

COMPARISON AND CHARACTERISATION OF FUNGI PRESENT IN CASSAVA BREAD AND GINGER CASSAVA BREAD

Submitted in partial fulfilment of the requirements for the award of Bachelor of
Technology in biotechnology

by

R PAVITHRA (37230055)



**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited with Grade "A" by NAAC

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI – 600 119

APRIL – 2021



SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with "A" grade by NAAC

Jeppiaar Nagar, Rajiv Gandhi Salai, Chennai – 600 119

www.sathyabama.ac.in

DEPARTMENT OF BIOTECHNOLOGY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bona-fide work of R. PAVITHRA (37230055) who carried out the project entitled "Comparison and characterization of fungi present in cassava bread and ginger cassava bread" under our supervision from January 2021 to March 2021.

Internal Guide

Dr. S. USHA NANDHINI

External Guide

Dr. R. KALPANA

Head of the Department

DR. V RAMESH KUMAR

Submitted for Viva voce Examination held on **16-04-2021**

Internal Examiner

External Examiner



Founded by Shri C Subramaniam

NAF/FSD-SP/256 /632/20-21
March 12, 2021

BONAFIDE CERTIFICATE

This is to certify that **R. PAVITHRA**, B. Tech Biotechnology, Sathyabama Institute of Science and Technology, Chennai has carried out the Project titled **"COMPARISON AND CHARACTERIZATION OF FUNGI PRESENT IN CASSAVA BREAD AND GINGER CASSAVA BREAD"** under the guidance of Dr. R. Kalpana, Head – R&D Centre, National Agro Foundation, Taramani, Chennai – 600113 during January 2021 to March 2021.

A handwritten signature in black ink, appearing to be "Dr. R. Kalpana".



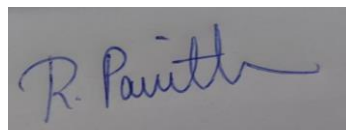
Dr. R. Kalpana
Head- R&D Centre
National Agro Foundation

Registered Office : 2, "River Side", River View Road, Kotturpuram, Chennai - 600085, India. Phone : +91 44 42131237
Research and Development Centre : Anna University Taramani Campus, CSIR Road, Taramani, Chennai - 600 113, India.
Phone : +91 44 - 22542803, 22542598, E-mail : nationalagro@gmail.com, www.nationalagro.org

DECLARATION

I, R PAVITHRA (37230055) hereby declare that the Project Report entitled **“Comparison and characterization of fungi present in cassava bread and ginger cassava bread”** done by me under the guidance of **Dr. S Usha Nandhini** and **Dr. R Kalpana** at National Agro Foundation – R&D Center, Taramani, Chennai is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

DATE: 16-04-2021



PLACE: Chennai

SIGNATURE OF THE CANDIDATE

ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to the Board of Management of **SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY** for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to **Dr. V Ramesh Kumar** and **Mr. G Narendra Kumar** Head of the Department, Dept. of Biotechnology for providing me necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Guide **Dr. S Usha Nandhini** and **Dr. R Kalpana** for their valuable guidance, suggestions and constant encouragement, which paved way for the successful completion of my project work.

I would like to thank **Mrs. Divya Bharathi**, National Agro Foundation-R&D for helping me in carry out the analytical works in laboratory.

I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of Biotechnology, Sathyabama Institute of Science and Technology who were helpful in many ways for the completion of the project.

ABSTRACT

Bread is a staple food in the world and it is recognized as a semi perishable commodity. The spoilage of bread is mostly because of the improper storage and presence of bread mold. Two bread samples were prepared one with cassava flour and another with ginger and cassava flour. The bread samples were exposed to 3 different environmental conditions and the shelf life was determined. Once the yeast and mold growth starts forming, the bread is taken as sample and made to grow on potato dextrose agar plate to check the yeast and mold growth. The colony characteristics was determined. Isolation of fungi was done using slide culture technique and the morphological characteristics of the fungi was determined. The fungi present in both the bread samples was compared by the colony and morphological characteristics.

Key Words: Cassava, Ginger, Bread, Shelf life, Colony Characteristics, Morphology Characteristics.

LIST OF CONTENT

CHAPTER NO.	TITLE	PAGE NO.
	Abstract	i
	List of Abbreviation	V
	List of Figures	vii
	List of Tables	X
1	Introduction	1
1.1	Bread and its Evolution	1
1.2	Cassava	1
1.3	Nutritional composition of cassava	2
1.4	Health benefits of cassava	2
1.5	Ginger	3
1.6	Nutritional composition of ginger	3
1.7	Health benefits of ginger	3
1.8	Shelf Life of bread	4
1.9	Fungi present in bread	4
1.10	Fungi present in cassava	5
2	Literature Review	6

3	Aims and Objectives	11
3.1	Aim	11
3.2	Objectives	11
4	Materials and Methodology	12
4.1	Materials required	12
4.1.1	Ingredient required for cassava and Ginger-Cassava bread	12
4.1.2	Chemicals and reagents required for Nutritional Analysis	12
4.1.3	Media and reagents required for Fungi isolation	13
4.1.4	Apparatus required	13
4.1.5	Instruments	14
4.2	Methodology	15
4.2.1	Formulation of cassava bread and ginger -cassava bread	15
4.2.2	Formulation of standard	16
4.2.3	Formulation of cassava bread	18
4.2.4	Formulation of Ginger- cassava bread	19
4.2.5	Bread sample preparation for nutritional analysis	21
4.2.6	Nutritional Analysis	21
4.2.7	Shelf -Life Analysis	31
4.2.8	Total plate count procedure	33
4.2.9	Yeast and mold for fungi isolation	34
4.2.10	Isolation of fungi by slide culture technique	35
5	Results and Discussion	37
5.1	Formulation of bread	36
5.1.1	Formulation of standard	36
5.1.2	Formulation of cassava bread	37

5.1.3	Formulation of ginger-cassava bread	38
5.2	Nutritional Analysis Result	38
5.2.1	Cassava bread proximity and Nutritional Analysis	38
5.2.2	Ginger cassava bread proximity and Nutritional Analysis	39
5.2.3	Mineral analysis result for cassava Bread	44
5.2.4	Mineral analysis result for Ginger-cassava Bread	44
5.3	Shelf-life analysis	45
5.3.1	Shelf life at Room temperature	45
5.3.2	Shelf life in Refrigerator	46
5.3.3	Shelf life in stability chamber	46
5.4	Microbial analysis	47
5.4.1	Total plate count of cassava bread at Room Temperature	47
5.4.2	Total plate count of Ginger-cassava bread at Room Temperature	48
5.4.3	Yeast and mold method (cassava bread)	48
5.4.4	Yeast and mold method (Ginger-cassava bread)	50
5.4.5	Microscopic view of fungi present in Cassava bread	52
5.4.6	Microscopic view of fungi present in Ginger-Cassava bread	54
6	Summary and Conclusion	58
	References	59

