18.98±0.025[b]
Crude fat (% db)	9.87±0.015[a]	11.75±0.036[b]
Crude fiber (% db)	0.56±0.015[a]	0.60±0.015[a]
Ash (% db)	1.75±0.015[a]	1.97±0.020[b]
Iron (mg/100 g)	4.05±0.025[a]	7.26±0.036[b]
Calcium (mg/100 g)	10.64±0.015[a]	12.10±0.015[b]
Polyphenols (mg GAE/g)	0.97±0.07 [a]	13.46±0.40 [b]
Antioxidant activity (DPPH % inhibition)	12.10±0.5 [a]	34.33±0.25 [b]

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

The moisture content of product A and product B were 35.08 and 39.04 respectively. The moisture content of product B increased than that of control bread with increase in the proportion of oat flour. There was increase in moisture content of bread after incorporation of oat flour. This could be due to higher amount of soluble dietary fiber (SDF) in flour than wheat flour. SDF of oat flour has β-glucan, a hydrophilic component which binds water, resulting higher moisture retention in oat flour incorporated bread (Dawkins *et al.*, 1999). The high moisture content makes it very prone to microbial attack which could decrease the shelf life of the product but it also gives the characteristic firmness to the bread.

The protein increased with addition of oat flour to the control bread. Protein of oat flour was higher in lysine, aspargine and arginine, as well as valine, alanine, glycine and tyrosine, as compared to proteins of bread flours (Gambuś *et al.*, 2011).

The increased in crude fiber and crude fat was seen. This may be due to high crude fiber in maize and oats flour than that of wheat flour. One of the studies carried out by Salehifar and Shahedi (2010) showed that protein and fat content of bread increased with oat fortifications. It should be stressed, that oat fat is rich in polyunsaturated fatty acids, and its digestibility is higher than in other cereals (Gambuś *et al.*, 2011). The carbohydrate content decreased with increase in the amount of oats flour as maize flour is kept constant. The ash content of bread increased after oat flour incorporation, this increase in ash content may be due to the high mineral content in the oat flour such as phosphorus, potassium, magnesium, calcium, iron etc. as reported by Youssef *et al.* (2016). The incorporation of maize flour in bread resulted in a increased in carbohydrate content while incorporation of oat flours in bread resulted in a decrease in the carbohydrate content. These results are in good agreement with Habbal and Samaan (2012).

The bread with incorporation of oat flour found increased in iron and calcium content than white bread. This was found similar to the work done by Sharma *et al.* (2012). The total phenolic content and antioxidant activity of product B was found higher than that of control bread. This is due to the incorporation of oats flour to the control bread, as it is known that oat is rich in antioxidant activity and contain high phenolic content than that of wheat flour. The increase in antioxidant in composite flour bread was also revealed by various authors (Villarino *et al.*, 2014). Ingestion of food rich in antioxidant activity is associated with improvement of health status.

4.5 Cost of the bread

The cost of the developed bread was NRs. 7.95 per 100 g including overhead cost and profit of 10% (calculation is given in Appendix D).

PART V

Conclusions and recommendations

5.1 Conclusions

On the basis of this research work, the following conclusions could be drawn:

1. Composite bread from wheat, oat and maize flour can be prepared successfully. The statistical analysis showed that formulation with 10% oats along with constant 5% maize flour was significantly superior in terms of color, crumb, flavor, texture, taste and overall acceptability among formulations.
2. The proximate composition of optimized bread is superior in crude protein, crude fat, crude fiber, crude ash as compared to the control bread.
3. Iron content and calcium content of the composite bread were increased due to the supplementation of oat flour and maize flour was higher than control bread.
4. Polyphenol content and antioxidant activity of oat and maize four supplemented bread is superior than that of control bread and their retention was found to be high in bread.

5.2 Recommendations

On the basis of the study done here, following recommendations can be given from the study of composite bread of wheat, oats and maize flour:

1. Composite biscuit from this formulations could be prepared and studied.
2. Composite bread other than oat and maize flour like barley, millet, soybean etc. could be prepared and studied.

