FORMULATION OF *GUNDRUK* SOUP MIX AND ITS EVALUATION

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

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Approval Letter

This ***dissertation*** entitled ***Formulation of Gundruk Soup Mix and Its Evaluation*** presented by **Keshav Bogati** has been accepted as the partial fulfilment of the requirement for the **B.Tech. degree in Food Technology**

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(Keshav Bogati)

Abstract

Gundruk, an indigenous fermented food of Nepal, is known for its unique appetizing flavor. The main aim of the present work was to formulate and optimize *gundruk* soup mix using Design Expert® v. 7.125 for the DOE. Four types of *Brassica juncea* species (*rayo sag*) viz., Marpha, Marpha wide, Tankhuwa and mixed were collected from Parkhibas Agriculture Research Center and 4 types of *gundruks* made by adding 10% cabbage (hybrid T21) and fermenting for 9 days at 24±1ºC. The best *gundruk* was selected based on sensory score for soup mix preparation. Nine formulations were prepared by using *gundruk* powder, chilli powder, black pepper, in various amounts while keeping the amounts of tomato powder, corn flour, SMP, salt constant. The best sample was selected on the basis of sensory score (9 points hedonic rating for color, smell, flavor, taste, and overall) and analyzed for physicochemical properties (moisture content, crude fat, crude fiber, protein, total ash, Fe, Ca, Total plate count, Yeast mold and coliform).

The soup mix with 45.45% *gundruk,* 1.14% chilli powder, 1.14% black pepper and 1.14 garlic powder received the highest mean sensory score, and hence the best formulation. The best soup had 9.5% moisture content, 5.37% crude fat, 12.2% protein, 6.53% crude fiber, 19.0% total ash, 52.74% carbohydrate, 1325.6 mg/100 g Ca, 12.0 mg/100 g Fe, 7.2×10^3 cfu/g TPC, and 65 cfu/g yeast & mold.

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List of Abbreviations

Abbreviation	Full form
ADP	Adenosine Di-Phosphate
ANOVA	Analysis of Variation
AOAC	Association of Analytical Communities
ATP	Adenosine Tri-Phosphate
LAB	Lactic Acid Bacteria
SMP	Skimmed Milk Powder
STC	Salt Trading Corporation

Part I

Introduction

1.1 General introduction

Fermented foods have been one of the major nutrient items used widely in Nepalese kitchen. Fermentation of vegetables dairy products, cereals and beans and also alcoholic beverages, has been in practice in Nepal since a long time. A significant amount of fermented food is produced and consumed throughout the country (Karki, 1984).

Gundruk is one of the most significant fermented foods of Nepal which is consumed by almost all the households. It is popular for its uniquely appetizing taste or flavor. It is an ethnic fermented vegetable, a dry and acidic product indigenous to the Nepali living in the Himalayan regions of India, Nepal, and Bhutan. From nutritional standpoint, *gundruk* can be considered as a concentrated source of minerals, vitamins and therapeutically active compounds. It has been found that the regular consumption of *gundruk* has several nutritional benefits (Upadhaya, 2002).

The word '*gundruk*' is derived from the Newari word '*gundru*' (the Newaris being one of the ethnic groups of the Nepalese). Nepalese traditionally use it. The fermenting substrate for *gundruk* is usually '*rayo*' (*Brassica campestris* L var *cumifolia* Roxb) leaves. Other leaves such as radish (*Raphanus sativus* L) shimarayo (*Cardamine hirsute* L var *sylvatica*), cauliflower (*Brassica oleracea* L var *botrytis*), etc. are also used. *Gundruk* is usually prepared during the months of December to February when weather is less humid and there is an ample supply of vegetables. Prepared in other seasons, particularly during the monsoon, it is said to decay rapidly and to have an unpleasant flavor (Rao *et al*., 2005).

The preparation takes about week to a month. Leaves are dried in the sun (1-2 days depending on the weather). The dried leaves, after mild crushing, are soaked briefly in hot water and hand pressed in a perforated tin or earthen jar with heavy article such as a large stone to move surplus water or directly subjected to fermentation after crushing. The traditional methods differ from one ethnic community to another. They are then kept in warm and dry place for fermentation. The leaves are allowed to ferment in situ until a fermentation color develops. The *gundruk* is taken out and sun-dried for 2-4 days. It has shelf life of about one year (Rao *et al*., 2005).

Due to the demand of special foods by consumers and also because of time factor people now a days are attracted towards easy food. Convenience is a multifaceted concept and is often listed as the most important factors that determine the food of choice apart from cost, health, sensory acceptability and related concerns (Grunert, 2005). The demand of ready to eat or ready to cook minimally processed products has noticeably increased during recent years. Soup mix is becoming popular and better option for the population in this busy world. So, in this scenario, *gundruk* soup mix can serve as easy food in the kitchen which provides the necessary nutrients, preserves the precious time as well as provides the traditional appetizing taste to the people.

1.2 Statement of the problem

Traditional fermented foods are of greater importance from both nutritional as well as sociocultural point of view. Minerals, vitamins and several therapeutically active compounds can be obtained from these foods. *Gundruk* is one of the traditional fermented foods with above mentioned qualities. Besides, it has got the appetizing flavor and unique taste loved by the people of Nepal since ancient period of time (Tamang *et al*., 1988). Nowadays traditional foods *(gundruk*) are gradually disappearing from the diet as people have shifted towards more attractive and so-called hygienic foreign packed foods. Besides, due to hygienic questions of the products such as *gundruk* and its preparation materials and methods as well as containers, consumers are demotivated towards its consumption. *Gundruk* is a good source of nutrient but its consumption among the urban population is low because of the methodology adopted in preparation (Khadka, 2005). If the assurance of product quality can be given with proper methods and technological improvements in the traditional practices consumption can be increased. Besides, the time factor and presentation of the food plays important role towards the popularity of certain food product in today's context. They always have limited time for cooking and are searching for foods which take lesser time in cooking and have the desired flavor as well as quality. Also, the presentation and appearance of food has direct connection in the food choice of people (Grunert, 2005).

Literature survey shows that so many researches have been already done on formulation of soup mix of several commodities and its quality evaluation. Several researches have been done for the optimization of traditional method of *gundruk* making, selection of the best container for fermentation and other aspects of *gundruk* making but it is not found that research has been done for making *gundruk* soup mix. *Gundruk* being one of the popular fermented foods that has been consumed by the people since ancient period of time, has potential towards development of a soup mix along with other ingredients.

Thus, *gundruk* soup mix can be a better option and may have higher priority in peoples' kitchen than simply dried *gundruk*.

1.3 Objectives

The general objective of this dissertation is to prepare different formulations of *gundruk* soup mix using *gundruk* powder and other ingredients and find out the best formulation through sensory and chemical analysis.

Specific objectives include the following:

1. Preparation of *gundruk* using mustard leaves (*rayo sag)* and cabbage.
2. Preparation of *gundruk* powder and other ingredients powder.
3. Formulation of *gundruk* soup mix by incorporating different quantities of the powders.
4. Preparation of soup by adding the soup mix in hot water and sensory evaluation of the different formulations.

1.4 Significance of the study

This dissertation will use the knowledge from other literatures and researches done in the field of *gundruk* making and further use the prepared *gundruk* for making soup mix by blending the powdered *gundruk* with the powder of other ingredients like tomato, chili, spices, etc. This research will focus on the formulation of optimum blend of the ingredients with *gundruk* in their powdered forms. The successful completion of this work will provide us with the instant *gundruk* soup mix that can be easily be reconstituted into soup by simply adding the powder in boiling water just within seconds. This can help people to consume their traditional flavor of *gundruk* in attractive form of powdered mix by making soup in a very short period of time than cooking the dried *gundruk* and making soup.

1.5 Limitations and delimitations

a) Rheological properties of powders and soup were not studied.
b) Shelf-life study of soup mix was not done due to lack of time.

Part II

Literature Review

2.1 Fermented foods

A food is considered fermented when one or more of its components has been acted upon by microorganisms to produce a considerably altered final product acceptable for human use. Traditional fermented products are those products which are indigenous to people and people of the local area prepare them with the help of their ethnic knowledge from the ancestors. Traditional fermented food preparation is one of the oldest biotechnological processes around the world (Marshall and Mejia, 2011). Fermentation is a bioprocess technology which is practiced since time immemorial. Fermentation is mainly done with the help of microorganism, specifically lactic acid bacteria (LAB). The microorganism involved in fermentation are generally probiotic in nature which are good for health. When these microorganisms are grown in the food product, they enhance the nutritional property, with increasing the therapeutic property of food (Sekar and Mariappan, 2007).

Preservation of foods by fermentation is thought to have originated in the Orient before any recorded history. Basically, fermented foods are agriculture products, which have been converted by enzyme activities of microorganisms into desirable food products whose properties are considered attractive. In addition to the external properties, its nutritional value and keeping quality are in many cases better than the original. Moreover, if the manufacturing procedures are properly followed, the foods are safe for consumption. All of these beneficial properties of the final product increase the economic value of the original agriculture commodity. Fermentation is therefore an inexpensive and effective means of food production that could be utilized in alleviating world food problem. The term 'fermentation' may be defined as a process in which chemical changes are brought about in an organic substrate through the action of enzymes elaborated by microorganism (Prescott and Dunn, 1959). Stated differently, fermentation is an enzyme-induced chemical alteration in food. The enzymes involved in the fermentation may be produced by microorganism or they may be indigenous to the food. All fermentations are complex, but they vary considerably in their degree of complexity (Pederson, 1971).

The character of fermented food will be determined by the nature and quality of the food itself, the changes that occur as a result of the action of its inherent enzymes, the alterations that occur as a result the microbial fermentation, and the interactions that occur between the products of these activities and the constituents of the food. Fermentation by desirable microorganisms imparts characteristic flavor and texture to the fermented food. 1n most lactic acid fermentations, the high acidity, low pH and low oxidation-reduction potential attained are responsible for inhibition of other organisms, and of undesirable chemical changes. 1n fact, lactic acid bacteria carry on essential metabolic biological processes without oxygen by means of a complex series of intramolecular oxidation and reduction. These organisms are sometimes referred to as microaerophilic. The activities of these bacteria do not result in decomposition of the foods to their basic components, such as carbon dioxide, water, simple nitrates and sulfates: instead, the most commonly recognized end product of their metabolism is lactic acid derived from sugar. They alter other components to minor extent and some species produce other products from sugar. Because of the ability to convert carbohydrates to lactic acid, acetic acid, alcohol and carbon dioxide with only minor changes in other food components, this group of bacteria is considered very important to mankind in the preservation of edible and nutritious food (Battcock and Azam-Ali, 1998).

Carbohydrates, in particular the simple sugars, are the most readily available source of energy. Lactic acid is the most obvious product of lactic acid fermentation while alcohol and carbon dioxide are the common product of yeast fermentation. Although sugars furnish the energy for metabolic process, proteins, lipids, vitamins, nucleic acids and minerals are essential in the synthesis of cell protoplasm. 1n general, these must be supplied for growth since the fermentative organisms, particularly the bacteria have relatively poor synthetic abilities. Most foods contain sufficient amounts of these substances to permit active growth of fermentative microorganisms. Pure culture fermentation seldom occurs naturally. The requirements for growth supplied by natural constituents of food are so similar for both yeasts and many species of lactic acid bacteria that mixed fermentation normally occurs. The activity of enzymes indigenous to the food cannot be entirely ignored from a discussion of fermentation. Generally, such activity is associated with curing, ripening and aging rather than fermentation (Pederson, 1971).