LIST OF ABBREVIATION

SL.NO	ABBREVIATION	FULL FORM
1.	NaOH	Sodium hydroxide
2.	H ₂ SO ₄	Sulphuric acid
3.	g	grams
4.	mg	milli gram
5.	ml	milli litre
6.	°C	Degree Celsius
7.	mm	milli metre
8.	PDA	Potato dextrose agar
9.	SDA	Sabouraud dextros agar
10.	Hrs	hours
11.	N	Normality
12.	(v/v)	Volume/volume
13.	Mins	Minutes
14.	Pre	previously
15.	W	Weight
16.	Rh	Relative humidity
17.	rpm	Revolution Per Minute
18.	Spp.	Species in plural form
19.	CuSO ₄	Copper sulphate

20.	Na_2SO_4	Sodium sulphate
21.	CFU	Colony forming unit
22.	TPC	Total plate count

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE NO.
1.1	Cassava and cassava flour	2
1.2	Ginger	3
1.3	Bread Mold formation in bread (Maida bread)	4
1.4	Bread Mold formation in bread (cassava-bread)	5
4.1	Activated Yeast	15
4.2	Dough after second proofing	16
4.3	Flow chart for standard preparation	17
4.4	Flow chart for cassava bread preparation	18
4.5	Flow chart for Ginger cassava bread Preparation	20
4.6	Powered bread sample	21
4.7	Moisture test	22
4.8	Sample placed in hot plate	23
4.9	Ash of the sample cooled in desiccator	23
4.10	Kjeldahl apparatus setup for protein test	25
4.11	Receiver solution before titration	25
4.12	Receiver solution after titration	26
4.13	Soxhlet apparatus for fat extraction	27
4.14	Mixture of solution filtered using vacuum Pump	28
4.15	Residue in the filter paper after drying in hot air oven	29
4.16	Filtration of alcoholic extract	30
4.17	Alcohol after titrated against sodium hydroxide	30
4.18	Samples stored in refrigerator	32
4.19	Samples stored in room temperature	32
4.20	Serial diluted test tubes for total plate count	33
4.21	Petri plates allowed to solidify	34

4.22	Serial dilution and spread plate for yeast and mold	34
4.23	Stained using lactophenol cotton blue	35
5.1	Standard bread	36
5.2	Standard bread sliced	36
5.3	Cassava bread	37
5.4	Ginger cassava bread and cassava bread	37
5.5	Ginger cassava bread	38
5.6	Graphical representation of moisture content of cassava bread and ginger cassava bread	39
5.7	Graphical representation of Ash % of cassava bread and ginger cassava bread	40
5.8	Graphical representation of protein content of cassava bread and ginger cassava bread	40
5.9	Graphical representation of Fat content of Cassava bread and ginger cassava bread	41
5.10	Graphical representation of Crude Fibre % of cassava bread and ginger cassava bread	41
5.11	Graphical representation of alcoholic acidity of cassava bread and ginger cassava bread	42
5.12	Graphical representation of Carbohydrate% of cassava bread and ginger cassava bread	43
5.13	Ginger cassava bread at room temperature	45
5.14	Cassava bread at room temperature	45
5.15	Ginger cassava bread in refrigerator	46
5.16	Cassava bread in refrigerator	46
5.17	Ginger cassava bread in stability chamber	47
5.18	Cassava bread in stability chamber	47
5.19	Yeast and mold 10^{-1} Dilution -Cassava bread	48
5.20	Yeast and mold 10^{-3} Dilution- Cassava bread	49
5.21	Yeast and mold 10^{-5} Dilution -Cassava bread	49

5.22	Yeast and mold 10^{-1} Dilution- Ginger Cassava bread	50
5.23	Yeast and mold 10^{-3} Dilution – Ginger Cassava bread	50
5.24	Yeast and mold 10^{-5} Dilution- Ginger Cassava bread	51
5.25	Microscopic view of Mucor spp.in Cassava bread	52
5.26	Microscopic view of Rhizopus spp.in Cassava bread	52
5.27	Microscopic view of Aspergillus spp.in Cassava bread	53
5.28	Microscopic view of Phoma spp.in cassava bread	53
5.29	Microscopic view of Rhizopus spp.in Ginger-cassava bread	54
5.30	Microscopic view of Aspergillus spp. In Ginger-cassava bread	54
5.31	Microscopic view of Mucor spp.in Ginger-cassava bread	55

LIST OF TABLES

TABLE NO.	TITLE	PAGE NO.
4.1	Standard formulation for 200 grams of bread	17
4.2	Cassava bread formulation for 200grams	19
4.3	Ginger cassava bread formulation for 200 grams	20
5.1	Proximity analysis result of cassava Bread	38
5.2	Proximity analysis result of Ginger-cassava bread	39
5.3	Mineral quantity of Cassava bread	44
5.4	Mineral quantity of Ginger- Cassava bread	44
5.5	Colonies counted on cassava bread sample diluted plates	47
5.6	Colony forming unit of Cassava bread	48
5.7	Colony and morphological characteristics of cassava bread	56
5.8	Colony and morphological characteristics of Ginger- Cassava bread	57

CHAPTER 1- INTRODUCTION

Bread has become the stable food in many countries. Cereals and bakery products act as a valuable source of nutrients in the diet of people. Bread provides nutrients such as carbohydrates, lipids, vitamins, minerals and protein. The basic process involves kneading wheat flour, water, yeast, salt, and other ingredients until it becomes a dough that will rise easily, baked to give good quality bread. Yeast is used in dough, because it utilizes the nutrients in the substrate, releases CO₂ and it makes the bread spongy.

Spoilage in bread is due to post baking contamination. Loaves fresh out of the oven are free from molds or mold spores due because of thermal inactivation during the baking process. (Ponte and Tsen,1978). Spoilage of bread is mainly because of microbial contamination from the surrounding atmosphere during cooling, slicing, packaging, transporting and storage. The common molds of breads are *Aspergillus*, *Penicillium*, *Rhizopus* and *Mucor*. Bread molds like *Mucor*, *Rhizopus* are found to grow first and followed by *Aspergillus* and *Penicillium*. (Nirmala Ravimannan, *et al.*,2016).

