# PRODUCTION AND EVALUATION OF SAUERKRAUT WITH ENHANCED NUTRIENTS

**THESIS**

**Submitted to**

**Department of Biotechnology and Bioinformatics**

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*In the partial fulfillment of the requirement for the degree of*

**Master of Science in Biotechnology**

**By**

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**MSc Biotechnology [4th semester]**

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*Under the supervision of*

**Dr. Anil Kant Thakur, Associate Professor**

**JUIT Solan (H.P)**

# CERTIFICATE

This is to certify that thesis entitled **"Production and evaluation of sauerkraut with enhanced nutrients",** submitted by Ashish Guleria in partial fulfillment for the award of a degree of Master of Science in Biotechnology to the Jaypee University of Information Technology, Waknaghat, Solan has been made under my supervision.

This report has not been submitted partially or fully to any other university or institute for the award of this or any other degree or diploma.

**Date** **Dr. Anil Kant Thakur**

**Place** **Associate Professor**

## ACKNOWLEDGMENT

The project's final output required a lot of guidance and assistance from many people, and I am grateful to have received this all along with the completion of my project work. Whatever I have accomplished is solely due to such guidance and assistance, and I would want to express my gratitude to everyone who assisted me throughout the project

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## DECLARATION

I hereby declare that the present work on "Production and evaluation of sauerkraut with enhanced nutrients", is a record of original work done by me under the supervision of Dr. Anil Kant Thakur Associate Professor at JUIT Solan (H.P), from February 2022 to May 2022 at Food lab of Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Solan.

I also declare that no part of the thesis has previously been submitted to any university or examining body for acquiring any degree.

Date: Ashish Guleria

Place: MSc Biotechnology

# CONTENTS

## ABBREVIATION

LAB - Lactic acid bacteria

WHO – World health organization

FDA – Food and drug administration

BC – Before Christ

AD – Anno domini

CFU – Colony-forming unit

pH – Potential of hydrogen

GLS – Glucosinolates

ROS – Reactive oxygen species

CRP - C-reactive protein

BSA – Bovine serum albumin

SK – Standard Sauerkraut

SKMB – Sauerkraut with sprouted mung beans

SKTL – Sauerkraut with turnip leaves

W/V – Weight / volume

ml – Milli liter

µl – Microliter

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# CHAPTER -1

# INTRODUCTION

## Introduction

Many people in today's world suffer from Nutrition deficiencies and stomach issues. Malnutrition in the children and adult population is a major problem in India. In India, around 36% of women are underweight, while 56% of women and 56% of teenage girls between the ages of 15 and 19 suffer from iron deficiency anemia owing to malnutrition[1]. Low weight, stunted growth, and muscle atrophy are all symptoms of malnutrition. Malnutrition has been observed to hinder the growth of over half of Indian infants under the age of five[2]. poor gut health and unable to absorb nutrients are also major causes of malnutrition in India.

Malnutrition and nutritional deficiencies can be treated with high-nutrient foods that are easily absorbed by the body. Fermented food is traditionally blended with health benefits, but it has recently been used to introduce specific health qualities, such as vitamin enrichment or the introduction of probiotics, which are "live bacteria that impart a health benefit on the host when provided in suitable proportions." These probiotic microorganisms strengthen gut health and enhance nutrition bioavailability in the gut[3]. From ancient procedures and scientific investigations, fermentation technology has evolved into an industrial, life-science-driven process. Fermented foods and beverages are thought to account for about approximately a quarter of the average human diet. Microbial activity has an impact on the composition and nutritional quality of food, which can enhance specific health benefits[4].

Sauerkraut is a common fermented vegetable product that is abundant in nutrition and high in probiotics, which help to improve gut health. Sauerkraut comprises only two ingredients: cabbage and salt, and once they are combined properly, the producer has very little to do until the fermentation is complete.[5] sauerkraut is more nutritious than raw cabbage because of the probiotics it contains, which aid in the absorption of these elements. Probiotics are the living bacteria that provide a health benefit to the host when administered in sufficient concentrations[6]. Two species of genus Lactobacillus i.e *L. paraplantarum SF9* and *L. Brevis SF15* which were isolated from sauerkraut were shown to be capable of surviving in an acidic gastrointestinal environment as well as competitively excluding enteropathogens[7]. Furthermore, these bacteria were able to adhere to Caco2cells, which is a necessary attribute for probiotic characteristics.

Similarly, several Lactobacillus bacteria obtained from sauerkraut survived well in a simulated environment[6].

The following objectives were set in consideration of the potential and health advantages of sauerkraut.

1. Preparation of standard sauerkraut and its Evaluation.
2. Preparation of sauerkraut supplementing with sprouted mung beans and its protein content evaluation.
3. Preparation of sauerkraut supplementing with Turnip leaves and its nutrient evaluation.

# CHAPTER – 2

# REVIEW OF LITERATURE

## 2.1 Fermentation -Introduction, and History

Fermented foods have experienced considerable biochemical changes as a result of microbes and enzymes acting on them throughout the manufacturing process. Fermentation is one of the earliest among those technologies, relying on the biological activity of microbes to produce a variety of compounds that might inhibit the proliferation of undesirable microorganisms in foods [8]. Fermentation has been used to preserve food for thousands of years. The technique of cheese-making is said to have originated in Iraq at that time(Table1).[9]. The Egyptians and Sumerians are claimed to have invented alcoholic fermentation technique used in the winemaking and brewing between 2000 and 4000 BC. Dough fermentations, which are utilized in manufacturing leavened bread, were also created by the Egyptians. Although fermentations have been used to preserve beverages and food for thousands of years.[8]. Pasteurization was invented in 1861, and the importance of microbes in the fermentation process was recognized for the first time. It was also the period of the industrial revolution, which as a consequence simultaneous population concentration in urban areas. As a outcome, food production was shifted from a smaller to the larger scale, which was very important to sustain the enhanced demand of the distant market. subsequently fermentation methods operating ata large scale were grown for the purpose of commercial production of alcoholic drinks and fermented foodstuffs.[10]. In each instance, t beverage or raw foodstuffs supplie substrates to synthesize a variety of metabolites produced by microbes enhancing he product's shelf-life extension and quality [10]. Fermented food manufacturing has benefited from scientific advances and industrialization. Traditional methods of natural fermentation or substrate backslopping were insufficient as a foundation for any industrial process on a vast scale. To maintain consistent quality and safety of fermented foods, characterization of microorganisms began in 19th century, particularly for milk-derived products, to isolate starter cultures that can be manufactured in vast quantities and supplied to industries that are engaged in the manufacture of these products [10]. The usage of specific strain starters in the industries has

