PROCESS OPTIMIZATION OF SOYBEAN AND MUSHROOM INCORPORATED STEAMED BREAD

by

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Process Optimization of Soybean and Mushroom Incorporated Steamed Bread

A dissertation submitted to the Central Department of Food Technology, Institute of Science and Technology, Tribhuvan University, in partial fulfillment of the requirements for the degree of M. Tech. in Food Technology

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Approval Letter

This *dissertation* entitled *Process Optimization of Soybean and Mushroom Incorporated Steamed Bread* presented by Subash Chandra Osti has been accepted as the partial fulfillment of the requirements for the M. Tech. degree in Food Technology

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Abstract

Steamed bread was prepared with incorporation of oyster mushroom powder and defatted soybean powder, aiming to minimize wheat flour and maximize soybean and mushroom powder content in the mix. Design expert software, mixture design was used for experimental design, in which level of soybean and mushroom powder within a range from 0 to 20 parts of each for formulation of steamed bread with varying refined wheat flour content by 80 to 100 parts. Similarly, Response surface methodology, central composite design was used to design another experiment for optimization of fermentation time within a range from 30 to 90 min at 35±2°C and steaming time for 10 to 20 min at normal atmospheric condition. For microbiological study, steamed bread samples were packaged in 40 μ LDPE zip lock bags and stored at ambient and refrigerated storage conditions at 30±2ºC and 5±1ºC respectively. Both samples were subjected to microbiological assessment daily until the microbial load had reached to a critical value of 5 log cfu/g.

The optimum level of wheat flour, soybean powder and mushroom powder were found to be of 87.89, 10.03 and 2.08 parts respectively with magnitude of hardness, specific volume, spread ratio and L* (whiteness) were 2801.73 g, 1.75 ml/g, 1.58 and 73.87 respectively. Fermentation time of 67.04 min at 35±2°C and steaming time of 15.05 min under normal atmospheric pressure as optimized process conditions with magnitude of hardness, specific volume, spread ratio and L* (whiteness) were 2806.63 g, 1.74 ml/g, 1.49 and 75.07 respectively. Moisture, crude fat, crude protein, total ash, crude fiber and total carbohydrate content of optimized product on dry weight basis were found to be 41.20±0.23, 2.95±0.09, 18.16±0.17, 3.98±0.11, 0.99±0.09 and 73.13±0.47% respectively. Results of sensory evaluation showed that there was no significant difference ($p>0.05$) between soybean and mushroom incorporated steamed bread and the control product containing 100 parts refined wheat flour in terms of color and appearance, flavor, texture, taste and overall acceptability. Microbiological analysis of steamed bread packaged in 40 μ zip lock LDPE bags showed that the product was found to be safe for consumption within the first 5 days of storage under refrigerated storage at 5±1ºC and only a day at ambient storage at 30±2ºC. The cost and calorific value of the optimized product per 100 g was found to be NRs. 12 (as of 2021) and 230.35 Kcal respectively.

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List of Abbreviations

Abbreviation	Full form
µ	Micron
AACC	American Association of Cereal Chemists
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BF	Banana Flour
BU	Brabender Units
CCT	Central Campus of Technology, Dharan
CDFT	Central Department of Food Technology, Dharan
CSB	Chinese Steamed Bread (Bun)
CSL	Calcium Stearoyl Lactylate
DATEM	Diacetyl Tartaric Acid Ester of Mono- and Diglycerides
Db	dry basis
DFTQC	Department of Food Technology and Quality Control, Kathmandu
EADSF	Enzyme Active Defatted Soybean Flour
EAFFSF	Enzyme-Active Full-Fat Soybean Flour
Expt.	Experiment
FAO	Food and Agriculture Organization of the United Nations
FBD	Fluidized Bed Drying
FTQC	Food Technology and Quality Control Office, Biratnagar
FWA	Farinograph Water Absorption

Continued

List of Abbreviation

Abbreviation	Full form
HTFFSF	Heat-Treated Full-Fat Soybean Flour
KMS	Potassium metabilsulphite
LDPE	Low Density Polyethylene
LSD	Least Significant Difference
Mb	Moisture basis
MP	Mushroom (oyster) Powder
NFE	Nitrogen-Free Extract
OA	Overall Acceptability
OMP	Oyster Mushroom Powder
RSM	Response Surface Methodology
SP	Soybean Powder
SMP	Skimmed Milk Powder
SPIs	Soybean Protein Isolates
SR	Spread Ratio (of bread)
SSL	Sodium Stearoyl Lactylate
SV	Specific Volume of bread
TLD	Thin Layer Drying
Wb	Weight basis
WF	Wheat Flour
WHC	Water Holding Capacity

Part I

Introduction

1.1 General introduction

Steamed bread is a major use of wheat in China and often consumed as breakfast meal by many peoples in other Asian countries (Rubenthaler *et al.*, 1990). Chinese steamed bread (CSB) is believed to be originated from China, CSB is a traditional wheat product in Chinese cuisine which has been successfully commercialized as frozen steamed bread (Keeratipibul and Luangsakul, 2012; Loong and Wong, 2018). There are three major types of steamed bread in China and other Asian countries: northern, southern and Guangdong style (Huang *et al.*, 1998). According to (Sha *et al.*, 2007), steamed bread (*Mantou*) has long been considered as a nutritious and healthy food in the Chinese society, with the rapid urbanization in China, as a ready-to-eat food and it has been consumed in some parts of China especially, in Northern China as a staple food over two thousand years, and the popularity is increasing worldwide.

According to Hou and Popper (2006), Chinese steamed bread is a fermented wheat flour product that is cooked by steaming in a steamer. It is often made from a wheat flour of low or medium protein, depending on the its type. The preparation process is similar to that of western pan bread, but the final product is steamed, not baked in an oven, so there are some differences in appearance and shape between pan bread and steamed bread. Steamed bread is white in color and has a soft, shiny surface. The common types of steamed bread weigh about 30-120 g and are either pillow or round in shape. The original steamed bread was a filled bread, but it has gradually changed in the course of time. Nowadays, steamed bread always means unfilled bread (called *mantou*) and filled steamed bread is now called steamed buns (*baozi*).

Chinese steamed bread is made from wheat flour, which is mixed into a dough and then fermented before being cooked by steaming. The most common types of steamed breads are either round or roughly cylindrical in shape. Northern type steamed breads weigh about 130-150 g and have a white, smooth, shiny skin and no crust. The flavor depends on local preferences, and texture varies from compact to open (Huang and Miskelly, 1991). Different fillings can be added into steamed bread according to the local taste preference. However, the basic steamed bread consists of elementary ingredients such refined wheat flour, water and commercial yeast (Loong and Wong, 2018).

Soybean (*Glycine max*) is rich source essential nutrients, including protein, fat, dietary fiber, vitamins and minerals, soybean saponins and isoflavones. It has a great potential to be developed further as a protein source for vegetarians, and also for people who don't consume animal proteins for ethical or religious and environmental reasons (Nishinari *et al.*, 2018). The production of edible soybean flours and grits may take place either as an independent industrial activity or as a by-product of oil-mill operations. Soybean grits are similar to soybean flour except that the soybeans have been toasted and cracked into coarse pieces. Defatted flour and grits have the oils removed during processing, contains less than 1% oil (Singh *et al.,* 2008).

Mushrooms are an edible fungus typically grown above the soil; the three majorly grown mushrooms in India are button mushrooms, milky mushrooms and oyster mushrooms. Mushrooms are nutritious, medicinal and functional food; considered as a health food as it contains low calories, high protein, dietary fiber, vitamins and minerals (Barros *et al.,* 2008). Mushroom can be sliced and dried and/or made into powders to be used as additive to increase content of dietary fibers in various foods and as a partial substitute for wheat flour in the bakery products or the dried slices are used directly in soups, biscuits, nuggets and snacks preparation (Jahan and Singh, 2019).

1.2 Statement of the problem

Steamed bread is considered as healthy food due to the absence of toxic Maillard reaction products such as acrylamide and furan and also possible low oil and sodium contents. The relatively low steaming temperature (<100°C) during production may render better retention of diverse endogenous and added nutrients as compared to the baked counterpart (Su, 2005). Steamed bread (*Mantou)* has long been considered as a nutritious and healthy food in China. With the rapid urbanization in China, steamed bread, as a ready-to-eat food has high demand. Hence, the current small-scale production of the bread is being upgraded to a larger industrial scale (Sha *et al.*, 2007).

Mushrooms are healthy foods, low in calories and fat, rich in vegetable proteins, chitin, vitamins and minerals and constitute an increasing share in the diet (Manzi *et al.*, 1999). Edible mushrooms are a potential source of dietary fiber as fungal cell walls contain chitin, other hemicelluloses, mannans, the most other functional components such as beta glucans are scientifically justified to play a key role in human health. Its role in enhancement of macrophage function and host resistance to many bacterial, viral, fungal and parasitic infections, activation of a non-specific immune stimulation, reduction of blood cholesterol and blood glucose levels (Cheung, 2010; Rajarathnam *et al.*, 1998).

Oyster mushroom can be are produced for all year round, the consumption of oyster mushroom, however, is limited because fresh mushroom is very perishable as it has high moisture content as well as well as active enzymes. Hence, the development of processed foods incorporating oyster mushroom having physiologically active compounds could be the best way to increase its consumption (Hong *et al.*, 2005).

Food proteins, both animal and plant proteins, play critical roles in human nutrition (Lamsal *et al.*, 2007). Plant proteins, especially from grain legumes, as one of the most cultivated plants in the world, have been widely utilized due to their highly nutritious and vital functional properties (Torrezan *et al.*, 2007) in such food industry as breads, cakes, noodles, soups and a variety of nutritional foods and supplements (Yuan *et al.*, 2012).

Soybean seeds contain approximately 36–38% protein on a dry-weight basis and the protein contains eight essential amino acids, especially lysine. (Chen *et al.*, 2013). Soybean protein isolate (SPI) is widely used in the food industry because of its favorable water-holding capacity, oil-binding capacities and other functional properties. Many researchers have also discovered the functions of soybean protein in reducing osteoporosis and inhibiting hyperlipidemia and other physiological health functions (Shenoy *et al.*, 2013).

Various ingredients can be added in bread products to improve wheat flour processing and/or nutritional value of bread. It is known that wheat flour proteins are of a lower nutritional quality than the milk, and legume proteins like soybean and is deficient in essential amino acids such as lysine, tryptophan and threonine (Jideani and Onwubali, 2009). Quality of bread can be improved by incorporation of new types of raw materials to produce composite breads as functional foods, made from blend of wheat flour and non-wheat or pseudo cereals flours (Dewettinck *et al.*, 2008).

Oyster mushrooms are rich in protein, carbohydrates, fiber, minerals, vitamins (B_1, B_2, B_3, C and D_2) and low fat. Various bioactive compounds have been isolated and identified from the fruiting bodies of oyster mushrooms, which exhibit antioxidant activity. These mushrooms are shown to have all the three properties of food, i.e., nutrition, taste and physiological function (Deepalakshmi and Mirunalini, 2014). There are limited studies have been found regarding incorporation of soybean and oyster mushroom powder in preparation of steamed bread.

1.3 Objectives

1.3.1 General objective

The general objective was formulation and process optimization of soybean and oyster mushroom incorporated steamed bread.

1.3.2 Specific objectives

The specific objectives were to

i. Optimize wheat flour, soybean and mushroom powder content by determination of specific volume, spread ratio, whiteness and texture (hardness) of steamed bread.
ii. Optimize steamed bread processes as fermentation time and steaming time with the measurement of specific volume, spread ratio, whiteness and texture (hardness).
iii. Determine shelf-life of optimized product, based on total mesophilic microorganism count.

1.4 Significance of the study

From the nutritional point of view, bread is a source of vitamins, minerals, dietary fiber and proteins (Steer *et al.*, 2008). During milling and processing many of the nutrients from the wheat are destroyed, affecting from the nutritional point of view, the quality of the wheat flour as raw material. Also, some vitamins such as A, C, D, and B_{12} lacked from wheat flour, decreasing it nutritional quality (Delcour and Hoseney, 2010). The quality of raw materials used in bread formulation play an important role, influencing not only the nutritional value of bread but also functional, sensory and physicochemical quality of the final bread product (Duodu and Minnaar, 2011).

Steam bread is rarely consumed in Nepal, similar product (*ting* momo) in its traditional form is found to be prepared and consumed in *Boudhha* area in Kathmandu, Nepal (Gurung, 2019). It is regarded as healthy food in comparison to its baked counterpart due to the absence of carcinogenic compounds like acrylamide and furan that can be produced during baking operation. Although, oyster mushroom production in Nepal is hiking to its commercial level, being highly perishable in nature cannot be consumed fresh for a long time. Such mushrooms contain functional properties beyond its contribution to basic nutrients in human diet. Soybean is one of the most valued functional foods, which can provide several essential nutrients and bioactive components for an active and healthy human life. With the optimization the recipe with these valuable functional ingredients, steamed bread can be prepared and consumed as a healthy dish at homes, restaurants and commercialized as frozen steamed bread.

1.5 Limitations of work

The limitation of the study is:

- Texture profile analysis (TPA) cannot be carried out due to lack of facilities.

Part II

Literature review

2.1 Steamed bread

Chinese steamed bread, or simply steamed bread has long been a traditional staple food of the wheat-growing areas in China. Western breads, which are baked at high temperatures in an oven but, steamed breads are cooked in a steamer, giving a product with a thin, soft skin and creamy white color. Steamed bread can be made with and without fillings, steamed breads without fillings but consisting of rolled sheets of different colored and/or flavored doughs are known as steamed rolls. Steamed breads, rolls, and buns have long been part of Asian cuisine, not only in China but also in other Asian countries, where they have been modified as per local preferences. The popularity of steamed breads is growing worldwide due to the availability in restaurants and as frozen steamed bread in supermarkets (Huang and Miskelly, 2016).

Steamed bread has been considered a staple food in northern China, just like baked bread in the diet of the western countries. However, steamed bread is very different from baked bread in that it is cooked by steam at atmospheric pressure, and thus the cooking temperature is much lower than for baking bread in an oven. Steaming results in a thin soft crust without a browning effect. During the bread baking process, Maillard reactions take place, forming a golden yellow crust. This reaction also causes the loss of soluble amino acids (mainly lysine), which reduces the nutritional value of the bread. In this respect, steamed bread is more nutritious than baked bread. Furthermore, acrylamide is formed as a secondary product of the Maillard reaction during baking, but not during steaming (Hou and Popper, 2006).

Traditionally, steamed bread was made at home by hand. Today, in cities, almost all steamed bread, buns and rolls are made in small factories. These modern factories use mechanical mixing, sheeting, and even rounding. According to (Huang, 2014a), Steamed products without fillings are called steamed bread (*mantou* or *moo*). Typically, steamed bread weighs about 65 or 130 g, and has either a round or cylindrical shape and small-sized steamed bread, weighing only 25 g is also in practice. A steamed product with a filling is often called steamed bun, which are frequently garnished with sweet or savory fillings. There are three types of steamed bread in China and in east and south-east Asia: northern, southern, and Guangdong styles (Huang and Miskelly, 2016).

For health reasons, steamed bread has been produced using blends of wheat and other flours. These flours include maize, millet, sorghum, sweet potato, buckwheat, oat, and black rice. Up to 30% of maize (or millet, or sorghum, or buckwheat) flour was mixed with wheat flour (70%), sugar (4%), dry yeast (0.2%), and water (40%) to make steamed bread (Huang 2014c). According to Huang and Miskelly (2016), steamed bread made from a mixture of wheat flour and other grain flours are smaller, darker, and denser. Green banana flour (BF) incorporated CSB had lower volume and a lower elasticity (Aziah *et al.*, 2012) and BF incorporated muffins had reduced volume and increased hardness. These significant differences in appearance, color, and texture may affect the overall acceptability (Ng *et al.*, 2012). Hence, in the product development of Chinese steamed bread using BF, it is important to ensure that various sensory properties are either maintained or improved to increase and ensure its consumer acceptability in the future market (Aziah *et al.*, 2012).

In bread formation, gluten is formed through hydration of bread proteins gliadin and glutenin. These proteins are responsible to provide the viscoelastic properties of bread hence supporting its structure, preventing it from collapsing (Swanson, 2004). Besides, a significant amount of water will be absorbed by the increased starch for the gelatinization reaction. As the water holding capacity of dough increases, the dietary fiber competes with gluten and starch for water in hydration hence causing the gluten structure to be incompletely formed as well as causing poor gelatinization process (Zhou and Therdthai, 2007) and therefore affecting the sensory properties of CSB. In another study on steamed bread, it was also found that adequate water level in the dough is needed to achieve high quality steamed bread (Rubenthaler *et al.*, 1990).

Functional foods are regarded as innovative and promising products, which can provide additional health benefits beyond the basic nutrition. Although the functional foods have no formal definition, some groups define the primary category of functional foods as modified foods that claim to have been fortified with nutrients or enhanced with phytochemicals or botanicals to provide specific health benefits (Hasler, 2002).

Soybean foods can be best described as uniformly high in protein but low in calories, carbohydrates, and fats, entirely devoid of cholesterol, high in vitamins, easy to digest, tasty, and wonderfully versatile in the kitchen, which positions them as irresistible new food staples for the evolving spectrum of health diets (Singh *et al.*, 2008).

Yen *et al.* (2015) incorporated Enokitake (*Flammulina velutipes*) into steamed bun. Quality attributes including specific volume, color and sensory evaluation, and taste components in enokitake supplemented steamed bun were analyzed and compared with those in white steamed bun. Specific volumes were 4.00, 3.45, 3.28 and 2.98 cm^3g^{-1} for white steamed bun, for 2%, 5% and 10% enokitake supplemented steamed buns, respectively. All steamed buns had a comparable profile in proximate composition. Enokitake supplemented steamed buns contained more total soluble sugars and total free amino acids. Enokitake supplemented steamed buns showed lower lightness and became browner as more enokitake flour added. Although their sensory results were lower than those of white steamed bun, 2% and 5% enokitake supplemented steamed buns were moderately acceptable. Overall, enokitake could be incorporated into steamed bun to provide its beneficial health effects.

Otegbayo *et al.* (2018a) prepared four types of soybean-bread samples (5%, 10%, 15% and 20%), the inclusion of 5% soybean flour produced bread, which was nutrient dense with morphological and geometric characteristics similar to wheat bread and was more acceptable and preferred by the consumers. Soybean flour inclusion at 5% level can therefore be advantageous due to the increased nutritional value and acceptable consumer attitude in sensory characteristics.

Hong *et al*. (2005) prepared bread from composite flour containing 1%, 2%, 3% and 4% oyster mushroom (*Pleurotus ostreatus*) flour. Taste and texture scores of breads had the highest score at 1% addition, and did not significantly different from those of control bread up to 2% addition, resulting in acceptable values except for 3 and 4% additions. In the mean scores of nine hedonic scale (1=extremely dislike 9=extremely likes), overall acceptability of breads ranged from 6.70 for 1% addition to 4.56 for 4% addition, indicating that 1% addition had acceptable breads similar to those obtained from wheat flour only. However, beyond 1% addition level, the overall acceptability scores of breads decreased significantly. From the sensory evaluation results, acceptable breads up to 1% addition level of oyster mushroom powder could be produced.

Mahmoodi *et al.* (2014) carried out a study in which blending of wheat flour with defatted soybean flour (DSF) altered the organoleptic properties of breads. Addition of DSF increased significantly the protein and ash content of the bread ($p<0.05$). Organoleptic test indicates that the best formulation is between 3 and 7% fortifications of DSF blends. In biological evaluation, rats fed the control diet had the lowest body weight gain and their food efficiency ratio was significantly different ($p<0.05$) in compare with 7% DSF-fortified blend.

Rubenthaler *et al.* (1990) reported to be use of 8% sugar, 1% instant active dry yeast with 58 min proofing and 10 min steaming for preparation of steamed bread using commercial soft wheat flour. Liu *et al.* (2016) prepared 0, 5, 10 and 15% buckwheat flour incorporated Chinese steamed bread and specific volume, loaf height, moisture, and texture were measured. Incorporation of buckwheat flour into wheat flour decreased specific volume, increased bread hardness, gumminess and chewiness of the final product. In another study, Farzana and Mohajan (2015) prepared biscuit using composite flour containing 5% mushroom and the composite flour was supplemented with 20, 15 and 10 percent of soybean flour. From biochemical analyses and organoleptic evaluation, good quality biscuits can be prepared by substituting wheat flour with 15% soybean flour.

Consumers require steamed bread that has a white, shiny skin, free from blemishes and with the desirable combination of textural properties and eating quality. In order to supply this need, steamed bread manufacturers perform within the framework of increasing automation requiring greater process control, well-documented product quality specifications and food safety programs. These trends will lead to tighter and more stringent flour quality specifications for flour millers supplying to the market sector, particularly with regard to maximum flour ash, range of protein content and range of acceptable dough properties including dough strength consistency of flour quality is essential. Depending on the manufacturing process used, steamed bread style and the regional preferences, flour quality requirements will differ, and change from time to time as new products are developed or according to change with consumer preferences (Huang and Miskelly, 2018).

2.2 Classification of steamed bread

There are three classes of steamed bread in China and in east and south-east Asia: northern, southern, and Guangdong styles. It should be emphasized that the three styles of steamed bread refer to steamed products without fillings. The differences in dough for steamed buns and rolls (products with fillings) consumed across China are much less than for steamed breads (Huang, 2014a). According to Su (2005), Chinese steamed bread can be classified into two categories: staple food and non-staple food, steamed bread consumed as staple food is sub-divided into three types based on firmness: soft, medium, and hard. A brief description is given in following paragraphs.

2.2.1 Steamed bread

Steamed bread is called *mantou* or *moo* in China and is made without filling. Steamed bread is more popular in China than in other Asian countries. Steamed bread usually has a round or cylindrical shape. In northern China, which is a semi-dry region, wheat is the main crop and steamed bread is a staple food (Huang and Miskelly, 2016).

Northern-style steamed bread with firm, elastic, and cohesive eating quality is preferred as it provides greater satiety. Round northern-style types commonly weigh approximately 130 g, whereas the southern-style type is produced in two sizes of 65 or 130 g and the Guangdong-style type is smaller, weighing only 25 g (Huang and Miskelly, 2016). Classification of steamed bread with their quality characteristics is given in table 2.1.

Table 2.1 Classification of steam bread with quality characteristics

Form	Style	Quality Characteristics				
		Texture	SV[a]	SR[b]	Structure	Eating quality
Staple food						
Steamed bread	Northern	Very firm	2.0	1.2	Very dense	Very firm and cohesive
		Firm	2.5	1.5	Dense	Firm and cohesive
	Southern	Soft	3.0	1.6		Soft, a bit cohesive
Non-staple food						
	Guangdong		3.2-3.4	1.7	Open	Very soft, not cohesive
Fancy steamed bread						Varies – depends on variety
Steamed buns and rolls					Open	Soft and slightly cohesive
Guangdong buns					Open	Very soft, not cohesive

Continued

Table 2.1 Classification of steam bread with quality characteristics

Char *shiew bao*	Open	Very soft, fluffy and not cohesive
Steamed cake	Open	Very soft, fluffy and low cohesive

$^{a}\mathrm{SV}$, Specific volume = volume/weight Source: Huang (2014a)

$^{b}\mathrm{SR}$, Spread ratio = width/height

In southern China, which has a warm climate, rice is staple food instead of wheat. Popular breakfast foods in the south include southern-style steamed bread and rice porridge. Southern-style steamed breads which are soft and a bit chewy are preferred over the traditional northern-style breads. In the Guangdong region of southern China, rice is the staple food, but a wider choice of food is available from elsewhere in China and abroad. In the Guangdong region, unique steamed products have been developed according to local eating habits and preferences (Huang and Miskelly, 2016).

Northern-style steamed bread is popular in northern China. Steamed bread in Taiwan is a rather sweet version of northern style steamed bread, which has a very cohesive texture and a slightly sweet taste. Southern style steamed bread used to be popular only in southern China, but now it is popular throughout China, which is prepared without fermentation or single stage fermentation process. When all ingredients are mixed dough is prepared, divided, molded, proofed and steamed without delay so that the steamed bread would have good chewing properties and a natural wheat flavor. (Huang, 2014a). Classification of Chinese steamed on the basis of different formulation type is given in Table 2.2.