Cassava flour is used in baking bread due to its health benefits and the cassava bread is baked as the same way as normal bread with all other ingredients. The spoilage of bread is generally because of fungal contamination. Ginger has an anti-fungal properties and it inhibits *Rhizopus* activity in bread.

1.1 BREAD AND ITS EVOLUTION

After the first ever bread was produced, there has been a constant improvisation that has resulted in the formation of health based and sensory based bread making methodologies. There is increased awareness amongst the people to consume healthy and nutritive foods. Cereals and bread became a stable food in around 10,000 years ago.

1.2 CASSAVA

Cassava is also known as *Manihot esculenta*. It is a tuberous edible plant and after rice is considered as the third largest source of carbohydrates. It is cultivated all around the world for its tuberous roots from which alcoholic beverages, cassava flour, bread,

starch are produced. Cassava is a rich and the best source of dietary fibre. (chiwona-karlton, *et al.*, 2002)



Figure 1.1 Cassava and cassava flour

1.3 NUTRITIONAL COMPOSITION OF CASSAVA

Cassava is a type of tuberous edible plant that grows in a tropical climate. They are rich in fiber, carbohydrates, protein and vitamins. Cassava contains significant quantity of vitamins such as Vitamin A, Vitamin B, Vitamin D and Vitamin C, and minerals such as calcium, iron, cobalt, magnesium, sodium, and potassium. It has anti-inflammatory and anti-oxidant properties. These properties in cassava can be used to prevent diseases like arthritis, cancer, diabetes, and diarrhea. (Tewe, *et al.*, 2004.)

1.4 HEALTH BENEFITS OF CASSAVA

Cassava can be used as a medicine for many diseases. It protects and repairs the body tissues as it has high protein content. Cassava helps in reducing blood cholesterol level as it has saponins which act as a binding agent by binding the bile acids and cholesterol. Thus, it can reduce the risk of heart related diseases. Cassava can promote osteo-trophic activity in bones as it has calcium and vitamin-K. The fiber present in the cassava can promote the growth of good bacteria in the gut and boosts up the immune system. (Jillian Levy, CHHC 2019)

1.5 GINGER

Ginger or *Zingiber officinale* comes under the family of *Zingiberaceae* which also includes turmeric, cardamon and galangal. It is a plant with leafy stems, and ginger spice comes from the root of the plant. Ginger is usually grown in warmer parts of Asia such as India, Japan and China. In recent years it is also cultivated in places like South America and Africa. (Kew Science,2017).



Figure 1.2 Ginger

1.6 NUTRITIONAL COMPOSITION OF GINGER

Ginger is good source of Vitamin-C, and it contains other vitamins like Vitamin-E, K, Thiamin, Riboflavin, Niacin, Folate, Pantothenic Acid and Choline. Around 79% of ginger is composed of water. (An K, Zhao D, et al.2016)

1.7 HEALTH BENEFITS OF GINGER

Ginger is most commonly used to treat nausea and vomiting. It is also used for menstrual cramps, diabetes, and migraine headaches. Ginger has 9 different types of substances that help in stimulating serotonin receptors in the gut which has good benefits for gastrointestinal system. Ginger plays a major role in curing osteo - arthritic pain and cancer. (Joe Leech, MS , 2020)

1.8 SHELF LIFE OF BREAD

The shelf life of the bread depends on the 3 major factors. They are, type of the bread, storage and climate.

Types of bread: Certain types of bread usually have longer shelf life and this includes both the bread with preservatives and sourdough bread. Lactic acid bacteria slows down the process of staling as the acidity of the bread inhibits the growth of microbes.

Storage: Bread should be stored in a proper environment with appropriate packaging. The bread wrapped with a paper bag or cotton bag can keep the bread fresh for long periods.

Climate: Climate also plays a major role in the shelf life of the bread. The place with dry climate usually keeps the bread fresh for longer days, while it becomes stale in a place with high humid condition

1.9 FUNGI PRESENT IN BREAD

The most common fungi associated with the bread spoilage is Rhizopus. The other fungi are Aspergillus, Mucor and Penicillium. (Journal of Applied & Environmental Microbiology.2019)



Figure 1.3 bread mold formed in bread(maida bread)

1.10 FUNGI PRESENT IN CASSAVA

The most common fungi found in cassava products belong to genera *Rhizopus*, *Aspergillus*, *Fusarium*, *Phoma* spp. and *Penicillium*.



Figure 1.4 bread mold formed in cassava bread

CHAPTER 2 - LITERATURE SURVEY

Bread is one of the oldest foods developed ever since the dawn of agriculture. Bread has become a staple food in several countries and is basically prepared by baking dough made from wheat flour and water. Several improvisations and value additions have been done to increase the nutritional value and quantity of bread in terms of increasing the shelf life and texture of the bread. Bread is usually made from wheat flour dough that is cultured with active dry yeast or wet yeast and allowed for rising and proofing, and baked finally in an oven. The elasticity of the bread is because of the gluten content. Wheat is the most common grain used in making bread. Wheat, in the form of bread, provides more nutrients to the world population than any other single food source (Pena, R,J october2016)

Bread is categorized as a source of grains within the organic phenomenon. Further, it is a good source of carbohydrates and nutrients such as magnesium, iron, selenium, B vitamins, and dietary-fibre (Dietary Guidelines of America,2010).

Bread making is based on six major ingredients, which include flour, water, yeast, sugar, salt, and shortening. Flour and water are the basic requirement for making bread. However, the quantity of water varies based on the protein content of the flour. Yeast is the mandatory ingredient which is used to ferment the sugar into carbon dioxide and ethyl alcohol. sodium chloride which is salt is added for the gas retention and taste enhancer. Shortening is added to improve the crumb grain and loaf volume the shortening is used based on the quality and quantity protein. (Finney,1984)

Manihot esculenta is usually referred to as cassava is woody shrub native to South America of the spruge family, Euphorbiaceae. It is major source of carbohydrates as it as a edible starch filled tuberous root. It is a perennial plant but cultivated as an annual crop in tropical and subtropical region. Cassava is the 3rd largest source of carbohydrates next to rice and wheat.(Fauquet Claude, Fargette Denis ,1990). Cassava is a major staple food in this developing world and it provides a basic dietary food to half a billion of people around the world.(Food and Agriculture Organization of United Nations, 1995)

Raw cassava is 60% water, 38% carbohydrates, 1% protein, and has negligible fat .(Tewe, Olumide, 2014). Raw cassava provides 160 kilocalories of food energy and 25% of the daily value of vitamin C. Cooked cassava features digestibility of over 75%.