**Table 1** Significant incidents in food fermentation [8].

| Time Period | Major Events in Food Fermentation |
|---|---|
| 6000 BC | Cheese production in Iraq. |
| 4000 BC | Egyptians discovered how to produce leavened bread and wine using yeast. |
| 1750 BC | Sumerians brewed beer from barley. |
| 500 BC | Moldy soybean curds are being used as an antibiotic in China |
| 1276 AD | In Ireland, the first whisky distillery was established. |
| 1500 AD | Fermentation of sauerkraut and yogurt |
| 1861 AD | Pasteurization was invented by Louis Pasteur. |
| 1928 AD | Rogers and Whittier discovered Nisin's discovery- antagonism of some *Lactococci* to other LAB. |
| 1947 AD | Mattick and Hirsch isolated and identified Nisin, a group N inhibitory chemical. |
| 1953 AD | Nisin was first introduced in England and has now been approved for usage in more than 48 countries. |
| 1968 AD | Nisin is recognized by the FAO/WHO as a safe and authorized biological food preservative. |
| 1988 AD | Nisin was approved by FDA |

substituted the undefined strain combinations that were traditionally used for the Traditionally, it's utilized to make fermented foods. The transition to specific strains has resulted in resulted in significant improvements in culture efficiency as well as product quality and consistency, while the food and beverage industries are now dependent on a fewer number of strains[8]. This overdependence on specialized starters, on the other hand, has certain disadvantages and can lead

to output issues and poor strain performance. Bacteriophage proliferation can alter the efficacy of cheese starters in lactococcal fermentations[11].

## 2.2 Lactic acid fermentation

Food fermentation has been performed for centuries to preserve and improve food quality. vegetables are preserved by spontaneous fermentation which is induced by the group of lactic acid bacteria present in the vegetable. When given the right conditions, the bacteria found in raw vegetables cause fermentation. Lactic acid bacteria are bacteria that induce most vegetables to ferment, and the acids they produce are essential for the preservation of many foods. Bacteria and, in some situations, yeast break down the sugars in the vegetables to produce acid and carbon dioxide during vegetable fermentation. The acid gives the vegetables distinctive tartness while also protecting them by preventing unwanted microorganisms from multiplying on them. Furthermore, a variety of desired products that affect food quality, such as the flavor components diacetyl and acetaldehyde, as well as chemicals with potential health benefits, such as vitamins, antioxidants, and bioactive peptides, may be synthesized[8]. *Lactococcus, Lactobacillus, Enterococcus, Streptococcus, Leuconostoc,* and *Pediococcus* are a bacterial family that is generally involved in lactic acid fermentation, and their main end product is lactic acid. These groups of bacteria during the fermentation are unable to synthesize the Heme. Resulting from them being catalase-negative and devoid of a terminal electron transport chain [5]. Lactic acid bacteria are mesophilic bacteria that may grow at temperatures as low as 5 °C and as high as 45 °C. Although most strains thrive at a pH of 4.0-5.0, some of these are active at pH 9.6 and others at pH 3.2. Preformed amino acids, purine and pyrimidine bases, and B vitamins are all required for strain growth. [10]. Lactococci for cheese production, Streptococcus salivarius subsp. thermophilus for cheese and yogurt production, and various Lactobacillus genus members for a variety of dairy, meat, and vegetable fermentations are the most common members of the group that are exploited for food utilization[10]. Based on their carbohydrate metabolism, members of the LAB can be divided into two different groups. *Lactococcus, Pediococcus, Enterococcus, Streptococcus,* and some *lactobacilli* are members

of the homofermentative group, which uses the Embden – Meyerhof –Parnas's pathway to synthesize 2 moles of lactate from 1 mole of glucose (Fig 1). On the other hand, Heterofermentative bacteria use the hexose monophosphate or pentose pathways to synthesize equimolar levels of lactate, CO2, and ethanol from glucose. [8].

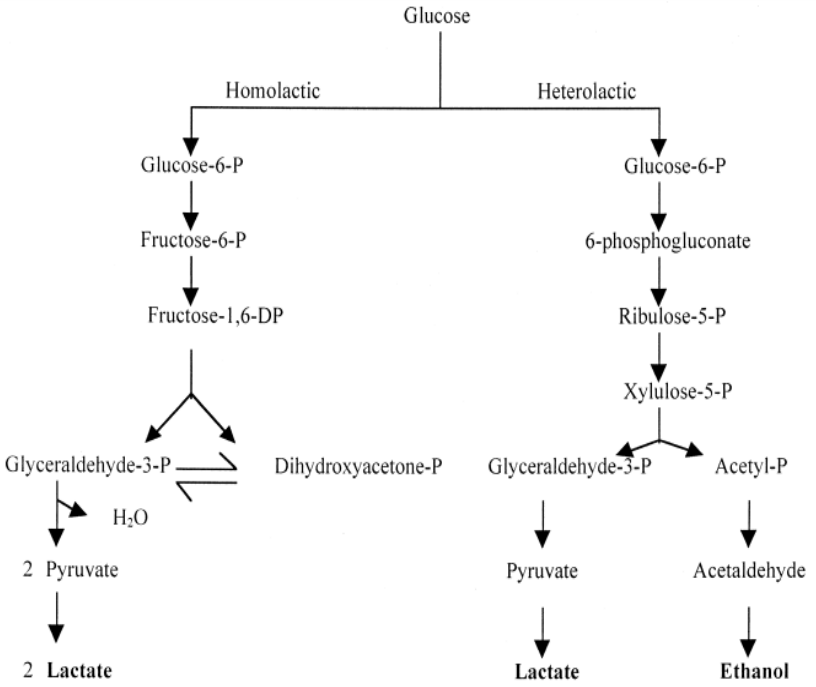


**Figure 1** Glucose fermentation in lactic acid bacteria [10]

## 2.3 Anti- Microbial compounds production during LAB fermentation

Lactic acid bacteria during the fermentation produce various types of antimicrobial compounds that help preserve the food. The cumulative effect of a variety of metabolites that are antibacterial and are synthesized during the fermentation process is related to the starter strains' preservation action on the food and beverage systems [12]. To establish an acidic environment, many organic acids are generated as end products, including propionic acid, acetic acid, and lactic acid which inhibits the proliferation of pathogenic and spoilage microorganisms. Acids are assumed to have

the antimicrobial properties which hinder with cell membrane potential maintenance, restricting active transportation, decreasing the pH within the cell, and disrupting a range of biosynthetic processes [8]. Propionic acid, which is produced by propionic acid bacteria, has been used as a basis for various bio preservatives