Table 2.2 Classification of CSB on the basis of different formulation

Basis for classification	Formulation type	Reference
Fermentation	1. Sourdough CSB 2. Yeast CSB	Keeratipibul *et al.* (2010)
Ingredients	1. Southern CSB 2. Northern CSB	Zhu (2014)
Processing procedure	1. One-step method 2. Two-step method 3. Sourdough method	Wu *et al.* (2012)
Shape	1. Pillow-like 2. Round	Keeratipibul *et al.* (2010)

2.2.2 Steamed buns

Steamed bread can be prepared with and without fillings and developed the terminology of steamed buns to describe the filled product. Steamed buns contain many different fillings. They can be divided broadly into two types: savory and sweet fillings (Huang and Miskelly, 2016).

2.2.3 Steamed rolls

Steamed rolls, those have condiments such as sesame oil spread between the layers of the dough. Differences in the cutting, stretching, rolling, and folding of the dough. Such breads result difference in texture, shapes and flavors when rest of the processes are terminated by steaming process (Huang and Miskelly, 2016).

2.2.4 Fancy steamed bread

There are numerous types of fancy steamed breads manufactured in China which are consumed as a snack. Popular examples are round steam breads filled with chopped pork and the sweet bread (Huang and Miskelly, 2016).

2.2.5 Guangdong-Style

It is a unique Guangdong-style steamed bun filled with barbequed pork (*char siew*). It has an extremely soft and fluffy texture and very sweet taste. It is characterized by a split on the top of the bun (which occurs during steaming) it resembles like steamed cake (Huang and Miskelly, 2016).

2.2.6 Frozen steamed bread

Frozen steamed bread, buns, and rolls have become readily available in supermarkets in China other Asian countries for a decade. Due to extended shelf life (12 months or more), frozen steamed products are also exported to markets of oversees. These are often high-quality, premium value products can be successfully stored in a home freezer and instantly reheated in a steamer or microwave before being eaten for breakfast or as a snack. The time taken for preparation will depend on the size and shape, and if the product is filled or unfilled (Huang and Miskelly, 2016). During freezing of steamed products, the center of the product needs to reach about -18°C as soon as possible. However, rapid staling of bread proceeds at +10 to -6°C, study revealed that a peak rate of staling at about 2°C (BRI, 1989).

2.2.7 Frozen doughs

Pre-proofed and un-proofed frozen dough are prepared usually prepared at the central level, which are then transported to retail outlets and chain stores. Frozen doughs are not considered suitable for domestic use. Products are steamed on demand to provide fresh buns for breakfast and snacks in cities. Use of frozen dough saves the retailers the need to purchase costly raw materials and equipment and employ highly trained staff (Yang, 2007).

2.3 Steamed bread ingredients

The description of major ingredients used for preparation of steamed bread is given as following:

2.3.1 Wheat flour

Wheat flour is the major ingredient of steamed bread. Its major components: protein, the water soluble fraction, and starch play a very important role in determining the quality of steamed bread (Huang *et al.*, 1996). The wheat grain, at maturity, consists of 85% carbohydrate, most (80%) being the starch of the endosperm. The non-starch carbohydrate is made up of mono-, di- and oligo-saccharides and fructans (7%), cell-wall polysaccharides (12%). The quality requirement of wheat flour for various bread products is presented in Table 2.3.

Table 2.3 Wheat flour quality for bread products

Product	Grain protein content	Grain hardness/ Softness	Water absorption of flour	Dough strength	Dough development time*
Pan bread (by method)					
Sponge-and-dough	>12%	Hard	High	Strong	High
Straight-dough	>12%	Hard	High	Medium	Medium
Rapid-dough	>11%	Hard	High	Medium	Short
Flat bread					
Middle Eastern	10.5–12%	Hard	High	Medium	Medium
Indian subcontinent	10–12%	Hard	High	Medium	Short
Tortillas	10.5–12%	Hard	Medium	Medium	Medium
Steamed bread					
Northern-China style	11-13%	Hard	High	Medium	Medium
Southern-China style	10-12%	Medium-hard	Low-medium	Medium	Medium
Cookies and Cakes	8–9%	Very soft	Low	Weak	Low

*In the Farinograph Source: Wrigley and Uthayakumaran (2010)

Carbohydrate is the predominant source for human diets, which constitutes 60–70% of the mass of wheat flour (Stone and Morell, 2009). In general, soft wheats have less protein and more starch than hard wheats, reflecting the respective use according to the specifications for each product.

Huang *et al.* (1996) investigated the relationship between flour quality and the quality of northern type steamed bread. There was a significant linear correlation between the protein content and the specific volume and spread ratio of steamed bread. But there was no significant linear correlation between protein content and the total quality score of northern type steamed bread. However, protein content below 10%, it was linearly and positively correlated with the total quality score of steamed bread. As protein content further increased, the total quality score was not significantly influenced by protein content and was more dependent on other flour quality attributes

2.3.1.1 Particle size

Zhou *et al.* (1994) indicated that flour particle size had an effect on the quality of steamed bread. Flour particle size of 160 μ is the most suitable for good steamed bread quality, The effect of flour particle size on the quality of steamed bread is given in Table 2.4. Flour that is too fine causes a decline in specific volume, flat steamed bread with a less attractive color, and more sticky eating quality, which results in a low quality score Zhou *et al.* (1994).

Table 2.4 Effect of flour particle size on the quality of steamed bread

Flour	Sieve	Flour	Flour	Dough	Steamed bread				
		Particle size	Whiteness	Water absorption	Specific volume [a]	Spread ratio [b]	Color	Non-sticky	Total quality
Sample	Size	(μm)	(%)	(%)	(ml/g)	score/15	score/10	Score/15	Score/100
1	54GG	≤ 318	73.3	60.5	2.33	14.5	8.5	13.5	90.0
2	CB30	≤ 181	75.0	61.9	2.25	14.5	9.1	13.1	90.8
3	CB36	≤ 160	76.9	63.2	2.2	14.5	8.3	13.0	88.6
4	CB42	≤ 123	76.8	62.9	2.19	10.0	7.5	12.0	85.7
5	CB54	≤ 105	79.2	67.2	2.11	10.0	6.6	10.8	76.1
6	150 mesh		83.4	74.2	1.92	9.0	6.9	10.6	72.4

^{a}SV, Specific volume = volume/weight Source: Zhou *et al.* (1994)
^{b}SR, Spread ratio = width/height

2.3.1.2 Color and extraction rate

White, shiny, smooth steamed bread with a bold shape are the most preferred features (Huang and Miskelly, 1991). The color of steamed bread not only depends on the color of the flour, but also on the addition of alkali (for neutralization of the dough after fermentation), processing procedures such as sheeting and steaming rate (Huang and Hao, 1994). A yellowish color is not acceptable to customers. Flour extraction rate is usually controlled to 60–70%. The steamed breads served in banquets utilize flour with an even lower extraction rate. The practice of bleaching flour is not liked by consumers, however, even though the steamed bread is extremely white (Huang, 2014a).

2.3.1.3 Water absorption

The water absorption of flour used for steamed bread varies and depends on the style of steamed bread. For northern style it is around 60–63% (Huang *et al.*, 1996); for southern style it is about 58–60% (Huang and Quail, 1996); and for Guangdong style it is about 58–63% when fat is included in the formula, and 54–57% when there is no fat in the formula (Huang and Quail, 2003). For Hong Kong style steamed buns with a Chinese barbecue pork filling, water absorption is about 50% (Limley, 2008).

2.3.1.4 Protein content and quality

Proteins are recognized as the most important exist in the slopes of these relationships between components governing bread-making quality. A high protein content is often related to good bread making quality. Quantity alone cannot explain all the variation in bread-making quality however, other factors such as protein 'quality', are also important (Weegels *et al.*, 1996).

With adding starch and gluten to a base flour, Huang and Quail (1999) investigated the effect of flour protein content on the quality of Guangdong style steamed bread. It was revealed that when the protein content was increased from 7.8 to 10.8%, the total quality score of steamed bread increased sharply and linearly. As protein content increased further, however, the total steamed bread quality score continued to increase, but at a much lower rate.

Protein content can vary greatly in wheat, from as low as 6% up to nearly 20%. The level depends on wheat class, soil fertility of the growing region, and environmental conditions encountered during the growing season. In global trade, the excessively low (<9.0%) and high (>15.0%) ranges of wheat protein, calculated on a 13.5% moisture basis (mb), are not often traded, but exporting countries can use high protein wheat to meet the specifications for protein content by blending it with wheat of lower protein content. Importing countries usually specify wheat protein minimum values based on nitrogen analysis ($N \times 5.7$) at a specified moisture content. Many nations may also require that the wheat produce flour with a specified minimum total wet gluten content (Carson and Edwards, 2009)

The quality of the gluten protein in wheat that confers good or poor baking properties at a given protein content. The gluten complex, which forms during the mixing of flour with water, is composed primarily of gliadin and glutenin. Glutenin is considered to confer the elasticity and gliadin the extensibility, or viscous flow properties, to dough. The presence of specific high molecular weight glutenin subunits and the proper balance between gliadin and glutenin quantities has been identified as corresponding with superior baking quality (Carson and Edwards, 2009).

A large number of studies show that protein quality and quantity are related to loaf volume. The importance of bulk rheological properties on foam structure has been visualized by magnetic resonance imaging, studies revealed that a high degree of dissimilarity in growth, as a consequence of stress in dough, was related to a coarser crumb structure (Duynhoven *et al.*, 2003).

Production of leavened bread from non-wheat sources is difficult, even though such a product is attractive to those who must avoid wheat gluten because of their dietary requirements, such as coeliac disease or other forms of wheat intolerance. Mixed soybean and wheat flour breads have been shown to produce an acceptable alternative bread product when there are economic constraints on using 100% wheat flour (Maforimbo *et al*., 2006).

2.3.1.5 Starch

Starch is the least important fraction influencing the quality of steamed bread in comparison to the gluten and water-soluble fractions of flour (Huang and Quail, 1999; Lin, 1983). Fan (1983) pointed out that steamed bread made from flour with high amylose content had poor eating quality, smaller volume, and low cohesiveness and sticky texture. Steamed bread made from flour with low amylose content had better eating quality, bigger volume, and a more cohesive and non-sticky texture. Huang *et al.* (1996) indicated that rapid visco-analyzer (RVA) viscosity parameters were significantly correlated with northern style steamed bread quality.

At starch damage values less than 50%, a significant positive linear correlation ($\alpha = 0.01$) was found between damaged starch content and steamed bread quality scores (Zhou *et al.*, 1994). When starch damage value increase to 50% or more, the steamed bread quality scores found decreased. Furthermore, an increase in damaged starch levels caused a decrease in bread volumes. Flour with damaged starch levels within the range 20–50% produced satisfactory steamed breads (Zhou *et al.*, 1994).

2.3.1.6 Water-soluble components

The water-soluble fraction of flour is important to the quality of steamed bread. The water-soluble fraction of flour contains albumins and globulins as well as water soluble pentosans. Wheat flour contains about 2–3% pentosans, of which about 20–25% are water soluble. The water-soluble pentosans are most frequently conjugated with proteins and possibly other polymers. Studies showed that there was varietal difference in the water-soluble fraction of flour (Huang and Quail, 1999).

2.3.1.7 Fiber content

The total dietary fiber content of wheat is reported to range from 11 to 12.7% and is composed of both soluble and insoluble fiber. The majority of dietary fiber in wheat is found in the bran layers. When wheat is milled, the resulting white flour contains only 2.0-2.5% total fiber (Carson and Edwards, 2009). The proximate composition with its crude fiber content is given in Table 2.5.

Table 2.5 Proximate composition of wheat flour

Characteristics	Percentage
Moisture	12.50±0.43
Ash	0.35±0.01
Crude protein	9.45±0.03
Crude fiber	0.30±0.01
Crude fat	1.25±0.04
NFE (Nitrogen-free extract)	76.15±2.66

Source: Majeed *et al.* (2017)

2.3.2 Soybean

The soybean plant (*Glycine max*) is the world's most widely cultivated and economically successful legume. Most soybeans cultivated are used as the raw materials for oil milling, and the residues are mainly used as feedstuffs for domestic animals as good dietary source of protein and oil. It requires considerably more cooking time than other legumes to have a palatable texture, which may be part of the reason for the use of soybean as processed foods in East Asian countries. As a healthy food, several approaches have been undertaken to get more soybean in the diet (Sugano, 2006) . In 2017, the total cultivated area of soybean in the world was 123.55 million ha and the total production was 352.63.5 million MT (FAO, 2019). The data of world soybean production including major soybean producing countries and in Nepal is summarized in Table 2.6.

Table 2.6 Soybean production data

Area	Element	Unit	Value
Nepal	Area harvested	Ha	23,563.00
	Yield	hg/ha	12,333.00
	Production	Tonnes	29,061.00
China	Area harvested	Ha	7,343,963.00
	Yield	hg/ha	17,910.00
	Production	Tonnes	13,152,688.00
USA	Area harvested	Ha	36,228,660.00
	Yield	hg/ha	32,990.00
	Production	Tonnes	119,518,490.00
World	Area harvested	Ha	123,551,146.00
	Yield	hg/ha	28,542.00
	Production	Tonnes	352,643,548.00

ha = hectare; hg/ha = hectogram (100 g)/hectare Source: FAO (2019)

2.3.2.1 Composition and benefits of soybean

The protein content in soybean seed is approximately 40% and the oil content is approximately 20%. This crop has the highest protein content and the highest gross output of vegetable oil among the cultivated crops in the world with vitamins and minerals which are beneficial to human health. The constituents of soybean are given in Table 2.7.

Table 2.7 Proximate composition of soybean

Component	Content (g/100 g)[a]	Mineral	Content (g/100 g)[a]	Vitamin	Content (g/100 g)[a]
Energy (kcal)	417 (433)[b]	Na	1 (1)	Retinol (µg)	0 (0)
Moisture	12.5 (11.7)	K	1900 (1800)	Carotene (µg)	6 (7)
Protein	35.3 (33.0)	Ca	240 (230)	Retinol Eq. (µg)	1 (1)
Fat		Mg	220 (230)	Vitamin D (µg)	0 (0)
Carbohydrate	28.2 (30.8)	P	580 (480)	Vitamin E (mg)	3.6 (3.4)
Ash	5.0 (4.8	Fe	9.4 (8.6)	Vitamin K (mg)	18 (34)
Dietary fiber	19.0 (21.7)	Zn	3.2 (4.5)	Vitamin B_1 (mg)	0.83 (0.88)
Total	17.1 (15.9)	Cu	0.98 (0.97)	Vitamin B_2 (mg)	0.30 (0.30)
Water insoluble	15.3 (15.0)	Mn	1.90 (-)	Niacin (mg)	2.2 (2.1)
				Vitamin B_6 (mg)	0.53 (0.46)
				Vitamin B_{12} (µg)	0 (0)
				Folic acid (µg)	230 (220)
				Vitamin C (mg)	Tr (Tr)

[a] Data from Resources Council, Science and Technology Agency, Japan, *Standard Tables of Food Comp. in Japan* 5th rev. ed., Printing Bureau of Ministry of Finance, Tokyo, 2000.
[b] Values in parentheses are for products in the United States.
Tr = trace

Source: Sugano (2006)

Soybean is the most important legume in relation to total world grain production and the most frequently used because of its high protein content and relatively low price. The addition of soybean ingredients to bread can improve the protein quality of the product. Consumption of soybean foods is increasing because of its beneficial effects on nutrition and health, such as lowering of plasma cholesterol, prevention of cancer, osteoporosis, heart diseases, type 2 diabetes, obesity and protection against bowel and kidney diseases. At present, use of soybean protein is probably a suitable approach to increasing soybean consumption (Friedman and Brandon, 2001; Shakya, 2012). Most of the nutritional and physiological functions of those components have been studied extensively, as summarized in Table 2.8.

Table 2.8 Soybean components and their physiological functions

Components	Functions
Protein	Hypocholesterolemic, antiatherogenic, reduces body fat
Peptides	Readily absorbed, reduces body fat
Lectins	Body defense, anticarcinogenic
Trypsin inhibitor	Anticarcinogenic
Dietary fiber	Improves digestive tract function, prevents colon cancer, regulates lipid metabolism
Oligosaccharides	Bifidus factor, improves digestive tract function
Phytin	Regulates cholesterol metabolism, anticarcinogenic, interferes with mineral absorption
Saponin	Regulates lipid metabolism, antioxidant
Isoflavone	Estrogenic function, prevents osteoporosis, anticarcinogenic
Linoleic acid	Essential fatty acid, hypocholesterolemic
α-Linolenic acid	Essential fatty acid, hypotriglyceridemic, improves cardiovascular function, antiallergenic
Lecithin	Improves lipid metabolism, maintains neurofuctions (memory and learning abilities)
Tocopherols	Antioxidants, prevents cardiovascular diseases
Plant sterols	Hypocholesterolemic, improves prostate cancer
Vitamin K	Promotes blood coagulation, prevents osteoporosis
Mg	Essential mineral, prevents cardiovascular disorders

Source: Sugano (2006)

2.3.3 Mushroom

Mushroom, an edible fungus, has been used as a food item since ancient times. It grows on decomposed organic matter and produce edible portion above the surface of the substrate.

Out of large varieties of mushrooms, less than 25 species are accepted as food and few of them have assumed commercial significance (Angle and Tamhane, 1974).

Mushrooms are the members of higher fungi belonging to class Basidiomycetes and some are Ascomycetes. They are spore bearing fleshy organ of fungi and characterized by heterotrophic mode of nutrition (Aryal, 2008). Edible mushrooms once called the “food of the gods” and still treated as a garnish or delicacy can be taken regularly as part of the human diet or be treated as healthy food or as functional food (Chang and Miles, 1989). Mushroom is a delicate fleshy fungus having high nutritional value. Mushrooms are saprophyte belonging to the lower plant group. Wild species often grow in the humus deposing during the rainy season being a temperature optimum (Hayes and Haddad, 1976).

According to Srivastava and Kumar (2002), mushrooms are a rich source of proteins, vitamins and minerals. Their low content of carbohydrate and fat makes them an ideal food for diabetics and persons who wish to maintain ideal body weight. They are also a good source of energy; about 454 g of fresh mushroom provides 120 Kcal. Mushrooms are highly perishable so producers are compelled to sell at lower price at their peak yielding time. This situation is due to lack of several mushroom processing methods. According to Rahman (2007) value-added food products can give better-quality foods in terms of improved nutritional, functional, convenience, and sensory properties. Consumer demand for healthier and more convenient foods also affects the way food is preserved. Eating should be pleasurable to the consumer, and not boring. People like to eat wide varieties of foods with different tastes and flavors.

Nowadays, mushroom cultivation is being introduced as a popular program for income generation in development projects of government of Nepal, NGOs, and INGOs in different parts of the country. Oyster mushroom production is a most appropriate technology for the poor landless farmers and women farmers in Nepal. Mushrooms can be grown in the small space of farmer’s own house for small scale production and generate income that aids in the family support. Mushroom cultivation is a most popular activity for development programs targeting income generation among women in Nepal because it is suitable for the women’s life style (Manandhar, 2004). Komanowsky (1970) investigated air drying of mushroom and excessive blanching has found to produce very dark products, presumably owing to the loss of soluble compounds which inhibit non-enzymatic browning but improvement was achieved by mild sulfite treatment.

2.3.3.1 Chemical composition of mushroom

With respect to proximate composition, there are wide variations in their values as reported by different workers for the same species and for different species of mushrooms. Table 2.9 and Table 2.10 show the proximate and chemical composition of two varieties of oyster mushroom.

Table 2.9 Proximate composition of *Pleurotus ostreatus*

Parameters	Value
Moisture	73.7–90.8
Crude Protein (N×4.38)	10.5–30.4
Crude Fat	1.6–2.2
Carbohydrate (Total)	57.6–81.8
Carbohydrate (N-free)	48.9–74.3
Crude Fiber	7.5–8.7
Ash	6.1–9.8
Energy Value	345–367

Note: All data are presented as percentage of dry weight, except moisture (percentage of fresh weight) and energy value (Kcal per 100 g dry weight)

Source: Crisan and Sands (1978)

Table 2.10 Proximate composition of *Pleurotus sajor-caju*

Parameters	Value
Moisture	90.1
Crude Protein (N×4.38)	26.6
Crude Fat	2.0
Carbohydrate (Total)	50.7
Carbohydrate (N-free)	-
Crude Fiber	13.3
Ash	6.5
Energy Value	300

Note: All data are presented as percentage of dry weight, except moisture (percentage of fresh weight) and energy value (Kcal per 100 g dry weight)

Source: Chang *et al.* (1981)

2.3.3.2 Varieties of mushroom

There are around 38,000 mushroom varieties known to exist, however about100 of them are considered to be edible. Of the edible varieties, ostpopularones are the *Agaricus bisporus* (the European or white button mushroom), *Lentinus edodus* (Shitake) or Japanese mushroom, *Pleurotus* species like *Pleurotus ostreaus* (American oyster mushroom) and the *Pleurotus sajorcaju* (Indian Oyster mushroom), *Volvariella volvacae* (the Chinese or paddy straw mushroom) (Chang and Miles, 2004).

Among thousands of mushroom species fewer than a hundred are toxic. Most fungal toxins cause mild or moderate poisoning. It is, however, the ingestion of a few species of extremely poisonous fungi that define the medical dimension of the problem. Mushroom poisoning is mostly accidental and the result of a mix-up between edible and toxic fungi, but intentional ingestion of psychotropic (magic) mushrooms is also a problem (Beck and Helander, 1998).

The most dreaded poisonings are those caused by cytotoxic agents e.g. amatoxins in death cap and destroying angel (severe gastroenteritis and liver damage) or orellanine in *Cortinarius* spp. (kidney damage). Dramatic, but rarely lethal, effects are caused by fungi-holding neurotoxins like muscarine (*Clitocybe* and *Inocybe* spp.), psilocybin (*Psilocybe* and *Panaeolus* spp., magic mushrooms), isoxazoles (fly agaric and panther cap) and gyromitrin (false morels) many poisonous species cause gastroenteritis only (Resinsky and Besl, 1990).

2.3.3.3 Mushroom farming in Nepal

Mushroom cultivation is relatively new in Nepal. The research for mushroom cultivation began in 1974 under Nepal Agriculture Research Council (NARC). Cultivation of white button mushroom in 1977 was first mushroom farming done by farmers. Plant pathology division in NARC began distribution of spawn. Oyster mushroom was introduced to farmers in 1984. In the beginning a few of farmers started this farming in Bhaktapur and Kathmandu district. After successful production of oyster mushroom, the number of farmers increased day by day. At present there are about 5000-6000 mushroom farmers in Kathmandu alone. The average production is about 8000- 10000 Kilograms per day. Pokhara and Chitwan are other major mushroom producers. Other districts also produce these two species but in very less amount, barely enough to meet local demand (Poudel and Bajracharya, 2011).

Oyster mushrooms are often grown without any environmental control. *P. sajor-caju* is cultivated for the summer crop at Kathmandu (25-30°C and 80%) and in the hills of Nepal while it is cultivated in the Terai regions during the winter season (22-26°C and 70%). *P. ostreatus* is grown during the winter season in Kathmandu and other cool places (5-20°C and 70%). Some mushroom growers try to grow these two species together. Of course, oyster mushrooms cannot be grown in Terai during the summer (30-40°C and 70%). The mid hills of Nepal are the most appropriate areas for oyster mushroom production and therefore the mushroom technology has been expanded widely in those villages (Manandhar, 2004).

The total production of fresh mushroom was only 1,530 metric ton in 2011/12, which rose to 1650 MT in 2012/13, and 1900 MT in 2013/14 and then 2,700 in 2014/15 and by 2015/16 the production rose to 9300 MT. This clearly shows a 600 percent rise in production of mushroom since 2011 to 2016. Similar to the production of mushroom, there is a significant rise in the production of Mushroom seeds since 2011. There has been over 500% rise in the production of mushroom seeds between 2011 and 2016. In 2011/12 there were only 269 thousand bottles of mushroom seeds, which grew to 290 bottles in 2012/13. The production grew by 115% in 2013/14 to 334 thousand bottles and 425 thousand bottles in 2014/15. 2015/16 saw the highest production with 1,488 thousand bottles of seeds. Hence, mushroom cultivation has great potential and is one of the most income generating activity. Nepal's remarkable diversity has immense potential for cultivation of many kinds of mushroom which can contribute to the society (Verma, 2017).