China, India, Africa, the Caribbean, and other warm climates are the best place for the growth of ginger. Ginger plant used as a spice and flavoring. It's been a standard remedy in many cultures for thousands of years. (Fundukian, L.J.,2009)

Ginger may be a good source of nutrients, vitamins and minerals. Consuming 24 gram of ginger offers 0.054 mg of Copper,4.26 g of Carbohydrate, 0.038 mg of vitamin B6, 0.055 mg of Manganese,10 mg of Magnesium,100 mg of Potassium and 0.14 mg of Iron. (www.healthbenefitstimes.com/ginger/)

There are differening types of bread, each with its own time period. Generally, bread lasts approximately 5-7 days. Refrigerating and freezing bread can extend its lifespan. However, it's not recommended, because the bread can lose its nutritional value, taste, and switch stale faster. Packaged bread can last for 2-3days at humid and moist climate, 5-7 days at room temperature. Packaged bread can lasts 2-3 days in the refrigerator with packing. But storing it for longer days zip lock pouch or air tight containers works well. (Susan Grey,2019)

Bread may be a source of nutrients, like macronutrients and micronutrients that are all essential for human health.Bread and other bakery products are subjected to varied spoilage problems, viz., physical, chemical and microbial; the latter is that the most serious one particularly bacterial (*Bacillus* sp.) and mold growth. *Rhizopus*, *Mucor*, *Penicillium*, *Eurotium*, *Aspergillus* and *Monilia* are types of molds involved in the spoilage of bread. (Saranraj P, Sivasakthivelan P, 2015).

The research method within the isolation of fungi begins with sampling of the banana waste that is already retted, whereas to isolate the fungus is thru Dilution Series. It is expected that fungus contained in the sample can be grown, separated, isolated and subsequently cultured on PDA and SDA medium. (Noor Arida Fauzana,2011)

Identification of Fungi: After the isolates are obtained, the fungus is identified through the moist chamber method. Fungi identification is then performed by observing colony

characteristics and morphology of fungi with the assistance of fungi identification book. (Noor Arida Fauzana,2011)

The isolation of micro-organism can be done using a spread plate. Serial dilution is done by adding 1ml of sample transferred to test tube containing 10 ml of sterile water. 1ml of solution is added from one solution to another which is 10^{-1} dilution. Serial dilution is made up to 10^{-9} dilutions. The molten PDA agar is added in a sterile petri plate and 10^{-4} , 10^{-6} , 10^{-8} dilution was added in a respective sterile petri plate, and incubated at 37°C for 4-5 days.

Different colonies were taken from a mixture of colonies grown on culture plate, streaked on separate plate, and incubated at 37°C for 48 hrs. (AOAC - Official methods of analysis,2005)

The colonies with different colours were noted and they were used for the isolation of fungi. 7x7mm sized agar block is cut with the help of sterile blade with enough to cover a cover slip. Flip the agar block up onto the surface of the agar plate. Inoculate the four sides of the agar block with colony of the fungus to be grown. Coverslip is flamed and placed centrally on the agar block. Incubate the plate at 37°C until growth and sporulation have occurred. Remove the cover slip from the agar block. Apply a drop of 95% alcohol as a wetter. Gently lower the coverslip onto a little drop of Lactophenol cotton blue on a clean glass slide. Viewed under microscope for the morphological characteristics. (Haris JL., 1986)"

The bread samples were exposed different types of environment and observed for the daily spoilage. The breads were handled in environments like wet bread in dark room, in sunlight, in refrigerator, airtight container, and at normal room temperature. The spoilage is checked in daily basis and the shelf life is noted in all these breads in different environmental conditions. (Unachukwu, 2015)

COLONY AND MORPHOLOGICAL CHARACTERISTICS

ASPERGILLUS spp.

Colony characteristics:

Yellow or yellowish green colonies with distinct margin. (Tobias, 2007)

Morphological characteristics:

Conidiophores arise from a foot- cell. Club shaped vesicles are at top of the conidiophores. Conidia are found in chains. (Nirmala Ravimannan,2016)

RHIZOPUS spp.

Colony characteristics:

White cottony colonies with black dots covers the entire plate. (Nirmala Ravimannan,2016)

Morphological characteristics:

stolons and pigmented rhizoids are present, generally globose sporangia will be present and formation of sporangiophores,. The Rhizopus species can be categorised .After spore release the apophyses and columella often collapse to form an umbrella-like structure .(Domsch K.H et al. (1980)

MUCOR spp.

Colony characteristics:

Larger white colony turns to black in colour .(Nirmala Ravimannan,2016)

Morphological characteristics:

Erect sporangiophores are formed.sporangiophore swells at the tip to form sporangia which are globular shaped. Columella is present. .(Nirmala Ravimannan,2016)

PENICILLIUM spp.

Colony characteristics:

colonies which grows faster in green colour with dense conidia .(Nirmala Ravimannan,2016)

Morphological characteristics:

Branched conidiophores with chains of conidia seems like a brush. .(Nirmala Ravimannan,2016)

PHOMA spp.

Colony characteristics:

Colonies of *Phoma* grow rapidly. They are flat, spreading, powdery to velvety, and sometimes largely submerged within the medium. The colour of the colony will be initially white which later turns to olive grey with an occasional tint of pink. From the reverse, it's dark brown to black. (de Hoog, G. S., 2000)

Morphological characteristics:

Pycnidia do have openings called ostioles, present on their surface from which the conidia are released outside. Conidia are unicellular, hyaline, and oval-shaped. Each conidium typically has two oil droplets inside. (Larone, D. H. 1995)

CHAPTER 3 – AIMS AND OBJECTIVES

3.1 AIM

Comparison and characterization of fungus present in cassava bread and ginger cassava bread.

