COMPARATIVE STUDY OF RATE OF FERMENTATION FOR DIFFERENT FLOUR SAMPLES

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ABSTRACT

Fermentation has historically been used to preserve food. The primary goal of food fermentation nowadays is to create a diverse range of fermentation products with various flavors, aromas, fragrances, and textures. We can produce hundreds of different types of dairy products (cheese, fermented milk products), vegetables (sauerkraut, pickles, olives), meat (fermented sausages), breads, alcoholic beverages (wine, beer, cider), vinegar and other food acids, and oils using various microbial strains, fermentation conditions (microorganisms, substrates, temperature, time of fermentation, etc.), and chemical engineering innovations. There are mainly two forms of fermentation, lactic acid and ethanolic and through them only a vast range of goods, flavors, and textures can be yielded. Both seek to alter the environment in order to prevent the growth of pathogenic or undesirable germs that can ruin or degrade food. Historically, the majority of items produced by fermentation were used in the food business, but in recent years, there has been increased interest in the production of bulk chemicals like ethanol and other solvents, specialized chemicals, biofuels, medicines, and industrial enzymes. Fermentation techniques are employed in agriculture(in production of normal silages, microbial insecticides , single-cell proteins, etc,) as well. As a result, it is crucial to compare the rates at which different flours ferment, which is exactly what we will do in this study paper, along with some other significant facts on the subject.

***Keywords**: fermentation, flour, yeast, pharmaceuticals, chemicals*

INTRODUCTION

Induced fermentation (such as the process of generating bread dough by adding baking yeast to flour) is a type of fermentation that can be contrasted with spontaneous fermentation, which occurs naturally. The fact that the products of fermentation still contain some amount of chemical energy indicates that they have not been completely oxidized; hence, oxygen is necessary for their mineralization to be finished. In terms of energy efficiency, fermentation is inferior to oxidative phosphorylation (only substrate-level phosphorylation can result in the production of ATP), which is the more common metabolic pathway. In comparison, the fermentation of one molecule of glucose results in the production of two molecules of ATP, whereas the aerobic respiration of one molecule of glucose results in the production of 36 molecules of ATP. The last stage of fermentation, in which pyruvate is converted into end products, does not result in the production of usable energy but does result in the production of NAD+, which is necessary for glycolysis.

A number of different phenolic compounds, such as folates, sterols, and benzoxazinoids, were subjected to fermentation and germination, and the results of these processes were analyzed. These phenolic compounds consist of phenolic acids, lignans, and alkylresorcinols, among other phenolic derivatives. Phenolic compounds, like lignans, alkylresorcinols, and phenolic acids, can act as bioactive molecules because they are antibacterial, anti-oxidative, and anti-carcinogenic. Consumption of whole grains necessitates, first and foremost, the processing of the grains themselves. However, there is currently a paucity of studies about the effects that activities such as milling, baking, malting, and extruding have on the levels of bioactive compounds in grains and the availability of those molecules [3]. When it comes to the preparation of meals that are constructed from grains, the information in question plays a function that is of the utmost significance. Rye has traditionally been used in a number of bread recipes, including both soft and crisp breads, all of which are based on using wholemeal flour as the primary ingredient. Rye is an essential component of diets since it provides whole-grain meals.

History

Fermenting food is a practice that has been used by people ever since the beginning of time as a method for preserving the food as well as altering it. This method was used all the way back in ancient times. It is conceivable to state that it has been carried out more by accident than by conscious efforts, which means that researchers have been working since the 19th century without having a true knowledge of the mechanisms that are involved in microbes [9]. In Babylon around the year 5000 B.C., in Ancient Egypt around the year 3150 B.C., in Medico around the year 2000 B.C., and in Sudan around the year 1500 B.C., the first evidence of fermented beverages was discovered.

Grain processing is essential for maintaining both the grain's sensory and nutritional qualities. In the case of whole grains, it is especially possible for it to alter the amounts of the bioactive components as well as their bioavailability. After germination and fermentation of the rye flour, the levels of folate were increased up to sevenfold and that of free phenolic acids by up to tenfold. The tailored fermentation presents an opportunity to further enhance the bioactive potential of wholemeal rye [3].