Part VI

Summary

Composite bread is the baked product made from the composite flour to make bread more nutritious in reasonable price. The overall objective of this research was to prepare the composite bread from wheat, oat and maize in the development of a nutritious bread in reasonable price for the nutritional benefit of poor people of Nepal and other developing countries.

Response Surface Methodology was used for the formulation of recipe and for this, Design Expert software was used. Seven different bread formulations with constant 5% maize namely A (95% wheat flour), B (85% wheat flour and 10% oats flour), C (82% wheat flour and 13% oats flour), D (75% wheat flour and 20% oats flour), E (68% wheat flour and 27% oats flour), F(65% wheat flour and 30% oats flour) and G (55% wheat flour and 40% oats flour) were prepared by straight dough process with the incorporation of yeast 2%, salt 0.4%, butter 4% and sugar 20% per 100 parts of flour mixture. The seven different bread samples were prepared and subjected to sensory evaluation. The sensory analysis was carried out based on crumb appearance, color, texture, taste, flavor, and overall acceptance. Sample B got the highest mean sensory score. The proximate composition of best bread according to which the moisture content, protein, fat, fiber, and ash content are found to be 38.98, 18.98, 11.75, 0.60 and 1.97 respectively.

It was concluded from the present research that the composite bread was nutritionally superior as compared to control bread. There was significantly increased in protein, crude fiber, total ash content and weight whereas significantly decreased the loaf volume and specific loaf volume of bread. Similarly, the substitution of oats flour with constant maize flour in control bread significantly increased the iron and calcium content. The iron, calcium, polyphenols and antioxidant activity of the control and best bread formultion are (4.05 mg/100 g, 10.64 mg/100 g, 0.9 mg GAE/g and 12.36 DPPH inhibition%) and (7.26 mg/100 g, 12.10 mg/100 g, 13.46 mg GAE/g and 34.33 DPPH inhibition%) respectively. Also, the result of iron, calcium, total polyphenol content and antioxidant activity of different bread formulations showed that these components were retained at baking temperature of bread.

References

AACC. (2016). Approved methods of the AACC. Retrieved from http://methods.aaccnet.org/summaries/10-05-01.aspx. [Accessed 21 May, 2018].

Abdel-Kader, Z. M. (2000). Enrichment of Egyptian 'Balady'bread. Part 1. Baking studies, physical and sensory evaluation of enrichment with decorticated cracked broadbeans flour (*Vicia faba* L.). *Mol. Nutr. Food. Res.* **44** (6), 418-421.

Ahmad, M. and Zaffar, G. (2014). Evaluation of oats (*Avena sativa* L.) genotypes for β-glucan, grain yield and physiological traits. *Appl. Biol. Res.* **16** (1), 1-3.

Ahmed, W. S., Rouf, S. T., Bindu, B., Khalid, M. and Pradyuman, K. (2014). Oats as a functional food. *Universal J. Pharmacy*. **3**, 14-20.

Al-Saleh, A. and Brennan, C. S. (2012). Bread wheat quality: some physical, chemical and rheological characteristics of Syrian and English bread wheat samples. *Foods*. **1**, 3-17. [doi:10.3390/foods1010003].

Arendt, E. K. and Zannini, E. (2013). "Cereal Grains for the Food and Beverage Industries". Woodhead Publishing. [ISBN 978-0-85709-413-1].

Arora, S. M. (1980). "Handbook of Baking Products". SIRI World Renowned Inst. Publications. Roopnagar, Delhi.

Baker, P. G. and Ream, A. E. (1976). Oats and barley toxicity in celiac patients [Report]. 52. USA, USA. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/959100.

Barro, F. and Real, A. (2017). Characterization of celiac disease related oat proteins. *Eu. J. Clinical Ntri.* **67**, 310-317.

Beloshapka, A. N., Buff, P. R., Fahey, G. C. and Swansom, K. S. (2016). Compositional analysis of whole Grains, Processed Grains Co-products, and other Carbohydrate sources with applicablility to Pet animal Nutrition. *Foods*. MDPI. [Accessed 25 March, 2016].