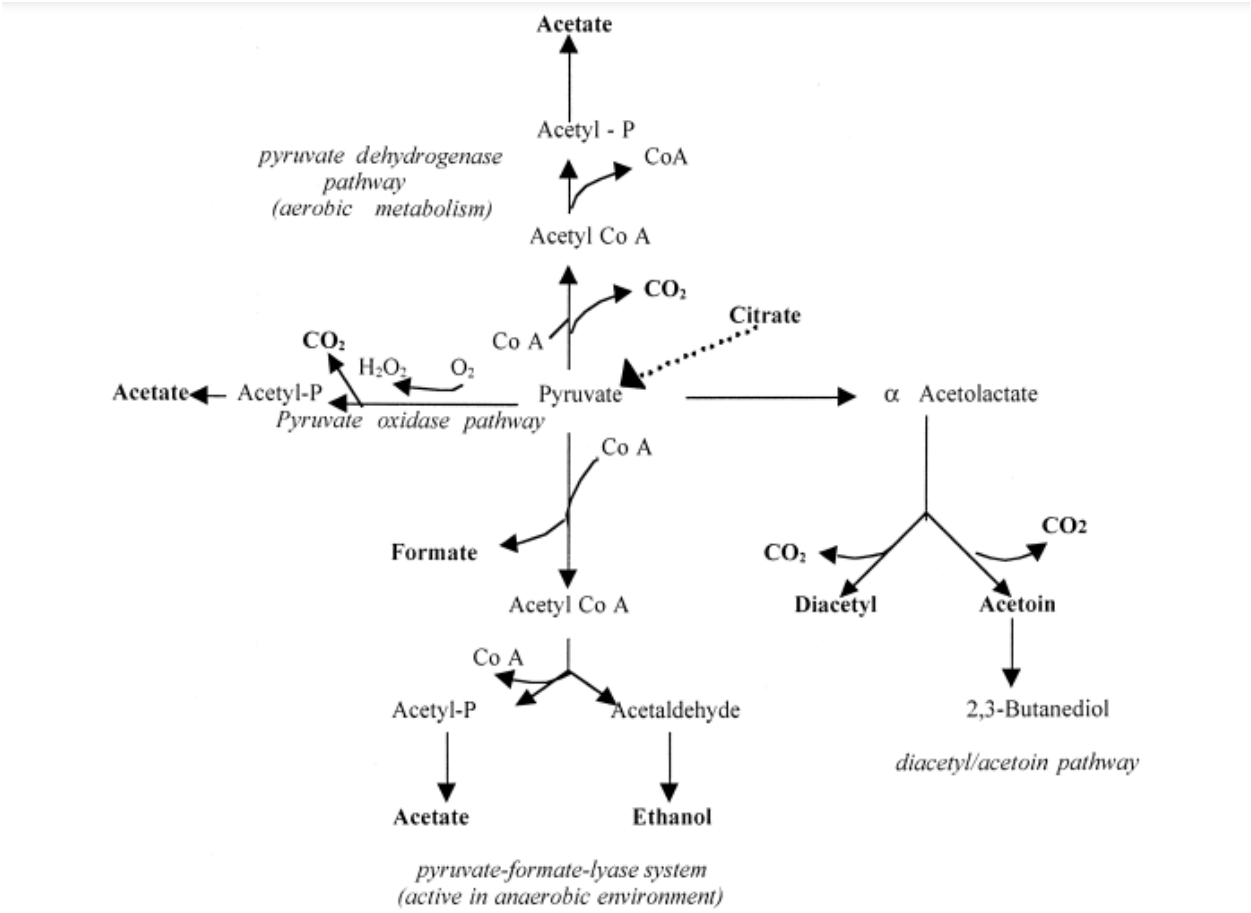


**Figure 2** In lactic acid bacteria, pyruvate is converted to essential metabolic products [10].

products due to their antimicrobial activity against microorganisms such as yeast and molds. The Propionibacterium freudenreichii subsp. shermanii, an FDA-approved fermentate, containing propionic acid and which is used for the around 30% of production of cottage cheese in the U.S.A[8]. Other antimicrobial metabolites produced by starting strains $H_2O_2$ synthesized while aerobic growth, and diacetyl is manufactured from the excess pyruvate from citrate are all products of the heterofermentative route. The flavoprotein oxidoreductases NADH peroxidase, NADH oxidase, and a-glycerophosphate oxidase create $H_2O_2$, which can cause severe oxidative damage

to membrane lipids and cellular proteins.[13]. The chemical reuterin and the recently found antibiotic reuterocyclin, both produced by Lactobacillus reuteri strains, are examples of secondary metabolites that are produced by the lactic acid bacteria with antagonistic activity[14]. In the Lactic acid bacteria fermentation, various proteins are synthesized which inhibit the growth of food pathogens which are known as bacteriocins. Bacteriocins are synthesized by a variety of lactic acid bacteria (LAB). Despite the fact that these bacteriocins are made by LAB and might be identified in a wide range of fermented and non-fermented foods, nisin is the only bacteriocin normally utilized as a food preservative at the present.[15]. Bacteriocins are classified into three categories: Class I: the lantibiotic family, Class II: small non-modified peptides, and Class III: large heat-labile proteins[15]. The wide variety of dairy and nondairy products around the world, including liquid egg, cottage cheese and dairy desserts shelf – life is extended by using bacteriocins nisin, the lantibiotic. Bacteriocin has also been shown o be efficient in preventing the growth of spoilage microorganisms during the fermentation of beer and wine, and Various vegetable fermentations have been demonstrated to benefit from the use of nisin-producing strains. Over the last two decades, Due to the sheer effectiveness of nisin, numerous research groups are looking for new bacteriocin-producing strains. These compounds are utilized as a natural preservative in the food system by directly incorporating strains producing bacteriocin into food as starter or protection cultures to using concentrated bacteriocin preparations as food additives[8].

## 2.4 Sauerkraut – History

Sauerkraut is among the most often consumed fermented vegetable foods, with a vast history in human nutrition dating back to ancient times. White cabbage, shredded and salted, fermented with lactic acid produces sauerkraut, a classic meal in Europe, America, and Asia (Brassica oleracea var. capitata) [16]. Sauerkraut is a German word that means "sour cabbage." Sauerkraut was first mentioned in China in ancient time when the cabbage was fermented in rice wine and were exported to Europe one thousand years later by Genghis Khan after he invaded China[17]. Early civilizations were well-aware of the health benefits of sauerkraut. Sauerkraut was recommended by Hippocrates, a Greek physician, to help people lose weight, and Romans ate it to keep their intestines healthy. Furthermore, on his long travels, Captain James Cook, an English navigator,

and explorer, restocked his ships' food supplies with sauerkraut, It does not need to be refrigerated and hence avoided scurvy in sailors [18].