2.3.3.4 Nutritional and medicinal value of mushroom

Mushrooms are consumed in a variety of ways because of their delicious flavor. Experts have realized and appreciated the food value of mushroom because of the low calorific value and very high content of protein, vitamins, and minerals. Normally mushrooms contain from 20-40% proteins on dry basis and thus surpass many foods, in terms of protein content. The proteins of mushroom are of high quality and rich in various amino acids (Crisan and Sands, 1978).

Crisan and Sands (1978) have reported that mushrooms contain more proteins than other vegetables and its digestibility in fresh form is reported to be as high as 90%. They have low carbohydrate content, no cholesterol and are almost fat free (0.2 g/100 g) in nature. Therefore, they form an important constituent for a balanced diet.

There are 1600 mushroom species out of which 100 species have been accepted as food. More than 33 species of mushroom are under commercial cultivation throughout the world and 3 species are popularly grown in India viz, white button mushroom, oyster mushroom, and paddy straw mushroom. These mushrooms are rich in protein, vitamins, minerals and excellent source of thiamine, riboflavin, niacin and folic acid etc. The digestibility of mushroom protein is 71-90%. Major contribution to Indian mushroom production comes from white button mushroom (*Agaricus bisporus*) i.e 90-92%, while rest comes from oyster mushroom (*Pleuroteus* spp.) and paddy straw mushroom (*Volvarieua* spp.). For centuries, people across the world have been using wild mushrooms for food, medicine and cosmetics as well as for other economic and cultural purposes. Mushrooms are devoid of starch and low in calories and other carbohydrates. Apart from their nutritional value, mushrooms have potential medicinal benefits; they are an ideal food for diabetics and over- weight people

According to FAO (2006), mushrooms are edible fungi of commercial importance and their cultivation and consumption have increased substantially due to their nutritional value, delicacy and flavor. It is rich in vitamins C, D, B, and Mg, P, Ca, dietary fibers and amino acids. Another important ingredient of mushroom is the polysaccharide compound beta-glucan, which enhances cellular immune function. Mushroom protein can serve as food contributing protein in developing countries, where the population mainly depends on cereal based foods. The oyster mushrooms are rich in protein, minerals, devoid of starch or low in calories and carbohydrates. These are ideal food for diabetic and heart patient and those who do not want to put on weight (FAO, 1970).

Cultivation of oyster mushroom with agricultural residues, such as rice and wheat straw is a value added process to convert these materials into human food (Pokhrel *et al.*, 2013).

Fruiting bodies as well as active mycelia of *Pleurotus* species also possesses a number of therapeutic properties like anti-inflammatory, immuno-stimulatory and immuno-modulatory, anticancer activity, ribonuclease activity and possessed higher concentration of antioxidants than other commercial mushrooms (Yanga *et al.*, 2002). These mushrooms also content antimicrobial (antibacterial, antiviral), anti-tumor, anti-neoplastic, anti-mutagenic, anti-lipidemic hepato-protective, hyperglycemic, hypotensive and anti-ageing (Patel *et al.*, 2012).

Ikekawa (2001) reported that the intake of mushrooms proved to be effective in cancer prevention, growth inhibition also, has high anti-tumor activity and a preventive effect in tumor metastasis. Ikekawa (2001) on his study found that the substance extracted from the mushrooms can reduce blood pressure, blood cholesterol and blood sugar level as well as inhibition of platelet aggregation. Mattila (2001) reported that mushroom rich in antioxidant activity have been shown to play an important role in prevention of cancer also, mushrooms have functional properties such as vitamin B-complex, vitamin D and antitumor, anticancer and antiviral activities due to lentinan.

Jin *et al.* (2012) reported that the use of *G. lucidum* mushrooms as a first-line treatment for cancer. It remains uncertain whether *G. lucidum* helps prolong long-term cancer survival. However, *G. lucidum* could be administered as an alternative adjunct to conventional treatment in consideration of its potential of enhancing tumor response and stimulating host immunity. Mushrooms are the leading source of the essential antioxidant selenium in the produce aisle. Antioxidants, like selenium, protect body cells from damage that might lead to chronic diseases. They help to strengthen the immune system, as well. In addition, mushrooms provide ergothioneine, a naturally occurring antioxidant that may help protect the body's cells.

2.3.4 Anti-nutritional factors in legumes and cereals

There are a number of components present in soybeans that exert a negative impact on the nutritional quality of the protein. Among those factors that are destroyed by heat treatment are the protease inhibitors and lectins. Protease inhibitors exert their anti-nutritional effect by causing pancreatic hypertrophy/ hyperplasia, which ultimately results in an inhibition of growth. The lectin, by virtue of its ability to bind to glycoprotein receptors on the epithelial cells lining the intestinal mucosa, inhibits growth by interfering with the absorption of nutrients (Liener, 1994).

According to Liener (1994), there is less significance of the antinutritional effects produced by relatively heat stable factors, such as goitrogens, tannins, phytoestrogens, flatus-producing oligosaccharides, phytate, and saponins. The processing of soybeans under severe alkaline conditions leads to the formation of lysinoalanine, which has been shown to damage the kidneys of rats. This is not generally true, however, for edible soybean protein that has been produced under milder alkaline conditions. Also meriting consideration is the allergenic response that may sometimes occur in humans, as well as calves and piglets, on dietary exposure to soybeans (Liener, 1994).

2.3.4.1 Protease inhibitors

Enzyme inhibitors can occur naturally in the human body includes pancreatic, proteolytic inhibitors, blood clotting enzymes inhibitors, liver nicotinamide deamidase inhibitors and hyaluonidase inhibitions. Trypsin inhibitors are the most widely distributed among the protease inhibitors. In addition to trypsin inhibitors, chymotrypsin, subtilin, elastase, plasmin, Kallikrein and papain inhibitors are also present in legumes. Two main trypsin inhibitors Bowman Birk and Kunitz are isolated from legumes (Whitaker and Feeccy, 1973).

Trypsin inhibitors present in some of the common foods are soybean, limabean, *Phaseolus aureus*, navybean, Kidney and black bean, broad bean (*Vicia faba*), double bean, field beans (*Dolichos lablab*), Jackbean (*Canavalia ensifermis*), Pigeonpea (*Cajanus cajan*), cereals (wheat flours), tubers, vegetables, nuts and eggs (Matthews, 1989; Pusstai, 1967). The trypsin inhibitors adversely affect the digestion of dietary protein by proteolytic enzymes present in the intestinal tract. The growth depression caused by the trypsin inhibitors may be consequence of an endogenous loss of essential amino acids being secreted by a hyperactive pancreas since pancreatic enzymes such as trypsin and chymotrypsin particularly rich in sulphur containing amino acid resulted in pancreatic hypertrophy and amino acid requirements (Salunkhe *et al.*, 1985).

2.3.4.2 Amylase inhibitors

Protein inhibitors of various amylases have been reported in beans, colocasia, unripe mangoes, unripe bananas and leaves of rice plants infected with insect pest. The inhibitors from colocasia roots were non dialyzable and stable at boiling temperature, it strongly inhibited salivary alpha amylase but had no activity on pancreatic amylase, soybean beta amylase, takadiastase and bacterial alpha amylase (Whitaker and Feeccy, 1973). They reported that amylases of human saliva and hog pancreases were inhibited by the alpha amylase inhibitors from colocasia, turmeric and cassava. These inhibitors have been reported to be responsible for underutilization of bean starches which leads to the lower calorific values of dry bean carbohydrates (Salunkhe *et al.*, 1985). The amylase inhibitors from different legumes vary in their activity (Singh *et al.*, 1982).

2.3.4.3 Oxalates

Oxalate is a common constituent of plants and present as soluble oxalate in form of sodium or potassium salts and insoluble calcium oxalate or a combination of two forms (Libert and Fransceschi, 1987; Fassett, 1973). Oxalic acid is considered as a toxic constituent in many foods. Consumption of high oxalate content food can increase the risk of renal calcium oxalate formation in certain groups of people and it may affect calcium absorption. The acute toxic effect of excessive oxalic acid intake leads to vomiting, diarrhea, muscular cramp, cardiovascular collapse, lowers blood coagulability caused by low level of calcium in the body fluid (Doyle *et al.*, 1993). Oxalates are very poorly absorbed under normal non fasting conditions in humans. If the foods containing excess oxalates are ingested, they may be eliminated as insoluble calcium salts. Excess ingestion of oxalate may interfere with calcium metabolism and may result in chronic diseases such as renal damage and stone formation (Reddy and Pierson, 1994).

2.3.4.4 Phytate

Phytic acid, the hexametaphosphate of myoinositol is found in various roots and tubers, cereals, nuts; legumes contain higher amounts of phytates. The phytate of seed is concentrated primarily in the bran and germ. Phytate has been generally regarded as the primary storage form of both phosphorous and inositol in almost all seeds. In food such as grains and legumes, a large proportion (60-80%) of phosphorous is present in the form of phytic acid primarily as a complex salt of divalent minerals such as zinc, calcium, magnesium and iron or as complexed with proteins and starch (Dahal, 2005).

It has been shown that phytate is involved in lowering bioavailability of minerals(Reddy *et al.*, 1978) especially on calcium, zinc and iron. Phytate also interacts with protein resulting reduced protein solubility which reflects in the functional properties of proteins. It has been shown that phytate inhibits general enzymes including pepsin, alpha amylase, trypsin and beta galactosidase (Reddy and Pierson, 1994).

Other antinutrients include lectins (hemaglutinins), flatulence factor, lathyrism, allergens, saponins, goitrogens, cyanogens, toxic amino acids (mimosine, djenkolin and dihydroxyphenyl alanine), toxic enzymes (urease) and toxic peptides (Matthews, 1989). Lectins are proteins that have the ability to clump or agglutinate red blood cells in a fashion similar to antibodies. The phyto hemaglutinins are heat labile. Normal cooking destroys their specific action, cooking of soaked beans for 1 h at 100^0C destroys toxicity and haemoglutinating activity completely. Legume seed contain flatulence providing oligosaccharides such as raffinose, stachyose and verbascose. They escape digestion while passing through the small intestine to the large intestine and providing the substrate for bacterial fermentation. This results in production of gas (Salunkhe *et al.*, 1985).

2.3.4.5 Effect of processing on antinutrients

According to (Chua *et al.*, 2014) the effects of three different cooking methods (boiling, microwave and pressure cooking) on the antioxidant activities of six different types of oyster mushrooms (*Pleurotus eryngii, P citrinopileatus, P. cystidiosus P. flabellatus, P. floridanus and P. pulmonarius*) were assessed. Free radical scavenging: 2,2-diphenyl-1-picrylhydrazyl (DPPH) and reducing power: trolox equivalent antioxidant capacity (TEAC) were used to evaluate the antioxidant activities and the total phenolic contents were determined by Folin-Ciocalteu reagent. Pressure cooking improved the scavenging abilities of *P. floridanus* (>200%), *P. flabellatus* (117.6%), and *P. pulmonarius* (49.1%) compared to the uncooked samples. On the other hand, the microwaved *Pleurotus eryngii* showed 17% higher in the TEAC value when compared to the uncooked sample. There was, however, no correlation between total phenolic content and antioxidant activities. There could be presence of other bioactive components in the processed mushrooms that may have contributed to the antioxidant activity. These results suggested that customized cooking method can be used to enhance the nutritional value of mushrooms and promote good health.

Asamoa *et al.* (2018) reported phytochemicals and antioxidant activity of two mushroom varieties (*Termitomyces schimperi* and *Volvariella volvacea*) during processed and unprocessed condition. The steamed extract was the strongest scavenger of DPPH with 50 % inhibitory concentration (IC_{50}) value of 3.03±0.40 mg/ml whereas the unprocessed extract had the least effect with IC_{50} value of 9.35±0.42 mg/ml. Similarly, the steamed extract recorded the highest total phenol content with value of 1644±39 mg GAE/100 g whereas the unprocessed extract was the lowest with value of 1336±93 mg GAE/100 g respectively. The present findings suggest that steamed mushrooms possess the highest antioxidant activity.

Ramos *et al.* (2016) studied the influence of culinary treatments (boiling, microwaving, grilling, and deep frying) on bioactive components. Boiling improved the total glucans content by enhancing the β-glucans fraction. A significant decrease was detected in the antioxidant activity especially after boiling and frying, while grilled and microwaved mushrooms reached higher values of antioxidant activity.

Several methods of processing beans which include dehulling, milling, soaking, cooking, germination, fermentation, autoclaving, roasting, frying and parching and protein extraction will reduce the antinutrients depending on the type of bean. Generally adequate heat processing inactivates the protease inhibitors (Liener, 1994).

2.3.5 Mushroom flour uses in bread

Mushroom is a versatile vegetable food and can be used in different ways, by mixing with other vegetables or serving separately. Mushroom can be used as a main ingredient and also for flavoring agent in other products; they can be eaten cooked or raw. Different types of products can be prepared from mushroom including mushroom sauce, mushroom flan, and mushroom omelet (Camrass, 1977).

Mushroom can also be utilized for the preparation of weaning foods, biscuits and soup powders. The recipe of different preparations of mushroom dishes like mushroom kidney fry, mushroom curry, mushroom salads, mushroom sandwiches, mushroom stuffed capsicum, stuffed morels, mushroom fritters, meat stuffed mushroom, mushroom hot dogs, mushroom burgers are available (Srivastava and Kumar, 2002). Other various products can be made from the different form of mushroom like dehydrated mushroom, mushroom powder, mushroom paste, canned mushroom, frozen mushroom etc. (Arora *et al.*, 2003).

Kotwaliwale *et al.* (2007) found that oyster mushroom when exposed to sulphitation treatment with potassium) in 1% aqueous solution and dipped for 15 min following by hot air drying at 50ºC preserved the white color of mushroom than drying at 55ºC and 60ºC with other treatments like blanching.

Oyster mushrooms were dried at a temperature of 45°C for 2 days and ground to powder, which was used in bread making. OMP of 3%, 6% and 9%, 12% and 15% were used to make composite flour with wheat flour from which bread was prepared. Bread having 6% OMP was found acceptable bread with improved protein content.

Gothandapani *et al.* (1997) dried oyster mushroom in three methods viz, sun drying, thin layer drying and fluidized bed drying. It was found that fluidized bed drying with a temperature of 50ºC for a period of 80-120 min was having a lower browning index. From the results it was found that different drying methods did not have any effect over the biochemical constituents of mushrooms. But, treatment with KMS and blanching reduces the nutritive quality due to the removal of water-soluble nutrients. Browning index showed much variation. Treatment with chemicals and blanching improved the color of the mushroom when compared with sundried samples. The microbial mass of oyster mushroom was considerably reduced by drying. The rate of microbial deterioration was higher in mushroom stored after sun drying compared to mushrooms stored after TLD and FBD. Storage of dried mushroom after pretreatment with KMS at higher concentration of 1.5% had better effect on reducing the microbial spoilage.

Majeed *et al.* (2017) prepared the composite flour by blending straight grade flour with OMP in different proportions for the baking of bread. Results showed that OMP increased water absorption capacity of dough with highest absorption recorded in T_5 (66.4%) and minimum in T_0 (56.4%). Dough development time increased with the increasing amount of OMP. It was minimum in T_0 (1.5 min) and maximum in T_5 (4.6 min). The capacity of dough to get soft also increased with increasing treatment level. Dough stability also decreased with increasing level of OMP. Mixing tolerance index was also significantly affected by OMP. Results of amylograph indicated that peak viscosity increased from 1595 to 1700 Brabender Units (BU) from T_1 (3% mushroom powder) to T_5 (15% mushroom powder) with the increase of OMP. Mushroom powder may be supplemented up to 6% in wheat flour to get acceptable bread with improved protein content. The proximate constituents of oyster mushroom powder are given in Table 2.11.

Table 2.11 Proximate constituents of mushroom powder

Constituents	Percentage
Moisture	7.67±0.31
Ash	7.07±0.28
Crude protein	29.60±1.21
Crude fiber	8.33±0.34
Crude fat	1.78±0.07
NFE (Nitrogen-free Extract)	45.55±1.86

Source: Majeed *et al.* (2017)

2.3.6 Use of soybean in bakery

The main aim for the use of soybean flour in the production of steamed products is its high biological value in terms of digestibility and amino acid composition. Soybean contains high lysine content which is one of the indispensable amino acids justifies the effectiveness of soybean protein as a nutritional supplement in comparison to cereal proteins (Huang, 2014a). Chruickshank (1996) found that the addition of soybean flour (full fat) significantly improved the total quality score of steamed bread at 0.6% soybean flour. Higher levels of soybean flour not only reduced specific volume and total quality score, but also produced noticeable soybean aroma and flavor to the steamed bread.

According to Subhash (2015), increasing soybean and finger millet flour in composite flour the flour moisture, protein, crude fiber, calcium and iron increases whereas total sugars decreased. The loaf volume of bread decreased gradually with increasing soybean and finger millet flour and crust color, crumb color, texture, taste, flavor and overall acceptability were highest at 10% soybean and 10% finger millet flour. The force required for bread cutting and penetration increased with addition of soybean and finger millet flour.

Ndife *et al.* (2011) carried out a study in which soybean was roasted, winnowed, milled using attrition mill and sieved into fine flour of uniform particle size, by passing them through a 2 mm mesh sieve. The use of whole wheat and soybean flour blends in the production of functional breads was studied. The flour blends of whole wheat and soybean were composites at replacement levels of 10, 20, 30 and 40% while the whole wheat flour bread (sample A) served as control. It was concluded that a substitution of 10% soybean flour into wheat flour gave the bread with the best overall quality acceptability.

In a study by Ribotta *et al.* (2005), soybean products were blended with wheat flour at different levels to reach similar protein contents in the flour mixtures (12.6 ± 0.2 and 14.6 ± 0.2% were the lower and higher protein levels reached respectively). Enzyme-active full-fat soybean flour (EAFFSF), and heat-treated full-fat soybean flour (HTFFSF: 120 μm particle size) were added at 6 and 12% wheat substitution levels, enzyme active defatted soybean flour (EADSF) was added at 5 and 10% wheat substitution levels, and commercial soybean protein isolates (SPIs) were added at 3 and 5% wheat substitution levels.

Dhingra and Jood (2004) used soybean (full-fat and defatted) and barley flour and wheat flour at 5, 10, 15 and 20% (both in equal parts) substitution levels for bread making. Soybean seed was cleaned and ground to make fine powder without any pretreatments. The gluten content, sedimentation value and water absorption capacity of the flour blends and the mixing time of the dough decreased with increase in the level of soybean and barley flour separately and in combinations. Protein and glutelin contents increased significantly on blending of soybean flour (full-fat and defatted) to bread wheat flour. The bread volume decreased with increasing amount of non-wheat flour substitution. The crumb color changed from creamy white to dull brown and a gradual hardening of crumb texture was observed as the addition of soybean (full-fat and defatted) and barley flours increased. At the higher levels, the acceptability declined because of the compact texture of the crumb and the strong flavor of the product. The addition of 10% of soybean flour (full -fat and defatted) or 15% of barley flour, full-fat soybean + barley or defatted soybean + barley flour to bread flour produced acceptable bread.

Sana *et al.* (2012) prepared bread from composite flour containing wheat flour and soybean flour (7%, 12%, 17% and 22%). From farinograph and sensory studies it was revealed that bread can be prepared up to 22% soybean flour, but organoleptically acceptable bread can successfully be prepared from composite flour containing 7% soybean flour and 93% wheat flour.

In a study for the preparation of bread, Otegbayo *et al.* (2018b) used soybean seeds which were cleaned to remove dirt and foreign material. The seed was then weighed and soaked in hot water for 1 h to soften the hulls. The soybean seeds were then dehulled, boiled at 99°C for 20-25 min and dried in a cabinet dryer at 60°C for 72 h. The dried soybean seeds were cooled, milled, sieved with a 250 μm mesh, packed and stored in polyethylene bags until needed.

Singh *et al.* (2008) prepared bun by adding wheat flour, soybean flour water and chestnut flour in different formulations. Among the recipe prepared, 80% wheat flour with 10% water chestnut flour and 10% soybean flour was found to be more acceptable in term of chemical evaluation as well as sensory quality.

Lin (1983) carried out a sensory evaluation of steamed bread samples produced using 7.5% full fat and defatted soybean composite flour and reported that their acceptability was significantly less than the control. Zheng *et al.* (2006) reported that up to 2% addition of defatted soybean flour improved the quality parameters such as color and specific volume of steamed bread.

Zheng *et al.* (2006) prepared baked bread from composite flour containing whole wheat flour and full fat roasted soybean flour. In the study, wheat flour was replaced by 10%, 20%, 30% and 40% with soybean flour and physicochemical and sensory analysis was performed. Bread volume, dough expansion and specific volume were found decreased linearly with the increment of soybean flour. The scores for organoleptic attributes like taste, aroma, texture (mouth feel), except for color were generally inferior to that of whole-wheat. There was no significant difference observed between the whole wheat bread and the soybean bread samples in the sensory attributes of crust color and crumb appearance, while significant difference ($p<0.05$) was observed in texture, flavor and overall preference respectively. It was concluded that a substitution of 10% soybean flour into wheat flour gave the bread with the best overall quality acceptability.

In a similar study, Otegbayo *et al.* (2018b) reported that soybean enrichment of bread could be used to produce a nutrient dense food with proposed great health benefit. Out of the soybean-bread samples (5%, 10%, 15% and 20%), the inclusion of 5% soybean flour produced bread which was nutrient dense with morphogeometric characteristics similar to wheat bread and was more acceptable and preferred by the consumers. Soybean flour inclusion at 5% level can therefore be advantageous due to the increased nutritional value and acceptable consumer attitude in sensory characteristics.

According to Fleming and Sosulski (1978), the protein content of ordinary white bread ranges from 8 to 9%. Special breads can be made with 13–14% protein by including soybean protein. However, incorporating high levels of soybean protein result decrease in loaf volume, gives poor crumb characteristics and decreases overall acceptability.

2.3.7 Fat and edible oils

Fat has a significant effect on the softness of steamed bread. It found that for strong flour, up to 8% fat significantly increased the softness of steamed bread. Over that amount, softness was significantly reduced. For medium flour, up to 2% fat significantly softened the crumb of steamed bread. The total quality score of steamed bread was significantly increased up to 4% fat addition for strong flour. The optimal level of fat addition to Guangdong style steamed bread was 2–4%. Too much fat was detrimental to most quality attributes of Guangdong style steamed bread (Huang and Quail, 1997).

Pomeranz *et al.* (1991) pointed out that 2% shortening produced the best steamed bread: replacing shortening by soybean or corn oil produced comparable bread. Li and Cheng (1999) studied the effect of lard on the quality of steamed bread. They found that addition of lard improved the volume and color of steamed bread, softened the texture, and increased the eating quality, also prolonged the shelf-life of steamed bread.

2.3.8 Sugar

Sugar can be used to aid extended fermentation in flour which has a low maltose sugar and levels of 8–10% effectively improved specific volume and the total quality score of steamed bread, but above 10% were detrimental to the quality of steamed bread (Huang and Quail 1997). Kruger *et al.* (1992) claimed that negligible changes in external or internal appearance, volume, width to height ratio or texture were observed when 0.5, 1.5 and 3.0% sugar were incorporated in the standard formulation for the control flour.

2.3.9 Milk and dried milk powder

Milk and dried milk powder are often used in Guangdong-style steamed bread and buns to increase the flavor of the products and enhance nutritional quality. Milk and dried milk powder are added during the first dough mixing stage (Huang and Miskelly, 2016).

2.3.10 Salt

An addition of 0.25% salt significantly improved the softness of steamed bread crumb. At salt levels over 0.5%, however, crumb softness was significantly decreased. Salt also affects the water absorption of dough. The addition of 2% salt will decrease water absorption of the dough by 3% (Liu, 2005).