Bennion, M. (1967). Effect of batter ingredients on changes in fatty acid composition of fats used for frying. *Food Technol.* **21** (12), 94-110.

Beressani, R. and Breuner, M. (1989). Acid and neutral detergent fiber content and minor mineral in maize and its tortilla. *Latin America Nutr. Files*. 382-396.

Berghofer, E. (2000). Brot als Funktionelles Lebensmittel. *Getreide, Mehl und Brot*. **54** (3), 175-179.

Berski, W. and Ptaszek, A. J. (2011). "Carbohydrate Polymer". Vol. 3. St.Paul. USA.

Bhaduri, S. (2013). A comprehensive study on physical properties of two gluten-free flour fortified muffins. *J. Food Process. Technol.* **4**, 5.

Bibiana, I., Grace, N. and Julius, A. (2014). Quality evaluation of composite bread produced from wheat, maize and orange fleshed sweet potato flours. *Am. J. Food Sci. Technol.* **2** (4), 109-115.

Biel, W., Bobko, K. and Maciorowski, R. (2009). Chemical composition and nutritive value of husked and naked oat grains. *J. Cereal Sci.* **49**, 413–418.

Campbell, G. M. and Martin, P. J. (2012). Bread aeration and dough rheology: an introduction. *In:* "Breadmaking" (2nd ed.). (S. P. Cauvain, Ed.). pp. 299-336. England. Woodhead Publishing. [ISBN 978-0-85709-060-7].

Capouchová, I., Petr, J. and Krejčířová, L. (2006). Protein composition of sorghum and oat grain and their suitability for gluten-free diet. *J. Agric. Food Chem.* **93** (4), 271-284.

Cauvain, S. P. and Young, L. S. (2006). "The Chorleywood Bread Process". Woodhead Publishing. England. [ISBN 1845691431].

Cauvain, S. P. and Young, L. S. (2007). "Technology of Breadmaking" (2 ed.). Springer Science. New York, USA. [ISBN 978-0-387-38565-5].

Chan, J. (2017). Oats production in 2016 [Report]. 55. Vol. 16. Greenwell. USA. Retrieved from https://www.cso.ie/en/releasesandpublications/er/aypc/areayieldandproductionofcrops2016/. [Accessed 23 March, 2017].

Chauhan, D., Kumar, K., Kumar, S. and Kumar, H. (2018). Effect of incorporation of oat flour on nutritional and organoleptic characteristics of bread and noodles. *Curr. Res. Nutr. Food Sci. J.* **6** (1), 148-156.

Clarke, C. I. and Arendt, E. K. (2005). A review of the application of sourdough technology to wheat breads. *Adv. Food Nutr. Res.* **49** (1), 137-161.

Das, M. and Das, S. K. (2002). Analysis of moisture sorption characteristics of fish protein myosin. *Int. J. Food Sci. Technol.* **37** (2), 223-227.

Dawkins, N. L., Phelps, O., McMillin, K. W. and Forrester, I. T. (1999). Composition and physicochemical properties of chevon patties containing oat bran. *J. Food Sci.* **64** (4), 597-600.

Devkota, N. R., Upreti, C. R., Paudel, L. N. and Joshi, N. P. (2015). Production potentials of promising oat (*Avena sativa*) varieties in combination with legumes at farmers' field condition. *Nepalese J. Agric. Sci.* **13**.

Dooshima, I. B., Julius, A. and Abah, O. (2014). Quality evaluation of composite bread produced from wheat, defatted soy and banana flours. *Int. J. Nutr. Food Sci.* **3**, 471-476.

EFSA. (2009). Scientific opinion on substantion of health claims related to betaglucans and maintenance of normal blood cholesterol concentrations and maintenance or achievement of a normal body weight. *Eur. Food Safety Authority J.* **7** (9), 1254.

Fance, W. J. (1972). Student's technology of breadmaking and flour confectionery. *Agric. FAO. Org*.