## 2.5 Sauerkraut Production

Sauerkraut is made up of only two ingredients: cabbage and salt once they have been thoroughly combined, the producer has very little to do until the fermentation is finished. The selection of fresh cabbage with high sugar content is the first step in the sauerkraut manufacturing process. Fresh cabbage is removed from its outer leaves, then the head and cores are removed. Cabbage is shredded and salted into 0.7–2-mm thick strips[19]. The salt and cabbage are thoroughly mixed. Salt has a key function in sauerkraut fermentation (Fig 3). After the salt and shredded cabbage are combined, simple osmosis allows water to diffuse out of plant tissue relatively soon. In the brine, nutrients and sugars diffuse out with the water, supplying the media for microbial activity to microorganisms. Anaerobic conditions are created by salt during the sauerkraut fermentation which prevents the growth of spoilage microorganisms, providing lactic acid bacteria a major growth advantage and also inhibiting the endogenous pectinolytic enzymes activity that makes cabbage soft during fermentation The microbiota and sensory characteristics of sauerkraut are affected by the amount of salt added. sodium chloride concentration used is generally determined by the fermentation temperature and the preferences of the market [20]. The salt and lactic acid mixture formed aids in the long-term preservation of the final product. Finally, salt adds flavors to the product while also keeping the tissues from softening, resulting in a crisp texture. If there is insufficient mixing of salt, some regional pockets may have more or less salt than was added to the overall mixture. This could result in either insufficient or excessive inhibitory control of the organisms in that microenvironment. If spoiling organisms can grow in these pockets, their products (such as slime, colors, and off-flavors) will infect the entire batch of sauerkraut when it is mixed again before packaging[21]. After thoroughly mixing the salt with the cabbage, start squeezing the cabbage immediately until some brine formation occurs. serve After mixing and squeezing, the mixture is placed in the tanks, and the cabbage mixture is pressed until the cabbage mixture is submerged in the brine. The outer removed leaves are densely packed but softly, and

weights are placed on the surface of the cabbage mixture until enough brine has extruded to entirely cover the surface [21].

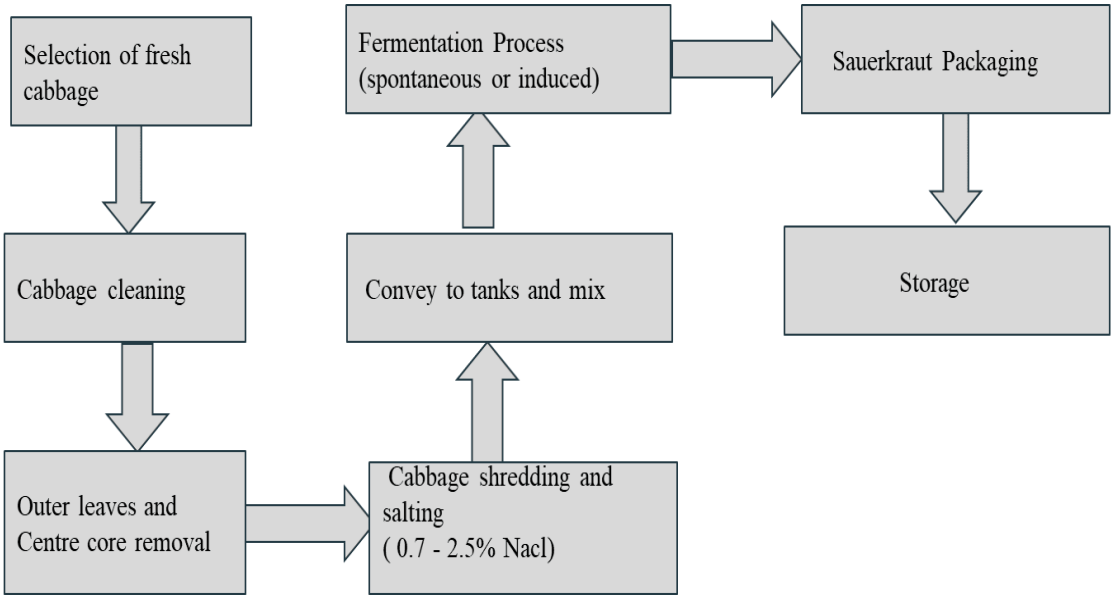


**Figure 3** Schematic representation of sauerkraut production [16]

## 2.6 Spontaneous fermentation of sauerkraut

The fermentation of sauerkraut has long attracted the interest of food microbiologists and microbial ecologists. Several metabolic and microbiological aspects of sauerkraut fermentation have been documented since the 1930s. The fact that the fermentation process comprises numerous different naturally existing bacteria functioning as part of a complex ecosystem has aroused the public's attention [21]. The fermentation of sauerkraut involves several naturally occurring microorganisms. Sauerkraut is made by a group of microorganisms interacting. Many microorganisms developed initially in the process of fermentation, perform a specific function, and then disappear from the final product. on the other hand, microorganisms develop later in the fermentation process and persist at moderate to high levels throughout. Microorganisms found in