Applications in the real world:- In Indonesia, the Gnetum gnemon tree is most commonly referred to by its local name, melinjo. Crackers and cookies made from ground melinjo seeds are a common way in which these seeds are used. These seeds have a high bioavailability and can serve as an appropriate dietary supplement. After fermentation with Lactobacillus fermentum, the antioxidant activity of fermented melinjo flour was shown to be significantly improved in this investigation. In addition, the fermentation process resulted in the production of certain beneficial chemicals. The fermented products have the potential to be utilized as nutritional supplements for the purpose of preventing and managing degenerative diseases. As a result, the findings of this study present a method that is both straightforward and efficient for enhancing the nutritional content of melinjo flour [5].

Vegetables contain a lot of different nutrients, including macronutrients, antioxidants, vitamins, and mineral components. However, they also contain significant levels of anti-nutrients, which decrease the bioavailability of some nutritionally essential components such as zinc or iron. This is due to the presence of many anti-nutritional compounds such as phytates and myo-inositol.. Tannins and phytates are either reduced or neutralized during the processing of vegetables, which results in an increase in the bioavailability of vitamins and minerals. This processing can include microbial fermentation. A second result of bacterial processing is an increase in the ability to fight free radicals. This has been linked to the release of polyphenols from complexes with anti-nutritional components. This effect occurs as a result of bacterial digestion. In addition, bioactive compounds such as beta-aminobutyric acid and inhibitors of an angiotensin-converting enzyme were discovered in fermented vegetables. These chemicals may be helpful in the treatment of lifestyle disorders like depression and hypertension. On the other hand, there is not yet any information regarding the bioavailability of these compounds following the consumption of fermented vegetables. In addition, fermented foods contain a high quantity of beneficial probiotic microorganisms, the majority of which belong to the lactic acid bacteria family. The fermentation process has a unique impact on each individual vegetable. This should serve as motivation for us to increase our level of understanding as more novel and unproven fermented goods are introduced to the market [4].

Because whole wheat flour dough (WWFD) has a high quantity of dietary fiber, it has an adverse influence on the rheology of the dough, which results in whole wheat bread which is of an undesirable quality. In light of this, the goal of developing processing solutions that can assist WWB in overcoming its quality issues is one of extraordinarily significant importance. It has been hypothesized that yeast and lactic acid bacteria work together in a process known as cooperative fermentation by yeast and lactic acid bacteria (CFYL) in order to alter the dough's rheology, retention, and stabilization of gas cells in preparation for a WWFD. This method is referred to as the WWFD dough stabilization procedure. The bread making capabilities of WWFD have been altered as a result of the structural changes that the CFYL treatment brought about in wheat bran, gluten proteins, and wheat starch. These structural changes were brought about by the wheat being subjected to the CFYL treatment. CFYL was able to counteract the wheat bran's possibly deleterious influence on the gluten network. In addition to this, CFYL assisted in the enhancement of the intermolecular interactions that are already present between starch and protein. The rheology of the dough was changed during CFYL as a result of the formation of exopolysaccharides (EPSs), enzymes, and organic acids. As a consequence, the overall quality of the bread was improved as a consequence of these changes. CFYL was also responsible for an increase in the amount of water-extractable arabinoxylans and soluble wheat proteins, while at the same time it caused the size of the starch granules to get smaller. This results in a greater strain hardening during the process of creating bread, as well as a stronger liquid coating of the dough; both of these things promote the retention and stabilization of gas cells in a WWFD. As a consequence of this, CFYL has exceptional potential for use in the production of WWB. This will make it possible to produce goods that have a quality level that is desirable as a whole and an acceptability level that is satisfactory among customers [6].

The incorporation of whole grains into one's diet has been advocated in the majority of countries, and numerous publications have focused on the positive impact that this has on one's health. The use of a methodical analysis of these articles, which cover a variety of research methodologies, geographical areas, and points of view, can yield a better understanding of the innovation pattern in this industry. The purpose of this bibliometric study is to conduct an analysis of the global publication features, hotspots, and frontiers of research on the health benefits of whole grains, as well as to discuss the trends and opportunities associated with this issue. The United States of America is the country that has contributed the most publications to the global rise in the total number of publications. The research that has been cited the most indicates that observational studies, systematic reviews, and meta-analyses are the methodologies that have been utilized the most frequently. Dietary fiber and bioactive compounds are the primary foci of research in this area, with a particular emphasis placed on the latter in recent years due to the growing body of knowledge surrounding them. The creation of foods made with whole grains and the amount of those foods that should be consumed regularly have gradually become hot topics in response to the growing prevalence of the problems of hidden hunger and chronic disease. In addition to the need to discover the mechanism behind the health benefits of whole grains, there is a need to achieve a consensus on the criteria and definitions for whole grain foods, and there is a need to pay attention to the preservation of flavor as well as healthy components throughout the processing of whole grains [7].