2.3.11 Emulsifiers

Emulsifiers are active surfactant compounds that are widely used in bakeries as dough strengtheners and anti-staling and crumb softeners. The effect of the emulsifiers diacetyl tartaric acid ester of mono- and diglycerides (DATEM), sodium stearoyl lactylate (SSL), and calcium stearoyl lactylate (CSL) on the quality of steamed bread. They found there was a positive effect on the quality of steamed bread if the emulsifiers were used alone. However, the effect became obvious when the emulsifiers were used in combination. With 3 g/kg DATEM, 1 g/kg CSL-SSL and 8 mg/kg lipase, the color and structure of steamed bread improved markedly (Wang and Wang, 2006).

2.3.12 Yeast

In China, instant active dry yeast is widely used in steamed bread factories. However, starter or sourdough left from the previous day has also been used for the production of steamed bread in some workshops and homes. When sourdough fermentation method is used, the Lactobacillus spp. makes the fermented dough very sour (pH 3.7–4.0) often needs to be neutralized by adding alkali solution (such as 40% Na_2CO_3). The pH of dough can be maintained between 6.4 and 6.7 if the dough has been properly neutralized (Huang and Miskelly, 1991). Baker’s yeast has largely replaced the use of sourdough or “starters” for fermentation in most fully or semi-automated steamed bread factories as the rate of fermentation is higher and more consistent (Kim *et al.*, 2009).

2.3.12.1 Types of yeast

There are three main types of yeast used in the manufacture of steamed products in Asia: compressed (fresh) yeast, active dry yeast, and instant active dry yeast. Instant active dry yeast is the most widely used by smaller manufacturers because it is convenient to use. Instant active dry yeast contains small amounts of emulsifier, enabling more rapid hydration and dispersion in the dough. Instant active dry yeast is more expensive by comparison with compressed yeast, due to drying and vacuum packaging after manufacture. Compressed yeast needs to be refrigerated at 0–6°C and has a shelf life of about 4 weeks compared with 12 months for sealed dried yeast. However, once the package is opened, the shelf life is 1–2 weeks, due to moisture absorption and oxidation (Luangsakul *et al.*, 2009).

Instant active dry yeast does not need to be activated before use. Active dry yeast requires activation according to manufacturer’s instructions. Compressed yeast is best suspended in water at about 38°C. Yeast is very sensitive to the changes in temperature. The rate of gas production in dough increases as temperature increases up to an optimum of about 40°C. It is advisable to avoid direct contact of yeast with salt, sugar, or alkali (Huang and Miskelly, 2016).

2.3.12.2 Rate of addition

Consistency of yeast activity is important so that once the usage rate is established, it can be maintained. In China, fresh yeast usage rate is about 1% by flour weight. As the yeast activity decreases, the rate of usage will need to increase. Any increase was determined by assessing product quality but was influenced by yeast aging and storage conditions. Less dried yeast is required when used instead of fresh yeast. Dried yeast is used at the rate of 50–100% of the fresh yeast and instant active dried yeast at the rate of 33% (Yeh *et al.*, 2009).

2.3.13 Baking powder

Baking powder is used only for Guangdong style steamed buns. Baking powder improved the skin color and total quality score of steamed bread at a low dosage, but over 1% it seemed to have a detrimental effect (Huang and Quail 1997). Baking powder addition above 1% resulted in bitter flavors. A double acting baking powder is used for char siew bao to ensure a continuous supply of carbon dioxide during steaming to aerate the products. Ammonium bicarbonate and/or a solution of potassium carbonate (known as lye water) are alkalis commonly used to neutralize the sourdough so that the end product has a neutral taste. Ammonium bicarbonate also helps with dough aeration during cooking as it decomposes and release carbon dioxide gas when the dough temperature reached 40°C (Huang and Miskelly, 2016).

2.4 Recipe formulation of steamed bread

2.4.1 Response surface methodology

Response surface methodology (RSM) is an effective statistical method for relating the relationship between dependent and independent parameters. RSM is particularly appropriate for product development work. The effectiveness of response surface methodology (RSM) in optimization of ingredient levels, formulations and processing conditions in food technology from raw to final products have been documented by different researchers. RSM consists of a group of mathematical and statistical procedures that can be used to study the relationships between one or more responses (dependent variables) and factors (independent variables) (Myers *et al.*, 2016).

2.4.1.1 Mixture design

Mixture design, a special type of RSM, is a very effective method of determining the proportions of variables (ingredients) of a blend. The output varies depending on the proportions but the total remains constant as 1 or 100. Although no multipurpose technique is known to be applicable to all situations, mixture designs have been successfully applied to scientific research and development and have been implemented successfully in real-world problems (Sahin *et al.*, 2015).

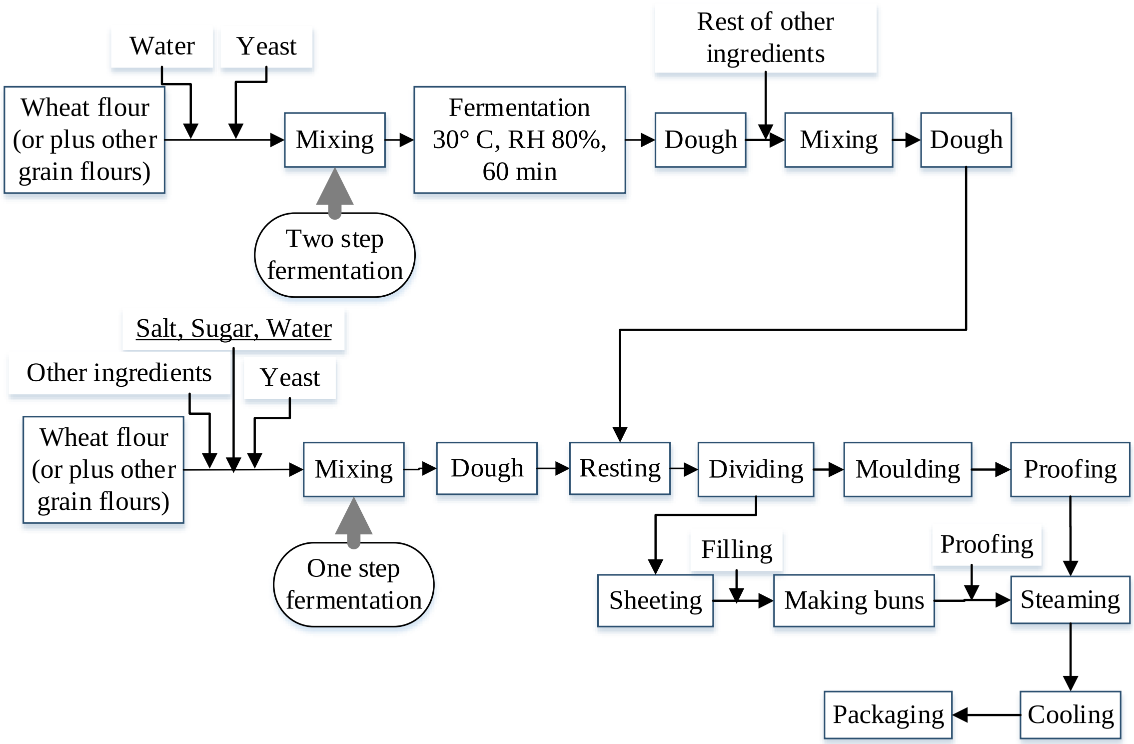
2.5 Technology of steamed bread

2.5.1 Raw materials preparation

The primary step for dough making process essentially the sifting of dry materials to break up lumps and to ensure that no foreign matter is included in the dough; weighing each ingredient so that it is in the correct ratio; and monitoring ingredient temperature to ensure the final dough temperature is within the required range. The installation of a bar magnet in the screening apparatus is required to remove any metal impurities. Small or trace amounts of ingredients need to be dissolved or suspended in water rather than added directly to flour. The sequence of adding ingredients is another factor which may affect the quality of steamed products. For the instance, if the two-step fermentation procedure is used, water, sugar, and improver are added and mixed. Milk powder and instant active dry yeast are added to flour and blended, and then the water is added. In this way the yeast will not come into direct contact with cold or very warm water and the milk powder will not become lumpy by direct contact with water. Fat is added when the dough has formed, but not developed. Salt is added 5–6 min before the completion of mixing when the gluten has not fully developed (Huang, 2014a).

2.5.2 Dough preparation

According to Huang and Miskelly (2016), there two methods for dough preparation for steamed bread. In one-step fermentation procedure, all the ingredients are mixed to the optimal extent, sheeted, divided, molded, proofed and steamed. This procedure includes one step fermentation. The common steamed bread making processes used in China is presented in Fig. 2.1.



Source: Huang (2014c)

Fig. 2.1 Common steamed bread making process

One of the steamed bread recipes consisted of wheat flour (2000 g), water (960 g) and dry yeast (20 g). Yeast was dissolved in lukewarm water before mixing it with other ingredients. After steaming, breads were placed in a container without a cover at room temperature (22±2°C) and humidity (32±2%) for 1 h. After cooling, they were packaged into polyethylene bags and kept at room temperature for further study (Sha *et al.*, 2007).

2.5.2.1 Water addition

Dough used for production of Chinese steamed bread contains less added water (10–15% less) than European-style bread (Huang and Moss, 1991). In traditional European style wheat flour bread, the total amount of water added to the dough usually varies between 55 and 65% of flour weight. For Chinese steamed bread, however, less water is added to the dough as Chinese prefer a rather chewy bread texture. Typically, the amount of added water is about 50% for southern style and 45% or lower for northern style Chinese steamed bread (Huang and Miskelly 1991; Su 2005). The addition of water for steamed bread also varies with flour properties and the final consistency of dough required by the different styles of steamed bread. The main factors that influence the water absorption capacity of the flour are protein content, degree of starch damage, and bran content (Huang, 2014a).

Huang and others (1993) verified that 70% of Farinograph water absorption (FWA) was the optimum for northern style steamed bread prepared using the optimized laboratory procedure, while 80% of FWA was the optimum water addition for southern style steamed bread (Huang and others 1998). Su (2005) reported that 80% of FWA, 72% of FWA and 65% of FWA were used for the preparation of soft firmness type steamed bread (southern style), medium firmness type of steamed bread (northern style) and hard firmness type of steamed bread, respectively. Liu (2005) stated that 36–42% water addition was used for both the rapid “no-time” dough procedure and the rapid “sponge and dough” procedure (two-step fermentation procedure) for industrial production of steamed bread.

2.5.2.2 Dough fermentation

Before the molded dough pieces can yield a light, aerated loaf of steamed product, they must be fermented for a period of time. During this process, the yeast cells act upon the available sugars, transforming them into carbon dioxide gas and alcohol as the principal end products. There are several fermentation procedures currently used in steamed bread factories in China: single stage fermentation and two stage fermentation (Huang, 2014a). There is a clear relationship exists between the stability of the gas cell wall and the bread making quality (Weegels *et al.*, 1996).

2.5.2.3 Single stage fermentation

This is also called the “no-time” fermentation procedure. All ingredients are mixed together, sheeted and molded, then proofed and steamed. Virtually all the fermentation takes place in this proofing stage, which takes about 50–80 min. This procedure has the advantage of reducing process time, labor, power, and equipment requirements. There is also a reduction in fermentation losses because of the short fermentation time. The disadvantage of this procedure is that more yeast is used. The steamed bread produced has a firmer texture and much less flavor than those made by traditional methods (Huang, 2014b).

2.5.2.4 Two stage fermentation

Two-step mixing and two steps of fermentation can be used to produce steamed bread. In the first mixing, about 70% of the flour and 80% of the total water and total yeast are added and mixed for 3–4 min with a final dough temperature of 28–32°C. After that the dough is fermented at 30–33°C and 70–80% relative humidity for 50–80 min depending on yeast level, ratio of flour used in the first mixing, temperature, and relative humidity in the fermentation cabinet. The fermented dough is then mixed again with the rest of the flour, water, and alkali solution (0.05–0.1% of alkali). Alkali is used to neutralize the acids produced during the fermentation. The second mixing takes 8–12 min with a final dough temperature of 33–35°C. The dough is then divided, molded, and proofed for about 60 min at 38–40°C and 80–90% relative humidity (Huang, 2014b).

2.5.2.5 Sourdough procedure

There is increased interest in steamed bread made by the sourdough procedure because of better flavor and the perceived health benefits (Wu *et al.*, 2012). There are a number of methods of production in practice. Generally, a starter dough is prepared from the dough of a previous batch. The leftover dough is mixed with flour; water is added and allowed to ferment overnight at ambient conditions for use in the next day's production. In Thailand, preparation of a starter dough can take several days and varies according to region (Keeratipibul and Luangsakul, 2012).

In the first dough mixing stage, 5–15% of sourdough is mixed with 33% of the total flour and about half of the water (the total water used is about 37–42% by flour weight). Instant dried yeast may be used as a supplement. The dough is mixed until fully developed. After fermentation, the dough becomes sour because of the organic acids produced by *Lactobacillus* spp. The fermented dough is transferred to the mixer and the remaining flour and water are added (Huang and Miskelly, 2016).

2.5.2.6 Moulding

Dough moulding is very important to the quality of the finished steamed products. Correct moulding will ensure a fine and even crumb structure. A number of moulding techniques for steamed bread, buns, and rolls are possible using hand or machine methods. Dough can also be rolled into a long cylinder and cut to the required length for individual pieces by hand using the same technique as is used traditionally in small manufacturing facilities. Hand molding is traditional for most plain and fancy homemade steamed breads, buns, and rolls. Most mechanical molders incorporate sheeting rolls, which will result in a reduction of the number and size of gas bubbles, and in turn reduce surface faults such as blisters and dimples. Further dough development occurs during sheeting, leading to an improvement in underdeveloped doughs and dough color (Huang and Miskelly, 2016).

2.5.3 Proofing

Correct proofing is crucial for the manufacture of quality steamed products. During intermediate proofing, the dough becomes more relaxed however, in final proofing the dough not only relaxes, but also expands due to the carbon dioxide released during fermentation that creates the internal structure in the dough piece. Commercial batch wise proofers have controlled temperature and humidity rooms or cabinets of varying size. Dough pieces are loaded onto trays and stored on racks in the proofer. Very-large-scale facilities may install equipment to enable continuous production (Huang and Miskelly, 2016).

2.5.3.1 Control of proofing conditions

Homemade steamed products are often left in steamers at ambient temperature and humidity so proofing times will vary according to ambient conditions. For industrial proofers it is essential that proofing time, temperature and humidity are carefully controlled. Proofers should have good internal air circulation so that they maintain even temperature and humidity throughout (Huang and Miskelly, 2016).

2.5.3.2 Temperature

According to Huang and Miskelly (2016), high temperatures accelerate yeast fermentation, and may cause surface cracking if the products are over-proofed. Low temperatures will prolong the proofing time, causing a flattened appearance after steaming. The optimum proofing temperature is usually 38–40°C.

2.5.3.3 Relative humidity

Humidity can be controlled in the proofer using a fine water spray or steam injection coupled with controlled heating. The optimum relative humidity in the proofer is 70–90%. Below 60%, a skin will form and the expansion of the dough pieces is inhibited. This could cause problems with appearance and surface cracking. If the humidity is too high (over 95%), condensation will occur, resulting in a wet and sticky surface and after steaming, products may have a dull color and rough surface (Huang and Miskelly, 2016).

2.5.3.4 Proofing time

According to Huang and Miskelly (2016), under-proofed doughs will result in products with a small volume and a dense crumb texture. Products manufactured from over-proofed doughs may have a flat shape, dull and rough skin, and an open and rough cell structure and are prone to shrinkage. Products manufactured by the two-step procedure need a shorter proofing time (about 60 min), while products prepared using the one-step dough procedure need a longer proofing time, in the range of 50–80 min. Some adjustments to yeast level, temperature, and/or water addition may be required to achieve a proofing time within the desired range.

2.5.4 Steaming

Proofed dough pieces are cooked by steaming in bamboo baskets over boiling water at home or in a steaming chamber or cabinet in factories, instead of being baked in an oven. This cooking process resulted in huge differences between steamed bread and baked bread (Huang, 2014a).

2.5.4.1 Changes in moisture

During steaming, the moisture content of the steamed bread increases, particularly during the first 10 min of steaming. Steam increases the moisture content of steamed bread, while the increasing bread temperature in turn accelerates evaporation of moisture from steamed bread (Huang and Miskelly, 2016).

2.5.4.2 Changes in volume

The volume of steamed bread increases drastically in the first 5 min of steaming. It was observed that small bubbles appear on the surface of steamed bread during the first 5 min of steaming if the air bubbles trapped during mixing were not removed by sheeting. These small bubbles ruptured when the steamed bread was removed from the steamer, leaving blisters on the skin of steamed bread (Huang and Miskelly, 2016).

2.5.4.3 Control of steaming conditions

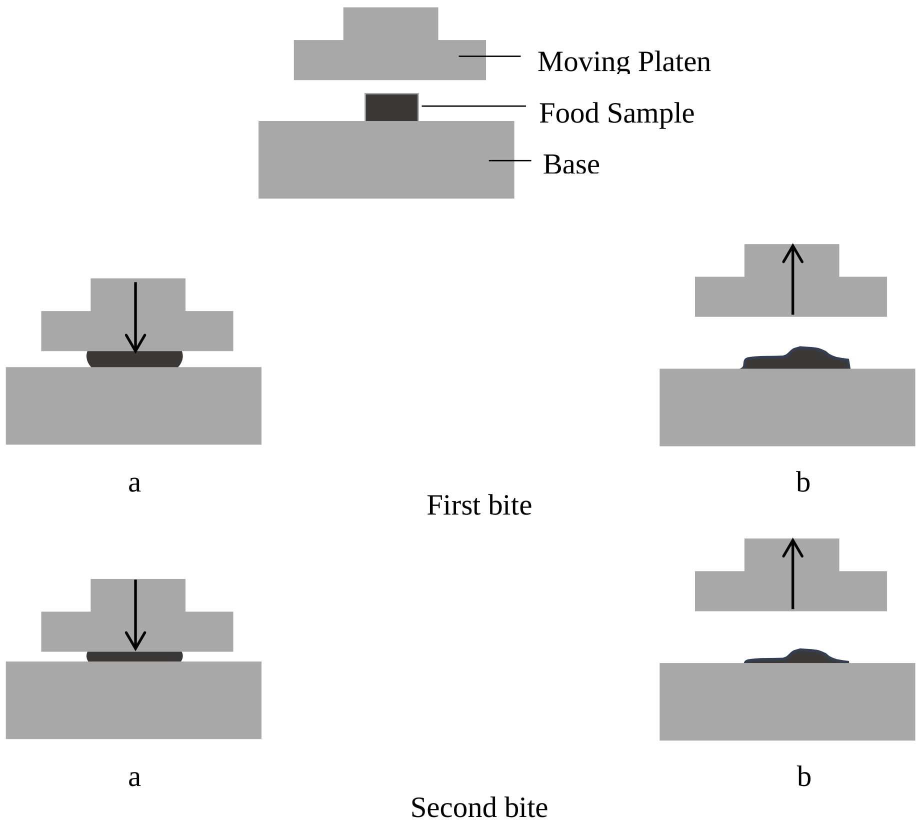
It was found that steam generation rate is very important to the quality of steamed bread. Steaming rate that was too low did not produce good-quality steamed bread; the bread had a smaller volume, poor texture, and sticky eating quality. However, a steaming rate that was too high also resulted in quality problems such as blisters, dimples, and jelly port on the surface of steamed bread and even shrinking of the steamed bread. These problems are more obvious in steamed bread made from flours with strong dough strength (Huang *et al.*, 1993).

Huang and Miskelly (2016) pointed out that the steam generation rate had great influence on the quality of steamed bread. A high steam generation rate resulted in the shrinking of steamed bread, particularly for steamed bread made from flour with high and medium dough strength, but not to that made from flour with weak dough strength.

For industrial production, Liu (2005) recommended that a steaming rate between 0.01 and 0.06MPa was suitable for a steam chamber in factories. Steaming times vary according to the size of the steamed bread, steam rate, and variety of products. Generally, 26–27 min steaming time is enough for steamed bread production of 135 g dough if the steaming rate is between 0.02 and 0.04 MPa.

2.6 Texture profile of steamed bread

The standard TA.XT2 Texture Analyzer is a single screw machine that was developed especially for food work. It has a force capacity of 250 N and crosshead speeds of 6–600mm min-1. Heavy-duty twin screw models are available up to 5000 N force. The TA.XT2 Plus model offers speeds up to 2400 mm /min. Stable Micro Systems and their US distributor, Texture Technologies Corp., have developed an extensive library of food applications and have established a reputation for personally helping customers with their day-today texture measurement problems and to work with them to develop new test methods and applications in operating their instruments (Bourne, 2002).

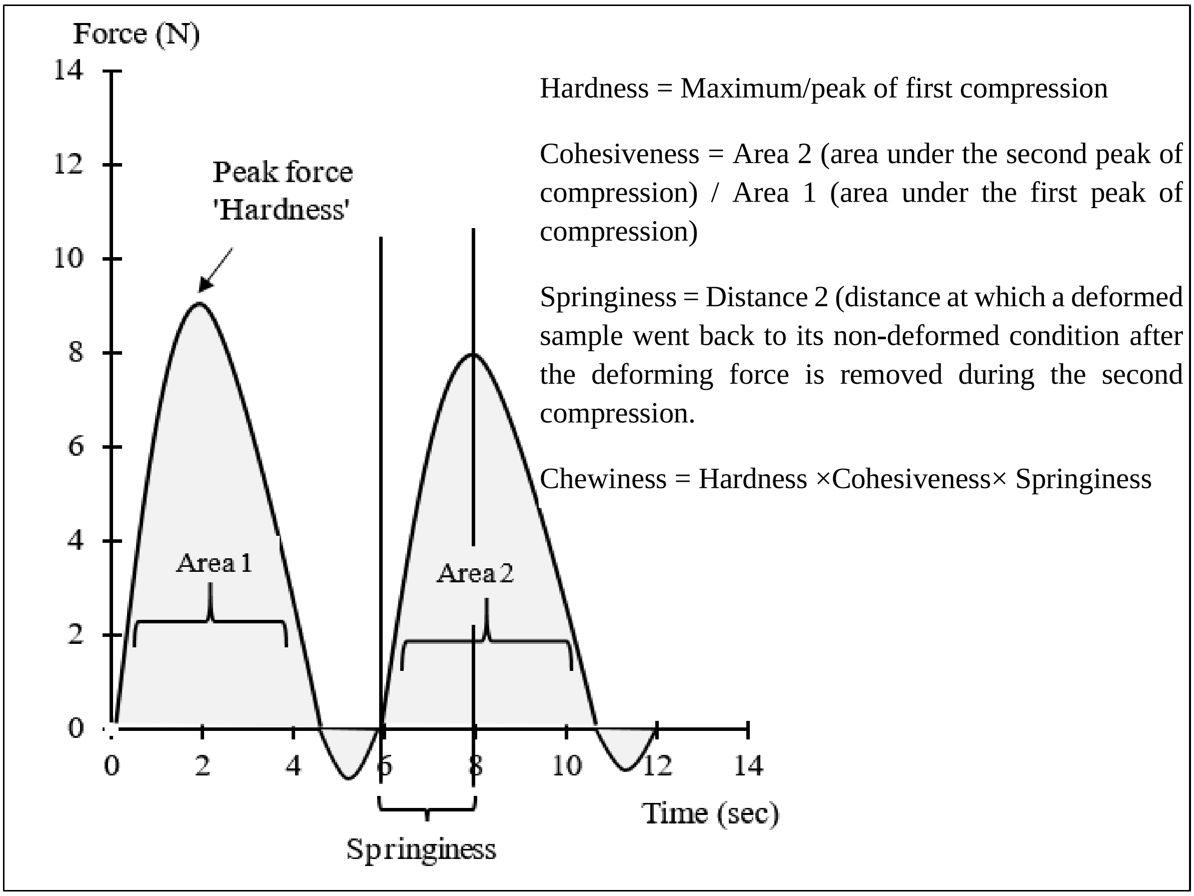


Source: Bourne (2002)

Fig. 2.2 Schematic diagram of texture profile analysis test. (a) Downstroke actions during the first and second bites; (b) upstroke actions during the first and second bites.