fresh, raw cabbage include Pseudomonas, Enterobacter, and other aerobic spoilage bacteria, and, as well as yeasts and molds [22]. These aerobic mesophilic microbes have populations of $10^4$–$10^6$ colony forming units (CFU) per gram, however, in the raw cabbage the LAB population is fewer, at $10^2$- $10^3$ CFU/g.[23] The atmosphere is initially aerobic, but due to the of physical exclusion of air and consumption by plant cells and bacteria, the environment quickly becomes anaerobic. As a result, pseudomonads, fungi, and other essential aerobic microorganisms with a high starting population have minimal development opportunities. Some of these species are also salt-sensitive, making survival in this environment much more difficult. Many salt-tolerant, mesophilic, facultative organisms grow initially, along with *Erwinia, Enterobacter, E. coli* and other coliforms, These microorganisms growth is inhibited and only survive for a short time, due to the inhibiting effects of the acids produced and competition with lactic acid bacteria. [16]. In sauerkraut fermentation, two types of microbial fermentation occur depending on lactic acid bacteria species-homofermentative and heterofermentative LAB species. Heterofermentative LAB initiate fermentation, especially Leuconostoc mesenteroides. This bacterium initially controls the microbiome. Because it has relatively low pH tolerance and microaerophilic properties, as well as a relatively short production period at the temperature range of 18–20°C[24]. These LAB species synthesize a significant amount of acetic and lactic acid that causes the pH to drop, as well as $CO_2$, which creates an anaerobic environment So when acid content reaches 0.6–1% and the pH falls below 4.5, Leuconostoc species are replaced by acid-tolerant homofermentative *Lactobacillus Brevis and Lactobacillus Plantarum* [25]. *L. Brevis and L. Plantarum* synthesize lactic acid at a higher concentration and control the later stage of sauerkraut fermentation when pH decreases from 3.5 to 3.7 [22]. The major end product in the sauerkraut fermentation is lactic acid and other metabolic end products are also produced. As many of these compounds contribute to the overall flavor and aroma of sauerkraut. The finished sauerkraut may contain up to 0.2 percent acetic acid and 0.6 percent ethanol. These bacteria also produce diacetyl, acetaldehyde, and other volatile texture and flavoring components in small amounts. Mannitol is also major end product that is accumulated during sauerkraut fermentation. To achieve sauerkraut of acceptable quality during fermentation, the correct succession of these bacterial communities is crucial. At the completion of the fermenting process, sauerkraut contains roughly more than 2% lactic acid and 1% acetic acid. [26].

## 2.7 Sauerkraut Spoilage

In sauerkraut fermentation spoilage microorganisms are mainly responsible for the sauerkraut spoilage and defect. The chemicals and physiological factors are also responsible for sauerkraut spoilage. Temperature and salt are the two most significant factors that affect fermentation and can cause product defects. The optimum temperature for sauerkraut fermentation is 18℃ to 20℃ and a Temperature of more than 30℃ can lead to sauerkraut spoilage. Salt concentration also impacts the quality of sauerkraut, salt concentration of more than 3% can lead to sauerkraut spoilage. *L. mesenteroides* growth is suppressed due to high and high salt concentration, and there will be no heterofermentative end-products, and the taste of the sauerkraut will not be up to the mark. Worse yet, excessive salt levels might delay acid generation, allowing salt-tolerant yeasts to proliferate. *Rhodotorula sp*. proliferation is concern in the sauerkraut fermentation because of the unwanted pink color this yeast creates [21]**.** on the contrary, Gram-negative bacteria, such as *Pseudomonas, Enterobacter and Flavobacterium, and,* may develop if somehow the temperature is less than 10℃ or too little salt is given (2%). Some of these bacteria can produce pectinolytic enzymes, which induce a pectin hydrolysis defect known as "soft kraut"[21].

## 2.8 Nutrition composition and Benefits of sauerkraut

Sauerkraut is considered a beneficial for health-promoting fermented food due to its abundant nutritional content and high quantities of bioactive chemicals. Carbohydrates and soluble fiber are the most important components of fermented white cabbage, followed by proteins, minerals, fat, and vitamins, particularly vitamin C [27]. Cabbage is also high in phytochemicals, particularly glucosinolates (GLS) and phenolic compounds. GLS is a collection of sulfur- and nitrogen-secondary metabolites from the plant that give Brassica vegetables their distinctive flavor and odor [28]. During LAB fermentation the chemical composition of cabbage changes, resulting in a fermented product that is higher in the carbs, proteins, lipids, dietary fiber, minerals, and vitamins. Sauerkraut contains primarily lactic and acetic acids (1–2%) and also organic acids, as well as

ethyl acetate, acetaldehyde, ethanol, and CO2. [29]. Vitamin C content ranges from 14.7 to 75 mg/100g, while phenolic substances are known to be particularly high in sauerkraut.

Sauerkraut is abundant in micronutrient such as vitamins C and E, as well as phenolic compounds, which are strong free radical scavengers that protect against oxidative stress[30]. Oxidative stress, defined as when the production of reactive oxygen species (ROS) exceeds the antioxidant capacity of an organism, is becoming more widely recognized as a factor in aging and the pathogenesis of a variety of chronic health problems, including cardiovascular disease, neurodegenerative disease, and cancer[31]. Vitamin C lowers C-reactive protein (CRP), a protein linked to inflammation and atherosclerosis, and works for eight human enzymes as an electron donor , neutralizing superoxide and hydroxyl radicals (in conjunction with phenolic substances) [32]. sauerkraut is more nutritious than raw cabbage because of the probiotics it contains, which aid in the absorption of these elements. Probiotics are live bacteria that provide a health benefit to the host when administered in sufficient concentrations[33]. Two species of genus Lactobacillus i.e *L. paraplantarum SF9* and *L. Brevis SF15* which were isolated from sauerkraut and shown to be capable of surviving in an acidic gastrointestinal environment as well as competitively excluding enteropathogens[7]. Furthermore, these bacteria were able to adhere to Caco2cells, which is a necessary attribute for probiotic effects. Similarly, several Lactobacillus bacteria obtained from sauerkraut survived well in a simulated environment [6].

# CHAPTER -3

# MATERIAL AND METHODS

## 3.1 Materials

Cabbage (6 kg) was purchased from the local market Waknaghat, Himalayan pink salt was from chef Urbano, Turnip leaves (1kg) were purchased from the local market Shimla, Mung Beans (1kg), Fermentation Jar, Duran bottles (500ml), Plastic tray, Muslin cloth, Falcon tubes (50ml), Test tubes, Sodium hydroxide, Sodium chloride, Distilled water, Bradford reagent, BSA stock (10mg/ml), Sodium sulfate, 2-propanol, Hexane, Methylene chloride, Methanol, Ethanol, Phenol, Sulfuric Acid, Glucose, Vitamin K1 internal standard was purchased from SRL Diagnostics.