FAO. (1992). "Maize in Human Nutrition". David Lubin Memorial Library. Rome, Italy. [ISBN 92-5-103013-8].

Fasano, A. and Catassi, A. S. (2001). "Gastroenterology" (2 ed.). Vol. 6. PubMed.

Fast, R. B. and Caldwell, E. F. (2000). "Breakfast Cereals and How They are Made". American Association of Cereal Chemists. [ISBN 1891127152].

Flynn, G. and James, A. W. (1981). An Industrial Profile of Bread-making (Report of the Tropical Products Institute) [Report]. Natural Resources Institute. U.S.A,

Frazier, W. C. and Westhoff, D. C. (2005). Contamination, preservation and spoilage of cereals and cereal products. *In:* "Food Microbiology". (G. Banwart, Ed.). pp. 173-186. New Delhi. Tata McGraw-Hill Publishing Company Limited. [ISSN 1740-1534].

Gambuś, H., Gibiński, M., Pastuszka, D., Mickowska, B., Ziobro, R. and Witkowicz, R. (2011). The application of residual oats flour in bread production in order to improve its quality and biological value of protein. *Food Sci. Technol.* **10** (3), 317-325.

George, E. L. (1973). "Wheat Production and Utilization". The AVI Publishing Company. Westport.

Habbal, H. and Saman, J. (2012). Utilization of multivariate statistical analysis in studying chemical, rheological and processing properties of wheat flour fortified by oat flour *Damascus Univ. J. Agric. Sci.* **28** (2), 361-374.

Henkey, V. J. (2009). Concerning the composition and labeling of foodstuffs suitable for people intolerant to gluten. *Off J. Eur Union*. **16**, 3.

Janatuinen, E. K., Pikkarainen, P. H., Kemppainen, T. A., Kosma, V., Järvinen, R., Uusitupa, M. and Julkunen, R. (1995). A comparison of diets with and without oats in adults with celiac disease. *J. Med. Sci.* **333** (16), 1033-1037.

Johnson, A. H. and Peterson, M. S. (1974). "Encyclopedia of Food Technology and Food Science". Vol. 2. AVI Publishing Co., Inc. USA. [0870551574].

Kent, D. W. and Amos, A. J. E. (1967). "Modern Cereal Chemistry" (6th ed.). Trade Press Ltd. London.

Kent, N. L. and Evers, A. D. (1994). "Technology of Cereals: An Introduction for Students of Food Science and Agriculture" (4th ed.). Woodhead Publishing. UK. [ISBN 0080408346].

Khanal, T. R. (1997). Preparation of bread using wheat malt flour. B. Tech. Dissertation. Tribhuvan Univ., Nepal.

Khatkar, B. S. and Schofield, J. D. (1997). Molecular and physico-chemical basis of breadmaking-properties of wheat gluten proteins: a critical appraisal. *J. Food Sci. Technol.* **34** (2), 85-102.

Kim, J. C. and Ruiter, D. (1969). Bakery products with non-wheat flours. *Bakers Digest.* **43** (3), 58-63.

Klose, C., Schehl, B. D. and Arendt, E. K. (2009). Fundamental study on protein changes taking place during malting of oats. *J Cereal Sci.* (49), 83-91.

Koenig, R., Dickman, J. R., Kang, C., Zhang, T., Chu, Y. and Ji, L. L. (2014). Avenanthramide supplementation attenuates exercise-induced inflammation in postmenopausal women. *Nutr. J.* **13** (1), 21.

Kumar, P. J. and Farthing, M. G. J. (1995). Oats and celiac disease. *N. Engl. J. Med.* **2**, 1075-1076.

Lamsal, A. (2018). Preparation and quality evaluation of oats flour incorporated muffin. B. Tech. Dissertation. Tribhuvan Univ., Nepal.

Lapvetelainen, A. (1994). Protein composition and functionality of high protein oats flour derived from intergrated starch-ethanol process. *In:* "Cereal Chemistry" (Vol. 2). (T. Aro, Ed.). pp. 133-139.