3.2 OBJECTIVES

3.2.1 To formulate cassava bread and ginger -cassava bread.

3.2.2 To determine the shelf life of the bread samples.

3.2.3 To determine its nutritional factors.

3.2.4. To isolate the fungus present in bread samples.

3.2.5 Comparison of fungi present in cassava bread and ginger cassava bread.

CHAPTER 4- MATERIALS AND METHODOLOGY

4.1 MATERIALS REQUIRED

4.1.1 INGREDIENTS REQUIRED FOR CASSVA AND GINGER-CASSAVA BREAD

- 1.Maida
- 2.Cassava Flour
- 3.Gluten
- 4.Water
- 5.Salt
- 6.Sugar
- 7.Vanaspati
- 8.Active dry yeast
- 9.Milk powder

4.1.2 CHEMICALS AND REAGENTS REQUIRED FOR NUTRITIONAL ANALYSIS

- 1.Distilled water
- 2.Sodium sulphate
- 3.Copper sulphate
- 4.H₂SO₄
- 5.Gel crystal
- 6.50% NaOH
- 7.0.1N H₂SO₄
- 8.Methyl Red
- 9.Petroleum Benzene
10. (1.25%) H₂SO₄
11. (1.25%)NaOH

12. Neutral Alcohol 90 % (v/v).

13. Standard NaOH- 0.05 N

14. Phenolphthalein Solution

4.1.3. MEDIA AND REAGENT FOR FUNGI ISOLATION

1. Plate Count Agar

2. Potato Dextrose Agar

3. Agar-Agar

4. Peptone water

5. Sterile distilled water

6. Lactophenol cotton blue

4.1.4 APPARATUS REQUIRED

1. Silica crucible

2. Moisture dish (Aluminum)

3. A1 filter paper

4. Whatman filter paper

5. Long neck round bottom flask

6. Beaker

7. Round bottom flask

8. Standard measuring flask

9. Petri Plates

10. Test Tubes

11. Test Tube rack

12. Centrifuge tubes

13. Glass slides

- 14.Cover slips
- 15.Measuring cylinder
- 16.1ml Pipette
- 17.Burette
- 18.Stopper conical flask
- 19.Desiccator
- 20.Bread mould

4.1.5 INSTRUMENTS

- 1.Hot Air Oven
- 2.Hot Plate
- 3.Muffle furnace
- 4.Soxhlet apparatus
- 5.Kjeldahl apparatus
- 6.Vacuum pump
- 7.Water bath
- 8.Incubator
- 9.Laminar Air flow chamber
- 10.Autoclave
- 11.Magnetic stirrer
- 12.Stability chamber
- 13.Refrigerator
- 14.Baking oven
- 15.Microscope
- 16.Weighing machine

4.2 METHODOLOGY

4.2.1 FORMULATION OF CASSAVA BREAD AND GINGER-CASSAVA BREAD

The formulation is based on the general bread making procedures which includes adding maida (refined wheat flour), salt, sugar, milk powder, fat. These ingredients along with yeast and water, added to the cassava flour are mixed to form a dough. The dough is then allowed to ferment for around 45 minutes to 1 hour. (Ali ,2012)The mixture is again mixed and folded and kept in bread loaf mould and allowed for second proofing for 30 -35 minutes. The dough is baked in the oven which is already pre heated for 10 mins at 180°C , for 25-30 minutes.

The procedure is followed, and ginger extract is added for mixing the dough.

Ginger extract: about 10 grams of ginger is grated, added in 90 ml of water and boiled and left to cool overnight and the extract is filtered to obtain a ginger extract.(Singh 2013)



Figure 4.1 Activated yeast

4.2.2 FORMULATION OF STANDARD

Refined wheat flour, milk powder, yeast, salt, sugar, fat were used to prepare the standard. These ingredients were added in the defined proportions. Activated yeast and water were added, kneaded for 10 minutes and made into a dough. The dough is allowed to ferment for 45 minutes. The dough is folded and kept in bread-mould and allowed for proofing for 45 minutes. Then the dough is baked in preheated oven at 180°C for 40 minutes. (Plessas,2005)



Figure 4.2 dough after second proofing

Figure 4.3 Flow chart for standard preparation

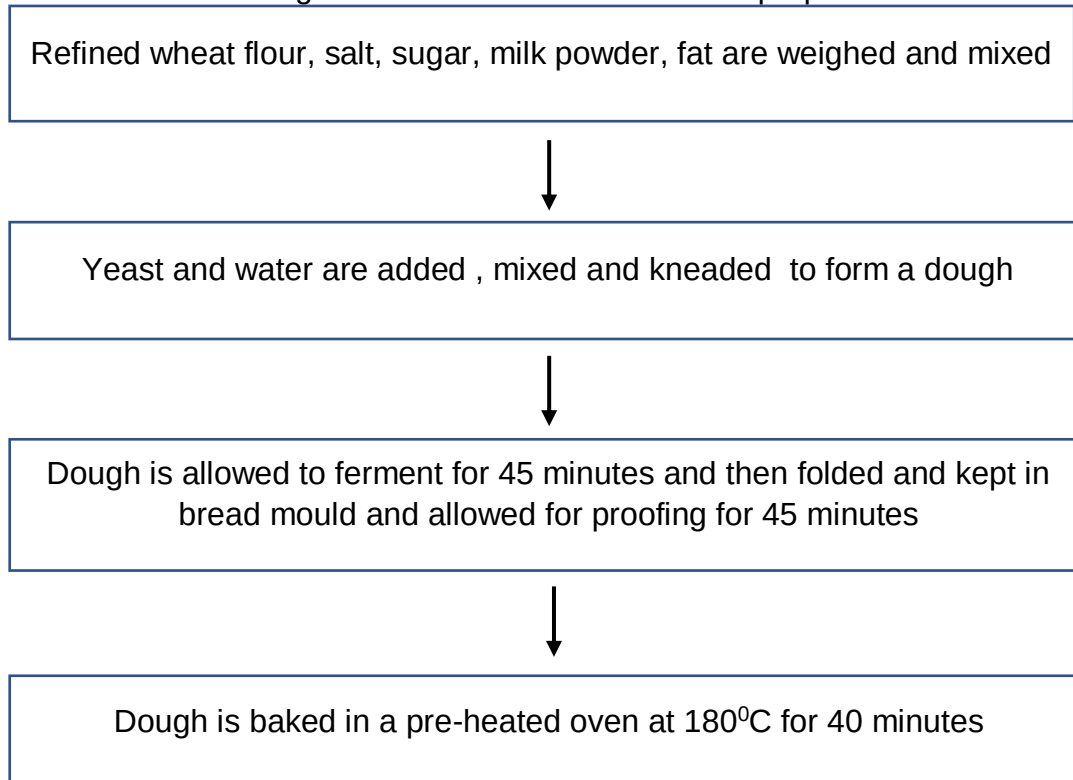


Table 4.1 Standard formulation for 200 grams of bread

Ingredients	Quantity
Refined wheat flour	117g
Salt	1
Sugar	2.5g
Milk powder	5g
Yeast	2g
Fat(vanaspati)	5g
Water	55ml
Bio gluten	1.5g

4.2.3 FORMULATION OF CASSAVA BREAD

Refined wheat flour, milk powder, yeast, salt, sugar, fat were used to prepare the standard. These ingredients were added in the defined proportions along with cassava flour. Activated yeast and water were added, kneaded for 10 minutes and made into a dough. The dough is allowed to ferment for 45 minutes. The dough is folded and kept in bread-mould and allowed for proofing for 45 minutes. Then the dough is baked in preheated oven at 180°C for 25-30 minutes.