## 3.2 Procedure

### 3.2.1 Standard Sauerkraut Production

This study used cabbage from a local store. Fresh cabbage was purchased from the Waknaghat market**.** Fresh cabbage was selected, cleaning of cabbage was done, the outer leaves and center cores of fresh cabbage is removed, cutting of cabbage of length 1- 2 mm thick, 40g of salt (2% w/v) is mixed in 2 kg chopped cabbage and squeeze the mixture for 10-15 minutes until some brine formation take place (Table 2). Convey the mixture of cabbage into the fermentation jar and press the cabbage continuously with the pestle until the cabbage is fully submerged in the brine. Place the outer leaves of cabbage as cover on top and place the weight on top to exclude air. close the fermentation jar tightly and place it in a dark place. At room temperature, the sample was fermented for 30 days (Fig 4) (15℃ to 23℃) until pH reaches less than 4. The sample is stored at 4℃ in a cold room [16], [21]

### 3.2.2 Preparation of sauerkraut supplementing with turnip green

Fresh cabbage was purchased from the local market waknaghat and turnip leaves were purchased from the local market Shimla. Selection of fresh cabbage and turnip leaves, clean the cabbage and turnip leaves with water, cabbage and turnip are shredded of length 1-2 mm thick, cabbage and turnip leaves were mixed and 40g of salt (2% w/v) is added to the mixture of 2 kg cabbage and turnip leave mixture (Table 2). Squeeze the mixture for 10-15 minutes until brine formation takes place. Transfer the mixture into the fermentation jar and press the mixture with a pestle until the mixture is fully submerged in the brine. Place the outer leaves of cabbage as cover on top and place the weight on top to exclude air. Tightly close the fermentation jar and keep it in a dark place. The sample was fermented for 30 days (Fig 5) at room temperature (15°C to 23°C) until the PH was less than 4, then stored at 4°C in a cold room.

**Table 2** Composition of supplementation and salt concentration.

| Sample | Cabbage weight | Supplementation product weight | Salt % (w/v) | Sauerkraut Ratio |
|---|---|---|---|---|
| Standard sauerkraut | 2 kg | 0 | 2% (40g) | |
| Sauerkraut with turnip leaves | 1.6 kg | 400 g | 2 % (40g) | 80:20 |
| Sauerkraut with sprouted mung Beans | 1.6 kg | 400 g | 2% (40g) | 80:20 |

### 3.2.3 Preparation of sauerkraut supplementing with Sprouted Mung Beans

Mung beans were purchased from the local market waknaghat. Mung beans were picked out and soaked overnight in the bowl. The next day mung beans were rinsed and packed into a moist muslin cloth. Place the mung beans in a dark place for 2 days at room temperature[34]. Pick out sprouted mung beans and supplement them with shredded cabbage for fermentation. 40g of salt(2%w/v) was added to the 2 kg mixture of sprouted mung beans and cabbage (Table 2). Squeeze the mixture for 10-15 minutes, or until brine is formed. Place the mixture in the fermentation jar and pound it down with a pestle until it is completely submerged in the brine. Place the outer cabbage leaves on top as a cover and a weight on top to keep the air out. Close the fermenting jar tightly and store it somewhere dark. The sample was fermented at room temperature (15°C to 23°C) for 30 days until the pH was less than 4, then stored in a cold chamber at 4°C.

### 3.2.4 Protein extraction from standard sauerkraut

Sauerkraut samples were dried for two days in a hot air oven at 40℃ and Homogenized with a grinder into powder form. 100 mg samples were dissolved in 10 ml 0.1M NaOH in 3.5 % NaCl buffer and incubated for 24 hours at 37℃ and exposed to constant shaking. Centrifuge the sample at 4000g for 15 minutes at 4℃. The supernatant was collected for further protein analysis [35].

### 3.2.5 Protein Estimation of standard sauerkeaut by Bradford Assay

BSA stock solution was prepared (1mg/ml) from stock solution(0.1mg/ml) working solution was prepared. Make a dilution of the BSA standard with a concentration of 0.2,0.4,0.6,0.8,1.0 mg/ml and a blank test tube containing 1ml water. Make up volume to 1ml. After that Bradford reagent (1ml) to each test tube including blank was added and incubated test tubes were at room temperature for 15 minutes. OD at 595 was taken[36].

### 3.2.6 Sample Preparation for Carbohydrates Analysis

2 g of dried samples were homogenized in 80 percent aqueous ethanol using a mortar and pestle. The supernatant was collected after centrifugation at 4℃ for 15 minutes at 10,000 g. In 80% ethnaol residue was reextrated two time and supernatant was collected. The collected supernatant was dried at room temperature and dried sample was weighed (40 mg). The sample was dissloved in 5 ml of distilled water and sample was centrifuged at 4℃ for 15 minutes at 10,000 g.[37]

### 3.2.7 Estimation of carbohydrates from the phenol-sulfuric method

Glucose stock solution was prepared (1mg/ml) from stock solution (0.7mg/ml) working solution was prepared. Make a dilution of glucose with a concentration of 0.1,0.2,0.3,0.4,0.5,0.6,0.7 mg/ml and blank test tube containing 1ml water. Make up the total volume of 1ml. Add 80% phenol and 2.5 ml of concentrated $H_2SO_4$. Mix well and incubate at room temperature for 25 minutes and OD at 490 was taken [33].

### 3.2.8 Extraction of phylloquinone from sauerkraut

Sauerkraut samples were dried for two days in a hot air oven at 40℃ and Homogenized with a grinder into powder form. Weight 0.5 sample and grind with 5g sodium sulfate by mortar and pestle. Add 15 ml 2-propanol: hexane (3:2) for extraction and 32 ml of distilled water is added to the sample. Sonication is done for the 30s and followed by vortexing for 10 minutes. Centrifuge the sample at 1000g for 10 minutes. The upper hexane layer is transferred into a 15 ml falcon tube. The sample is transferred to a glass beaker for evaporation or the sample is evaporated in a rotary evaporator. ACN is added to the evaporated sample and freeze the sample at -80℃. The sample was lyophilized and dissolve the residue in 30µl of 100% methylene chloride [39].