LI-BIRD. (2007). Enhancing the contribution of neglected but underutilized species to food security and to the incomes of the rural poor: Nepal component finger millet. Retrieved from http://libird.org/index.php?option=com_content&task=view&id=15&Itemid=80. [Accessed 28 April, 2019].

Liangli, L. Y., Tsao, R. and Shahidi, F. (2012). "Cereals and Pulses: Nutraceutical Properties and Health Benefits". John Wiley & Sons Ltd. Newfoundland, Canada. [ISBN 1118229460].

Maforimbo, E., Skurray, G., Uthayakumaran, S. and Wrigley, C. (2008). Incorporation of soy proteins into the wheat–gluten matrix during dough mixing. *J. Cereal Sci.* **47**, 380-385.

Menon, R. and Watson, J. (2016). "Advances in Food and Nutrition Research". Vol. 77. Elsevier.

Meyer, L. H. (1987). "Food Chemistry". CBS Publication. New Delhi.

Mickee, A. (2015). Baking with oat flour vs. wheat flour. Retrieved from www.livestrong.com. (Last update Oct 03, 2017).

Ndife, J., Abdulraheem, L. O. and Zakari, U. M. (2011). Evaluation of the nutritional and sensory quality of functional breads produced from wheat and soya bean flour blends. *Afr. J. Food Sci.* **5** (8), 466-472.

Noorfarahzilah, M., Lee, J. S., Sharifudin, M. S., Fadzelly, M. A. B. and Hasmadi, M. (2014). Applications of composite flour in development of food products. *Int. Food Res. J.* **21** (6), 2061.

Ohimain, I. E. (2014). The prospects and challenges of composite flour for bread productionin Nigeria. *Global Jr. Human Social Sci. Res.* **14**.

Orhun, G. E. (2013). Maize for life. *Int. J. Food Sci. Nutr. Eng.* **3** (2), 13-16.

Paton, D., Breasciani, S. and Hart, J. (1995). The potential Use of cereal beta-D-glucans as functional food ingredients. *J. Cereal Sci.*

Pederson, C. S. (1971). "Microbiology of Food Fermentations". AVI Publishing. Westport, USA. [ISBN 087051043].

Peterson, D. M., Hahn, M. J. and Emmons, C. L. (2002). Oat avenanthramides exhibit antioxidant activities in vitro. *J. Food Chem.* **79** (4), 473-478.

Pomeranz, Y. (2005). Grain structure and end-use properties. *J. Food Struct.* **1**, 45-59.

Prasad, R., Jha, A., Kumar, A., Unnikrishnan, V. S. and Sabhik, L. (2015). Nutritional advantages of oats and oppurtunities for its processing as value added foods- a review. *J. Food Sci. Technol.* **52**.

Ranganna, S. (1986). "Handbook of Analysis and Quality Control of Fruit and Vegetables". Tata McGerw Hill Pub. Co. Ltd. New Delhi.

Rao, H. P. and Rao, H. M. (1991). Effect of incorporating wheat bran on the rheological characteristics and bread making quality of flour. *J. Food. Sci. Technol.* **28** (2), 92-97.

Rasane, P., Jha, A., Sabikhi, L., Kumar, A. and Unnikrishnan, V. S. (2015). Nutritional advantages of oats and opportunities for its processing as value added foods. *J. Food Sci. Technol.* **52** (2), 662-675.

Ripsin, C. M., Keenan, J. M., Jacobs, D. R. and Elmer, P. J. (1992). "Oats Products and Lipid Lowering" (6 ed.). Vol. 1. AVI Publishing. USA.

Robert, L. S. and Nozzolillo, C. (1985). "Cereal Chemistry" (1 ed.). Vol. 3. Chapman and Hall. USA.

Salehifar, M. and Shahedi, M. (2010). Effects of oat flour on dough rheology, texture and organoleptic properties of taftoon bread. *J. Agric. Sci. Technol.* **9**, 227-234.

Sandhu, K. S., Godara, P., Kaur, M. and Punia, S. (2017). Effect of toasting on physical, functional and antioxidant properties of flour from oat (*Avena sativa* L.) cultivars. *J. Saudi Soc. Agric. Sci.* **16** (2), 197-203.