Research Gaps:

1. Literature did not explore the fermentation process of rice, wheat etc. rather explained about grains fermentation i.e., types of material for fermentation was not justified.
2. Literature explored the fermented flour characteristics rather than studying grains and testing them.
3. Literature also focused on the fermentation process of vegetables and their characteristics but the same study was missing for grains.
4. Literature used whole wheat flour dough and its characteristics but the fermentation process was missing completely.

Objectives of the Research:

This research aims at comparison of fermentation of different types of flour samples and thus study the fermentation process.

MATERIALS & METHODS:

Materials:

For this research, we have selected following materials for the experimental works:

1. FLOUR SAMPLES OF FOLLOWING GRAINS:-
    - Wheat
    - Rice
    - Finger millet (Ragi)
    - Pearl millet (Bajra)
    - Maize
    - Buckwheat(Kuttu)
    - Gram
2. 1% aqueous sodium chloride solution
3. 10% (w/v) Barley malt extract
4. Iodine Solution

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Procedure:

1. Preparation of flour Extract:

3 grams of each sample flour was dissolved in 300 milliliters of distilled water while being stirred constantly. The boiling process was then started for each of the mixes. After the contents had been brought to a proper boil, they were allowed to cool down at room temperature without being disturbed. Following the cooling process, each of the mixes was filtered with a suction pump, and the filtrate that was collected was the flour extract which was used throughout the experimental process (Flour extract ought to have a consistency that is extremely watery and be completely homogenous.)

2. Fermentation Process :

a) Iodine flasks of same sizes were each filled with 80 mL of the respective flour extract.
b) Then, 3 mL of 1% sodium chloride solution was added to each iodine flask.
c) After placing all the flasks on the thermostat (regulated at the temperature range of 50-60 degree Celsius). 2 mL of malt extract with 10% (w/v) concentration was added to each iodine flask.
d) Then, immediately after adding the malt extract, a stopwatch was started, and then 2 mL of each sample was taken out on the groove's tile at (t≈0), and then 1 mL of diluted iodine solution was aadded to each tile containing different test samples. A blue-violet complex was formed.
e) The procedure described in step (d) was carried out for each sample after every ten minutes until the formation of a blue-violet complex upon the addition of iodine solution stopped. When there was no longer any sign of a bluish color, this signified that the fermentation process was complete of a particular sample.
f) The total time taken for the completion of fermentation for each sample was recorded.

Figure 1: Results on addition of iodine in respective samples at t≈0 .

RESULTS and DISCUSSIONS OF THE FERMENTATION PROCESS:

Dark Blue Violet complex fromed on addtion of iodine

•DISCUSSION

Starch makes up the majority of the components in most cereal flours. In the presence of the enzyme diastase, this starch undergoes fermentation, which causes the starch that was put into solution to produce maltose. A blue-violet complex is known to form when starch and iodine solution are mixed. If iodine is added to the mixture, the starch found in flours will also produce a blue-violet solution. Iodine solution on reaction, however, gives a blue-violet tint until the fermentation process is over and the product (maltose) is formed completely. By comparing the period of time required for the fermentation process to be completed with equal amounts of each type of flour, it is possible to ascertain the rate of fermentation of different starch-containing flours.

1. Lactic Acid Fermentation:

There is no requirement for heat in the manufacture of lactic acid when yeast strains and bacteria transform starches or sugars into lactic acid. Pyruvic acid undergoes a series of events in the absence of oxygen that result in the formation of lactic acid and NAD+. These reactions require nicotinamide adenine dinucleotide hydrogen (NADH). Pickles, yogurt, and sourdough bread can all be produced using this approach.