Figure 4.4 Flow chart for cassava bread preparation

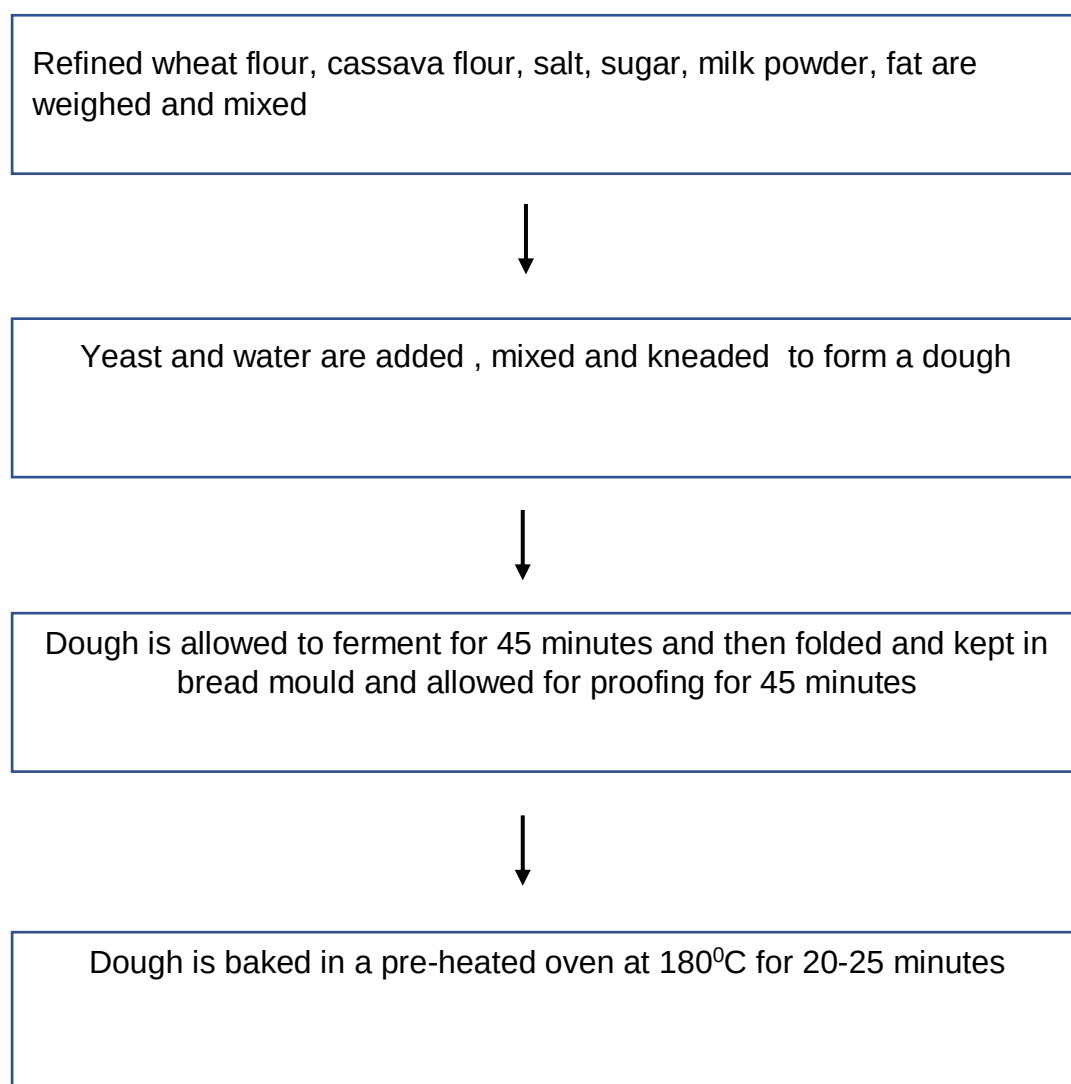


Table 4.2 cassava bread formulation for 200 grams

Ingredients	Quantity
Refined wheat flour	58.5g
Salt	1g
Sugar	2.5g
Milk powder	5g
Yeast	2g
Fat(vanaspati)	5g
Water	55ml
Bio gluten	1.5g
Cassava flour	58.5g

4.2.4 FORMULATION OF GINGER-CASSAVA BREAD

Refined wheat flour, milk powder, yeast, salt, sugar, fat were used to prepare the standard. These ingredients were added in the defined proportions along with cassava flour. Activated yeast, Ginger extract and water were added, kneaded for 10 minutes and made into a dough. The dough is allowed to ferment for 45 minutes. The dough is folded and kept in bread-mould and allowed for proofing for 45 minutes. Then the dough is baked in preheated oven at 180°C for 25-30 minutes.