2.2 *Gundruk*

Gundruk is one of the major fermented foods of Nepal. Fresh leaves of mustard, radish, and cauliflower are wilted for 2-3 days. The leaves are shredded, pressed into an earthen jar and covered with lukewarm (30-35°C) water and fermented at 16°C-20°C for 5-7 days. After fermentation, leaves are removed from the jar and sun dried for 3 days. *Gundruk* preparation is usually done in winter, when the weather is less humid. For large scale production of *gundruk*, pit fermentation is practiced in villages. A 2-3 ft deep pit of same diameter is dug in a dry place, and the pit is cleaned, plastered with mud, and warmed by burning. After removal of the ashes, the pit is lined with bamboo sheaths and paddy straw. Crushed withered leaves are dipped in lukewarm water, squeezed and pressed tightly into the pit, and then covered with dry leaves that are weighted down by stones or heavy planks. The top of pit is plastered with mud and left to ferment naturally for 15-22 day. Freshly fermented *gundruk* is removed, sun dried for 3-5 days, and stored at room temperature for future use (Tamang, 2010).

Gundruk can also be considered as a good source of minerals, vitamins and other therapeutically active compounds. It contains organic acids such as lactic acid, acetic acid, citric acid and malic acid. Cyanides and isothiocyanides are the main flavor components, followed by alcohols, esters and phenyl acetaldehyde. Increase of palmitic acid, alanine, leucine, lysine, and threonine have been observed in *gundruk* (Karki *et al*, 1983). Some LAB isolated from *gundruk* showed strong acidification, antimicrobial properties, and the ability to degrade antinutritional factors and also showed probiotic character (Tamang and Tamang, 2009).

2.3 History of *gundruk* making in Nepal

From time immemorial, *gundruk* has been made in the country. Whether in the village or in big cities, *gundruk* is relished by most Nepalese. *Gundruk* preparation is widespread in Nepal. This is basically because the traditional technology is rather straight forward, it does not demand extra requirements and raw materials are easily available (Upadhaya, 2002).

Gundruk is also a kind of leafy vegetables and is indigenous to Nepal. It is served as a side dish with the main meal and is also used as an appetizer. *Gundruk* is also important source of minerals particularly during the off season when the diet consists of mostly starchy tubers and maize which tend to be low in minerals. *Gundruk* is a non-salted fermented acidic vegetable product indigenous to Nepal, commonly prepared during winter when perishable leafy vegetables are plenty. The most common raw materials used for the preparation of *gundruk* in the country is mustard leaves. However, depending on the availability of raw materials, *gundruk* has been prepared in the country using various other leaves, e.g., raddish (*Raphanus sativus*), rapseed (*Brassica campestris* var. *toria*), cauliflower (*Brassica oleracea*) etc. (Upadhaya, 2002).

2.4 Technology of *gundruk* preparation

The green leaves are the principle materials for preparation of *gundruk*. Customarily, *gundruk* is prepared from the leaves of mustard, radish and *rayo*. However, depending on food habit and availability of leaves, various other leaves may be used. So far as the type of raw material is concerned, it has been found that *gundruk* made from mustard leaves are of superior quality, and in particular those made in hilly regions, where *gundruk* is made along with sinki. The process of *gundruk* making is quite simple. However, the preparation of *gundruk* merits superior hand. The green matured leaves are manually cleaned to remove the foreign matters. Then the leaves are withered in sun. Sun drying makes the leaves tender, which in turn facilitates filling in container.

The withered leaves are then crushed, as the crushing releases the juice on which fermentation occurs. This is soon followed by packing operation. *Gundruk* prepared with improper packing results in poor quality of *gundruk*. In tribal practice, *gundruk* is prepared by tamping crushed vegetables in earthern pots of dhungro (vessel made from bamboo tube). It has been reported that *gundruk* prepared by tight packing has relatively superior quality. The surface of the packed mass is tightly covered and the whole system is kept undisturbed in a warm place for several days (usually 5 to 15 days depending on the weather). It has been found that most villagers prefer to draw off the fermented juice that leaches out of the container. However, it is very unscientific practice as it removes all the nutritional attributes of *gundruk* (Upadhaya, 2002).

Fermentation usually completes within 5-15 days. However, it is the temperature that determines the rate of fermentation. Villagers usually work out the terminating point of *gundruk* fermentation by smelling the typical flavor. The fermented *gundruk* is then removed and finally dried in the sun until desired dryness is obtained. In some part of country, *gundruk* is made along with sinki, the process implies tamping of leaves of radish along with radish in alternate layers. The tamping is usually done in pits that have been previously cleaned and burnt. This variation of *gundruk* making is particularly common in the hilly region of the country (Upadhaya, 2002).

A general flowchart for traditional method for *gundruk* preparation is given in Fig. 2.1.

Leafy vegetable

↓

Wilting (1-2days)

↓

Crush mildly, soak briefly in warm water

↓

Pack into a pot, press tightly

↓

Ferment naturally for 15-22 days

↓

Take out freshly fermented *gundruk* and sundry for 2-4 days

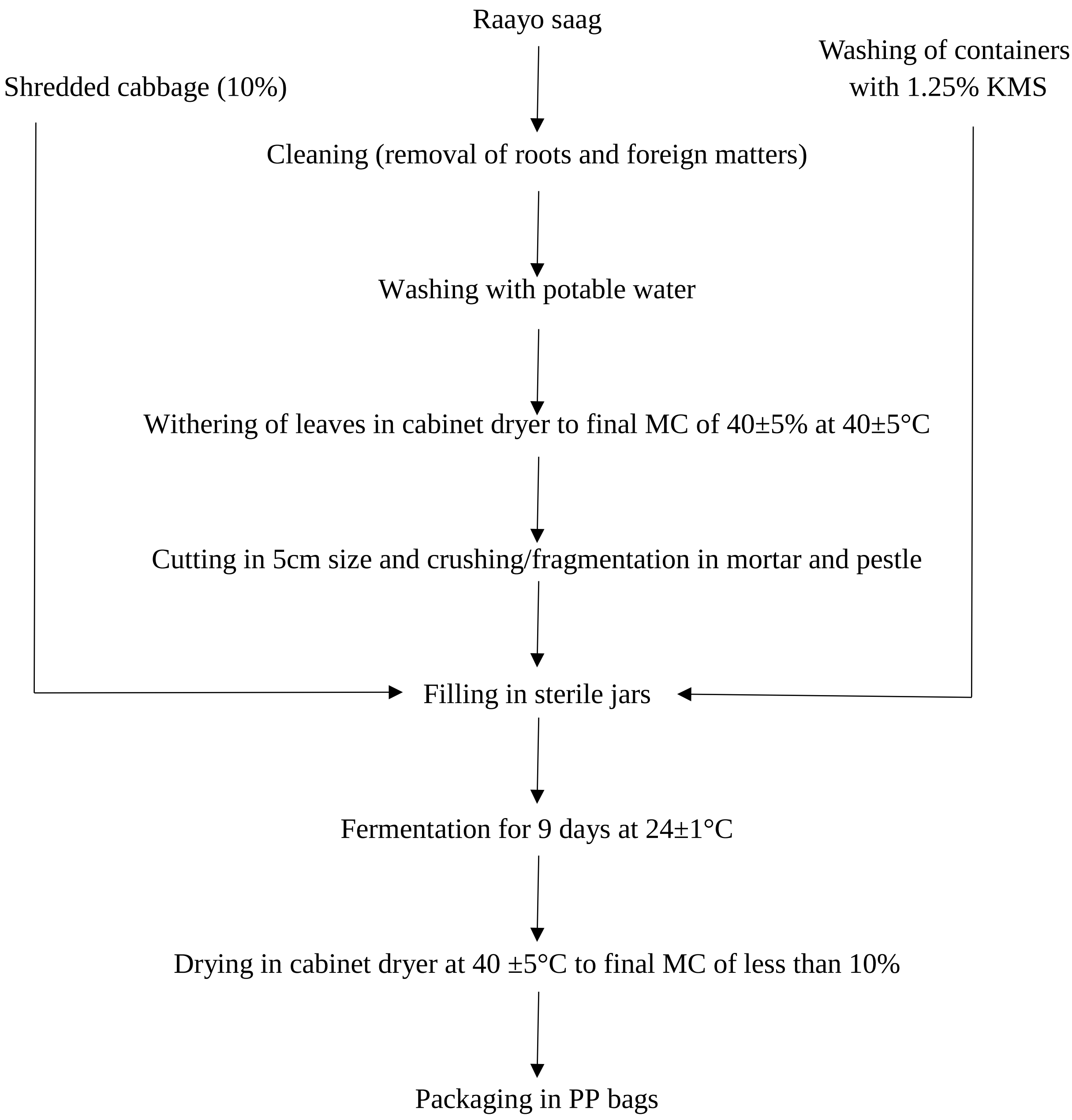
↓

Gundruk (dry)

Source: Tamang (2010)

Fig. 2.1 Traditional method of *gundruk* preparation

Several studies have been made on the improvement of *gundruk* quality by process modification. Focus has been given on the fermentation containers, pre-treatments of raw materials, method of drying, temperature control, etc. Fig. 2.2 shows an optimized method of *gundruk* preparation devised by Katwal *et al.* (2012).



Source: Katwal *et al*. (2012)

Fig. 2.2 Optimized method of *gundruk* preparation

2.5 Microorganisms during fermentation

Fresh vegetables contain a numerous and varied epiphytic microflora, including many potential spoilage microorganisms and an extremely small population of lactic acid bacteria (Battcock, 1998). The natural or spontaneous fermentation of vegetables is therefore the result of concerted actions of these microorganisms.

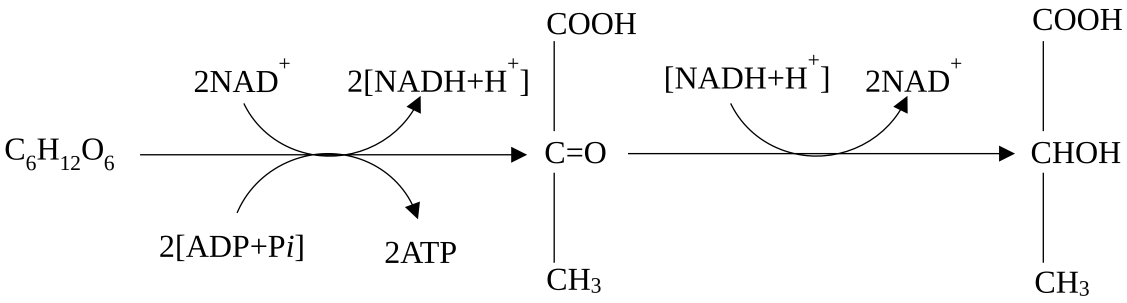
During natural fermentation there is distribution of homo-lactic and hereto-lactic flora, the homolactics form lactic acid whereas heterolactics form acetic acid, CO_2 and ethanol in addition to lactate which imparts typical and desirable flavor. The quality of *gundruk* mainly depends on the balanced production of lactic acid (about 50%) and acetic acid (about 35%), which is highly desirable to maintain stability in the production (Karki *et al.*, 1984).

Gundruk fermentation is primarily initiated by heterolacic *Lactobacillus cellobiosus* and homofermentative *Pedioocus pentosaceous*, and subsequently completed by more acid producing homolactic Lactobacillus plantarum (Karki *et al*, 1983). Pediococcus and lactobacillus species are the predominant micro-organisms during *gundruk*-fermentation. The fermentation is initiated by *L. cellobiosus* and *L. plantarum*, and other homolactics make a vigorous growth from the third day onwards. *Pediococcus pentosaceous* increases in the number on the fifth day and thereafter declines. During fermentation, the pH drops to a final value of 4.0 and the amount of acid (as lactic) increases to about 1% on the sixth day (Shrestha, 2002).

2.6 Lactic acid fermentation

Gundruk fermentation is based on lactic acid fermentation, which is the production of lactic acid and other compounds by the action of some microorganisms in sugary or starchy substrate. On the basis of the end product formed, the lactic acid fermentation may be homo-lactic and hetero-lactic. The lactic acid bacteria taking part in this fermentation are designated as homolactic (homofermentative) and heterolactic (heterofermentative). Heterolactic bacteria produce acetic acid, ethanol, formic acid and carbondioxide in addition to lactic acid. Fig. 2.3 is a repulative diagram of the lactic acid fermentation. For lactic acid fermentation, heterolactics are preferred since the end product (acetaldehyde and diacetyl) are responsible for the flavor of the product (Upadhya, 2002).