# CHAPTER – 4

# RESULTS AND DISCUSSION

## 4.1 Preparation of Sauerkraut

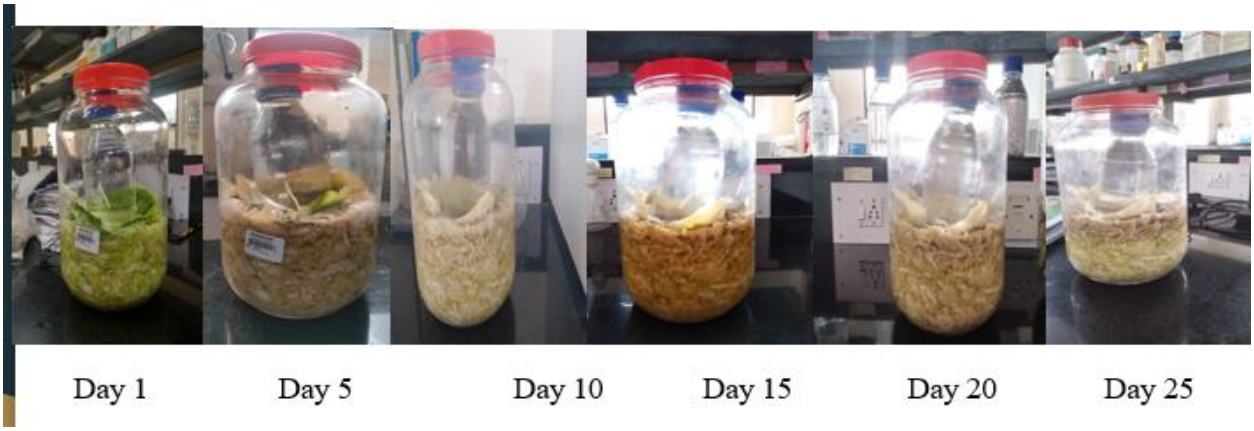


**Figure 4** Standard sauerkraut production.

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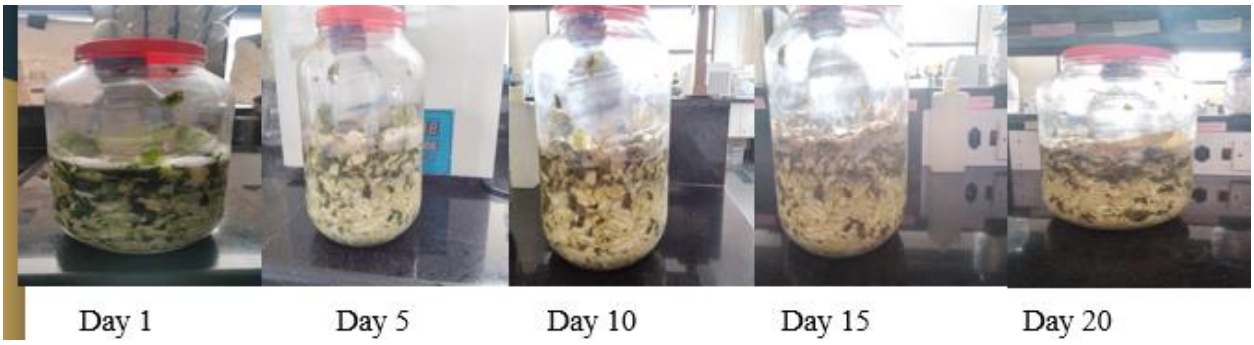


**Figure 5** Sauerkraut with turnip leaves

As illustrated in Figs 4 and 5, the color of the sauerkraut gradually changed from green to light yellow throughout fermentation. As there was no pink color appearance, which indicates microbiological deterioration from yeasts owing to unequal salt mixing, the fermentation is proceeding appropriately.

## 4.2 Physical Test

At the start of fermentation of sauerkraut, the pH of all 3 samples was 6.8 almost near neutral. As the fermentation proceed the production of lactic and acetic acid decreased the PH of the sample below 4 (Table 3). The pH was measured at 27°C after 30 days of fermentation.

**Table 3** pH of the sauerkraut sample after 30 days of fermentation

| Sample | pH |
|---|---|
| Standard sauerkraut | 3.8 |
| Sauerkraut with Turnip leaves | 3.6 |
| Sauerkraut with sprouted mung beans | 3.8 |

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## 4.3 Protein Quantification using Bradford Assay

To determine the Protein concentration of fermented sauerkraut samples, a standard plot of BSA protein was plotted with distilled water as a blank. (Fig 6)

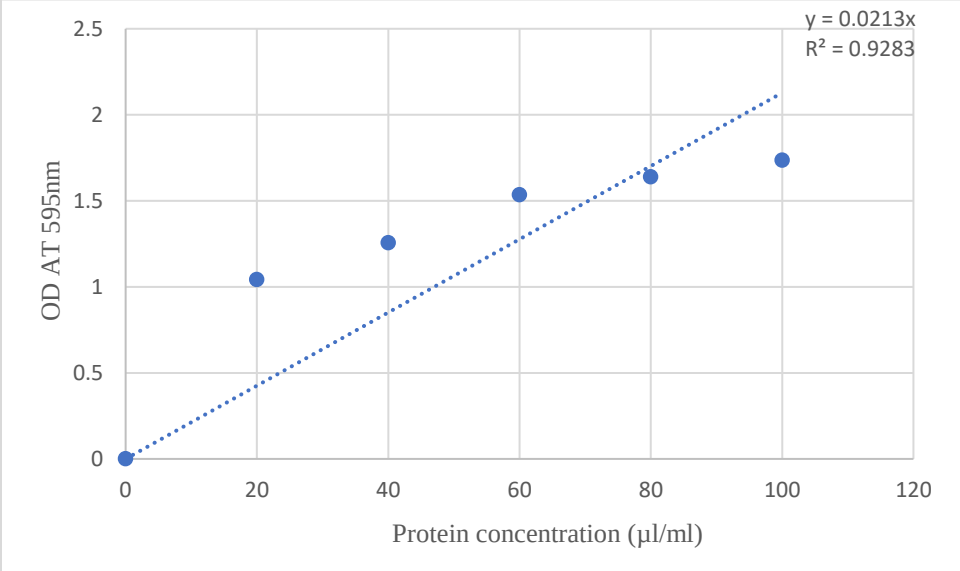


**Figure 6** Standard curve for BSA protein estimation using Bradford Assay

The OD at 595nm is represented on the x-axis, while the protein concentration in µg/ml is represented on the Y-axis.

The Bradford assay standard plot was obtained with an $R^2$ value of 0.9283 and y=0.0213x.

Hence, x=y/0.0213

The protein concentration in sauerkraut samples can be determined using the equation above (because here, X is protein concentration and y is OD at 595)

The above equation was used to calculate protein concentration (mg/ml).

x= y/0.0213* dilution factor

**Table 4** Protein concentration of Sauerkraut samples. (The results of triplicate testing are expressed as means.)

| Samples | Protein Concentration (mg/ml) |
|---|---|
| Standard sauerkraut | 0.574 |
| Standard sauerkraut brine | 0.628 |
| Sauerkraut with sprouted mung bean | 0.656 |
| Sauerkraut with sprouted mung bean brine | 1.606 |


**Figure 7** Comparison between Protein concentration of sauerkraut samples.

SK= standard sauerkraut
SKB= standard sauerkraut brine
SKMB= sauerkraut with sprouted mung beans
SKMBB= sauerkraut with sprouted mung beans brine

The protein composition of sauerkraut samples is shown in Table 4 and Fig7. Protein concentration differed only significantly between standard sauerkraut and sauerkraut with sprouted mung beans. Sauerkraut combined with sprouted mung beans slightly enhanced the protein content. The protein composition of standard sauerkraut brine and sprouted mung bean brine differs drastically, as seen in Table 4. Fermenting sprouted mung beans with cabbage greatly increases the protein content of the brine. Protein concentrations varied substantially in a previous study of this type. In that study, fermented mixed vegetables were compared to sauerkraut[38]