Figure 4.5 Flow chart for Ginger cassava bread preparation

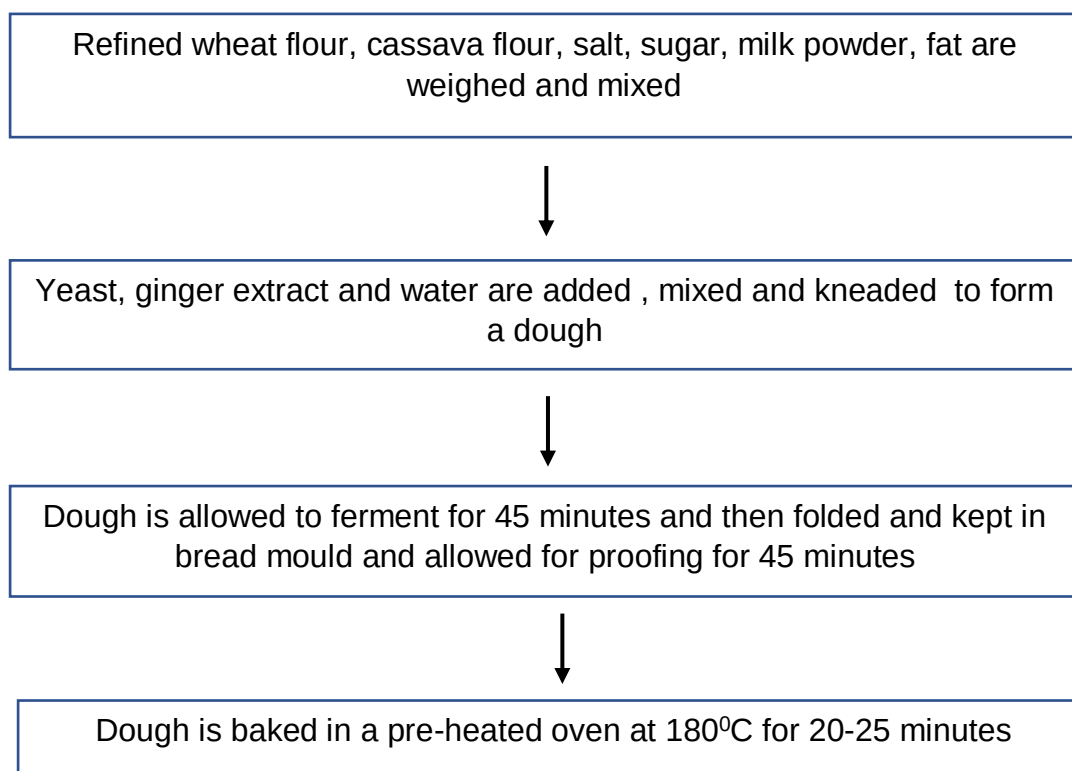


Table 4.3 Ginger cassava bread formulation for 200 grams

Ingredients	Quantity
Refined wheat flour	58.5g
Salt	1g
Sugar	2.5g
Milk powder	5g
Yeast	2g
Fat(vanaspati)	5g
Water	35ml
Bio gluten	1.5g
Cassava flour	58.5g
Ginger extract	20ml

4.2.5 BREAD SAMPLE PREPARATION FOR NUTRITIONAL ANALYSIS

The samples were sliced into pieces and blended into a fine powder, and stored in an air tight container. This can be used as a sample for all the analysis in an appropriate quantity.



Figure 4.6 Powdered bread samples

4.2.6 NUTRITIONAL ANALYSIS

MOISTURE TEST:

Weigh approximately about 5 gram of sample in a moisture dish previously dried in an oven at 105⁰C. The weight of the empty dish and the dish weighed with 5 g sample is noted down. The dish is kept in a Hot Air Oven for 4 hours at 105±2⁰C. Cool in a desiccator and weigh. From the loss in mass calculate the moisture percentage of the sample. (fssai -manual of methods of analysis of foods- cereal and cereal products, 2012)



Figure 4.7 Moisture test

Calculation:

$$\text{Moisture percent by weight} = 100 \frac{(W1 - W2)}{(W1 - W)}$$

W1-weight in gram with material before drying

W2-weight in gram of dish with material after drying to constant weight

W-weight in gram of empty dish

Cassava bread:

W1=64.182g

W2=62.833g

W=58.974g

Ginger cassava bread:

W1=63.618g

W2=62.238g

W=58.553g

ASH TEST:

Weigh about 5 gram of sample in a silica crucible which is weighed previously. The empty weight of the crucible is noted down. The crucible with sample is kept in a hot plate until it is burned completely. Then the crucible is transferred into a muffle furnace to make it into an ash for 16 hours. Then it is cooled in a desiccator. And the weight of the ash in the crucible is noted down. (fssai -manual of methods of analysis of foods- cereal and cereal products, 2012)



Figure4.8 Sample placed in hot plate



Figure 4.9 Ash of the sample cooled in desiccator

Calculation:

$$\% \text{ash} = \frac{(\text{weight of crucible with sample} - \text{empty crucible weight})}{(\text{ash weight} - \text{empty crucible weight})} \times 100$$

Cassava bread:

weight of crucible with sample: 43.967g

empty crucible weight : 38.902

weight of crucible with ash : 38.992g

Ginger cassava bread:

weight of crucible with sample: 25.296g

empty crucible weight : 20.296g

weight of crucible with ash : 20.362g

PROTEIN TEST:

In a long neck round bottom flask 2 gram of sample is taken. To it is added 10g of sodium sulphate, 0.4 g of copper sulphate and 25ml of H₂SO₄ and kept for protein digestion in a heating mantle for 16 hours. The digested sample is dissolved with distilled water in a round bottom flask and gel crystal Is added in the mixture. Slowly, 50% NaOH is added until the solution turns blue in color. The Kjeldahl setup is done and the receiver solution is prepared with 20 ml of H₂SO₄ and 2-3 drops of methyl red added. Then the setup is kept undisturbed until the receiver solution in the beaker reaches 150ml. Then the receiver solution is titrated against 0.1% NaOH. Pink to yellow color as an indication. The titrate value is noted down. (fssai -manual of methods of analysis of foods- cereal and cereal products, 2012)



Figure 4.10 Kjeldahl apparatus setup for protein test



Figure 4.11 Receiver solution before titration



Figure 4.12 Receiver solution after titration

Calculation:

$$\text{Protein \%} = \frac{(\text{Blank value} - \text{Sample}) \times 0.14 \times \text{N} \times 6.25}{\text{Sample weight} \times 0.1}$$

$$\text{Sample weight} \times 0.1$$

Cassava bread:

Blank value=40.5

Titrate value =25

Sample weight=5g

Normality=0.14 N

Ginger cassava bread:

Blank value=40.5

Titrate value =25.7

Sample weight=5g

Normality=0.14 N

FAT TEST

2g of sample is packed in a filter paper, petroleum benzene is taken as solvent for the fat extraction. Then it is setup in the Soxhlet apparatus and it is kept undisturbed for 18 hours. After 18 hours the round bottom flask is taken and then it is kept in the hot air oven for 2 hours to remove the excess moisture present in it. Then the round bottom flask is weighed before and after the process. (fssai -manual of methods of analysis of foods- cereal and cereal products, 2012)



Figure 4.13 Soxhlet apparatus for fat extraction

Calculation:

$$\text{FAT}\% = (W_2 - W_1)$$

W1- Empty Weight of the flask

W2- Weight of flask with fat

Cassava bread:

$$W_1 = 127.656\text{g}$$

$$W_2 = 128.061\text{g}$$

Ginger cassava bread:

W1=128.591g

W2=129.067

CRUDE -FIBER TEST

For this crude fiber test, 2 gram of sample is taken in a round bottom flask. Add 200 ml of 1.25% H_2SO_4 . It is then boiled in the heating mantle for 30 minutes. Then this solution is filtered by adding a filter paper in a funnel which is connected in to a gooch crucible. The solution is filtered using a vacuum pump. Then the residue present in the filter paper is washed with water to remove the traces of acid. The residue is again added in the round bottom flask and 200 ml of NaOH is added in it and boiled in heating mantle for 30 minutes. The procedure of washing and collecting the residue is repeated and the filter paper is weighed before filtering. The filter paper with the residue is weighed is kept in a petri plate and placed in the hot air oven until it is completely dried. The dried filter paper with the residue is transferred to the silica crucible and kept in the muffle furnace for 3 hours for the ash. The ash is weighed and ash weight is divided from the sample weight which gives the fiber content of the sample. (fssai -manual of methods of analysis of foods- cereal and cereal products, 2012)