The principle of the TPA test is illustrated in Fig. 2.2 the curve obtained from TPA is given in Fig 2.3. Food sample of standard size and shape is placed on the baseplate and compressed and decompressed two times by a platen attached to the drive system. To imitate the chewing action of the teeth there should be a high compression. The height of the force peak on the first compression cycle (first bite) is defined as hardness. The ratio of the positive force areas under the first and second compressions (A2/A1) is defined as cohesiveness.

The distance that the food recovered its height during the time that elapsed between the end of the first bite and the start of the second bite is defined as springiness. Other parameters can be derived by calculation from the measured parameters: chewiness is defined as the product of hardness × cohesiveness × springiness (Bourne, 2002; Jalgaonkar *et al.*, 2018).



Source: Bourne (2002); Fujimoto *et al.* (2016); Jalgaonkar *et al.* (2018)

Fig. 2.3 Texture profile analysis (TPA) curve

2.7 Shelf-life of steamed bread

The shelf-life of products is affected by many different aspects of the product ingredients, processing, packaging and storage conditions. For example, preservatives, water activity, heat treatments, temperature control, oxygen ingress, light, humidity and pack integrity result in effects into intrinsic and extrinsic factors. Intrinsic factors are the properties of the final product (e.g., water activity, pH) whereas extrinsic factors are the external effects on the product such as the time–temperature profile of the product, exposure to light and handling through the supply chain. These factors can all interact often unpredictably and should be taken into account when planning a sensory shelf-life study: particularly in respect of food safety (Rogers, 2010).

In a study, Mohamed *et al.* (2010) prepared bread of 5 different formulations containing 10%, 15%, 20%, 25%, and 30% banana powder with varying amounts of base flour. In order to determine the effect of storage time and temperature on bread, firmness testing was done at 25, 4.0, and -20°C for 2, 5, and 7 days, respectively. The time and temperature were selected to imitate supermarket's storage conditions. At -20°C showed the least firmness, while the greatest firmness recorded was the control stored at room temperature for 5 or 7 days.

According to Sheng *et al.* (2015), shelf-life of CSB is mainly affected by microbial spoilage and staling. CSB has a high moisture content (40–45 g/100 g) and water activity (0.960–0.970), which makes it more prone to faster microbial spoilage. The microbial shelf-life of CSB at room temperature is 1–3 days, and it gets shorter at higher storage temperature and humidity. Mold growth is the most common source of microbial spoilage and their growth on the surface of CSB is responsible for the formation of unpleasant flavor and changes in color and texture.

Leng *et al.* (2010) reported that *mantou* mold is mainly caused by secondary contamination with microbes. It has been reported that after 20 h storage in an ordinary condition, the aerobic bacterial population of fresh steamed bread went up to 10^5 cfu/g, which was associated with a peculiar smell and sticky filaments. Mold could be seen clearly after 30 h storage.

Several methods have been tried to solve the problems, such as using longer heating treatment for better sterilization; using clean flour to avoid microbes and insect eggs which could cause rancidity and flavor loss in the flour and its products (Campolo *et al.*, 2013).

In a study, Laohasongkram *et al.* (2009), added 2.5% glycerol and 0.25% lactic acid as preservatives aiming to develop shelf-stable product, the sensory qualities were not significantly different from the control ($p>0.05$). Microbiological test revealed that the treated bun could be stored in a PVDC pouch for at least 16 days at 30±2°C, while the control bun could be stored for only 4 days. However, from a textural viewpoint, the hardness and gumminess of this treated bun increased during storage led to an unacceptable after 8 days by sensory evaluation.

Texture profile analysis and sensory evaluation showed that steamed bread was not a shelf-stable product as the textural properties and sensory acceptability of steamed bread decreased drastically within 24 h of storage time. Differential Scanning Calorimeter (DSC) results, amylopectin crystallization of steamed bread sample increased with storage time which was mainly responsible for steamed bread firming (Sha *et al.*, 2007).

Part III

Materials and methods

3.1 Collection of raw materials

Refined wheat flour (*Maida*) and oyster mushroom (*Pleurotus sajor-caju*) was procured from local market of Biratnagar. Defatted soybean grit was obtained from commercial soybean chunks production industry, Katahari, Morang. Skimmed powder was purchased from Biratnagar Milk Supply Scheme (DDC-BMSS). Refined sunflower oil, yeast, sugar and salt were purchased from a supermarket at Biratnagar Metropolitan city, Morang, Nepal. Procuring of raw materials was accomplished in a single lot to avoid variation and compositional differences so that quality differences will be ruled out.

3.2 Preliminary operations

Preliminary operations include preparation of mushroom and soybean power as well as modification of *momo* steamer (*moktu*) before preparation of steamed bread which are described in following sub-sections.

3.2.1 Preparation of mushroom powder

Unwanted parts of fresh oyster mushroom were separated manually, cleaned in tap water and drained. The cleaned mushroom was chopped into 3-4 cm long pieces and then blanched in boiling water for 5 min as described by Oguntowo *et al.* (2016). Blanched mushroom was allowed to cool for 30 min before it was subjected for sulphiting in 0.5% aqueous KMS solution for 15 min and drained the excess solution. After completion of blanching and sulphiting, it was dried intermittently in the cabinet drier at 55±5°C for 12 h. The dried mushroom was then cooled to room temperature and ground to fine powder using mixture grinder. After grinding, the powder obtained was sieved using 160 μ sieve and it was packed in airtight container.

3.2.2 Preparation of soybean powder

Defatted soybean grit was obtained from soybean chunks (soybean *masyaura* or *soybeanbadi*) manufacturing industry from Morang, Nepal. Unwanted foreign matters were removed manually and subjected to dehydration in a cabinet dryer at 55±5°C for 12 h. The dried grit was cooled to ambient temperature and ground using common mixture grinder then sieved using 160 μ sieve. Finally, the fine soybean powder obtained was packed in airtight container for further study.

3.2.3 Preparation of steaming equipment

Moktu was purchased from local market of Dharan and modification was made with the help of local utensil craftsman at *Purano Bazar*, Dharan Nepal. Commonly used *momo* steamer utensil (*moktu*) in which, the vessel was partitioned with four rectangular sub-partitions to obtain four units (average size: 70 mm×85 mm) using galvanized iron sheets to hold the dough.

3.3 Experimental design

Design Expert software (STAT-EASE Inc., USA, version 7.0) was used for experimental design in two successive studies; formulation of refined wheat flour, soybean powder and mushroom powder and also for optimization of fermentation time and steaming time as process parameters. The details are mentioned in following paragraphs.

3.3.1 Formulation of ingredients for steamed bread

Wheat flour, soybean powder and mushroom powder were used at their lower and upper level as 80–100, 0–20 g and 0-20 parts respectively to make 100 parts of composite flour. Stat-ease, Design Expert version 7, in which mixture D-optimal design with a three-factor was used in formulation stage of the study

Three quantitative controllable factors (independent variables): refined wheat flour (X1), soybean powder (X2) and mushroom powder (X3) were taken as factors for preparation of 100g composite flour. Similarly, four dependent variables were taken as responses for representing the main parameters of steamed bread quality were hardness (Y1), specific volume (Y2), spread ratio (Y3), and L* (whiteness) (Y4). Other ingredients namely, skimmed milk powder, refined vegetable oil, yeast and salt content were used in a fixed ratio on percentage basis for 100 g flour. The detail of the experimental design for formulation of steamed bread is given in Table 3.1. The experimental data for each response variable were fitted to the linear as well as quadratic model and regression parameters of the equations were calculated.

Table 3.1 Experimental design for formulation of steamed bread

Expt. No.	WF (parts)	SP (parts)	MP (parts)	SMP (%)	Sugar (%)	Oil (%)	Yeast (%)	Salt (%)
1	83.33	3.33	13.33	5	5	2	2	0.25
2	100.00	0.00	0.00	5	5	2	2	0.25
3	100.00	0.00	0.00	5	5	2	2	0.25
4	90.00	0.00	10.00	5	5	2	2	0.25
5	93.33	3.33	3.33	5	5	2	2	0.25
6	86.67	6.67	6.67	5	5	2	2	0.25
7	80.00	10.00	10.00	5	5	2	2	0.25
8	83.33	13.33	3.33	5	5	2	2	0.25
9	80.00	0.00	20.00	5	5	2	2	0.25
10	80.00	20.00	0.00	5	5	2	2	0.25
11	90.00	10.00	0.00	5	5	2	2	0.25
12	80.00	0.00	20.00	5	5	2	2	0.25

WF = Wheat flour, SP = Soybean Powder, MP = Mushroom Powder, SMP = Milk Powder

3.3.1.1 Response optimization constraints for formulation

A numerical multi-response optimization technique was applied to determine the optimum combination of wheat flour, soybean powder and mushroom powder with the determination of responses i.e., hardness, specific volume, spread ratio and whiteness of steamed bread. The assumptions made to develop a combination which would have minimum values of hardness and spread ratio as well as maximum values of specific ratio and whiteness of steamed bread. Table 3.2 shows the different condition of the constraints used for recipe optimization of steamed bread.

Table 3.2 Response optimization constraints for formulation of steamed bread

Name	Goal	Lower Limit (parts)	Upper Limit (parts)	Weight	Weight	Importance
Wheat Flour	Minimize	80	100	1	1	3
Soybean Powder	Maximize	0	20	1	1	1
Mushroom Powder	Maximize	0	20	1	1	1
L* (whiteness)	Maximize			1	1	3
Spread Ratio	Minimize			1	1	1
Specific Volume	Maximize			1	1	1
Hardness (g force)	Minimize			1	1	2

3.3.2 Optimization of fermentation time and steaming time

For process optimization of steamed bread, Stat-ease, Design Expert 7, trial version, in which Response Surface, Central Composite Design (CCD) with ratable design (k<6) was employed. Fermentation time (X1) and steaming time (X2) were the factors and hardness (Y1), specific volume (Y2), spread ratio (Y3) were taken as responses. The upper and lower level of the process parameter were set as 30 to 90 min for fermentation and 10 to 20 min for steaming process. The scheme for process optimization is shown in Table 3.2.

Table 3.3 Experimental design for process optimization

Expt. No.	Fermentation time (min)	Steaming time (min)
1	60.00	15.00
2	90.00	20.00
3	102.43	15.00
4	60.00	15.00
5	30.00	10.00
6	60.00	22.07
7	17.57	15.00
8	60.00	15.00
9	30.00	20.00
10	60.00	15.00
11	60.00	7.93
12	90.00	10.00

3.3.2.1 Response optimization constraints for processing

Similar technique that used in formulation process was applied to determine the optimum condition of fermentation time and steaming time for the determination of hardness, specific volume and spread ratio in steamed bread. The assumptions were to develop a combination which would have minimum value of hardness and spread ratio as well as maximum value of specific ratio of the steamed bread. Table 3.4 shows the different condition of the constraints used for process optimization.

Table 3.4 Response optimization constraints used for process optimization

Name	Goal	Lower Limit	Upper Limit	Weight	Weight	Importance
Fermentation Time (minute)	Minimize	30	90	1	1	1
Steaming Time (minute)	Minimize	10	20	1	1	1
L* (whiteness)	In range			1	1	3
Spread Ratio	Minimize			1	1	3
Specific Volume	Maximize			1	1	3
Hardness (g force)	Minimize			1	1	3

3.4 Preparation of steamed bread

Steamed bread was prepared by employing single stage fermentation procedure as described by Huang and Miskelly (2016). Raw materials were weighted separately on the basis of 300 g composite flour according to the formulation given in Table 3.1. Before mixing the ingredients, active dry yeast was rehydrated for 15 min at 40°C in reconstituted SMP. Mushroom powder, soybean powder, sugar, and salt were added and mixed well in their dry form, later on vegetable oil, activated yeast and water were added and well mixed with the use of electric hand mixer at medium speed for 15 min. The dough was then divided into 120 g portion for a single loaf and kept inside a previously oiled steamer vessel (*momo* steamer or *moktu*) containing 70 mm×85 mm rectangular mould and wax paper was placed at the bottom.

The whole assembly was then kept inside an incubator for fermentation at 35±2°C for 60 min covered with moistened muslin cloth. After fermentation, dough was steamed at normal atmospheric condition in a steamer vessel for 15 min, allowed to cool at ambient temperature for 30 min. Finally, steamed bread thus prepared were divided into two portions; one subjected for physiochemical analysis, rest was packed in LDPE, sealed and stored refrigerated at 4±1°C for further analysis. The flow chart of steam bread preparation is given in Fig. 3.1.

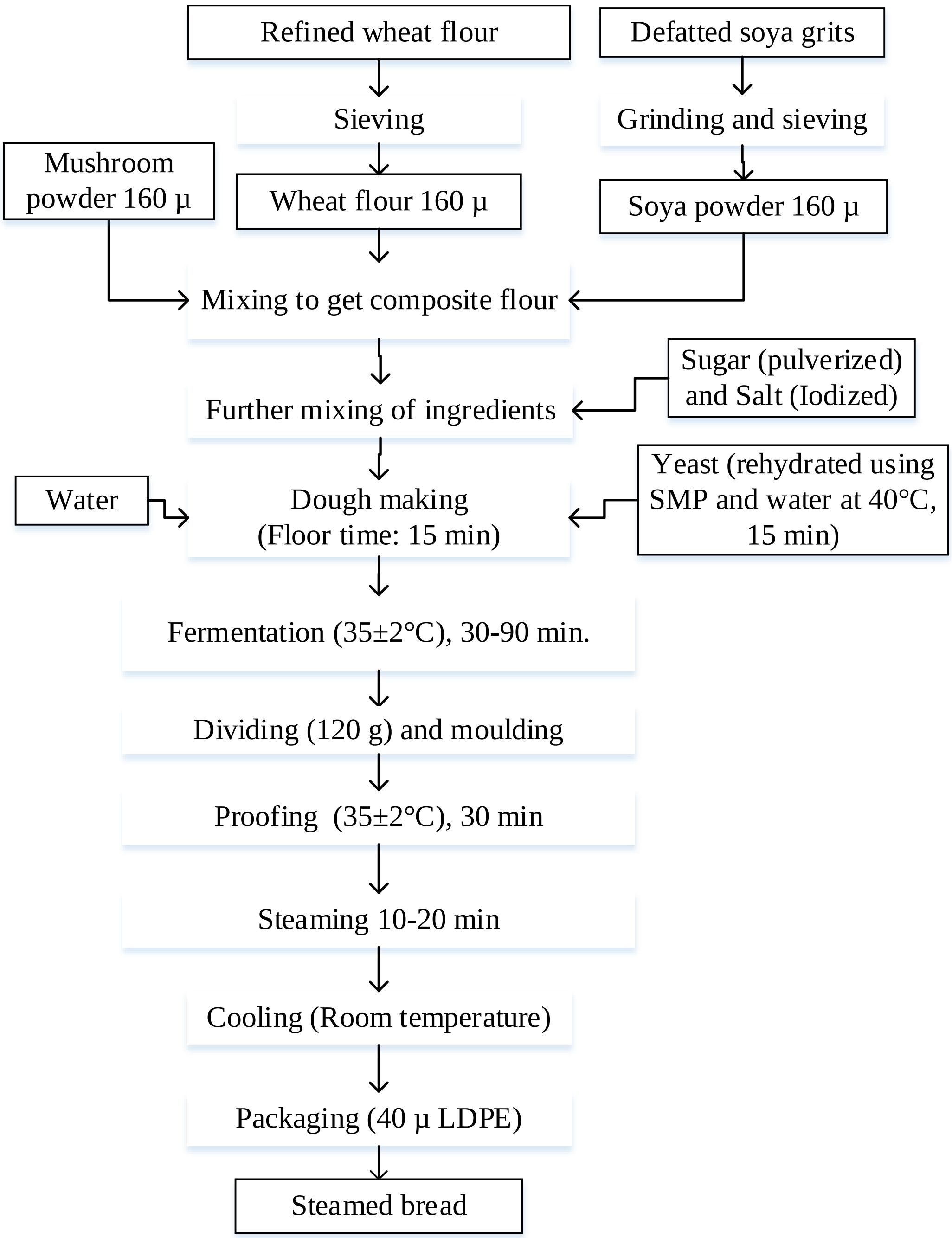


Fig. 3.1 Flow diagram for steamed bread preparation

3.4.1 Physiochemical and microbiological analysis

The glassware and equipment as used as per available in Central Campus of Technology (CCT), Central Department of Food Technology (CDFT), Dharan, Food Technology and Quality Control Department (DFTQC), Kathmandu and Food Technology and Quality Control Offices (FTQCO), Biratnagar and Bhairahawa. The list of chemicals, glassware and equipment used during the study is given in Appendix E.

Physical analysis was carried out to obtained the best suggested formulation as well as the best suggested process parameters for soybean and mushroom incorporated steamed bread. The determinations were conducted in triplicates for all parameters. Chemical analysis was carried out to compare best suggested product with control sample. Microbiological analysis was performed to predict the shelf-life of best sample under ambient and refrigerated storage conditions. The description of each analysis is presented as following:

3.4.1.1 Moisture

Moisture of raw material as well as steamed bread was determined by hot air oven method as described by Ranganna, 1986.

3.4.1.2 Specific volume

The specific volume of the steamed bread was determined using solid displacement method as per AACC, (2000) with slight modifications using mustard seeds instead of rape seeds as described by Rao *et al.*, (2007). Weight of steamed bread was measured using electronic balance and measured to the nearest of 0.001 g. Specific volume (ml/g) was calculated as the ratio of volume to weight of steamed bread as descried by Ren *et al.*, (2012). A rigid plastic container was taken and filled with sound mustard seeds. The container was then partially emptied and a bread piece of known weight was placed inside and again filled with mustard seeds. The extra rapeseed, which equal to the loaf volume, was measured with the use of graduated measuring cylinder. The specific volume of the loaf was calculated using the following equation

$$\text{Specific volume (ml/g)} = \frac{\text{Loaf volume (ml)}}{\text{Loaf weight (g)}}$$

3.4.1.3 L* (whiteness)

Samples were allowed to cool for 30 min before being tested. The L* (whiteness) of the steamed bread was measured using a Chroma meter, Model CR-200 (Manufactured by: Konica Minolta Inc., Japan) for whiteness (value of L*= 0 for black and L*=100 for white) according to the method described by Jha, (2010); Ren *et al.*, (2012).

3.4.1.4 Hardness of steamed bread

Hardness one of the important texture attributes of steamed bread and the test was carried out in DFTQC Laboratory using a TA-XT2i® Texture Analyzer (Stable Micro Systems, Surrey, England) equipped with a 35-mm-diameter aluminum cylindrical probe. Steamed bread was sliced horizontally at a thickness of 25mm, was compressed to 70% of its height using the method as described by Ren *et al.* (2012) with slightly modifications. The test conditions were as follows: pretest speed: 2 mm/s; test speed: 1.7 mm/s; post-test speed: 2 mm/s; rupture test distance: 4%; force: 100 g; time: 3 s; and load cell: 5 kg. The analysis was performed with 12 subsamples in triplicates.

From the data generated from texture analyzer, hardness (g) was obtained as maximum height/peak of the curve.

3.4.1.5 Protein

Protein of raw material as well as product was determined by micro-Kjeldahl method as per Ranganna (1986), using semi-automatic protein analyzer developed by FOSS, United Kingdom.

3.4.1.6 Fat

Fat was extracted using petroleum ether as described by Ranganna (1986) with the use of automatic fat analyzer.

3.4.1.7 Total ash

Ash was determined in muffle furnace at 450°C using dry ashing method as per Ranganna. (1986)

3.4.1.8 Crude fiber

The crude fiber content was determined by following the procedure as described by Ranganna (1986).

3.4.1.9 Carbohydrate

Carbohydrate content was determined by difference method.

Total carbohydrate (%) =100 – (moisture + crude protein + crude fat + crude fiber + ash) %

3.4.2 Microbiological analysis

Microbiological tests were performed to determine whether the product was safe or not for human consumption and is regarded as an important aspect to the shelf-life of the product. Standard plate count was carried out for microbial for the study as described by Aneja (2003). The samples were diluted serially and mixed with peptone buffer water in such a way that the colonies grown on the plates were countable. The changes in microbial load were taken as determinant of shelf-life of the product.

Twenty-five gram of steamed bread samples was removed aseptically on every day from the package and homogenized using a stomacher (Lab-blender 400, Seward Laboratory) for 1 minute. Serial dilutions were prepared using 0.85 g/100 mm aseptic physiological saline, and 1 ml of appropriate dilutions of the bread homogenate was spread on the surface of agar plates. Total plate count (TPC) was determined using plate count agar after incubation for 2 days at 37°C. Microbiological analysis was replicated twice on different occasions, and results were reported as logarithms of the number of colony-forming unit (log cfu/g) as described by Sheng *et al.* (2015).

3.4.3 Sensory analysis

Sensory quality of the selected product was evaluated against control by semi-trained panelist; including university faculties, food technologists and research students at Dharan and Biratnagar. The parameters selected for evaluation were appearance/color, shape, smell, taste, texture and overall acceptance. Nine-point hedonic rating test as suggested by Ranganna (1986) was adopted as a method of evaluation.

3.4.4 Statistical analysis

The experiment was arranged as a design of experiment (DOE) in Design- Expert software, version 7 developed by Stat- Ease, MN, USA with three replications. The sensory analysis data was analyzed by using one way and two- way ANOVA using IBM-SPSS Statistics version 23 at a significance level of $p<0.05$. Upon significant difference means were separated by using Tukey test. Data obtained from chemical analysis were analyzed using Microsoft Office Excel 2016.

3.4.5 Packaging and storage of steamed bread

LDPE 40 μ zip lock bags (pouch) were used for packaging of steamed bread and stored at ambient conditions 30±2°C and refrigerated temperature at 5±1°C.

Part IV

Results and discussion

Steamed bread was prepared using single stage fermentation method as described by Huang and Miskelly (2016) using various proportion of refined wheat flour, defatted soybean powder and oyster mushroom powder. Design Expert software was used to find best formulation and optimum processing parameters by determination of hardness, spread ratio, specific volume and L* (whiteness) as response variables steamed bread. The numerical optimization was performed for minimum values of hardness and spread ratio; maximum values of specific volume and L* of the steamed bread in each study with the employment of two separate set of experiment designs. The findings are described in the following sections.

4.1 Chemical composition of refined wheat flour

The proximate composition of refined wheat flour is shown in Table 4.1.

Table 4.1 Proximate composition of refined wheat flour

Constituents	Value
Moisture (%)	13.20±0.07
Gluten (% db)	10.01±0.06
Crude fat (% db)	0.79±0.01
Total ash (% db)	0.46±0.01
Crude fiber (% db)	1.23±0.02

Gluten content of the refined wheat flour used in the study was found to be 10.01±0.06% Table 4.1. According to Wrigley and Uthayakumaran, (2010), the wheat flour quality used in the current study falls within the category of medium-hard. Huang *et al.* (1996) reported that the quality scores for steam bread were found decreased significantly below gluten content of 10%, but not significantly increased above 10%.

4.2 Formulation of steamed bread

4.2.1 Effect of formulation on hardness of steamed bread

The hardness of soybean and mushroom incorporated steamed bread revealed with texture analyzer varied from 2471.20 g to 3366.63 g. Table 4.2 shows the coefficients of the model and other statistical attributes of steamed bread hardness. Regression model fitted to experimental results of hardness shows that the model F-value of 149.6 is significant ($p<0.05$). The lack of fit test was not significant ($p>0.05$). The chance of large model F-value due to noise is only 0.01%. The fit of model, also expressed by the coefficient of determination R^2, which is found to be 0.9894, indicating that 98.94% of the variability of the response can be explained by the model. The Adjusted R^2 is 0.9828 and adequate precision is 33.935 showed an adequate signal. A ratio greater than 4 is desirable and hence, this model may be used to investigate the design space (Myers *et al.*, 2016).

Considering all the above criteria the model, equation 4.1 is selected to represent the variation of hardness with the independent variables and further analysis. The quadratic model fitted for hardness obtained from regression analysis in terms of coded values of the variables is represented by equation 4.1.

Hardness = +2484.84*A +3157.85*B +3340.33*C -563.77*A*B +25.40*A*C +16.88*B*C …………………………………………………………..……………..….….. equation 4.1

Where, A, B and C are the coded values of wheat flour (parts), soybean powder (parts) and mushroom powder (parts) respectively.