## 4.4 Carbohydrate Quantification from phenol sulfuric acid method

A standard plot of Glucose was plotted with distilled water as a blank to estimate the carbohydrate concentration of fermented sauerkraut samples. (Fig 8)

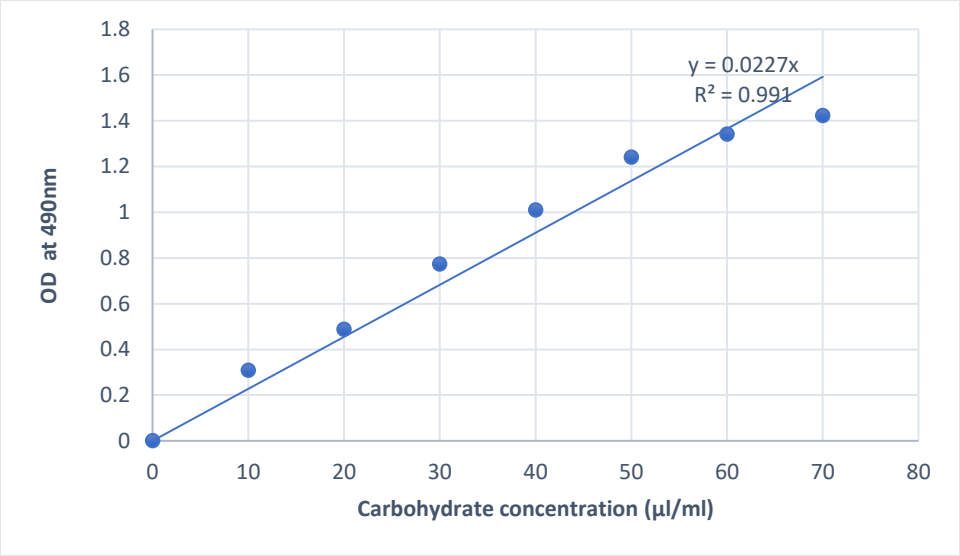


**Figure 8** Standard curve of glucose using Phenol sulfuric acid assay

The x-axis represents the OD at 490nm, whereas the Y-axis represents the glucose concentration in µg/ml.

The phenol sulfuric acid assay plot was obtained with $R^2$ with the value of 0.991 and 0.0227x.

Hence, x=y/0.0227

The protein concentration in sauerkraut samples can be determined using the equation above (because here, X is glucose concentration and y is OD at 490)

The above equation was used to calculate glucose concentration (mg/ml).

x= y/0.0227* dilution factor

**Table 5** carbohydrate concentration of sauerkraut samples (The results of triplicate testing are expressed as means.)

| **Samples** | Carbohydrate concentration (mg/ml) |
|---|---|
| Standard Sauerkraut | 16.541 |
| Sauerkraut with sprouted Mung beans | 7.231 |
| Sauerkraut with turnip leaves | 2.932 |

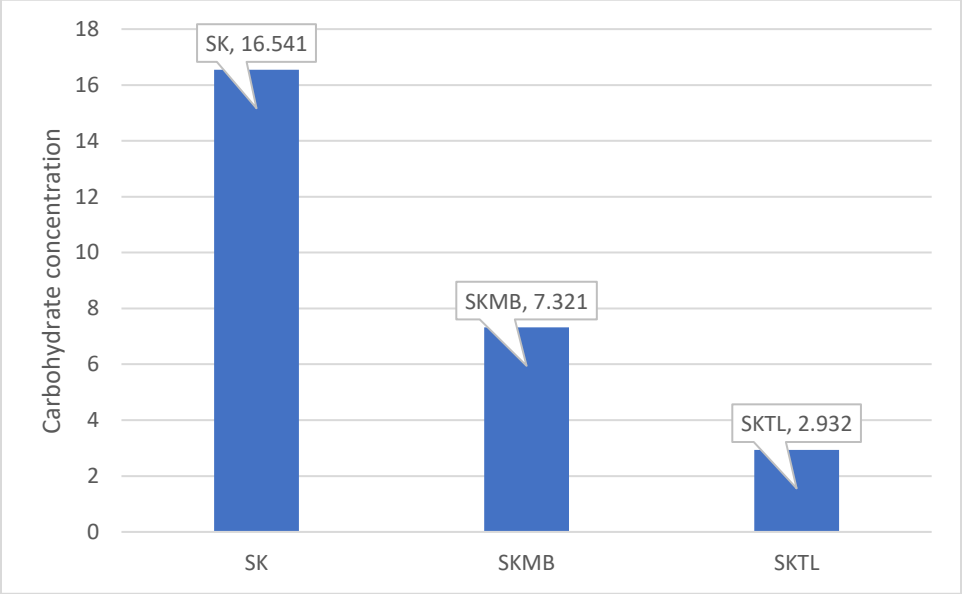


**Figure 9** comparison between carbohydrate concentration of sauerkraut samples.

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SK = standard sauerkraut
SKMB = sauerkraut with sprouted mung beans
SKTL = sauerkraut with turnip leaves

The highest concentration of carbohydrates was found in standard sauerkraut in the present research, as demonstrated in Fig. 9 and Table 5. Since the carbohydrate concentrations in all three samples differed significantly. Sauerkraut with turnip leaves had the lowest carbohydrate concentration, and sauerkraut with sprouted mung beans generally had a lower carbohydrate concentration when compared to standard sauerkraut (Fig 9 and table 5).

**Table 6:** Protein and carbohydrate composition in sauerkraut.

| Samples | Protein (mg/g) | Carbohydrate (mg/g) |
|---|---|---|
| Standard Sauerkraut | 57.4 | 206.76 |
| Sauerkraut with sprouted Mung beans | 65.6 | 90.38 |
| Sauerkraut with turnip leaves | - | 36.65 |

# CHAPTER - 5

# CONCLUSION

## Conclusion

The research focused on improving the nutritious content of sauerkraut by supplementing it with various nutrition-rich sources. Supplementing and improving the nutritional content of the sauerkraut was done with sprouted mung beans and turnip greens. Furthermore, the sauerkraut's nutritious content was improved as a result of these supplements.

The protein concentration was marginally increased when sauerkraut and sprouted mung beans were fermented. Standard sauerkraut brine and sprouted mung bean brine have drastically different protein concentration The protein content of the brine is considerably enhanced by fermenting sprouting mung beans with cabbage.

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