Two important genera of lactic acid bacteria are *Streptococcus* (Gram-positive cocci that tend to form chains) and *Lactobacillus* (Gram-positive rods that tend to form chains). In lactic acid fermentation pathways, pyruvate is reduced to lactic acid with the coupled re-oxidation of NADH to NAD^+. Outline of the pathway is shown in Fig. 2.3 and Fig. 2.4.



Pyruvate   Lactate

Fig. 2.3 Overall lactic acid fermentation pathway

The overall lactic acid fermentation pathway can be stoichiometrically shown as:

Glucose + 2ADP +Pi $\longrightarrow$ 2Lactic acid + 2ATP

When the Embden-Meyerhof scheme of glycolysis is used in the lactic acid fermentation pathway, the overall pathway is called homolactic fermentation because the only end product formed is lactic acid. The homolactic fermentation is carried out by *Streptococcus*, *Pediococcus* and various *Lactobacillus* species (Upadhya, 2002).

When the phosphoketolase pathway of glycolysis is used, the pathway is heterolactic, because ethanol and CO_2 are produced in addition to lactic acid. The ethanol and CO_2 come from glycolytic portion of the pathway. Fig. 2.4 depicts the scheme of heterolactic pathway (Upadhya, 2002).

The overall reaction of heterolactic fermentation can be written as:

Glucose +ADP +Pi $\longrightarrow$ Lactic acid + CO_2 + ATP

The heterolactic fermentation pathway produces only one molecule of ATP per molecule of glucose substrate metabolized. This fermentative pathway is carried out by *Leuconostoc* and various *Lactobacillus* species. Both the homolactic and heterolactic fermentations pathway have important practical applications. The homolactic fermentation pathway is quite important in dairy industry. It is the pathway that is responsible for souring of milk and is used in the production of numerous types of cheese, yoghurt and various other dairy products (Rao *et al*., 2005).

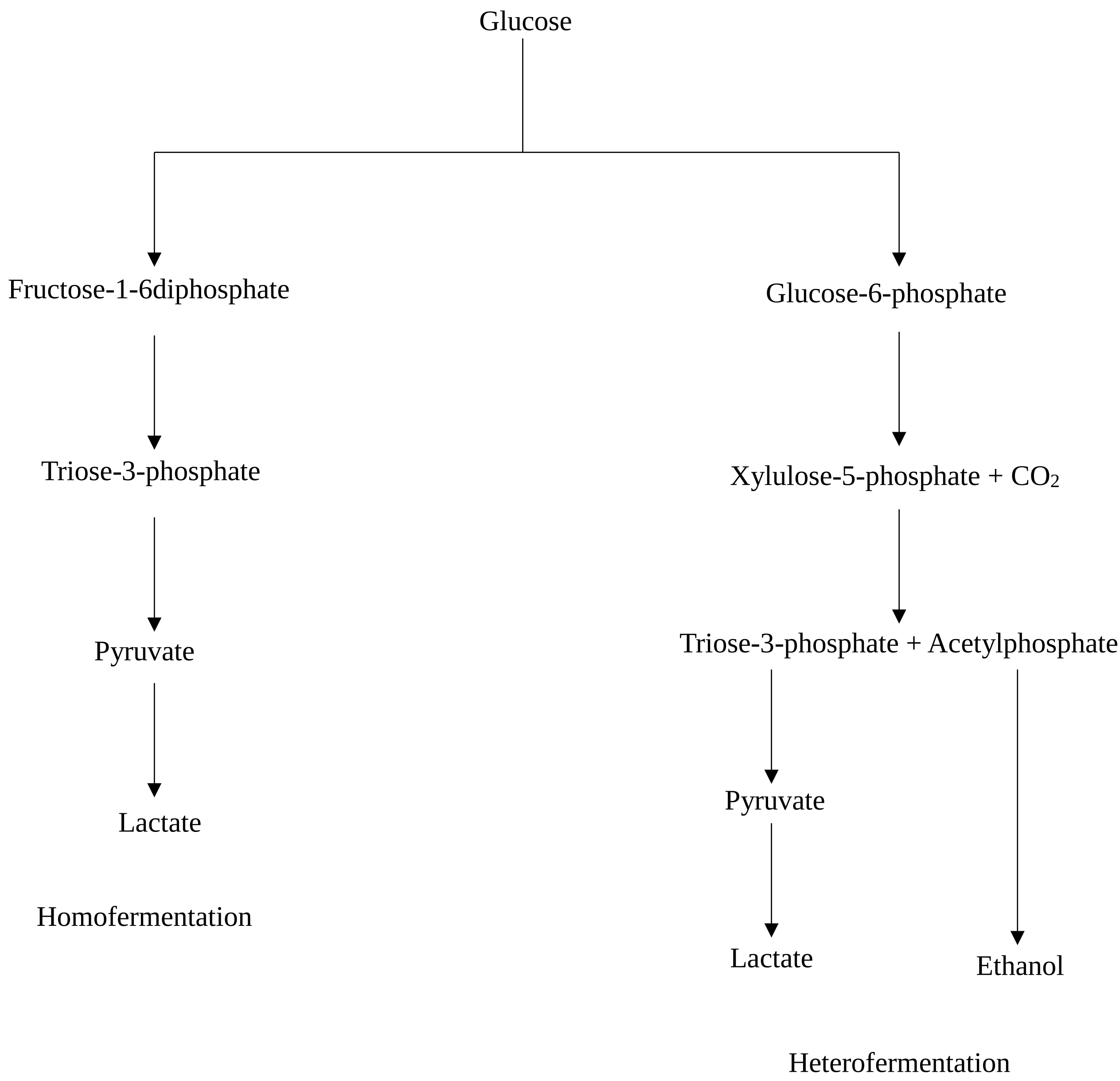


Fig. 2.4 Homo-lactic and Hetero-lactic fermentation pathways

2.7 Changes during *gundruk* fermentation

2.7.1 Chemical changes

The predominant chemical change in case of *gundruk* fermentation is conversion of sugar to organic acids, particularly lactic acid and acetic acid. In *gundruk* Fermentation, sugar present in leaves is converted into lactic, acetic and other minor acids and small amounts of alcohols. On the basis of end products formed, *gundruk* fermentation may be a homo or hetero-lactic fermentation. The LAB involved, by analogy, are designated as homo-lactic (homo fermentative) and hetero-lactic (hetero fermentative). The homolactics produce lactic acid mainly via Embden-Meyerhoff scheme of glycolysis and mainly involve *Streptococcus, Pediococcus* and various *Lactobacillus* species. The heterolactics consists of *lactobacillus* and *Leuconostoc* species. They produce acetic acid, ethanol, carbon-dioxide, etc., in addition to lactic acid. For lactic acid fermentation, heterolactics are preferred since the end products (acetaldehyde and diacetyl) are responsible for the flavor of the product. However, both homolactic and heterolactic fermentations have important practical implications (Karki *et al*., 1983).

During the course of fermentation, acidity increased by many folds. The final product contains about 0.5% acidity as lactic acid. The quality of *gundruk* mainly depends on the balanced production of lactic acid (about 50%) and acetic acid (about 35%). Organic acids not only contribute to the desired taste and flavor of the final product but also make the substrate unfavorable for the proliferation of spoilage and other undesirable microorganisms. At the same time the acid makes substrate more suitable for the growth of microorganisms that improve the properties of the food. The combined effect of these acids along with various metabolites, CO_2 and ethyl alcohol contributes to the characteristics flavor and texture of *gundruk* (Kharel *et al*., 2007).

2.7.2 Changes in amino acids, lipids and flavors

Various changes in amino acids occur during *gundruk* fermentation. The extent of changes in all the 20 amino acids varies with the type of vegetable used for fermentation. Glutamine, alanine, and leucine increase more whereas threonine, glycine, cysteine, methionine, isoleucine, phenylalanine, and lysine increase less during fermentation. Asparagine, glutamine, proline, tyrosine, histidine, and arginine decrease but aspartate, tryptophan, and valine remain almost constant during fermentation (Kharel *et al*., 2007).

The polar lipids constitute the major lipid component (Karki *et al*., 1983). Other important lipid components are mainly comprised of free fatty acids, triglycerides, diglycerides, hydrocarbon, and unknown fractions. The most pertinent alteration of lipids during *gundruk* fermentation is the substantial increase in free fatty acids fraction. The glycerides and some unknown fractions are hydrolyzed during *gundruk* fermentation., liberating free fatty acids fractions that may be eventually beneficial for the generation of desirable ester like flavor in *gundruk* (Upadhaya, 2002).

The main flavor components of mustard vegetable *gundruk* comprises of cyanides (15.7%), isothiocyanates (8.5%), followed by alcohols (12.5%) and esters (4.1%) (Upadhaya, 2002). Cauliflower *gundruk* consisted of alcohols (50%) as the major flavor components followed by cyanides (6.5%), isothiocyanides (6.1%) and esters (2.3%) (Karki *et al.*, 1983).

2.8 Natural preservation of *gundruk*

Gundruk possesses a good barrier system. Its shelf-life is 6 to 12 months (Karki, 1984). It is a self-stable product because of its remarkable natural properties that prevent the product from being spoiled and decomposed. The following are the main properties contributing to building of the natural preservative system in *gundruk.*

2.8.1 Depletion of nutrients (sugar)

At the end of fermentation, the sugar which is the source of carbon and energy for microorganisms is almost exhausted due to its conversion to acids and alcohols. As a result, various spoilage microorganisms including yeast and mold will not grow (Karki *et al*., 1983).

2.8.2 High acidity

Gundruk is highly acidic and it has been found that it attains acidity upto 1.0% (as lactic, wet basis) and the pH below 4, which is enough to prevent the growth of *Clostridium botulinum* (a pathogen) and other spoilage microorganisms (Upadhaya, 2002).

2.8.3 Low moisture

Low moisture content of *Gundruk* (dried *gundruk*) prevents the growth of mold and other spoilage organisms. The moisture is reduced to below 10%. Thus, these intrinsic factors make *gundruk* a shelf-stable product requiring no additional preservative (Dahal *et al*., 2007).

2.9 Improvement of *gundruk* processing

Hygienic and nutritional quality of *gundruk* can be improved by using selected strains of lactic acid bacteria. His study has revealed that *gundruk* fermentation is primarily initiated by heterolactic *Lactobacillus cellobiosus* and homofermentative *Pediococcus pentosaseous* and subsequently completed by more acid producing homolactic Lactobacillus plantarum. The quality of *gundruk* has been primarily judged on the basis of acidic taste and typical *gundruk* flavor. These two characteristics has been embodied as a key indicator of quality and therefore Lactobacillus plantarum, *Leuconostoc cellobiosus* and *Pediococcus pentosaceus* have been selected as important microorganisms for improvement of *gundruk* processing. The further study indicates the possibility of improvement in the quality of *gundruk* by synergistic action of *L. cellobiosus, P. pentosaceus* and *L.plantarum* (Karki *et al.*, 1983).

2.10 Beneficial effects claimed for lactic acid bacteria

It has been acknowledged that lactic acid fermented food products have several advantages, the more important of which are:

1. Nutritional improvement of food
2. Inhibition of enteric pathogens
3. Hypothetical esteemed value
4. Anticancer activity
5. Stimulation of immune system

2.11 Chemical composition of *gundruk*

From nutritional standpoint, *gundruk* can be considered as a concentrated source of minerals, vitamins and therapeutically active compounds (Upadhya, 2002). Tamang (2006) reported protein, fat, carbohydrate and ash of *gundruk* to be 38.7%, 2.1%, 38.3% and 22.1% reapectively on dry basis. Shrestha (2010) reported moisture, crude fiber, ash, calcium, and iron content of *gundruk* made from cabbage to be 7.92%, 14.65%, 0.68%, 2253 mg/100 g and 86.4 mg/100 g respectively. Shrestha, 2002 observed crude protein, fat, crude fiber and ash content as 33%, 2.1%,57.68%, and 0.68% respectively. Table 2.1, Table 2.2 and Table 2.3 represent the chemical composition of *gundruk* reported by different authors.