The absolute values of partial regression coefficient of one degree term are $C > B > A$ within the range of experimental design. The interaction term of wheat flour and soybean power (AB) had significant negative effect on hardness of the bread ($p<0.05$), whereas interaction terms of wheat flour and mushroom powder (AC) as well as soybean powder and mushroom powder (BC) were not significant.

Table 4.2 Analysis of variance of hardness for formulation of steamed bread

Source	Sum of Squares	Df	Mean Square	F Value	p-value
Model	1.107×10^6	5	2.213×10^5	149.26	< 0.0001***
Linear Mixture	1.086×10^6	2	5.430×10^5	366.18	< 0.0001***
AB	20518.58	1	20518.58	13.84	0.0059**
AC	59.82	1	59.82	0.040	0.8458
BC	18.39	1	18.39	0.012	0.9141
Residual	11863.25	8	1482.91		
Lack of Fit	9570.85	4	2392.71	4.18	0.0976
Pure Error	2292.39	4	573.10		
Total	1.119×10^6	13			
R^2	0.9894				
Adjusted R^2	0.9828				
Adeq. Precision	33.935				

***Significant at p < 0.0001, ** Significant at p<0.05, DF: degrees of freedom

Equation 4.1 and Table 4.1 and Fig. 4.1 show that magnitude of hardness increases when wheat four is gradually replaced with soybean and mushroom powder. This phenomenon is supported by Liu *et al.* (2016), who added tartary buckwheat flour and found increased the hardness of steamed bread, but there was no significant difference revealed between 5 and 10% levels. The main reason behind increase in hardness with displacement of wheat flour due to the rigid nature fibers incorporating into dough system that might interfere with the formation of gluten network (Chen *et al.*, 2016).

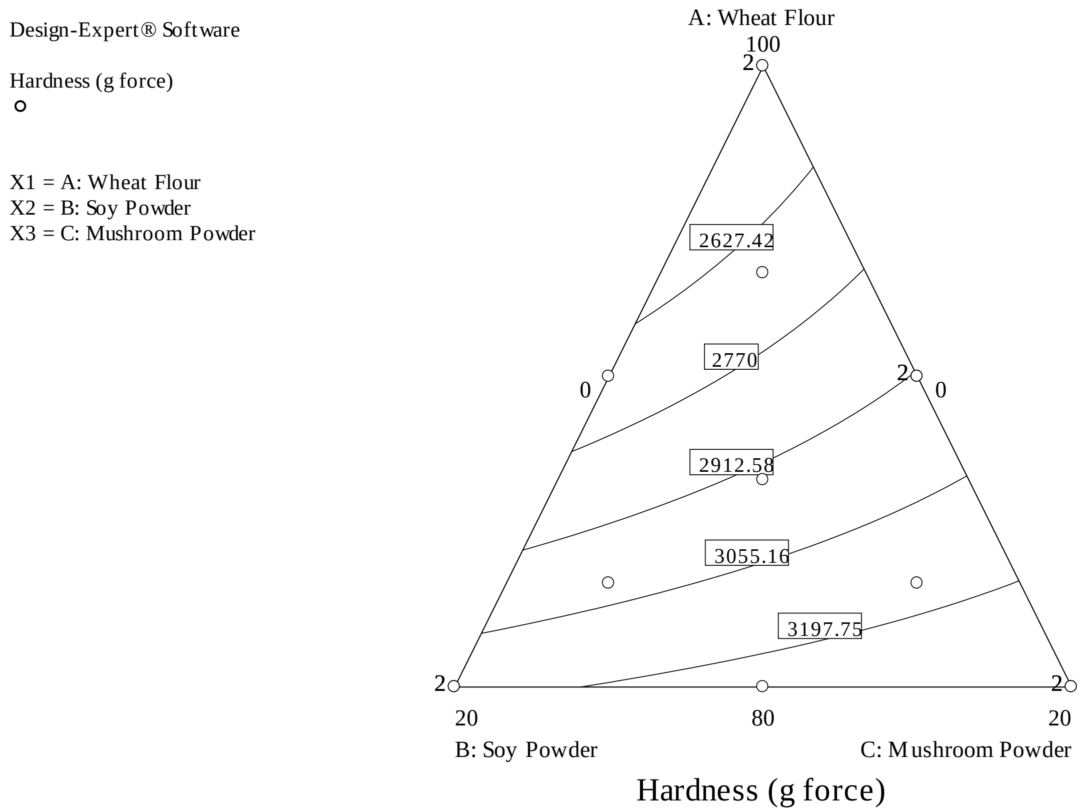


Fig. 4.1 Contour plot of hardness as a function of wheat flour, soybean powder and mushroom powder

Contour plot for the hardness of steamed bread as a function wheat flour, soybean powder and mushroom powder is given in Fig. 4.1. The graph shows the interaction between quantity of wheat flour, soybean powder and mushroom on hardness (g) as output (response) of soybean and mushroom incorporated steamed bread.

4.2.2 Effect of formulation on specific volume of steamed bread

The specific volume of steamed bread containing soybean and mushroom powder was determined within a range of 1.49 to 2.10 ml/g. Table 4.3 shows the coefficients of the model and other statistical attributes for specific volume. Regression model fitted to experimental results of specific volume shows that the model F-value of 154.63 is significant ($p<0.0001$). The lack of fit test was not significant ($p>0.05$). The chance of large model F-value due to noise is only 0.01%. The fit of model also expressed by the coefficient of determination R^2, which is found to be 0.9898, indicating that 98.98% of the variability of the response can be explained by the model. The adjusted R^2 is 0.9834 and adequate precision is 33.833 shows an adequate signal. A ratio greater than 4 is desirable and hence, this model may be used to investigate the design space. Considering all the above criteria the model equation 4.2 is selected to represent the variation of specific volume with the independent variables and further analysis. The linear model fitted for specific volume obtained from regression analysis in terms of coded values of the variables is represented by equation 4.2.

Specific volume = +2.08*A +1.55*B +1.48*C ……………………………..…. equation 4.2

Where, A, B and C are the coded values of wheat flour (parts), soybean powder (parts) and mushroom powder (parts) respectively.

The absolute values of partial regression coefficient of one degree term are $A > B > C$ within the range of experimental design. Equation 4.2, Table 4.3 and Fig. 4.2 show that value of specific volume (ml/g) of steamed bread was found decreased when quantity of soybean and mushroom powder is increased. The trend of specific volume of steamed bread was found decreasing when soybean and mushroom content increased in the study is supported by Lee (2010), added buckwheat flour to replace wheat flour by 0, 3, 6 and 9% respectively and effect on specific volume was noted. The value found decreased from 3.31 to 2.57 ml/g and observed no significant effect ($p>0.05$) up to 6%, whereas, specific volume of steamed bread was found increased significantly at 9% in comparison to 0, 3 and 6% buckwheat flour addition ($p<0.05$).

In present study, when 0 to 20% wheat flour was replaced by soybean and mushroom powder either singly or in combination, specific volume of steamed bread shrunk from 2.10 to 1.49 ml/g. The finding is comparable to a study carried out by Liu *et al.* (2016) who prepared steamed bread incorporating 0, 5, 10 and 15% buckwheat flour and noticed that specific volume was found decreased from 2.47 to 1.69 ml/g with significantly different ($p<0.05$). Moreover, the results noted in current study agree with the results reported by Giri and Sakhale (2019), decreased specific volume of oat, psyllium and barley fiber incorporated bread in comparison to control bread containing wheat flour only.

Table 4.3 Analysis of variance of specific volume for formulation of steamed bread

Source	Sum of Squares	Df	Mean Square	F Value	p-value
Model	0.54	2	0.27	127.83	< 0.0001***
Linear Mixture	0.54	2	0.27	127.83	< 0.0001***
Residual	0.023	11	2.1×10^{-3}		
Lack of Fit	0.020	7	2.89×10^{-3}	4.06	0.0969
Pure Error	2.85×10^{-3}	4	47.125×10^{-4}		
Total	0.56	13			
R^2	0.9587				
Adjusted R^2	0.9512				
Adeq. Precision	28.19				

***Significant at p < 0.0001, DF: degrees of freedom

Contour plot for the specific volume of steamed bread as a function wheat flour, soybean powder and mushroom powder is given in Fig. 4.2. The graph shows the interaction between quantity of wheat flour, soybean powder and mushroom powder on specific volume (ml/g) as output (response) of soybean and mushroom incorporated steamed bread.

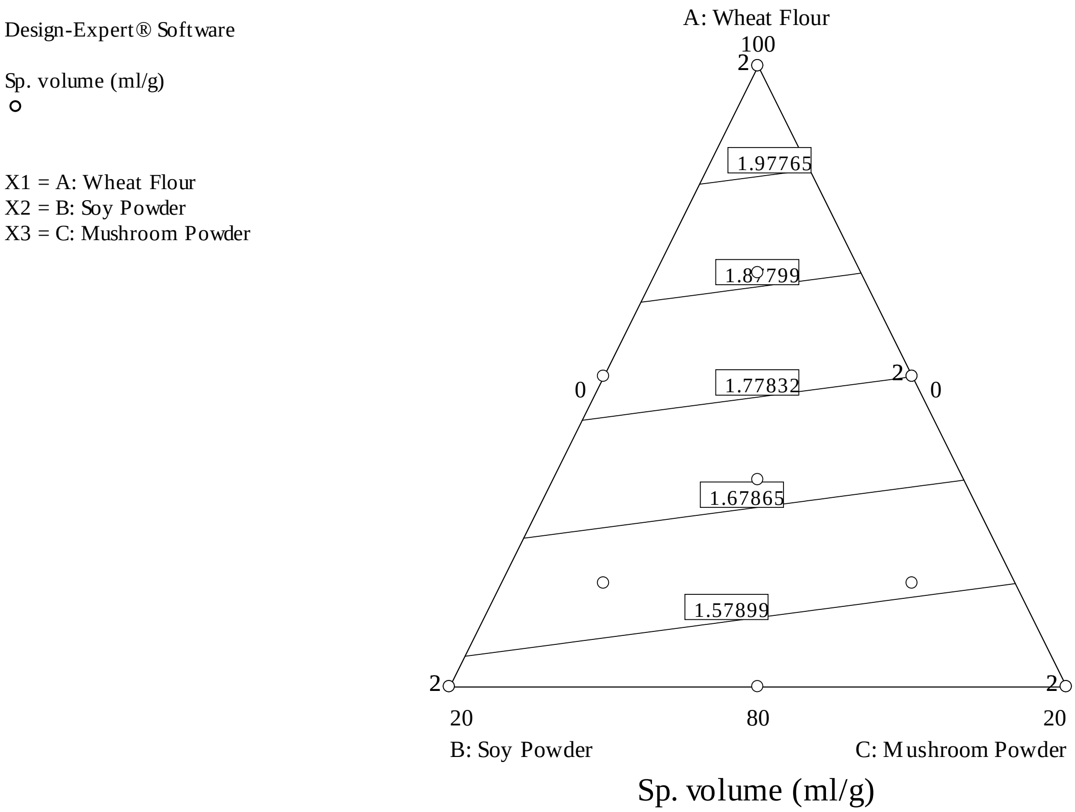


Fig. 4.2 Contour plot of specific volume as a function of wheat flour, soybean powder and mushroom powder

4.2.3 Effect of formulation on spread ratio of steamed bread

The spread ratio of steamed bread was found varied from 1.36 to 1.81. Table 4.4 shows the coefficients of the model and other statistical attributes for specific volume. Regression model fitted to experimental results of spread ratio shows that the model F-value of 50.90 is significant ($p<0.0001$). The lack of fit test was not significant ($p>0.05$). The chance of large model F-value due to noise is only 0.01%. The fit of model also expressed by the coefficient of determination R^2, which is found to be 0.9025, indicating that 90.25% of the variability of the response can be explained by the model. The Adjusted R^2 is 0.8847 and Adequate Precision is 17.314 shows an adequate signal. A ratio greater than 4 is desirable and hence, this model may be used to investigate the design space. Considering all the above criteria the model equation 4.3 is selected to represent the variation of spread ratio with the independent variables and further analysis.

The quadratic model fitted for spread ratio obtained from regression analysis in terms of coded values of the variables is represented by equation 4.3.

Spread ratio = +1.34*A +1.74*B +1.76*C ………………………..…..….... equation 4.3

Where, A, B and C are the coded values of wheat flour (parts), soybean powder (parts) and mushroom powder (parts) respectively.

The absolute values of partial regression coefficient of one degree term are $C > B > A$ within the range of experimental design, indicating that wheat flour (A), soybean powder (B) and mushroom powder (C) has highly significant positive linear effect on spread ratio of steamed bread ($p<0.0001$)

Table 4.4 Analysis of variance of spread ratio for formulation of steamed bread

Source	Sum of Squares	Df	Mean Square	F Value	p-value
Model	0.28	2	0.14	50.90	< 0.0001***
Linear Mixture	0.28	2	0.14	50.90	< 0.0001***
Residual	0.030	11	2.772×10^{-3}		
Lack of Fit		7	1.742×10^{-3}	0.38	0.8748
Pure Error			44.575×10^{-3}		
Total	0.31	13			
R^2	0.9025				
Adjusted R^2	0.847				
Adeq. Precision	17.314				

***Significant at $p < 0.0001$, DF: degrees of freedom

Equation 4.3, Table 4.4 and Fig. 4.3 show that L* (whiteness) of steamed bread was found decreased when more quantity of soybean and mushroom powder is added. Similar trend reported by Sun *et al.* (2015), who prepared steamed bread with incorporation of wheat germ flour (WGF) at various levels and noticed that there was a decrease in specific volume and an increment in spread ratio as wheat flour was gradually replaced with WGF. The spread ratio hiked as high as 24.2% when 12% of WGF was added to the dough during steamed bread preparation in comparison to control sample.

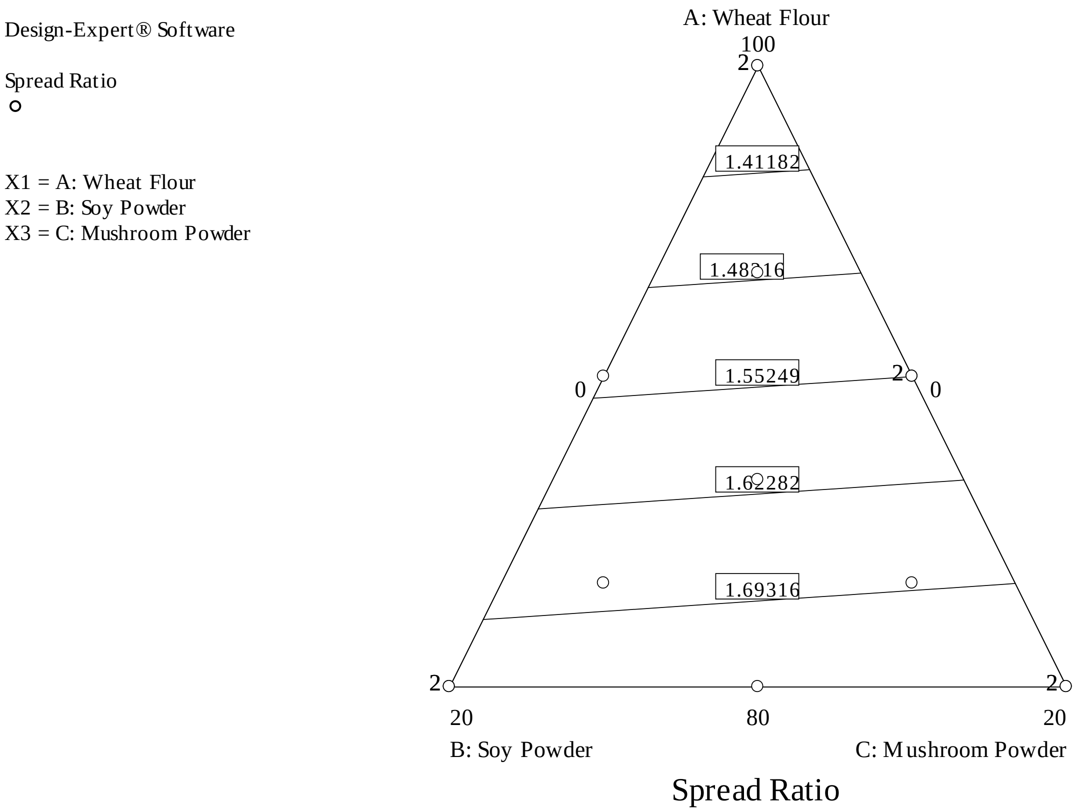


Fig. 4.3 Contour plot of spread ratio as a function of wheat flour, soybean powder and mushroom powder

Contour plot for the spread ratio of steamed bread as a function wheat flour, soybean powder and mushroom powder is given in Fig. 4.3. The graph shows the interaction between quantity of wheat flour, soybean powder and mushroom powder on spread ratio as output (response) of soybean and mushroom incorporated steamed bread.

4.2.4 Effect of formulation on L* (whiteness) of steamed bread

The L* of soybean and mushroom incorporated steamed bread was observed between 68.22 to 77.64. Table 4.5 shows the coefficients of the model and other statistical attributes for L*. Regression model fitted to experimental results of L* shows that the model F-value of 81.82 is significant ($p<0.0001$). The lack of fit test is not significant ($p>0.05$). The chance of large model F-value due to noise is only 0.01%. The fit of model also expressed by the coefficient of determination R^2, which is found to be 0.9808, indicating that 98.08% of the variability of the response can be explained by the model. The Adjusted R^2 is 0.9688 and adequate precision is 24.257 shows an adequate signal. A ratio greater than 4 is desirable and hence, this model may be used to investigate the design space.

Considering all the above criteria the model equation 4.4 is selected to represent the variation of whiteness with the independent variables and further analysis. The quadratic model fitted for hardness obtained from regression analysis in terms of coded values of the variables is represented by equation 4.4.

L*= +77.40*A +74.08*B +68.35*C -1.66*A*B -15.06*A*C +0.39*B*C .... equation 4.4

Where, A, B and C are the coded values of wheat flour (parts), soybean powder (parts) and mushroom powder (parts) respectively.

The absolute values of partial regression coefficient of one degree term are $A > B > C$ within the range of experimental design, indicating that wheat flour (A), soybean powder (B) and mushroom powder (C) has highly significant positive linear effect on L* of steamed bread ($p<0.0001$). The interaction term of wheat flour and mushroom power (AC) had significant negative effect on L* of the steamed bread ($p<0.0001$), whereas interaction terms of wheat flour and soybean powder (AB) as well as soybean powder and mushroom powder (BC) were not significant are presented in Table 4.5.

Table 4.5 Analysis of variance of L* (whiteness) for formulation of steamed bread

Source	Sum of Squares	Df	Mean Square	F Value	p-value
Model	132.71	5	26.54	81.82	< 0.0001***
Linear Mixture	111.47	2	55.74	171.81	< 0.0001***
AB	0.18	1	0.18	0.55	0.4792
AC	21.05	1	21.05	64.88	< 0.0001***
BC	9.709×10^{-3}	1	9.709×10^{-3}	0.030	0.8669
Residual	2.60	8	0.32		
Lack of Fit	1.86	4	0.46	2.53	0.1957
Pure Error	0.74	4	0.18		
Total	135.30	13			
R^2	0.9808				
Adjusted R^2	0.9688				
Adeq. Precision	24.257				

***Significant at $p < 0.0001$, DF: degrees of freedom

L* (whiteness) observed in the current study resembles with Hsieh *et al.* (2017), who investigated the effects of partial replacement of wheat flour with whole grain flours on the physical characteristics and starch digestion of steamed bread. CSB made with wheat flours partially substituted with brown rice or oat flours at the levels of 10% or 30%, steamed bread in which whole grain substitution reduced the degrees of L* from 73.9 to 63.3-68.6. Dose-dependent lower L* value indicated that whole grain incorporation darkened the appearance of CSB. In another study, Lin *et al.* (2012) prepared steamed bread with incorporation of barley flour into wheat flour at 10, 20 and 30% level and studied the effect on whiteness of steamed bread. There was significantly decreased the whiteness steamed bread *(*p<0.05).

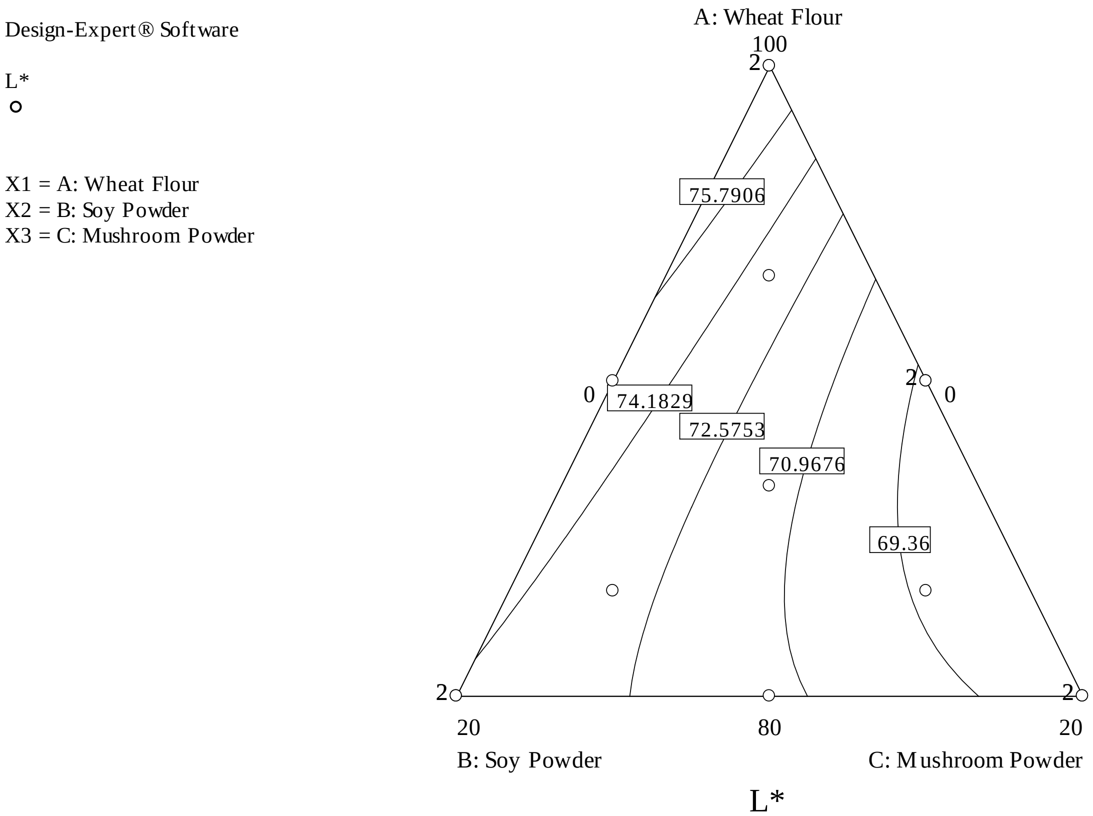


Fig. 4.4 Contour plot for L* as a function of wheat flour, soybean powder and mushroom powder

Contour plot for L* of steamed bread as a function wheat flour, soybean powder and mushroom powder is given in Fig. 4.4. The graph shows the interaction between quantity of wheat flour, soybean powder and mushroom powder on L*as output (response) of soybean and mushroom incorporated steamed bread

4.2.5 Verification of results for recipe optimization of steamed bread

The suitability of the model developed for predicting the optimum response values were tested using recommended optimum conditions of the variables and also used to validate experimental and predicted values of the response parameters. The results of the steamed bread recipe are shown in Table 4.6.

Table 4.6 Predicted and actual values of responses for formulation of steamed bread

SN	Responses	Wheat flour (parts)	Soybean powder (parts)	Mushroom powder (parts)	Predicted	Actual	Deviation (%)
1	Hardness (g)	87.89	10.15	2.08	2801.59	2918.02	3.99
2	Spread ratio	87.89	10.15	2.08	1.58	1.56	1.28
3	Specific volume (ml/g)	87.89	10.15	2.08	1.75	1.79	2.23
4	L* (whiteness)	87.89	10.15	2.08	73.87	72.44	1.97

4.3 Optimization of fermentation time and steaming time of steamed bread

The best formulation suggested by software as given in Table 4.6 was used optimization of process parameters as fermentation time and steaming time in min. Steamed bread was prepared using 87.89 parts of refined wheat flour, 10.15 parts of soybean powder and 2.08 parts of mushroom powder per 100 parts composite flour, keeping the other ingredients in a fixed ratio using single stage fermentation the same procedure as used in formulation stage. Fermentation time and steaming time were optimized with determination of hardness (g), specific volume (ml/g), spread ratio and hardness of steamed bread, which are presented in following paragraphs.