Schofield, J. D. and Booth, M. R. (1983). Wheat proteins and their technological significance. *Devlopment of Food Proteins*. **2**, 1-60.

Sharma, S., Gupta, J. P., Nagi, H. and Kumar, R. (2012). Effect of incorporation of corn byproducts on quality of baked and extruded products from wheat flour and semolina. *J.Food Sci. Technol.* **49** (5), 580-586.

Shewry, P. R., Halford, N. G. and Tathum, A. S. (1992). High mr subunits of wheat glutenin. *J. Cereal Sci.* **15**, 105-120.

Shukla, P., Kumar, R. and Raib, A. K. (2016). Detection of minerals in green leafy vegetables using laser induced breakdown spectroscopy. *J. Appl. Spectroscopy*. **83** (5), 872-877.

Suttie, J. M., Reynolds, S. G. and Batello, C. (2005). "Grasslands of the World". Food & Agriculture Org. [9251053375].

Upreti, C. R. and Upreti, S. (2013). "Livestock, Poultry and Fish Nutrition in Nepal" (2 ed.). Balika Upreti Published. Kathmandu, Nepal.

USDA. (1983). Classification for Kingdom Plantae Down to Species *Zea mays* L. NRCS. Retrieved from https://plants.usda.gov/java/ClassificationServlet?source=display&classid=ZEMA. [Accessed 16 April, 2019].

Villarino, C. B., Jayasena, V., Coorey, R., Chakrabarti-Bell, S. and Johnson, H. (2014). The effects of bread-making process factors on Australian lupin-wheat bread quality characteristics. *Int. J. Food Sci. Technol.* **49** (11), 2373-2381.

Youssef, M., Nassar, A. G., EL–Fishawy, F. A. and Mostafa, M. A. (2016). Assessment of proximate chemical composition and nutritional status of wheat biscuits fortified with oat powder. *Assiut J. Agric. Sci.* **47** (5), 83-94.

Zarh. (2010). Factors that affect in bread quality. Food Info. Retrieved from http://1st-foodinfo.blogspot.com/2010/02/factors-that-affect-in-bread-quality.html. [Accessed 4 January, 2019].

Zhao, Q., Hu, X. Z. and Zheng, J. (2014). Chemical composition and sensory characteristics of oat flakes. *J. Cereal Sci.* **60** (2), 297-301.

Zwer, P. (2010). Oats: characteristics and quality requirements *In:* "Cereal Grains Assessing and Managing Quality". (C. W. Wrigley and I. L. Batey, Eds.). pp. 163-182. New York. Woodhead Publishing Ltd. [ISBN 978-1-84569-952-9].

Appendices

Appendix A

Sensory evaluation score sheet for bread

Date:

Name of Panelist:

Name of the product: Composite bread of wheat, oats and maize

Dear panelist, you are provided 7 samples of composite bread on each proportion with variation on oats flour along with constant maize flour content. Please test the following samples of bread 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 slightly – 6	Dislike moderately – 3
Like very much – 8	Neither like nor dislike – 5	Dislike very much – 2
Like moderately – 7	Dislike slightly – 4	Dislike extremely – 1

Formulations / Attributes	A	B	C	D	E	F	G
Crumb Appearance							
Colour							
Texture							
Taste							
Flavor							
Overall acceptability							

Any Comments: Signature:……………….