Karki *et al.* (1983) reported 0.8% and 1% acidity as lactic acid in mustard *gundruk* and cauliflower *gundruk* , respectively, after fermentation for 7 days at 20–22 °C. Palmitic acid (26.8%) was found to be the dominant fatty acid followed by linoleic (13.9%), linolenic

(12.8%), and oleic (10.3%) in mustard *gundruk* . However, cauliflower *gundruk* consists of linolenic acid (28.9%) as the dominant fatty acid followed by palmitic acid (20.6%). The total nitrogen of mustard *gundruk* and cauliflower *gundruk* was found to be 3.38% and 1.56%, respectively. Glutamic acid (13.6%) was found to be dominant in mustard *gundruk*, whereas proline (15.2%) was dominant in cauliflower *gundruk.* The dominant flavor compounds in mustard *gundruk* are cyanides (15.7%) followed by alcohols (12.3%), isothiocyanates (8.5%), and esters (4.1%). However, cauliflower *gundruk* consists of alcohols (50%) as the major flavor compounds followed by cyanides (6.5%), isothiocyanates (6.1%), and esters (3.2%).

Acidity is the major factor that contributes to desired taste of *gundruk*. Higher acidity in *gundruk* is preferred by the people. The combined effect of the organic acids along with various metabolites, CO_2 and ethyl alcohol contributes to the characteristics flavor and texture of *gundruk* (Kharel *et al*., 2007).

Table 2.1 Chemical composition of cabbage *gundruk*

Parameters	Value
Moisture (%)	7.92
Crude fiber (g/100 g)	14.65
Ash (g/100 g)	0.68
Calcium (mg/100 g)	2253
Iron (mg/100 g)	86.4
Acidity (% as lactic acid)	1

Source: Shrestha (2010)

Table 2.2 Chemical composition of mustard *gundruk*

Parameters	Value
Moisture (%)	15
Crude Protein (%)	38.7
Crude Fat (%)	2.1
Crude Carbohydrate (%)	38.3
Acidity (% as lactic acid)	0.49

Source: Tamang (2006)

Table 2.3 Chemical composition of mustard *gundruk*

Parameter	Value per 100 g dry matter
Calories (cal)	19-30
Protein (%)	3.5
Fat (%)	0.1
Carbohydrate	1-2
Riboflavin (mg)	0.2
Niacin (mg)	0.5
Thiamine (mg)	0.07
Ascorbic acid (mg)	55.0

Source: Kharel *et al.* (2007)

2.12 Soup mix

2.12.1 Introduction

Convenience is a multifaceted concept and often listed as the most important factors that determine the food of choice apart from the cost, health, sensory acceptability and related concerns (Grunert, 2005). Many food manufacturers now use scientific approach in order to achieve the best product formulations. Convenience also decides to a greater extent when, where, what and how to eat foods. As a consequence, the demand of ready to eat or ready to cook minimally processed products has noticeably increased during the recent years (Brunner *et al*., 2010).

Instant food is very popular in modern society. Soup is one of the top instant foods which people like so much with other fast food item. It is actually a part of modern daily life. Soup is very much convenient to eat. It is now fulfilling the consumer's social requirements.

Soup is a liquid which is prepared from vegetables, fish or meat with water, juice or stock and some thickening agents and fall under heterogenous category of food. Usually there are two kinds of soups like thick soup and clear soup. Thick soups are prepared by mixing powder of cereal or pulse flour, cream and eggs. Thin soups are made from clear extracts of plant parts and animals which are edible (Radha *et al.*, 2015).

Soup mix is the blend of various dried and powdered ingredients in the optimum ratio so as to obtain the required flavor when made into soup with hot water. They are almost ready to eat and take less time to cook as they can be directly added in boiling water and stirred to get the soup within minutes. It has an important role for maintaining nutritional status of people by covering a wide range of dried foods. Soup mix is almost free from pathogenic attack and it can preserve its quality until one month under normal condition. Here major preservative action is due to the lowering of moisture content as all the components are dehydrated and powdered. There is a big demand for dry soup mixes in the global market. Generally commercial production of instant soup mixes largely depends on the physiochemical and rheological properties at the time of preparation. Rheological properties deal with the deformation and flow of matter. These properties are important to understand the behavior of the food structure during processing (Abeysinghe and Illeperuma, 2006).

2.12.2 Various soup mixes

Haleem and Omran (2014) gave the study on preparation of dried vegetarian soup supplemented with few legumes. Soup can be prepared with the combination of vegetables like potatoes, carrot, tomatoes, onion, garlic and coriander with few legumes such as hull-less barley flour, lentil, green pea and chicken pea. The result from chemical, physical, rheological and sensory evaluation indicated that supplemented vegetable soup with legumes improved the nutritional value of soup and especially enhanced good protein digestibility with other nutrient like carbohydrates, fats, iron and zinc, extended shelf-life and stability of dried soup by reduced content of moisture and water activity. Supplementation affected taste, color, flavor and overall acceptability but did not affect the thickness and appearance of the soup.

Rab is a traditional Rajasthani dish prepared from maize grits or flour and buttermilk. Value added instant rab mix was developed and its quality was evaluated. The soup made had thick consistency. For enhancing the nutritional value of rab, green gram dhal and spinach leaf powder was added to the mix. After value addition nutrient of mixes increased such as protein, ash and iron content. Shelf-life study for a period of 4 months showed negligible growth of Coliforms and S. aureus (Mogra and Choudhary, 2014).

Study has been made on the innovation of healthy vegetable soup powder supplemented with soy flour, mushroom, moringa leaf, etc. Supplementation of soy flour, mushroom and moringa increased the nutrient in soup like vitamin D, vitamin C, sodium, potassium, magnesium, zinc, iron, protein, and fiber and also decreased the amount of moisture, fat, and carbohydrates compared to the available soup powders in market. These supplemented food products could play important role combating protein energy malnutrition (Frazana *et al.*,2016).

Studies have been made on the development and storage stability of legumes and vegetable soup powders. Legumes which were unconventional such as motor dahl (pea) could be effectively used in the formulation of nutrients rich soup powder combination of other ingredients or using dried vegetables. Legumes are rich source of protein and energy and control the cholesterol level because these ingredients are plant products. The instant soup prepared from legume required only 5 min which was very helpful for the working women and easily prepared convenient food with good amount of nutrients which attracted more consumer. This soup mix which is rich in protein and calorie can be use as supplementary food for combating problems like malnutrition, undernutrition, hypertension, and is useful for different age groups (Rokshana *et al.*, 2007).

Study on "Development of instant food mixes from dehydrated pumpkin (*Cucurbita moschata*)" was carried out in the Department of Food Science and Technology Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan during the year 2013-2015. The objectives of the study were to develop instant food mixes by using dehydrated pumpkin to evaluate their quality during storage and to work out the cost of production. The raw material for the study was procured from the local market, Solan. The pumpkin powder and seed powder were prepared by following standard methods. The pumpkin powder, seed powder and broken wheat were analyzed for various quality characteristics. The recipes as well as the methods for preparation of Instant *Halwa* Mix (IHM), Instant Soup Mix (ISM) and Instant Porridge Mix (IPM) were developed and standardized. The instant food mixes were then packed in polyethylene pouches (PEP) and aluminum laminated pouches (ALP), labeled and stored at ambient temperature. The products were evaluated for chemical and sensory attributes at different storage intervals (0, 3 and 6 months). The cost of production of all the products was also worked out to see their economic feasibility (Devi, 2015).

Moringa pod soup powder has been prepared by optimizing the level of ingredients like Moringa pod powder, corn flour, spice mixture, salt, etc. Developed soup powder proved to be a rich source of protein and crude fiber. HPLC analysis of the developed soup powder shows that it contains high level of phenolic compounds and flavonoids. Thus, it could be used as rich source of nutrients and phytochemicals (Karishma *et al.*, 2019).

2.12.3 Ingredients of the soup mix

a. Major ingredient

It is the ingredient of soup mix whose taste and flavor is targeted to provide in the final soup made from the mix. Several soup mixes are available like mushroom soup mix, chicken soup mix, pumpkin soup mix, etc. where mushroom, chicken and pumpkin are the major ingredients respectively.

Mushroom powder was prepared by using microwave dried mushrooms which were pretreated with 0.5% KMS + 0.2% citric acid for 30 min before drying to prevent browning. Wheat flour, salt, fat, sugar, onion and garlic powder, dried pieces of mushroom and skimmed milk powder were mixed with mushroom powder in different proportions to formulate three recipes for soup powder and packed in poly propylene pouches (100 gauge) and stored at room temperature (21-35 ºC) (Frazana *et al*., 2016).

Dehydrated pumpkin soup mix was prepared where pumpkin is the major ingredient. Pumpkin powder and seed powder were prepared by following standard methods. Pumpkin powder, seed powder and wheat powder were analyzed for various quality characteristics. The recipes as well as methods for preparation of Soup mix were developed and standardized. Pumpkin powder is good source of beta-carotene (7.77 mg/100 g), fiber (2.91%) and protein (5.04%) (Devi, 2015).

Sisno soup powder can be prepared by blending Sisnu powder (67%), corn flour (17%), salt (10%), garlic powder (3.5%), citric acid (1.5%) and chili powder (1%). Good quality and nutritionally rich soup mix was prepared which was a good source of protein, fiber, minerals (like calcium, potassium), vitamin C, chlorophyll and phenols (Thapaliya *et al.*, 2014).

b. Thickener

Thickeners are substances which when added to an aqueous mixture, increases its viscosity without substantially modifying its other properties like taste. They provide body, increase stability and improve suspension of added ingredients. Some of the thickeners are polysaccharides (starches, vegetable gums, and pectin), proteins (eggs, collagen, blood albumen) and fats (butter, oil and lards). All-purpose flour is the most popular food thickener, followed by corn starch and arrowroot or tapioca (Anon., 2019).

Corn starch is actually a flour. It is the endosperm of corn kernels that has been dried and ground. Corn starch is used as thickening agent in soups and liquid-based foods, such as sauces, gravies and custard. It is sometimes preferred over flour because it forms a translucent mixture rather than an opaque one. As the starch is heated, the molecular chains unravel, allowing them to collide with other starch chains to form a mesh, thickening the liquid (Anon., 2019).

c. Other ingredients

Other ingredients mainly include the spices and seasoning agents. Spices are used extensively by soup manufacturers for providing full rounded flavor of each type of soup mix. They have important role in preservation of the food products, taste and nutritional point of view. Most commonly used spices are coriander, cumin, fennel, fenugreek, chili, black pepper, ginger, garlic, etc. These have a number of functional components such as phytochemicals and other bioactive chemical compounds which plays an important role in defense system and also used as fragrances and flavor compounds (Rathore *et al.*, 2013).

Tomato powder is also one of the ingredients of soup mix. Tomatoes are known to be good source of vitamins, minerals and carotenoids; especially vitamin C, phosphorous, potassium and lycopene. Besides it also has characteristic color and flavor. So, has become an ideal addition to different types of processed foods (Correia *et al*., 2015).

SMP is another ingredient of soup mix. It is the product resulting from the partial removal of fat and water from pasteurized milk. To give the effect of creamy texture on reconstitution WMP is used but under certain conditions instability of the butterfat fraction is obtained on storage giving rise to off flavors. Thus, SMP is used which has beneficial effect on body, creaminess and flavor of the soup mix (Anandharamakrishnan, 2017).