4.3.1 Effect of fermentation time and steaming time on hardness

The magnitude of hardness of steamed bread was found in a range from 2716.47g to 2973.22g. Table 4.7 shows the coefficients of the model and other statistical attributes for harness. Regression model fitted to experimental results of hardness showed that the model F-value of 41.13 is significant ($p<0.0001$). There is a chance of large model F-value due to noise is only 0.01%. The fitting of model also expressed by the coefficient of determination R^2, which is found to be 0.9014 indicating that 90.14% of the variability of the response can be explained by the model. The Adjusted R^2 is 0.8795 and adequate precision is 17.712

shows an adequate signal. A ratio greater than 4 is desirable and hence, this model may be used to investigate the design space (Myers *et al.*, 2016).

Considering all the above criteria the model equation 4.5 is selected to represent the variation of hardness with the independent variables and further analysis. The linear model fitted for hardness obtained from regression analysis in terms of coded values of the variables is represented by equation 4.5.

Hardness = +2823.06 -68.22*A -43.55*B …….………..…………………… equatin 4.5

Where, A and C are the coded values of fermentation time (minute), and steaming time (minute) respectively.

The absolute values of partial regression coefficient of one degree term are A > *B* within the range of experimental design. The effect of fermentation time (A) had significant negative effect on hardness of the steamed bread ($p<0.0001$), and steaming time (B) had significant negative effect on hardness of the steamed bread ($p<0.005$).

Table 4.7 Analysis of variance of hardness for process optimization of steamed bread

Source	Sum of Squares	Df	Mean Square	F Value	p-value
Model	52409.11	2	26204.56	41.13	< 0.0001***
A	37234.20	1	37234.20	58.44	< 0.0001***
B	15174.91	1	15174.91	23.82	0.0009 **
Residual	5734.52	9	637.17		
Lack of Fit	5734.52	6	955.75	0.2152	
Pure Error	0.00	3	0.00		
Total	58143.63				
R^2	0.9014				
Adjusted R^2	0.8795				
Adeq. Precision	17.712				

***Significant at $p < 0.0001$, ** Significant at $p < 0.05$, DF: degrees of freedom, A: Fermentation time (minute), B: Steaming time (minute)

Equation 4.5, Table 4.7 and Fig. 4.5 show that hardness of steamed bread was found highest at 30 min of fermentation and 10 min of steaming, decreased further towards optimum and hiked again when the process was continued further. Present study showed that optimum lowest value of harness was possible at somewhere in the middle of range of 30 to 90 minute of fermentation and 10 to 30 min of steaming. The result is comparable to Rubenthaler *et al.* (1990), who prepared steamed bread using commercial soft wheat flour with variations in sugar and yeast content and optimum time for proofing and steaming time were studied. The study showed that found 58 min of proofing time and 10 min of steaming time were sufficient to yield maximum loaf volume of steamed bread, however, studies regarding the hardness of soybean and mushroom incorporated steamed bread are not found so far.

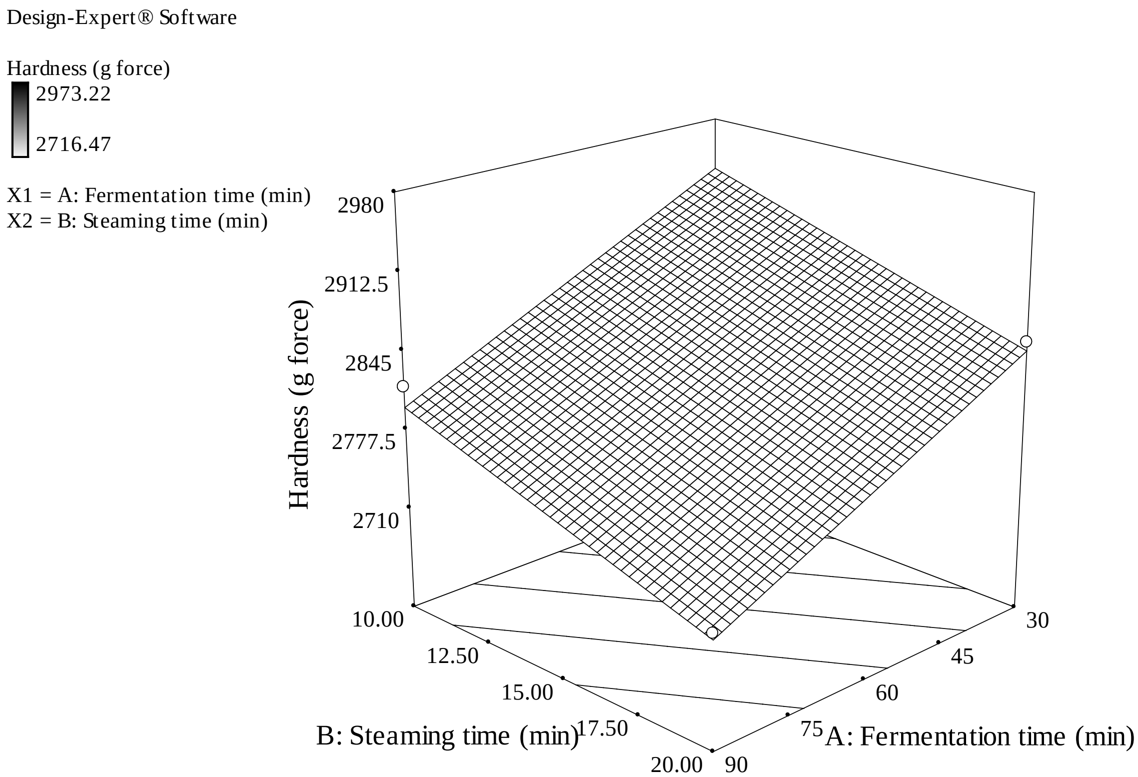


Fig. 4.5 Response surface plot for hardness as a function of fermentation time and steaming time

Three-dimensional plot for hardness of steamed bread as a function of fermentation time and steaming time of steamed bread is given in Fig. 4.5. This graph shows the interaction between fermentation time and steaming time (min) for the output hardness (g).

4.3.2 Effect of fermentation time and steaming time on specific volume

The specific volume of steamed bread varied from 1.58 to 1.74 Table 4.8 shows the coefficients of the model and other statistical attributes for specific volume. Regression model fitted to experimental results of it showed that the model F-value of 45.07 is significant ($p<0.0001$). The lack of fit test is not significant ($p>0.05$). The chance of large model F-value due to noise is only 0.01%. The fit of model also expressed by the coefficient of determination R^2, which is found to be 0.9741 indicating that 97.41% of the variability of the response can be explained by the model. The Adjusted R^2 is 0.9524 and adequate precision is 18.423 shows an adequate signal. A ratio greater than 4 is desirable and hence, this model may be used to investigate the design space (Myers *et al.*, 2016).

Considering all the above criteria the model equation 4.6 is selected to represent the variation of specific volume with the independent variables and further analysis. The quadratic model fitted for specific volume obtained from regression analysis in terms of coded values of the variables is represented by equation 4.6.

$$\text{Specific volume} = +1.74 + 0.021*A + 6.036\times10^{-3}*B + 0.000*A*B - 0.070*A^2 - 0.035*B^2 \quad \text{equation 4.6}$$

Where, A and C are the coded values of fermentation time (min), and steaming time (min) respectively.

From equation 4.6 the absolute values of partial regression coefficient of one degree term are A > *B* within the range of experimental design. The linear term of fermentation time (A) had significant positive effect on specific volume steamed bread ($p<0.005$), and term A^2 also was significant ($p<0.0001$). The square term of steaming time ($B^{2)}$ has significant negative effect on specific volume of steamed bread ($p<0.005$). Rest of the model terms were not significant.

Table 4.8 Analysis of variance of specific volume for process optimization of steamed bread

Source	Sum of Squares	Df	Mean Square	F Value	p-value
Model	0.038	5	$7.598x10^{-3}$	45.07	< 0.0001***
A	$3.397x10^{-3}$	1	$3.397x10^{-3}$	20.15	0.0042**
B	$2.914x10^{-4}$	1	$2.914x10^{-4}$	1.73	0.2366
AB	0.00	1	0.00	0.00	1.0000
A^2	0.031	1	0.031	186.02	< 0.0001***
B^2	$7.840x10^{-3}$	1	$7.840x10^{-3}$	46.50	0.0005**
Residual	$1.012x10^{-3}$	6	$1.686x10^{-4}$		
Lack of Fit	$8.115x10^{-4}$	3	$2.70x10^{-4}$	4.06	0.1401
Pure Error	$2.000x10^{-4}$	3	$6.667x10^{-5}$		
Total	0.039	11			
R^2	0.9741				
Adjusted R^2	0.9524				
Adeq. Precision	18.423				

***Significant at $p < 0.0001$, ** Significant at $p < 0.05$, DF: degrees of freedom, A: Fermentation time (minute), B: Steaming time (minute)

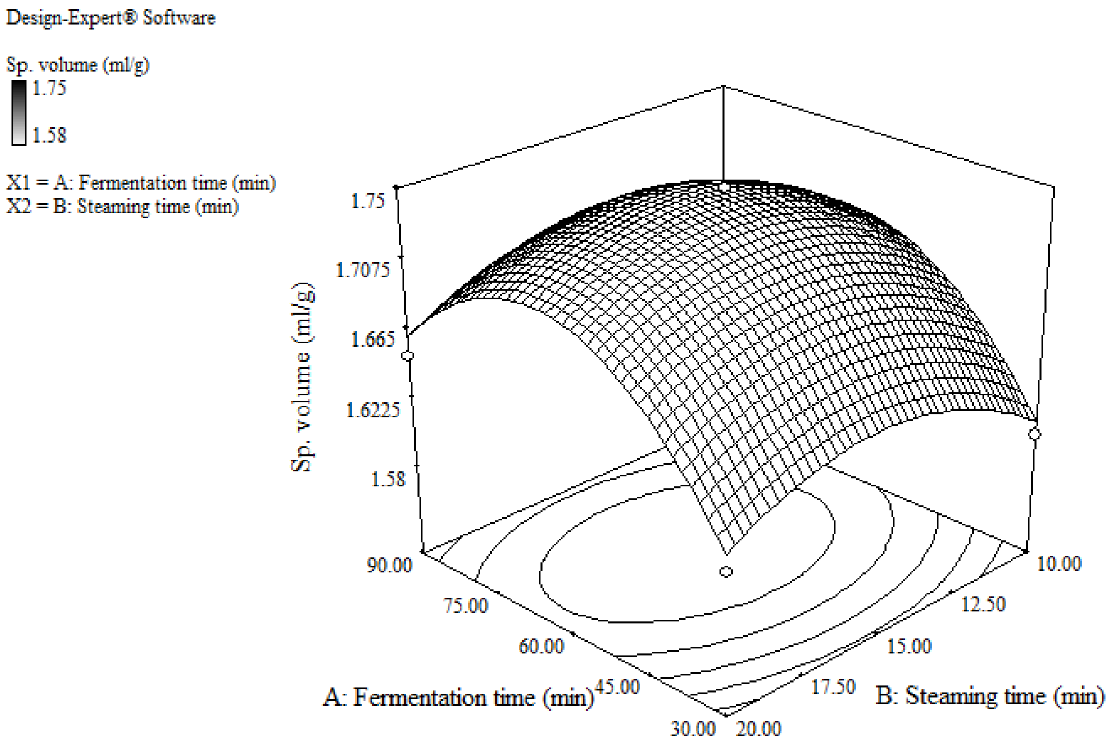


Fig. 4.6 Response surface plot for specific volume as a function of fermentation time and steaming time

Three dimensional plot for specific volume of steamed bread as a function of fermentation time and steaming time of steamed bread is given in Fig. 4.6. This graph shows the interaction between fermentation time and steaming time (min) for the output specific volume (ml g^{-1}).

Yue *et al.* (2019) studied the effect of fermentation time on quality of steamed bread and found the specific volume was increased at 40 and 60 min of fermentation time, but there was no significantly different (p>0.05). The values decreased when dough was fermented less than 40 min and greater than 60 min. The similar trend was also existed in current study but, however, study regarding effects on specific volume with variation in fermentation and steaming time for soybean and mushroom incorporated steamed bread were not found so far.

4.3.3 Effect of fermentation time and steaming time on spread ratio

The spread ratio of steamed bread varied from 1.49 to 1.64. Table 4.9 shows the coefficients of the model and other statistical attributes for specific volume. Regression model fitted to experimental results of it showed that the model F-value of 53.04 is significant (p<0.0001). The lack of fit test is not significant (p>0.05). The chance of large model F-value due to noise is only 0.01%. The fit of model also expressed by the coefficient of determination R^2, which is found to be 0.9779 indicating that 97.79% of the variability of the response can be explained by the model. The Adjusted R^2 is 0.9594 and Adequate Precision is 20.488 shows an adequate signal. A ratio greater than 4 is desirable and hence, this model may be used to investigate the design space (Myers *et al.*, 2016).

Considering all the above criteria the model equation 4.7 is selected to represent the variation of spread ratio with the independent variables and further analysis. The quadratic model fitted for spread ratio obtained from regression analysis in terms of coded values of the variables is represented by equation 4.7.

Spread ratio = $+1.50 -0.038*A -6.768x10^{-3}*B +0.000*A*B +0.048*A^2 +0.026*B^2$ .... equation 4.7

Where, A and C are the coded values of fermentation time (minute), and steaming time (minute) respectively.

From equation 4.7 the absolute values of partial regression coefficient of one degree term are A > *B* within the range of experimental design. The linear term of fermentation time (A) and its square term A^2 had significant negative effect on spread ratio of the steamed bread ($p<0.0001$). The square term of steaming time ($B^{2)}$ has significant negative effect on spread ratio of steamed bread ($p<0.005$). Rest of the model terms were not significant.

Table 4.9 Analysis of variance of spread ratio for process optimization of steamed bread

Source	Sum of Squares	Df	Mean Square	F Value	p-value
Model	0.028	5	$5.650x10^{-3}$	53.04	< 0.0001***
A	0.011	1	0.011	106.60	< 0.0001***
B	$3.664x10^{-4}$	1	$3.664x10^{-4}$	3.44	0.1131
AB	0.00	1	0.00	0.00	1.0000
A^2	0.015	1	0.015	139.13	< 0.0001***
B^2	$4.201x10^{-3}$	1	$4.203x10^{-3}$	39.35	0.0008
Residual	$6.392\ x10^{-3}$	6	$1.065x10^{-4}$		
Lack of Fit	$4.392x10^{-4}$	3	$1.464x10^{-4}$	2.20	0.2674
Pure Error	$2.000x10^{-4}$	3	$6.667x10^{-5}$		
Total	0.029	11			
R^2	0.9779				
Adjusted R^2	0.9594				
Adeq. Precision	20.488				

***Significant at p < 0.0001, ** Significant at p < 0.05, DF: degrees of freedom, A: Fermentation time (minute), B: Steaming time (minute)

Huang and Miskelly (2016) reported that 20 min fermentation at temperature 30-32°C can result in poorer spread ratios, inferior crumb texture, and lack of skin smoothness and shininess. The results of present study also shows that desirable low spread ratio is obtained at around 60 min of dough fermentation at 35±2°C and 15 min of steaming at normal atmospheric condition.

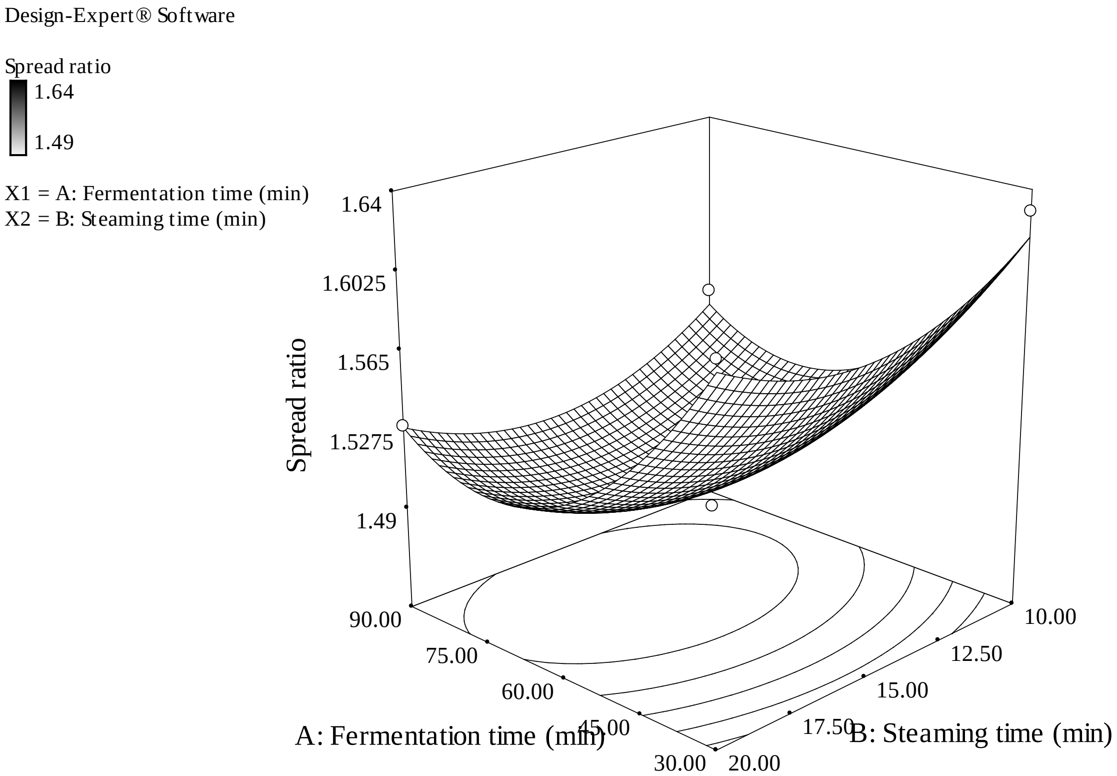


Fig. 4.7 Response surface for spread ratio as a function of fermentation time and steaming time

Three dimensional plot for spread ratio of steamed bread as a function of fermentation time (A) and steaming time (B) of steamed bread is given in Fig. 4.7. This graph shows the interaction between fermentation time and steaming time both in min for the output spread ratio.

4.3.4 Effect of fermentation time and steaming time on L* (whiteness)

L* (whiteness) of steamed bread varied from 1.49 to 1.64. Table 4.10 shows the coefficients of the model and other statistical attributes for specific volume. Regression model fitted to experimental results of it showed that the model F-value of 32.89 is significant ($p<0.0001$). The lack of fit test is not significant ($p>0.05$). The chance of large model F-value due to noise is only 0.01%. The fit of model also expressed by the coefficient of determination R^2, which is found to be 0.8797 indicating that 87.97% of the variability of the response can be explained by the model. The Adjusted R^2 is 0.8529 and adequate precision is 15.650 shows an adequate signal. A ratio greater than 4 is desirable and hence, this model may be used to investigate the design space.

Considering all the above criteria the model equation (4.8) is selected to represent the variation of L* with the independent variables and further analysis. The linear model fitted for L* obtained from regression analysis in terms of coded values of the variables is represented by equation 4.8.

L* (whiteness) = +75.08 -0.022*A -0.082*B ……………......................………equation 4.8

Where, A and C are the coded values of fermentation time (minute), and steaming time (minute) respectively.

From equation 4.8 the absolute values of partial regression coefficient of one degree term are B > A within the range of experimental design, indicating that steaming time (B) had highly significant negative linear effect on L* of soybean and mushroom incorporated steamed bread (p<0.0001). The linear term steaming time (B) has significant negative effect on L* of steamed bread (p<0.005). Rest of the model terms were not significant.

Table 4.10 Analysis of variance of L* (whiteness) for process optimization of steamed bread

Source	Sum of Squares	Df	Mean Square	F Value	p-value
Model	0.058	2	0.029	32.89	< 0.0001***
A	14.005×10^{-3}	1	14.005×10^{-3}	4.56	0.0615
B	0.054	1	0.054	61.23	< 0.0001***
Residual	7.906×10^{-3}	9	8.785×10^{-3}		
Lack of Fit	4.631×10^{-3}	6	7.719×10^{-3}	0.6724	
Pure Error	3.275×10^{-3}	3	1.092×10^{-3}		
Total	0.066				
R^2	0.8797				
Adjusted R^2	0.8529				
Adeq. Precision	15.650				

***Significant at p < 0.0001, DF: degrees of freedom, A: Fermentation time (minute), B: Steaming time (minute)

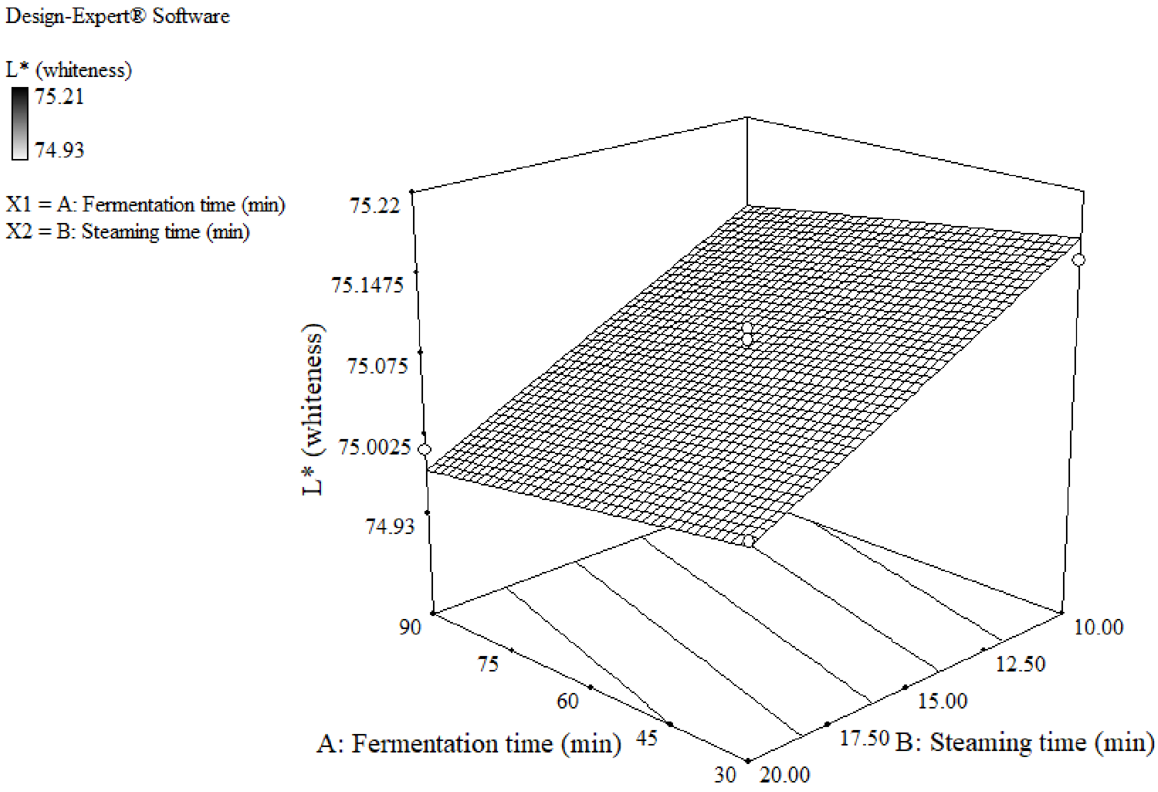


Fig. 4.8 Response surface for L* (whiteness) as a function of fermentation time and steaming time

Three dimensional plot for L* of steamed bread as a function of fermentation time and steaming time (min) of soybean and mushroom incorporated steamed bread is given in Fig. 4.8. This graph shows the interaction between fermentation time and steaming time both in min for the output L* (whiteness).