***2. Acetic Acid Fermentation*:**

When starches and sugars derived from grains and fruit undergo fermentation, the resulting products have a characteristic sour flavor. Apple cider vinegar and kombucha are two examples.

3. Ethanol / Alcohol Fermentation:

Wine and beer are made when yeast further breaks down pyruvate molecules into alcohol and carbon dioxide molecules.

NOTE: Pyruvate molecules are the byproduct of the process of glycolysis, which is the breakdown of glucose. ($C_6H_{12}O_6$).

During fermentation, carbohydrates such as glucose or starch are broken down into their component parts to produce cellular energy. This process also results in the production of ethanol and carbon dioxide as byproducts. This is an anaerobic process, in which yeasts typically carry out the conversion in conditions when oxygen is not present. Bread dough, ethanol fuel, and alcoholic beverages like beer and wine all require this particular form of fermentation to be produced. Natural sugars present in fruits like grapes, apples, pears etc. are used in production of fruit wines.

- The manufacture of beer, whiskey, rice wines, and other alcoholic beverages all need the fermentation of grain starches, which is accomplished with the assistance of the enzyme amylase.
- Ethanol is a clear, colorless liquid with a pleasant smell. Ethyl alcohol is the intoxicating component in beer, wine, and other alcoholic drinks.
- The naturally occurring sugars that are found in honey are utilized in the manufacturing of different varieties meads, sparkling wine, sherry with different flavors depending on the floral source of honey.

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The following microbes are utilized in ethanolic fermentation: -

•***Yeast***

- The yeast strain Saccharomyces cerevisiae
  and
- The yeast strain Schizosaccharomyces.

•Zymomonas mobilis (a bacterium)

The biofuel sector is interested in Zymomonas mobilis because of its excellent ethanol fermentation yields. Z. mobilis has undergone extensive basic and application research since it was originally identified as a bacterium isolate in fermented alcoholic beverages. Apart from ethanol, the potent Entner-Doudoroff glycolytic pathway of Z. mobilis has been the focus of meticulous biochemical studies and garnered attention for the production of high-value chemicals.[1]

4. Biotechnical Changes during Fermentation of Cereals:

People in many parts of the world believe that cereal grains constitute the single most significant source of a diverse range of nutrients, such as dietary proteins, carbohydrates, dietary fibers, minerals, and vitamins. On the other hand, the grains are deficient in terms of both their nutritional content and their sensory features. The process of fermentation is the method that has proven to be the most effective in enhancing these properties. The quantity of carb and certain non-digestible poly and oligosaccharides that were present in cereals typically decreases after the process. This occurs in the majority of instances. It is feasible for cereals to manufacture amino acids, as shown by the significant rise in the amount of lysine found in cereals like maize, millets, and other meals that are nutritionally comparable, as well as the increase in the amount of vitamin B that is readily available. The pH levels required for the enzymatic decomposition of phylate can be optimally produced by fermentation, which is a prerequisite for this process. The fermentation of cereals leads to an improvement in the flavor, aroma, and texture of the final product [8]. Additionally, the shelf life of the finished product is increased as a result of the fermentation process.

Enzyme Diastase (Malt Extract):

Enzymes found in malt extract are most active in neutral solutions. The activity is destroyed by the acidic environment. Starch is transformed into the disaccharide maltose by it. Heat deactivates the enzyme. Numerous heat-sterilized malt extracts are diastase-free. On standing, the aqueous solution exhibits flocculants precipitates. The enzyme diastase is produced by mixing water and sprouted barley seeds at a temperature of 15 degrees Celsius in the absence of light. The temperature is raised to 60 degrees Celsius once the germination process is complete in order to stop any further growth. The water is purified once the seeds are crushed up and added. The filtrate that contains the enzyme diastase is referred to as "malt extract." Malt extract can be used to assess the flavor, enzyme activity, and starch content of foods such as flours, malt vinegar, morning cereals, infant meals, confections, and baked goods. Malt whisky is also made with the aid of malt extracts.[2]