Common salt is an ingredient of all soup mixes. The amount used depends on the type of soup mix and consumer preference.

2.13 *Gundruk* as potential soup mix

Gundruk is the most common fermented foods in Nepal. It can be prepared easily in the local level and people have been preparing it with the traditional knowledge. It contains several nutritional components and has got therapeutic value. Besides the unique appetizing flavor adds to the popularity of *gundruk*. So, it is a potential item in terms of low price, easy processing, and good source of nutrients. Literature survey shows that so many researches have already done on formulation of instant soup mix of several commodities and its quality evaluation. But none was found based on *gundruk*. *Gundruk* being one of the popular fermented foods that has been consumed by the people since ancient period of time, has potential towards development of a soup mix along with other ingredients.

Part III

Materials and methods

3.1 Materials

3.1.1 Green leafy vegetable

Mustard green (*Brassica juncea)* of different varieties was brought from Pakhribas Agriculture Research Center (27°02’51.5” N 87°17’36.6” E). The different varieties were Marpha, Marpha wide, Tankhuwa and Mixed. The total amount of leaves brought was 10 kg and the leaves were of optimum maturity (about 40 days). Leaves were packed in a sack and brought by motorcycle. Leaves were immediately cleaned and kept in cabinet dryer for withering.

Cabbage of hybrid variety T21 was brought from Tarahara Agriculture Research Center (26°42’07.1” N 87°16’38.9” E). 2 kg of fully mature cabbage was brought in a polythene bag. Outer leaf was removed and it was subjected to further processing.

3.1.2 Tomato, corn flour, SMP, salt and spices

5 kg fully matured tomato was brought from the local market of Dharan. 1kg corn flour packed and marketed by Real Agro Foods Pvt. Ltd (Jharkhand, India) was bought from Bhatbhateni, Dharan. 1 kg SMP manufactured by Singhania Industries (Birgunj, Nepal) was bought from Bhatbhateni. Garlic powder, chili powder and black pepper powder packed and marketed by Bhatbhateni was used. Iodized salt marketed by STC (Salt Trading Corporation) was used.

3.1.3 Fermentation container

Food grade, air tight, odorless and non-breakable plastic jar of 1-liter capacity was used.

3.1.4 Equipment

The following equipments were used in this study:

a. Cabinet dryer (AISET YLD-2000)
b. Electronic balance (AMPUT Electronic Balance Model No-457, Sensitivity ± 0.01 g)
c. Incubator (VITCO)
d. Hot Air Drying Oven (VITCO)
e. pH meter (HANNA HI 96017, Sensitivity ± 0.01)
f. Sieves (ENDECOTTS LTD. LONDON)

3.2 Methods

3.2.1 Preparation of raw materials for fermentation

Fresh *rayo saag* (*Brassica juncea*) was brought and preliminary treatments was done viz. Cleaning, washing and removal of roots. Cabbage was cleaned and shredded to 5 cm length. The cleaned leaves were withered in cabinet dryer to moisture content of 40±5% at 40±5°C.

3.2.2 Fermentation

The withered leaves were cut into 5 cm length and crushed in a clean mortar. The leaves were tamped gently and uniformly (placing little amount at a time making alternative layer with shredded cabbage) in sterile fermentation jars. The mouth of jars were tightly closed by covering with clean polythene bags. The samples were fermented for 9 days at 24±1°C in incubator.

3.2.3 Drying

Drying of the product was done in cabinet dryer at temperature 40±5°C to the moisture content of less than 10%.

3.2.4 Flowchart for *gundruk* preparation

The flowchart for *gundruk* preparation is shown in Fig. 3.1.

Raayo saag

↓

Cleaning (removal of roots and foreign matters)

↓

Washing with potable water

↓

Withering of leaves in cabinet dryer to final MC of 40±5% at 40±5°C

↓

Cutting in 5cm size and crushing/fragmentation in mortar and pestle

↓

Shredded cabbage (10%) → Filling in sterile jars ← Washing of containers with 1.25% KMS

↓

Fermentation for 9 days at 24±1°C

↓

Drying in cabinet dryer at 40 ±5°C to final MC of less than 10%

↓

Packaging in PP bags

Source: Katwal *et al*. (2012)

Fig. 3.1 Outline of *gundruk* preparation

3.2.5 Selection of best *gundruk* by sensory analysis

Sensory analysis was performed for the selection of best *gundruk* according to 9-point Hedonic Scale as per Rangana (1986). The parameters for sensory evaluation were taste, flavor, smell, sourness and overall. The obtained data was analyzed statistically by GenStat Discovery Edition 3, for Analysis of Variance (ANOVA) at 5% level of significance. The data obtained from sensory evaluation were subjected to two-way Analysis of Variance.

3.2.6 Preparation of ingredients powder

Gundruk powder was made by grinding *gundruk* and sieving through 30-mesh screen. The ground powder is sieved through sieve of 60-mesh screen (Devi, 2015).

Tomato powder was prepared by drying tomato at temperature of 60°C for 48 h and grinding (Correia *et al*., 2015). Other ingredients were brought in powder form from the local market of Dharan.

3.2.7 Determination of threshold value of each ingredient

Threshold value for each ingredient was determined by Discriminatory testing in the lab of Central Campus of Technology through sensory. 100 ml of boiled water was taken in a beaker and each ingredient was added roughly by approximation. It was stirred properly and sipped. Lower and upper limit for each ingredient was determined in 100 ml boiled water (Sharif *et al*., 2017).

3.2.8 Determination of amount of fixed ingredients

For determination of amount of fixed ingredient Ranking test was performed but within the range obtained from threshold value determination. Several coded samples were provided and panelists were asked to rank the samples according to their desirability. An optimal value of the ingredients was determined that gave optimum sensory characteristics (Sharif *et al*., 2017).

3.2.9 Formulation of recipe using design expert

Design Expert v7.0.0 software was used to create the recipe. “User defined mixture design” was used to formulate the recipe as given in Table 3.1. Similar formulations were excluded to keep the work less complicated and result oriented. Those formulations having extreme ranges of the ingredients were also excluded. As the range was already determined and the optimum values were predetermined by sensory evaluation, those formulations having the values of ingredients in the extreme range need not be included in the research for optimization of the soup mix recipe.

Table 3.1 Recipe formulation for *gundruk* soup mix

Ingredients (g/100 g)	A	B	C	D	E	F	G	H	I
Gundruk	42.27	44.32	42.05	43.18	43.18	39.77	45.45	40.91	40.91
Garlic	3.41	2.27	1.93	1.14	2.27	3.41	1.14	3.41	2.27
Chili	1.14	1.14	1.14	2.27	2.27	2.50	1.14	1.14	2.27
Black pepper	2.05	1.14	3.41	2.27	1.14	3.41	1.14	3.41	3.41
Salt	11.36	11.36	11.36	11.36	11.36	11.36	11.36	11.36	11.36
SMP	22.73	22.73	22.73	22.73	22.73	22.73	22.73	22.73	22.73
Corn flour	11.36	11.36	11.36	11.36	11.36	11.36	11.36	11.36	11.36
Tomato powder	5.68	5.68	5.68	5.68	5.68	5.68	5.68	5.68	5.68

3.2.10 Selection of best soup mix by sensory analysis

The sensory analysis for overall quality of prepared soup mixes was carried out with semi-trained panelists which consisted of teachers and students of Central Campus of Technology. The parameters for sensory evaluation were taste, smell, color, flavor and overall acceptability. Sensory evaluation was performed according to the 9-point Hedonic Scale as described by Rangana (1986). The obtained data was analyzed statistically by GenStat Discovery Edition 3, for Analysis of Variance (ANOVA) at 5% level of significance. The data obtained from sensory evaluation were subjected to two-way Analysis of Variance.

3.2.11 Analytical methods

3.2.11.1 Proximate analysis

a) Moisture content

Moisture content was determined by Hot air oven method as described by K.C and Rai (2007).

b) Crude protein

Crude protein was determined following the method described by K.C and Rai (2007).

c) Crude fat

Crude fat was determined as described by K.C and Rai (2007).

d) Total ash

Total ash was determined as described by K.C and Rai (2007).

3.2.11.2 Determination of acidity

Acidity was determined by titrimetric method as described by Rangana (1986). The sample was prepared by grinding weighed amount of sample (10 g) and extracting the acid in water. The result was expressed as % anhydrous lactic acid.

3.2.11.3 Determination of reducing sugar

Reducing sugar was determined determined by the Lane and Eynon's method, as per K.C and Rai (2007) which involves the property of reducing sugars to reduce the copper in Fehling’s solution to red. The sugar content in food is estimated by determining the volume of unknown sugar solution required to completely reduce a measured volume of standardized Fehling’s solution. The sample was prepared and neutralized with dilute NaOH using phenolphthalein indicator if necessary, clarification was done with Carrez's solution. Then, after the standardization of Fehling’s mixture with a known concentration of sugar, Fehling’s mixture was titrated against sample titer on boiling condition using methylene blue indicator until brick red color appears or blue color vanishes.

3.2.11.4 Determination of vitamin C

Vitamin C was determined by dye reduction method as per K.C and Rai (2007). Briefly, first of all standardization of dye with standard ascorbic acid solution was carried out and then test sample was prepared in 3% HPO_3 solution to make volume 100 ml. Thus, prepared sample was mixed well and filtered to get clear solution. Finally, 2-10 ml of extract was titrated against dye to pink red color.

3.2.11.5 Mineral analysis (calcium and iron)

a) Calcium

Calcium content was determined by titrimetric method as described by K.C and Rai (2007). Ash solution was prepared by heating ash for 5 min in 10% HCl (25 ml) and, pH maintained to 5 with dilute ammonia and dilute acetic acid. This solution was heated to boiling point and left overnight and filtered and washed till chloride free with hot dilute H_2SO_4 and water. The filtrate and also filter paper were titrated with 0.01N $KMnO_4$ for determination of titer consumed. It was expressed in mg/100 g.

b) Iron

Iron content was determined spectrophotometrically as described by K.C and Rai (2007). To the ash solution prepared in similar manner for calcium determination, 5 ml sample was taken and reagents like saturated $K_2S_2O_8$ (1 ml), Conc. H_2SO_4 (0.5ml), 3N KSCN (2 ml) and distilled water (10 ml) was used for making sample solution for analysis. For standard iron sample (0.1 mg/ml) (1 ml) was used replacing 1ml distilled water whereas for blank, sample solution was replaced by distilled water. Observation was done in 480 nm. The result was expressed in mg/100 g.

3.2.11.6 Microbial analysis

a) Coliform count

Coliform count was performed as per the method given by IS 5887 (1977).

b) Yeast/Mold count

Yeast/mold count was done as per the method described by IS 5403 (1999).

c) Total Plate Count (TPC)

Total plate count was performed as per the method given by FAO (1997).

Part IV

Results and discussion

The process used for *gundruk* preparation was followed as described by Katwal *et al*. (2012). Different varieties of mustard green were used to make *gundruk* with 10% incorporation of cabbage of a single variety. The leaves kept for fermentation were taken out for drying in Cabinet dryer (40±5°C) till moisture content was less than 10%. Acidity and proximate components of each samples were determined and best *gundruk* was selected based on sensory evaluation. The best *gundruk* was powdered and used to make soup mix along with the powder of other ingredients as given in Table 3.1. Sensory analysis was performed for selection of best soup mix formulation. Proximate and microbial analysis of the best soup mix was performed.

4.1 Chemical composition of mustard leaves and cabbage leaves

Chemical composition of fresh mustard leaves (Marpha, Marpha wide, Tankhuwa and Mixed) and cabbage leaves were determined and the values are shown in Table 4.1.