From equation 4.8, Table 4.10 and Fig. 4.8 show that mean value of L* was found to be 75.08±0.15 which is comparable to the whiteness index of control sample 77.48±0.29 and that of predicted value 75.0658. Similar result is reported by Liu *et al.* (2012) where, the whiteness of steamed bread rose during the first 60 min of fermentation time with use of yeast as leavening agent.

4.3.5 Verification of results for process optimization.

The suitability of the model developed for predicting the optimum response values was tested using the recommended optimum conditions of the variables and was also used to validate experimental and predicted values of the responses. The predicted and actual values of the responses for fermentation time and steaming time are shown in Table 4.11.

Table 4.11 Predicted and actual values of the responses for process optimization of steamed bread

SN	Components	Fermentation time (min)	Steaming time (min)	Predicted	Actual	Deviation (%)
1	Hardness (g)	67.04	15.05	2806.61	2714.18	3.41
2	Spread ratio	67.04	15.05	1.49	1.51	1.32
3	Specific volume (ml/g)	67.04	15.05	1.74	1.72	1.16
4	L* (Whiteness)	67.04	15.05	75.07	74.86	0.28

4.4 Sensory evaluation of steamed bread

Instrumental methods of analysis are clearly defined, its signals or predictions are unambiguous in nature but regarding foods products, mere instrumental analysis cannot predict whether the product is acceptable or not among the intended consumer. Sensorial analysis by trained or semi-trained panelists is also frequently carried out specially when one or more ingredients are varied within a product or in the study of relatively novel foods products for a particular community. These qualities include flavor, body and texture, color and appearance and overall acceptability. Two steamed bread samples, were evaluated for the sensory quality, one was control and another was soybean and mushroom incorporated one. The results of sensory parameters of the steamed bread are discussed as following.

Coded samples were provided to 10 semi-trained panelist of food technology and quality control office, Biratnagar for sensorial evaluation of appearance and color, flavor, texture, taste and overall acceptance. The panelists were requested to provide scores in the score sheets as per their perception. Data were analyzed statistically and the statistical representation of the sensory analysis is given in Fig. 4.9.

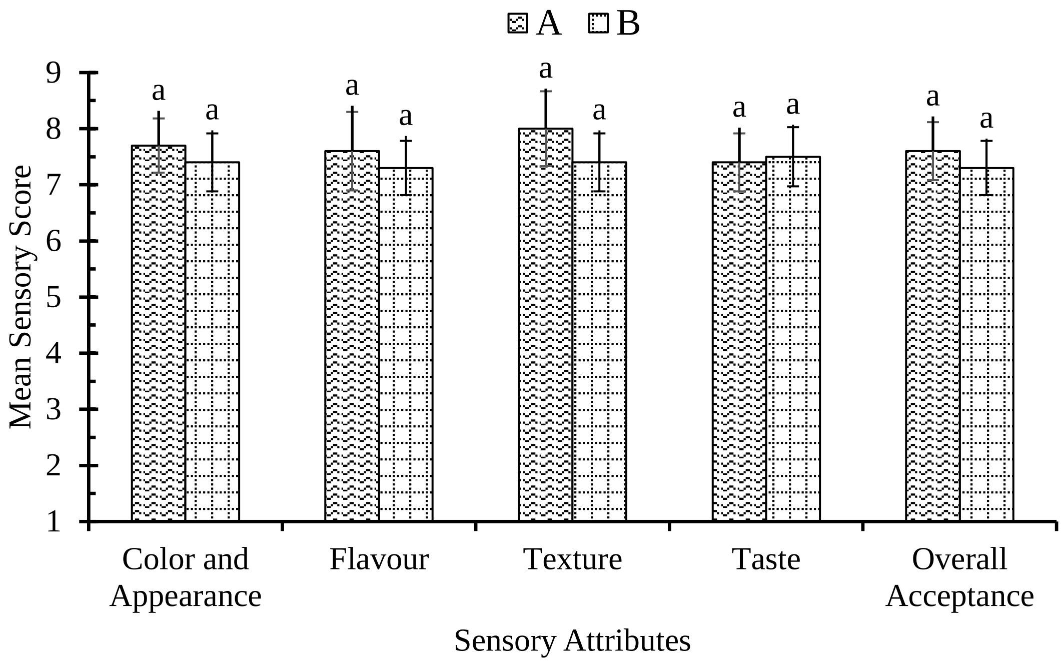


Fig. 4.9 Average sensory score for control and sample of steamed bread

Where A= Steamed bread with 100 parts wheat flour and B= Steamed bread with 87.89 parts wheat flour, 10.03 parts of soybean powder and 2.08 parts mushroom powder

The similar alphabet above the error bars indicate that the samples are not significantly different ($p \leq 0.05$) and the error bar represents the standard deviation.

The ANOVA at 95% level of confidence ($p \leq 0.05$) showed that the two steamed breads were not different from each other in terms of appearance and color, flavor, texture, taste and overall acceptance in sensory attributes.

4.4.1 Appearance and color

The average sensory score for appearance and color was 7.7 and 7.4 for sample A and B respectively. The analysis of variance showed that in case appearance and color, sample A and B showed no significant difference ($p > 0.05$). The soybean and mushroom incorporated steamed bread had no significant effect in appearance and color.

4.4.2 Flavor

The average sensory score for flavor was 7.6 and 7.3 for sample A and B respectively. The analysis of variance showed that in case of flavor, sample A and B showed no significant difference (p>0.05). The soybean and mushroom incorporated steamed bread had no significant effect in flavor.

4.4.3 Texture

The average sensory score for texture was 8.0 and 7.4 for sample A and B respectively. The analysis of variance showed that in case of texture, sample A and B showed no significant difference (p>0.05). The soybean and mushroom incorporated steamed bread had no significant effect in texture.

4.4.4 Taste

The average sensory score for taste was 7.4 and 7.5 for sample A and B respectively. The analysis of variance showed that in case of taste, sample A and B showed no significant difference (p>0.05). The soybean and mushroom incorporated steamed bread had no significant effect in taste.

4.4.5 Overall acceptance

The average sensory score for overall acceptance was 7.6 and 7.3 for sample A and B respectively. The analysis of variance showed that in case of overall acceptance, sample A and B showed no significant difference (p>0.05). The soybean and mushroom incorporated steamed bread had no significant effect in overall acceptance.

The analysis of variance showed that in case of appearance and color, flavor, texture, taste and overall acceptance, sample A (control) and B (superior sample) showed no significant difference (p>0.05). The soybean and mushroom incorporated steamed bread had no significant effect in appearance and color, flavor, texture, taste and overall acceptance.

4.5 Proximate composition of steamed bread

The control and superior formulation of steamed bread were analyzed for the determination of moisture, crude protein, crude fat, ash, crude fiber, carbohydrate content. The results obtained are tabulated in Table 4.12.

Table 4.12 Proximate composition of steamed bread

Parameters	Control	Sample
Moisture (%)	40.18±0.18	41.20±0.23
Crude Fat (%db)	2.94±0.10	2.95±0.09
Crude Protein (%db)	13.82±0.14	18.16±0.17
Ash (%db)	3.19± 0.08	3.98±0.11
Crude Fiber (%db)	0.40±0.07	0.99±0.09
Carbohydrate* (%db)	77.16± 0.54	73.13± 0.47
Energy (Kcal/100 g)	233.52± 0.51	230.35±0.57

* The carbohydrate is defined as the residue, excluding protein, lipid, fiber, and ash. Values are means ± standard deviations for three replications.

The value of moisture content, fat, protein, ash, crude fiber, carbohydrate and energy (per 100 g) of optimized formulation of steamed bread on dry weight basis was found to be 41.20%, 2.95%, 18.16%, 3.98%, 5.09%, 57.64% respectively. The moisture content of the control was slightly higher than that obtained by Mao and Singh (2019). High moisture content of the sample in comparison to control might be attributed to the hydration properties of proteins which increase the hydration properties of the dough during processing. Another possibility might be that, during steaming, the protein might have absorbed more moisture than the control sample (Kenny *et al.*, 2000).

As expected, the protein content of control and sample was found to be 13.82%±0.14 and 18.16±0.17% respectively. The higher protein content of the sample is due to addition of high protein soybean powder. With the increase of protein content, the carbohydrate content of sample found subsequently decreased.

4.6 Microbiological analysis of steamed bread

The optimized product samples were sealed in 40 µm LDPE zip lock pouches and stored at ambient condition at 30±2°C as well as refrigerated condition at 5±1°C. Growth of microorganisms in total plate count agar (TPCA) were noted daily until the count had reached a critical value of 6 log cfu/g. The results are presented in Fig 4.10.

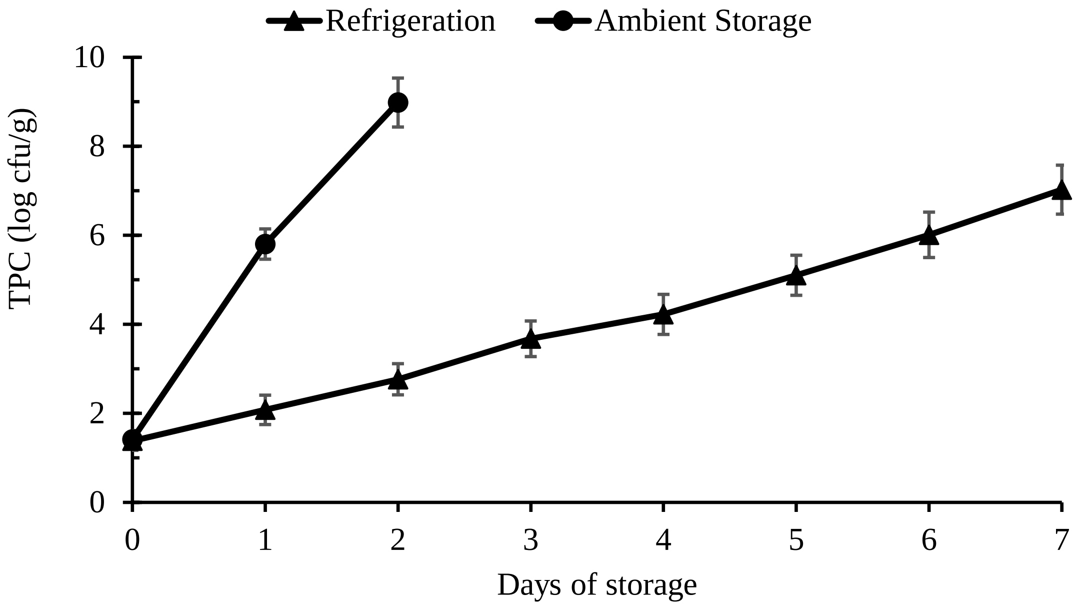


Fig. 4.10 Microbiological analysis of optimized product

Under refrigerated storage, TPC were found 1.38, 2.08. 2.77, 3.68, 4.22, 5.10, 6.01 and 7.03 log cfu/g from 0 to 7th days of storage and under ambient storage the counts were 1.41, 5.80 and 8.98 log cfu/g on 0, 1st, 2nd and 3rd days of storage.

Similar result has been reported by Sheng *et al.*, (2015), where steamed bread was found microbiologically safe only for a day at ambient temperature of 26°C. The initial yeast and mold load on the product was found in the current study to be below 1.5 log cfu/g, which is indicative of acceptable quality product.

In accordance with FSANZ (2018), the present study on microbiological analysis of bread revealed that the product is safe for consumption within the first five days when kept sealed in LDPE zipper bags of 40 µm thickness and stored at refrigerated storage at 5±1°C. After this period the product is considered as unsatisfactory as microbial count was found greater than 5 log cfu/g.

4.7 Cost and calorific value of steamed bread

Cost of soybean and mushroom incorporated steamed bread per 100 g was NRs 12 (as 0f 2021) including overhead cost and can contribute 230.35Kcal/100 g of product. The calculation is given in Appendix D.

Part V

Conclusions and recommendations

5.1 Conclusions

Based on results of current study, following conclusions are drawn:

1. The hardness and spread ratio were increased whereas, specific volume and L* (whiteness) of steamed bread were found decreased as refined wheat flour was gradually replaced with mushroom powder and soybean powder.

2. The hardness and spread ratio were decreased whereas, specific volume and L* of steamed bread increased when fermentation time increased up to certain level, the rate of change was found decreased afterwards.

3. Refined wheat flour, soybean powder and oyster mushroom powder ratio for steamed bread was found to be 87.89:10.03:2.08.

4. Optimum time for dough fermentation was found to be 67.07 min and steaming for 15.05 min for preparation of the steamed bread.

5. The values for protein and crude fiber content of the steamed bread were found increased in optimized product than that of control sample.

6. Steamed bread can safely be consumed within first 5 days of storage under refrigeration and only within 24 h when kept at ambient environment.

7. The cost and calorific value soybean and mushroom incorporated steamed bread per 100 g was found to be NRs. 12 (as of 2021) and 230.35 Kcal respectively.

5.2 Recommendations

From the present study following recommendation can be made;

1. Steamed bread can be prepared with incorporation of soybean and mushroom powder.
2. Effect of various preservatives and packaging materials for extension of shelf-life of steamed bread can be studied.
3. Staling of steamed bread over time with texture profile analysis (TPA) can be studied.

Summary

Steamed bread is made is usually prepared from wheat flour, which is mixed into a dough and then fermented before being cooked by steaming. The preparation process is similar to that of western-style pan bread, but the final product is steamed, not baked in an oven, so there are some differences in appearance and shape between pan bread and steamed bread. Steamed bread is white in color and has a soft, shiny surface.

The research work was mainly focused on formulation of ingredients (soybean and mushroom) and optimization of processes (fermentation and steaming time) of steamed bread. Soybean powder and mushroom powder were used to replace refined wheat flour within a range from 0 to 20 parts of each and in combination thereof. Fermentation time was varied from 30 to 90 min and steaming time by 10 to 20 min. With the use of two different experimental designs, best ingredients formulae and best process parameters suggested by design expert software, were taken for final product preparation. Proximate analysis, sensory analysis and microbiological study to find out shelf-life of the steamed bread were also studied.

The variation of ingredients for preparation of steamed bread were made by D-optimal mixture design using Design Expert version 7. The obtained ratio of wheat flour to soybean and mushroom powder for steamed bread were 100:0:0, 93.33:3.33:3.33, 90:10:0, 90:0:10, 86.67:6.67:6.67, 83.33:13.33:3.33, 83.3:3.33:13.33, 80:20:0, 80:0:20 and 80:10:10. Samples were analyzed for hardness, spread ratio, specific volume and L* (whiteness) as response variables. Increasing soybean and mushroom powder increased the hardness, spread ratio but decreased specific volume and whiteness of steamed bread, soybean powder showed less adverse effect on response parameters as compared to mushroom powder.

The variation in process parameters of steamed bread were made by response surface, central composite design using Design Expert version 7. Combination of fermentation to steaming time suggested by software were 102.43:15, 90:10, 90:20, 60:22.07, 60:7.93, 60:15, 30:20, 30:10 and 17.57:15. The magnitude of hardness found maximum at 30 min of fermentation reached a desired the lowest and gradually increased as process was continued further; L* (whiteness) of steamed bread was found decreased significantly ($p<0.0001$) when steaming time was increased. The effect of fermentation time was found pronounced on rest of response parameters in comparison with steaming time.

The experimental design allowed the fitting of a complete second order model to test main, interaction, linear and quadratic effects for all factors of interest as well possible to estimate a combination of factors to optimize formulation and process. The best combination was selected and based on Fisher's F-test, R^2, adjusted R^2, adequate precision and lack of fit which taken into account all these steps and contours/3-D surface plot were developed for selected response parameters.

Refined wheat flour, soybean powder and mushroom powder was found to be of 87.89, 10.03 and 2.08 parts respectively as optimized level of ingredients with the value of hardness, specific volume, spread ratio and L* (whiteness) of 2801.73 g, 1.75 ml/g, 1.58 and 73.87 respectively. Similarly, fermentation for 67.04 min and steaming for 15.05 min as optimized process conditions with value of hardness, specific volume, spread ratio and L* (whiteness) 2806.63 g, 1.74 ml/g, 1.49 and 75.07 respectively.

Physico-chemical composition of optimized steamed bread such as moisture, crude fat, crude protein, total ash, crude fiber and total carbohydrate in dry weight basis were found to be 41.20±0.23, 2.95±0.09, 18.16±0.17, 3.98±0.11, 0.99±0.09 and 73.13±0.47% respectively.

The sensorial analysis between sample A (control) and B (optimized sample) showed no significant difference ($p>0.05$) in terms of appearance and color, flavor, texture, taste and overall acceptance.

Storage stability of steamed bread packaged in 40 μ thickness LDPE zip lock bags was found to be 5 days on the basis of microbiological quality under refrigeration storage at 5±1°C and only a day at ambient storage of 30±2ºC. The cost and calorific value of the optimized product per 100 g was found to be NRs. 12 (as of 2021) and 230.35 Kcal respectively.

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Appendices

Appendix A

Sensory evaluation card

Hedonic rating test

Date:

Name of the judge: ………………………………….

Name of the product: Steamed Bread

Please test the following samples of steamed 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:

S.N.	Quality Parameters	Sample codes	
		A	B
1	Appearance and Color		
2	Flavor		
3	Texture		
4	Taste		
5	Overall Acceptance		

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

Like extremely-9 Like very much-8 Like moderately-7 Like slightly-6

Neither like nor dislike-5 Dislike slightly-4 Dislike moderately-3 Dislike verymuch-2

Dislike extremely-1

Any Comments:

Signature:

Appendix B

Table B Sensory Evaluation Score (A= control, B= sample)

Panelist	Formulation	Color and Appearance	Flavour	Texture	Taste	Overall Acceptance
1	A	7	8	8	7	8
2	A	8	8	7	7	7
3	A	8	7	8	8	7
4	A	8	7	8	7	8
5	A	8	8	7	8	7
6	A	8	7	9	8	8
7	A	8	7	8	8	8
8	A	7	7	8	7	7
9	A	8	8	8	7	8
10	A	7	9	9	7	8
1	B	8	8	8	8	7
2	B	7	8	7	7	8
3	B	7	7	8	8	7
4	B	8	7	7	8	7
5	B	7	7	8	7	8
6	B	8	8	7	7	7
7	B	8	7	7	7	8
8	B	7	7	7	8	7
9	B	7	7	8	8	7
10	B	7	7	7	7	7

Appendix C

ANOVA for sensory parameters

Table C.1 Multivariate tests[a]

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Panelist	Color and Appearance	2.450	9	.272	1.195	.397
	Flavour	3.450	9	.383	1.131	.429
	Texture	2.200	9	.244	.524	.825
	Taste	1.450	9	.161	.420	.894
	Overall Acceptance	1.450	9	.161	.475	.858
Formulation	Color and Appearance	.450	1	.450	1.976	.193
	Flavour	.450	1	.450	1.328	.279
	Texture	1.800	1	1.800	3.857	.081
	Taste	.050	1	.050	.130	.726
	Overall Acceptance	.450	1	.450	1.328	.279
Error	Color and Appearance	2.050	9	.228		
	Flavour	3.050	9	.339		
	Texture	4.200	9	.467		
	Taste	3.450	9	.383		
	Overall Acceptance	3.050	9	.339		
Total	Color and Appearance	1145.000	20			
	Flavour	1117.000	20			
	Texture	1194.000	20			
	Taste	1115.000	20			
	Overall Acceptance	1115.000	20			
Corrected Total	Color and Appearance	4.950	19			
	Flavour	6.950	19			
	Texture	8.200	19			
	Taste	4.950	19			
	Overall Acceptance	4.950	19			

Table C.2 Tests of between-subjects effects

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	1.000	3100.328[b]	5.000	5.000	.000
	Wilks' Lambda	.000	3100.328[b]	5.000	5.000	.000
	Hotelling's Trace	3100.328	3100.328[b]	5.000	5.000	.000
	Roy's Largest Root	3100.328	3100.328[b]	5.000	5.000	.000
Panelist	Pillai's Trace	2.029	.683	45.000	45.000	.897
	Wilks' Lambda	.038	.610	45.000	25.469	.928
	Hotelling's Trace	6.357	.480	45.000	17.000	.975
	Roy's Largest Root	3.997	3.997[c]	9.000	9.000	.026
Formulation	Pillai's Trace	.604	1.526[b]	5.000	5.000	.327
	Wilks' Lambda	.396	1.526[b]	5.000	5.000	.327
	Hotelling's Trace	1.526	1.526[b]	5.000	5.000	.327
	Roy's Largest Root	1.526	1.526[b]	5.000	5.000	.327

a. Design: Intercept + Panelist + Formulation

b. Exact statistic

c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Appendix D

Cost and caloric value of steamed bread

Table D.1: Cost estimation of steamed bread

Ingredients	Quantity (g)	Cost (NPR /1000 g)	Product cost (NPR)
Wheat flour	87.89	70	6.15
Soybean powder	10.03	90	0.90
Mushroom powder	2.08	1400	2.91
SMP	5	600	3.00
Sugar	5	80	0.40
Yeast	2	560	1.12
Vegetable oil	2	200	0.40
Salt	0.25	25	0.01
Water	40		
Total	152.25		14.89
Overhead cost*			2.98
Grand Total			17.87

The total cost 0f 152 g steamed bread = NPR 17.87

The cost of 1 g = NPR 0.116

Therefore, the cost of 100 g = NPR 11.6~12

* Overhead cost 20% of total cost

Table D.2: Calorific value estimation steamed bread

Parameters	Value (g/100g dry wt.)	Factor	Dry basis	41.2% moisture basis
Crude Protein	41.2	4	18.16	10.68
Crude Fat	2.95	9	2.95	1.74
Crude Fiber	18.16	4	0.89	0.52
Ash	3.98	0	3.98	2.34
Crude Fiber	0.99	0		-
Carbohydrate	73.13	4	73.13	43.00
Total (Kcal)	-	-	391.75	230.35

Appendix E

Table E.1 Materials used for product preparation

S. N.	Materials Used
1	*Momo* steamer (*Moktu*) with mould
2	Waxed paper sheet
3	Knives
4	Cabinet dryer
5	Heating vessels
6	Heating arrangement
7	Hygrometer, Thermometer
8	Muslin cloth
9	Packaging material: Zip lock LDPE
10	Semi-Automatic Kjeldahl protein determination set
11	Digital electronic balance
12	Hot air oven
13	Muffle furnace
14	Bacterial Incubator
15	Beakers
16	Volumetric flasks
17	Pipettes and micropipettes
18	Petri plates
19	Buchner funnel with vacuum pump
20	Refrigerator
21	BagMixer®, Autoclave
22	Electric hand dough mixture
23	Texture analyzer
24	Chroma meter
25	Serving trays
26	Disposable foil containers
27	Gloves
28	Apron
29	Hair net
30	Face masks

Table E.2 Chemicals used for product analysis

S. N.	Chemicals Used
1	Catalytic mixture (K_2SO_4 + $CuSO_4$)
2	Mixed indicator solution
3	Conc. H_2SO_4
4	Boric acid 4% aqueous
5	40% NaOH
6	0.1N HCl
7	Distilled water
8	Petroleum ether
9	Plate count agar
10	Potassium Metabisulfite (KMS)

Appendix F

List of typical words used and their meaning

Bouddha: One of the visiting sites of Kathmandu Nepal.

Mantou: Chinese steam bread or bun (In Chinese dialect).

Soybean *Masyaura/soybeanbadi:* A Texturized soybean product; soybean chunk.

Moktu: A utensil used for cooking/steaming *momo* or similar products.

Momo: Steamed dumpling stuffed with minced meat and/or vegetables with spices.

Purano Bazar: Traditional or old market (In Nepali dialect).

Appendix G

List of plates

Plate 1: Preparation of steamed bun (Trial)

Plate 2: Serving of steamed bun (Trial)

Plate 3: Drying of soybean and soybean

Plate 4: Soybean and mushroom powder

Plate 5: Partitioning of *moktu*

Plate 6: Preparation of steamed bread

Plate 7: Sample for physicochemical analysis

Plate 8: Sample for microbiological analysis

Plate 9: Shelf-life study of steamed bread

Plate 10: L* analysis with chroma meter

Plate 11: Hardness analysis with texture analyzer

Plate 12: Chemical analysis of steamed bread

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