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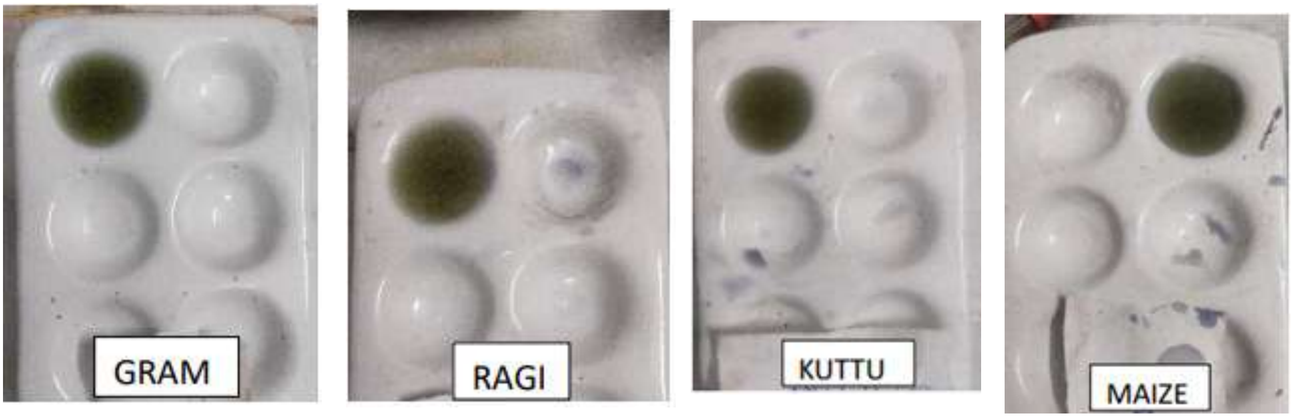
OBSERVATIONS:-

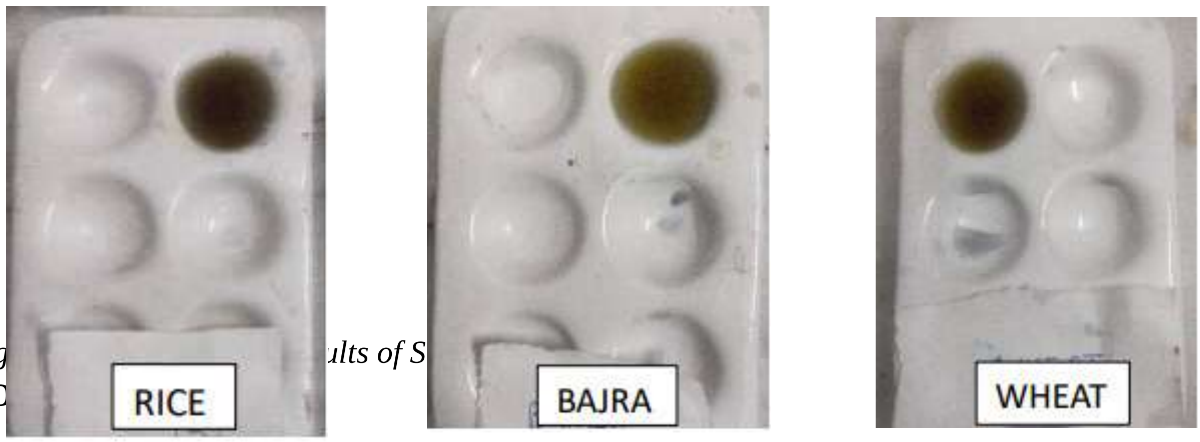
S.NO.	**FLOUR SAMPLE**	**TIME TAKEN FOR FERMENTATION**
1.	Maize	20 hrs 10 min
2.	Wheat	14 hrs 50 min
3.	Buckwheat(Kuttu)	13 hrs 40 min
4.	Pearl millet	13 hrs 30 min
5.	Rice	19 hrs 45 min
6.	Gram	13 hrs 20 min
7.	Finger millet	11 hrs 30 min

Table 1: Observations of Fermentation

The completion of fermentation of the selected samples has been shown in the figure given below:

COMPLETION OF FERMENTATION:





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Ragi flour took the least amount of time to get fermented, while maize flour took the maximum amount of time. Since Ragi flour has the least amount of starch content, it gets fermented earliest, whereas maize flour contains the maximum amount of starch, hence it took maximum time to get fermented. Hence it can be concluded that the rate of fermentation of flours used in this study varies proportionally with the amount of starch present in them. The same was deduced from the observations.

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Conflicts of interest

Nil.

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