Table 4.1 Chemical composition of fresh mustard leaves of different varieties and cabbage leaves

Parameters	Values (db)				
	Marpha	Marpha wide	Tankhuwa	Mixed	CabbageValues
Moisture (%)	87.15±0.40	90.56±0.65	85.24±0.71	88.59±0.26	88.33±0.42
Vitamin C (mg/100 g)	150±1.80	121±0.91	147±1.20	135±0.85	87.5±1.50
Total ash (%)	0.68±0.42	0.9±0.11	0.7±0.05	0.72±0.15	0.65±0.21
Reducing sugar (%)	1.88±0.31	1.41±0.08	1.89±0.18	1.53±0.71	2.95±0.06
Acidity (%)	0.06±0.047	0.047±0.003	0.042±0.006	0.05±0.008	0.048±0.17

[Values presented are means ± standard deviation of triplicates.]

Moisture content was found to be highest in Marpha wide (90.56%) and lowest in Tankhuwa (85.24%). Vitamin C was found to be highest in Marpha (150 mg/100 g) and lowest in Marpha wide (121 mg/100 g). There was variation among the mustard varieties in other parameters like total ash, reducing sugar and acidity. This variation might be due to genetic variations among the varieties. Climate, soil conditions and other external factors might have very little influence because these varieties were grown in research center in almost identical conditions.

4.2 Chemical composition of *gundruk* samples

Gundruk made from Tankhuwa, Marpha wide, Marpha and Mixed varieties of mustard leaves were coded as I, II, III and IV respectively. Chemical composition of each *gundruk* samples were determined and the values are shown in Table 4.2.

Table 4.2 Chemical composition of *gundruk* samples

Parameters			Values (db)	
	I	II	III	IV
Moisture (%)	8.5±0.725	7.8±0.17	6.25±0.01	9.5±1.2
Acidity (%)	0.7±0.24	0.75±0.12	0.92±0.35	0.6±0.5
Total ash (%)	0.68±0.13	0.78±0.05	0.67±0.42	0.69±1.12
Vitamin C (mg/100 g)	39.65±1.23	32.34±0.56	41.2±1.23	33.21±0.74
Reducing sugar (%)	0.8±0.14	0.75±0.04	0.61±0.95	0.98±0.25

[Values presented are means ± standard deviation of triplicates.]

Moisture content was lowest in sample III (6.25%) and highest in sample IV (9.5%). Acidity was lowest in sample IV (0.6%) and highest in sample III (0.92%). Variation was also observed among the samples in terms of total ash, vitamin C and reducing sugar. This variation in chemical parameters in *gundruk* samples is mostly due to the use of different varieties of mustard leaves. Besides other aspects like initial microflora, tamping extent, fermentation conditions, etc. might also have influenced.

4.3 Sensory analysis of gundruk samples

Statistical analysis of the sensory scores was obtained from 10 semi-trained panelists using 9-point hedonic rating scale (9= extremely like, 1= extremely dislike) for four *gundruk* samples. Sensory analysis was performed with the aid of different panelists evaluating taste, smell, flavor, sourness and overall acceptability of the *gundruk* samples.

4.3.1 Smell

The mean sensory score for smell of sample III was found to be 8.2 which was the highest score of all the *gundruk* samples. Statistical analysis showed that there was significant difference among the *gundruk* samples in terms of smell ($p<0.05$) at 5% level of significance. All the samples were significantly different from eachother. Cyanides, isothiocyanides, alcohols, esters, phenyl acetaldehyde and various other metabolites produced during the fermentation process contribute to the typical smell of *gundruk*. Optimum production of these compounds during *gundruk* fermentation leads to the typical desirable smell of *gundruk* (Kharel *et al*., 2007). The result of sensory analysis for smell is presented in Fig. 4.1.

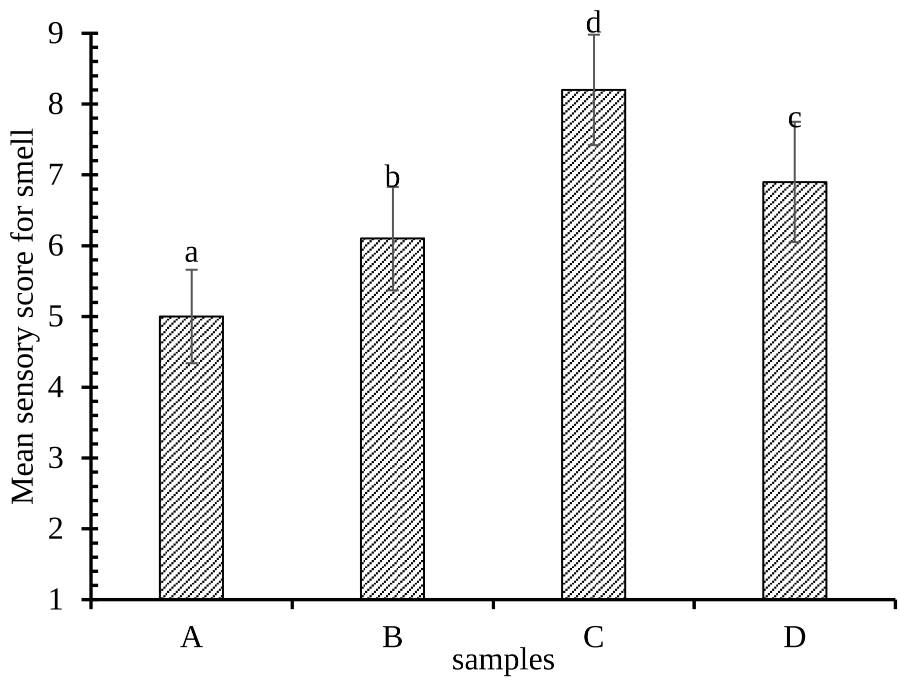


Fig. 4.1 Mean sensory score for smell of different *gundruk* samples

(Plotted values are the means of sensory scores. Vertical error bars represent ± standard deviations. Values on top of the bars bearing similar superscript are not significantly different ($p>0.05$) at 5% level of significance)

4.3.2 Taste

The mean sensory score for taste of sample III was found to be 8.3 which was the highest score of all the *gundruk* samples. Statistical analysis showed that there was significant difference among the *gundruk* samples in terms of taste ($p<0.05$) at 5% level of significance except II and IV. Sample III and I were significantly different among each other and also different than other samples of *gundruk*. Sample II and IV were not significantly different from each other but were significantly different from sample I and III. Acidity is the major factor that contributes to the characteristic taste of *gundruk*. Acidity of sample III was found to be 0.92% as lactic acid which was highest among the samples of *gundruk*. Besides various flavor components and metabolites also aid to typical taste of *gundruk*. Balanced production of these components might be the reason for superior taste of sample III (Karki *et al*., 2007).

The result of sensory analysis for taste is presented in Fig. 4.2.

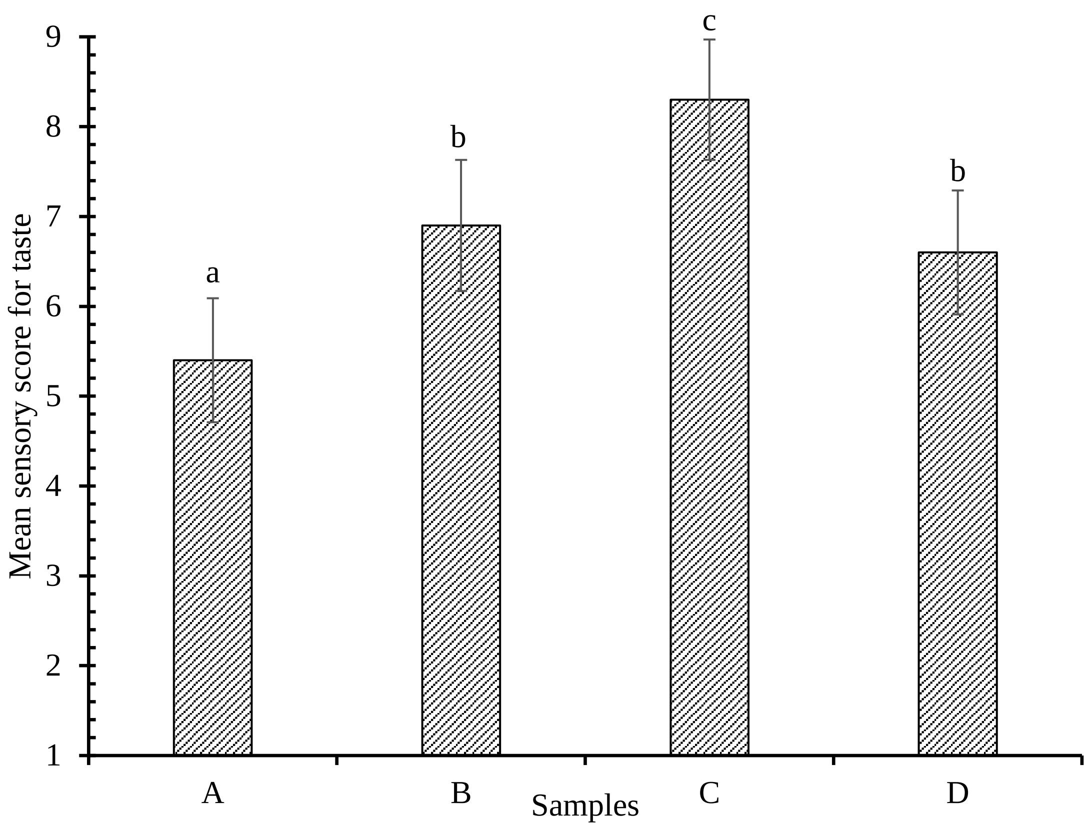


Fig. 4.2 Mean sensory score for taste of different *gundruk* samples

(Plotted values are the means of sensory scores. Vertical error bars represent ± standard deviations. Values on top of the bars bearing similar superscript are not significantly different ($p>0.05$) at 5% level of significance)

4.3.3 Sourness

The mean sensory score for sourness of sample III was found to be 7.8 which was the highest score of all the *gundruk* samples. Statistical analysis was done to determine significant difference among *gundruk* samples in terms of sourness ($p<0.05$) at 5% level of significance. Sample III and I were significantly different from each other. Sample II and IV were also significantly different from each other. There was no significant difference among sample II and I. Similar was the case with sample I and IV. Acidity of *gundruk* corresponds to its sourness. Sample III has highest acidity among the *gundruk* samples. This is the reason for higher score of sample III for sourness.

The result of sensory analysis for sourness is presented in Fig. 4.3.