Appendix B

ANOVA for physical analysis of samples

Table B.1.1 Two way ANOVA (No blocking) for Color

Variate: color					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
sample	6	63.0000	10.5000	11.71	<.001
Name_of_panelist	9	22.0714	2.4524	2.73	0.010
Residual	54	48.4286	0.8968		
Total	69	133.5000			

Table B.1.2 Two way ANOVA (No blocking) for Crumb appearance

Variate: crumb_appearance					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
sample	6	78.7429	13.1238	29.39	<.001
Name_of_panelist	9	11.4857	1.2762	2.86	0.008
Residual	54	24.1143	0.4466		
Total	69	114.3429			

Table B.1.3 Two way ANOVA (No blocking) for Texture

Variate: texture					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
sample	6	67.143	11.190	9.57	<.001
Name_of_panelist	9	26.057	2.895	2.48	0.019
Residual	54	63.143	1.169		
Total	69	156.343			

Table B.1.4 Two way ANOVA (No blocking) for Taste

Variate: taste					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
sample	6	108.971	18.162	14.39	<.001
Name_of_panelist	9	18.129	2.014	1.60	0.140
Residual	54	68.171	1.262		
Total	69	195.271			

Table B.1.5 Two way ANOVA (No blocking) for Flavor

Variate: flavor					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
sample	6	79.7714	13.2952	15.16	<.001
Name_of_panelist	9	18.2286	2.0254	2.31	0.028
Residual	54	47.3714	0.8772		
Total	69	145.3714			

Table B.1.6 Two way ANOVA (No blocking) for Overall acceptability

Variate: overall_acceptability					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
sample	6	100.6000	16.7667	17.14	<.001
Name_of_panelist	9	29.2714	3.2524	3.32	0.003
Residual	54	52.8286	0.9783		
Total	69	182.7000			

Appendix C

Table C.1 Summary of ANOVA of sensory evaluation of composite bread from wheat, oat and maize.

Sample code	Color	Crumb appearance	Texture	Taste	Flavor	Overall appearance
A	$7.5^{d}\pm1.04$	$7.3^{d}\pm1.09$	$6.8^{c}\pm1.23$	$7.5^{c}\pm1.19$	$7.3^{de}\pm0.76$	$7.3^{d}\pm1.12$
B	$8.2^{d}\pm0.83$	$8.4^{e}\pm0.99$	$8.1^{d}\pm1.19$	$8.1^{c}\pm0.43$	$8^{e}\pm1.11$	$8.4^{e}\pm1.16$
C	$6.5^{c}\pm0.64$	$6.5^{c}\pm0.76$	$6.7^{c}\pm0.49$	$7.6^{c}\pm0.43$	$6.9^{cd}\pm0.65$	$6.7^{cd}\pm0.43$
D	$6.3^{bc}\pm0.99$	$5.9^{b}\pm0.76$	$6.3^{c}\pm0.92$	$6.3^{b}\pm1.18$	$6.7^{cd}\pm0.94$	$5.9^{bc}\pm0.95$
E	$6.1^{abc}\pm0.91$	$6.0^{bc}\pm0.76$	$6.5^{c}\pm1.38$	$5.8^{b}\pm1.14$	$6.2^{bc}\pm1.43$	$6.0^{bc}\pm0.66$
F	$5.5^{ac}\pm1.53$	$5.3^{a}\pm0.86$	$5.4^{ab}\pm1.38$	$5.4^{ab}\pm1.09$	$5.6^{b}\pm1.09$	$5.3^{ab}\pm1.32$
G	$5.4^{a}\pm1.53$	$5.2^{a}\pm0.86$	$4.8^{a}\pm1.75$	$4.4^{a}\pm1.66$	$4.5^{a}\pm1.48$	$4.5^{a}\pm1.31$
LSD (5%)	0.849	0.599	0.970	1.007	0.840	0.887

Appendix D

Table D.1 Cost calculation of the product.

Particulars	Cost (NRs/kg)	Weight in a lot (g)	Cost (NRs)
Wheat flour	38	85	3.23
Oats flour	289	10	2.89
Maize flour	40	5	0.2
Sugar	65	20	1.3
Fat	310	4	1.24
Yeast	440	2	0.88
Salt	20	1	0.02
Raw material cost			9.76
Processing and labor cost (10% of raw material cost)			0.976
Profit (10%)			1.074
Grand total Cost			11.81
Total weight of product (g)		148.60	
Total cost of composite bread (NRs/100 g)			7.95

Color plates

Plate 1 Moulding

Plate 2 Bread samples

Plate 3 Semi-trained panelist carrying out sensory analysis