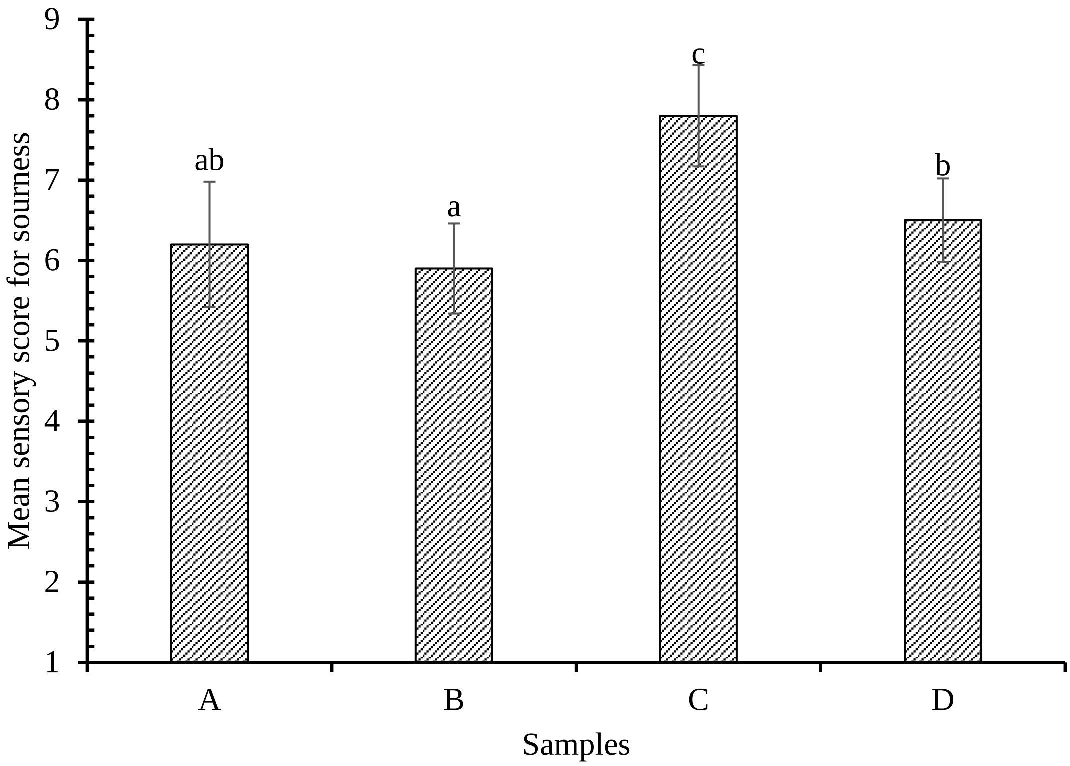


Fig. 4.3 Mean sensory score for sourness of different *gundruk* samples

(Plotted values are the means of sensory scores. Vertical error bars represent ± standard deviations. Values on top of the bars bearing similar superscript are not significantly different ($p>0.05$) at 5% level of significance)

4.3.4 Flavor

The mean sensory score for flavor of sample III was found to be 7.9 which was the highest score of all the *gundruk* samples. Statistical analysis showed that there was significant difference among the *gundruk* samples in terms of flavor ($p<0.05$) at 5% level of significance except II and IV. Sample III and I were significantly different among each other and also different than other samples of *gundruk*. Sample II and IV were not significantly different from each other but were significantly different from sample I and III. The combined effect of organic acids along with various metabolites like cyanides, isocyanides, esters, CO_2 and ethyl alcohol contribute to the characteristic flavor of *gundruk.* Better flavor of sample C might be due to the balanced production of these metabolites than other samples of *gundruk* (Karki *et al*, 2007).

The result of sensory analysis for flavor is presented in Fig. 4.4.

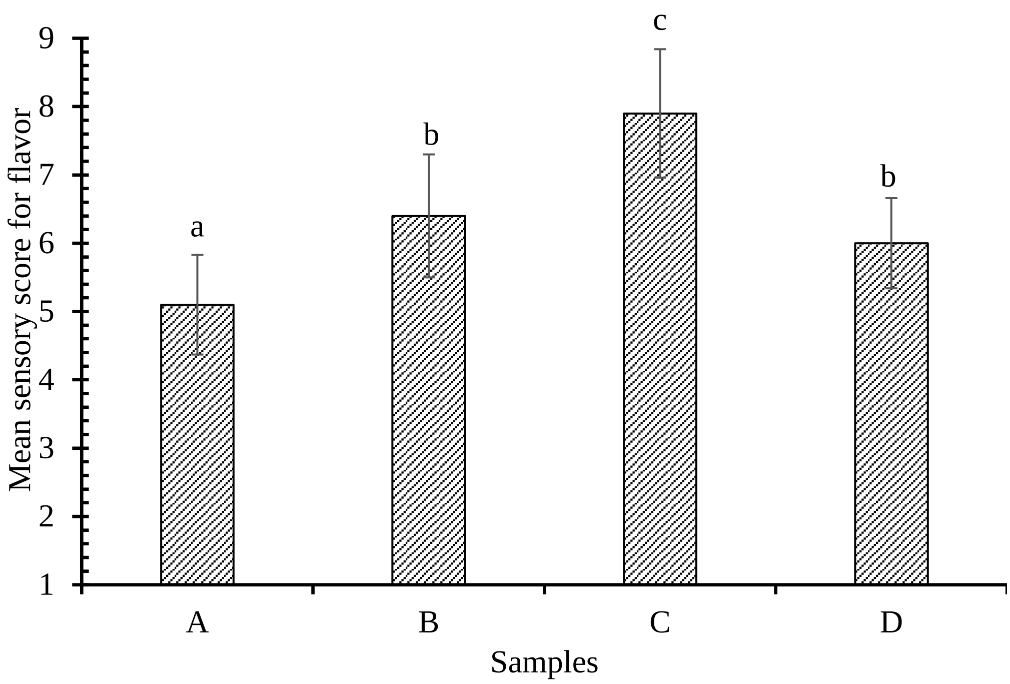


Fig. 4.4 Mean sensory score for flavor of different *gundruk* samples

(Plotted values are the means of sensory scores. Vertical error bars represent ± standard deviations. Values on top of the bars bearing similar superscript are not significantly different ($p>0.05$) at 5% level of significance)

4.3.5 Overall

The mean sensory score for overall acceptability of sample III was found to be 8.1 which was the highest score of all the *gundruk* samples. Statistical analysis showed that there was significant difference among the *gundruk* samples in terms of overall acceptability ($p<0.05$) at 5% level of significance except II and IV. Sample III and I were significantly different from each other. Sample II and III were also significantly different from each other. There was no significant difference among sample II and IV. Sample III was found to be the best *gundruk* which was further ground, sieved and used to formulate the soup mix. Acidity is the major factor that contributes to the taste and overall acceptability of *gundruk* according to Kharel *et al.* (2007). Table 4.2 suggests that sample III has highest acidity among all the *gundruk* samples. Besides, balanced production of flavor compounds during fermentation increases the overall acceptability. The glycerides and some unknown fractions are hydrolyzed during *gundruk* fermentation., liberating free fatty acids fractions that may be eventually beneficial for the generation of desirable ester like flavor in *Gundruk* (Upadhaya. 2002). These might be the reason for preference of sample III by the panelists.

The result of sensory analysis for overall acceptability is presented in Fig. 4.5.

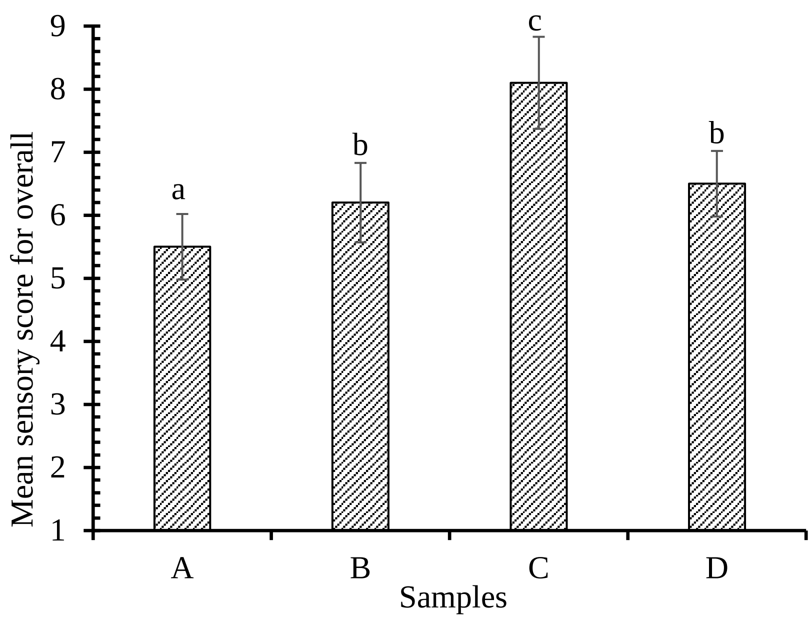


Fig. 4.5 Mean sensory score for overall acceptability of different *gundruk* samples

(Plotted values are the means of sensory scores. Vertical error bars represent ± standard deviations. Values on top of the bars bearing similar superscript are not significantly different ($p>0.05$) at 5% level of significance)

4.4 Range of ingredients

Upper and lower threshold for each ingredient was determined by discriminatory testing as described by Sharif *et al*. (2017). The range obtained is given in the Table 4.3.

Table 4.3 Range of each ingredients

Range (in gram)	*Gundruk*	Garlic	Chili	Black pepper	Salt	SMP	Corn flour	Tomato powder
Upper Threshold	5	0.3	0.3	0.3	2	4	3	1.5
Lower Threshold	2	0.1	0.1	0.1	0.5	1	1	0.5

4.5 Optimum amount of the fixed ingredients

Those ingredients whose amounts were kept same throughout the several formulations are fixed ingredients. The optimum amount of these ingredients was determined by ranking test as described by Sharif *et al*. (2017).

The optimum amount for each fixed ingredient is given in Table 4.4.

Table 4.4 Optimum amount of fixed ingredients

Ingredients	Optimum amount (g)
SMP	2
Salt	1
Corn flour	1
Tomato Powder	0.5

This recipe of fixed ingredients was used for making several formulations of *gundruk* soup mix along with the different amounts of variable ingredients. The amounts of variable ingredients whose range was already determined was obtained from Design Expert software using User Defined Mixture Design.

4.6 Sensory analysis of soup mix

Statistical analysis of the sensory scores was obtained from 10 semi-trained panelists using 9-point hedonic rating scale (9= extremely like, 1= extremely dislike) for nine *gundruk* soup mix samples. Sensory analysis was performed with the aid of different panelists evaluating taste, smell, flavor, color and overall acceptability of the *gundruk* samples. Summary of the statistical analysis of the data obtained from the sensory analysis of soup mix samples is presented in Table 4.5.

Table 4.5 Mean sensory scores for different samples of *gundruk* soup mix

Sample	Taste	Flavor	Smell	Color	Overall
A	$7.1^{a}\pm0.56$	$6.6^{a}\pm0.51$	$6.7^{a}\pm0.67$	$6.4^{a}\pm0.51$	$6.5^{a}\pm0.52$
B	$7.6^{b}\pm0.51$	$7.2^{b}\pm0.63$	$7.6^{cd}\pm0.51$	$7.3^{bc}\pm0.48$	$7.5^{cd}\pm0.52$
C	$7.4^{ab}\pm0.51$	$7.6^{bcd}\pm0.51$	$7.5^{cd}\pm0.52$	$7.5^{bc}\pm0.52$	$7.5^{cd}\pm0.52$
D	$7.4^{ab}\pm0.51$	$7.8^{cd}\pm0.78$	$7.8^{de}\pm0.42$	$8^{d}\pm0$	$7.8^{d}\pm0.42$
E	$7.5^{ab}\pm0.52$	$7.4^{bc}\pm0.51$	$7.3^{bc}\pm0.48$	$7.4^{bc}\pm0.51$	$7.4^{cd}\pm0.51$
F	$7.5^{ab}\pm0.52$	$7.3^{bc}\pm0.48$	$7.5^{cd}\pm0.52$	$7.5^{bc}\pm0.52$	$7.3^{bc}\pm0.48$
G	$8.2^{c}\pm0.63$	$8^{d}\pm0.66$	$8.1^{e}\pm0.73$	$8.1^{d}\pm0.56$	$8.4^{e}\pm0.51$
H	$7.1^{a}\pm0.56$	$7.2^{b}\pm0.63$	$6.9^{ab}\pm0.31$	$7.1^{b}\pm0.56$	$6.9^{ab}\pm0.31$
I	$7.4^{ab}\pm0.51$	$7.4^{bc}\pm0.51$	$7.2^{bc}\pm0.42$	$7.7^{cd}\pm0.48$	$7.4^{cd}\pm0.51$
LSD (5%)	0.478	0.542	0.486	0.437	0.439

[Values presented are means ± standard deviation of triplicates. Means in the same column with different superscript are significantly different ($p<0.05$).]

4.6.1 Color

The mean sensory score for color of sample G was found to be 8.0 which was the highest score of all the samples. There was significant difference among the samples A, G and H ($P<0.05$). There was no significant difference between the samples B, C, E and F in terms of color. Higher incorporation of *gundruk* powder gave more pronounced color in sample G which might be the reason for its preference in terms of color among the panelist.

4.6.2 Smell

The mean sensory score for smell of sample G was found to be 8.1 which was the highest score among all the samples. There was no significant difference between the samples B, C, F, I and E ($P>0.05$). There was significant difference between the samples A, G and I. Highest incorporation of *gundruk* in sample G gave more pronounced typical smell which might have resulted in preference of sample G in terms of smell.

4.6.3 Taste

The mean sensory score for taste of sample G was found to be 8.2 which was the highest score of all the samples. There was no significant difference among the samples C, D, I, E and F ($P>0.05$). G was significantly different from all the samples. Inclusion of *gundruk* powder was highest (45.45%) in sample G which might have resulted in more pronounced typical taste of *gundruk* in the soup. This might be the reason for preference of sample G by the panelist.

4.6.4 Flavor

The mean sensory score for flavor of sample G was found to be 8.0 which was the highest score of all the samples. There was significant difference among the samples A, B and G ($P<0.05$). There was no significant difference among the samples C and D, also among the samples E, F and I. *Gundruk* being the main ingredient of soup mix, flavor of soup is mainly influenced by its proportion. Spices added in the soup mix also contribute in the flavor. Small amount of spices has tendency to give strong spicy flavor. This spicy flavor might mask the typical flavor of *gundruk*. Sample G has lowest incorporation of spices and highest incorporation of *gundruk* among all the samples. This might have resulted in desirable flavor.

4.6.5 Overall

The mean sensory score for overall of sample G was found to be 8.4 which was the highest score of all the samples. There was significant difference among the samples A, D and G ($P<0.05$). There was no significant difference among the samples B, C, E, F and I. Sample G was found to be superior than all in terms of overall acceptability. This might be due to the highest inclusion of *gundruk* powder and moderate incorporation of spices like garlic, chili and black pepper. As Nepalese love the unique appetizing flavor of *gundruk*, there might be masking effect in other soup mixes due to higher incorporation of spices which lead to lower sensory scores (Personal communication, 2019).

4.7 Analysis of best soup mix

The best soup mix (sample G) was subjected to chemical analysis and results obtained is given in Table 4.6.

Table 4.6 Chemical composition of the best soup mix

Chemical components	Percentage
Moisture	9.5±0.25
Crude Fat	5.37±0.28
Crude Protein	12.2±0.35
Crude Fiber	6.53±0.15
Total Ash	19.06±0.20
Carbohydrate	52.74±0.38
Calcium (mg/100 g)	1325.6±4.85
Iron (mg/100 g)	12.0±0.23

[Values presented are means ± standard deviation of triplicates.]

Microbial analysis of best soup mix showed the results given in Table 4.7.

Table 4.7 Microbial analysis of the beat soup mix

Parameters	Values
Total Plate Count (cfu/g)	7.2×10^3
Yeast/Mold Count (cfu/g)	65
Coliform Count	Absent

Part V

Conclusions and recommendations

5.1 Conclusions

On the basis of this research following conclusions can be made:

1. *Gundruk* soup mix can be made by incorporating different parts of *gundruk* powder and other ingredients.
2. Typical appetizing taste of *gundruk* can be obtained from the soup mix without any adverse effect on the sensory qualities.
3. 40% to 45% of *gundruk* powder can be incorporated to obtain the best sensory results.
4. Moderate incorporation of spices should be done to prevent the masking effect of spices on typical taste of *gundruk*.
5. Best sensory results were obtained from spice incorporation of 3.42%.

5.2 Recommendations

The experiment can be further continued with the following recommendations:

1. Study on the incorporation of different herbal powders in *gundruk* soup mix can be carried out.
2. Study can be carried out on the instantization of the soup mix.
3. Study can be carried out on nutritional fortification and enrichment of the soup mix.

Part VI

Summary

Gundruk is a non-salted fermented food product prepared by spontaneous lactic acid fermentation of leaves of Brassica family such as mustard, radish, cauliflower, rape, etc. It is one of the most significant fermented foods of Nepal which is consumed by almost all households. It occupies an eminent place in the Nepalese diet and is eaten by great relish. It is one of the most prized typical indigenous vegetable products which is believed to have existed in Nepalese culture since time immemorial. It is a good source of minerals and vitamins during off-seasons when green vegetables are scarce. It is valued for its uniquely appetizing flavor and served in a number of ways.

Soup mix is a blend of various dried and powdered ingredients in the optimum ratio so as to obtain the required flavor when made into soup with hot water. Convenience is a multifaceted concept and often listed as the most important factor that determine the food choice apart from cost, health, sensory acceptability and related concerns. Due to the demand of special foods by consumers and also because of time factor people now a days are attracted towards easy foods. As a consequence, the demand of ready to eat or ready to cook minimally processed products has noticeably increased during recent years. Soup mix is becoming popular and better option for the population in this busy world.

This research was focused on preparing *gundruk* soup mix. Rayo leaves (mustard green) and cabbage leaves were brought from Pakhribas Agriculture Research Center and Tarahara Agriculture Research Center respectively. 4 varieties of mustard leaves were used for preparing 4 different *gundruk* samples with 10% incorporation of cabbage leaves following the optimized method given by Katwal *et al*. (2012). On 9th day samples were taken out for drying in cabinet dryer at 45±5°C till less than 10% moisture content. All samples were analyzed for moisture content, acidity, vitamin C and reducing sugar. Sensory analysis was performed for the selection of best *gundruk*. *Gundruk* made from the mustard variety “Marpha” was found to be best. It was further powdered and used for making *gundruk* soup mix. Acidity and moisture content of the selected *gundruk* was 0.92% (as lactic acid) and 6.25% respectively.

Design expert was employed for formulating the recipe of soup mix. The obtained 9 formulations with various amounts of *gundruk* powder, chili powder, garlic powder and black pepper powder as variable ingredients were prepared in lab. The amounts of tomato powder,

corn flour, SMP and salt (5.68%, 11.36%,22.73% and 11.36% respectively) was kept constant in all the formulations. Amounts of these ingredients was determined by discriminatory and ranking tests performed by semi-trained panelists at Central Campus of Technology. Tomato powder used for making soup mix was made by drying thin slices of tomato at 60°C for 48 hrs in cabinet dryer. Powder of other ingredients were brought from Bhatbhateni, Dharan. *Gundruk* powder was sieved through 30-mesh screen and other ingredients were sieved through 60-mesh screen before soup mix formulation.

All the formulations of soup mix were subjected to sensory evaluation for consumer acceptability. From mean sensory scores, formulation containing 45.45%, 1.14%,1.14% and 1.14% of *gundruk* powder, chili powder, garlic powder and black pepper powder respectively was selected as the best formulation and subjected for further proximate and microbial analysis. Moisture, crude fat, crude protein, crude fiber, total ash, carbohydrate (by difference), calcium and iron content were found to be 9.5%, 5.37%, 12.2%, 6.53%, 19.06%, 52.74%, 1325.6 mg/100 g and 12 mg/100 g respectively. Total plate count and yeast/mold count was found to be 7.2×10^3 and 65 cfu/g. No any traces of coliform was found during the microbial analysis of sample.

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Appendices

Appendix A

Sensory evaluation score sheet for *gundruk*

Date:

Name of Panelist:

Name of the product: *Gundruk*

Dear panelist, you are provided with 4 samples of *gundruk*. Please test the following samples of *gundruk* and check how much you prefer for each of the samples. Give the points for your degree of preferences for each parameter for each sample as shown below:

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

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

Parmeters	**Sample Code**			
	I	**II**	**III**	**IV**
Taste				
Smell				
Sourness				
Flavor				
Overall acceptability				

Any Comments:

Signature:

Appendix B

Sensory evaluation score sheet for *gundruk* soup mix

Date:

Name of Panelist:

Name of the product: *Gundruk* Soup Mix

Dear panelist, you are provided with 9 samples of *gundruk* soup mix. Please test the following samples and check how much you prefer for each of the samples. Give the points for your degree of preferences for each parameter for each sample as shown below:

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

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

Parameters	**Sample Code**								
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
Taste									
Smell									
Color									
Flavor									
Overall acceptability									

Any Comments:

Signature:

Appendix C

Mean sensory score of different samples of *gundruk*

Table C.1 Mean sensory scores of different samples of *gundruk*

Sample	Taste	Flavor	Smell	Sourness	Overall
A	5.4^{a}±0.70	5.1^{a}±0.74	5^{a}±0.67	6.2^{ab}±0.79	5.5^{a}±0.53
B	6.9^{b}±0.71	6.4^{b}±0.63	6.1^{b}±0.74	5.9^{a}±0.74	6.2^{b}±0.53
C	8.3^{c}±0.82	7.9^{c}±0.67	8.2^{d}±0.63	7.8^{c}±0.74	8.1^{c}±0.52
D	6.6^{b}±0.67	6^{b}±0.67	6.9^{c}±0.63	6.5^{b}±0.74	6.5^{b}±0.52
LSD (5%)	0.622	0.651	0.661	0.572	0.585

[Values presented are means ± standard deviation of triplicates. Means in the same column with different superscript are significantly different ($p<0.05$).]

Appendix D

ANOVA for *gundruk* samples

Table D.1 Two way ANOVA (no blocking) for flavor

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	40.9000	13.6333	27.07	<.001
Panelist	9	6.6000	0.7333	1.46	0.215
Residual	27	13.6000	0.5037		
Total	39	61.1000			

Table D.2 Two way ANOVA (no blocking) for overall

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	36.2750	12.0917	29.75	<.001
Panelist	9	2.5250	0.2806	0.69	0.711
Residual	27	10.9750	0.4065		
Total	39	49.7750			

Table D.3 Two way ANOVA (no blocking) for smell

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	54.5000	18.1667	35.04	<.001
Panelist	9	7.4000	0.8222	1.59	0.170
Residual	27	14.0000	0.5185		
Total	39	75.9000			

Table D.4 Two way ANOVA (no blocking) for sourness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	21.0000	7.0000	18.00	<.001
Panelist	9	4.1000	0.4556	1.17	0.351
Residual	27	10.5000	0.3889		
Total	39	35.6000			

Table D.5 Two way ANOVA (no blocking) for taste

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	42.6000	14.2000	30.92	<.001
Panelist	9	5.4000	0.6000	1.31	0.279
Residual	27	12.4000	0.4593		
Total	39	60.4000			

Appendix E
ANOVA for *gundruk* soup mix

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

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	8	20.4222	2.5528	10.59	<.001
Panelist	9	2.4444	0.2716	1.13	0.356
Residual	72	17.3556	0.2410		
Total	89	40.2222			

Table F.2 Two way ANOVA (no blocking) for flavor

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	8	12.8889	1.6111	4.35	<.001
Panelist	9	1.8333	0.2037	0.55	0.833
Residual	72	26.6667	0.3704		
Total	89	41.3889			

Table F.3 Two way ANOVA (no blocking) for taste

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	8	8.4000	1.0500	3.65	0.001
Panelist	9	3.2889	0.3654	1.27	0.268
Residual	72	20.7111	0.2877		
Total	89	32.4000			

Table F.4 Two way ANOVA (no blocking) for smell

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	8	15.0000	1.8750	6.30	<.001
Panelist	9	1.1556	0.1284	0.43	0.914
Residual	72	21.4444	0.2978		
Total	89	37.6000			

Table F.5 Two way ANOVA (no blocking) for overall

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	8	22.4889	2.8111	11.56	<.001
Panelist	9	1.7889	0.1988	0.82	0.602
Residual	72	17.5111	0.2432		
Total	89	41.7889			

Color plates

Plate 1 Tarahara agriculture research center

Plate 2 Pakhribas agriculture research center

Plate 3 Bhatbhateni supermarket (Dharan)

Plate 4 Local vegetable market (Dharan)

Plate 5 Preparation of raw material

Plate 6 Chemical analysis

Plate 7 Sensory analysis of *gundruk* samples

Plate 8 Sensory analysis of *gundruk* soup mix