Figure 4.14 Mixture of solution filtered using vacuum pump



Figure 4.15 Residue in the filter paper after drying in hot air oven

Calculation:

$$\text{Crude fiber\%} = \frac{\text{Residue weight} - \text{Ash weight}}{\text{Sample weight}} * 100$$

Cassava bread:

Residue weight:46.753g

Ash weight:38.189g

Sample weight: 2g

Ginger Cassava Bread:

Residue weight:48.923g

Ash weight:40.218g

Sample weight:2g

ALCOHOLIC-ACIDITY

Weigh about 5 grams of dried sample into a stoppered conical flask and add 50 ml of 90% neutral alcohol previously neutralized against phenolphthalein. The flask is closed using stopper and stand for 4 hours with occasional shaking. Filter the alcoholic extract using a dried filter paper. Titrate the conical flask against 0.05 N standard sodium hydroxide solution using phenolphthalein as an indicator. (fssai - manual of methods of analysis of foods- cereal and cereal products, 2012)

$$\text{Alcoholic Acidity} = \frac{\text{Titer value} \times 0.05 \times 100}{\text{Sample weight}}$$

Sample weight

Cassava bread:

Titer value: 1.2

Sample weight: 5.07g

Ginger Cassava bread:

Titer value:1.5

Sample weight:5.06g



Figure 4.16 Filtration of alcoholic extract

Figure 4.17 Alcohol after titration
against sodium hydroxide

CARBOHYDRATES CALCULATION:

$$\% \text{carbohydrates} = 100 - \text{protein}\% - \text{moisture}\% - \text{fat}\% - \text{ash}\% - \text{crude fiber}\%$$

MINERAL ANALYSIS:

Weigh about 5 gram of sample in a silica crucible which is weighed previously. The empty weight of the crucible is noted down. The crucible with sample is kept in a hot plate until it is burnt completely. Then the crucible is transferred into a muffle furnace to make it into an ash. Then it is cooled in a desiccator. The weight of the ash in the crucible is noted down. (fssai -manual of methods of analysis of foods- cereal and cereal products, 2012) . 10ml of 50% H₂SO₄ was added in the crucible and made to boil in the hot plate and content is transferred to a standard measuring flask and made up to 100 ml. This is taken as sample for the mineral analysis using an atomic absorption spectrophotometer.

4.2.7 SHELF-LIFE ANALYSIS

Breads exposed to different environments were checked for the yeast and mold growth. The Cassava Bread and Ginger- Cassava Bread were stored in 3 different environments.

1. Breads were stored in air tight container and kept at room temperature and checked for the yeast and mold growth and shelf life periodically.

(Temperature -31⁰C -33⁰C and Humidity-50-55)

2. Breads were stored in sterile zip lock pouches and stored in a stability chamber at temperature 40⁰C and humidity 80% and checked for the shelf life.

3. Breads were stored in an air tight container and kept in refrigerator and the shelf life checked.



Figure 4.18 Samples stored in refrigerator



Figure 4.19 Samples stored in room temperature

4.2.8 TOTAL PLATE COUNT PROCEDURE

The plate count was done for the cassava bread and ginger cassava bread to check for the bacterial and fungi count. 1gram of each sample is taken in a centrifuge tube and 9 ml of sterile distilled water added and centrifuged at 3000 rpm for 10 minutes. The aliquots were collected and 1ml of it is transferred to 9ml of water in a test tube and it is 10^{-1} dilution and the same process is continued and serial dilution is performed until 10^{-9} dilution. Potato dextrose agar or plate count agar was prepared at 3.9 grams of agar in 100 ml of distilled water or 2.3 grams agar in 100ml of distilled water; and sterilized in an autoclave at 15lbs. 1 ml of 10^{-2} , 10^{-4} , 10^{-6} , 10^{-8} dilutions were poured in a petri plate and molten agar is poured over. The pour plate is kept in incubator for 48 hours. The colonies were counted. (Nirmala Ravimannan.*et al.*,2016)



Figure4.20 Serial diluted test tubes for total plate count

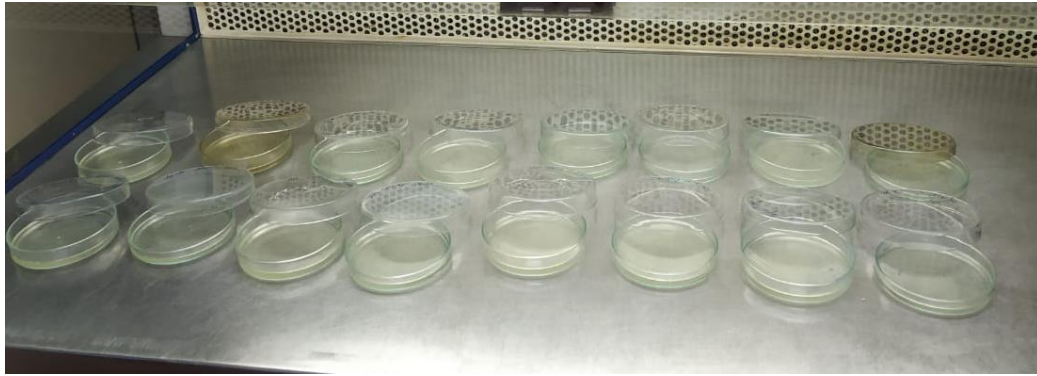


Figure 4.21 Petri plates allowed to solidify

4.2.9 YEAST AND MOLD METHOD FOR FUNGI ISOLATION

Once the yeast and mold were formed in the bread samples, 25 grams of sample was taken and 225 ml of sterile distilled water or peptone water was added, shaken on a rotary shaker for 120 oscillations in 30 minutes. After 30 minutes, the solution was serial diluted to 10^{-1} , 10^{-3} , 10^{-5} . 3.9 grams of potato dextrose agar was dissolved in 100 ml of distilled water and autoclaved it at 121C for 15 minutes. Agar was poured in a petri plate and allowed it to solidify completely. The spread plate technique was performed for the dilutions using a L-Rod in a petri plate and incubated at 37C for 4 days. (Nirmala Ravimannan *et al.*, 2016)

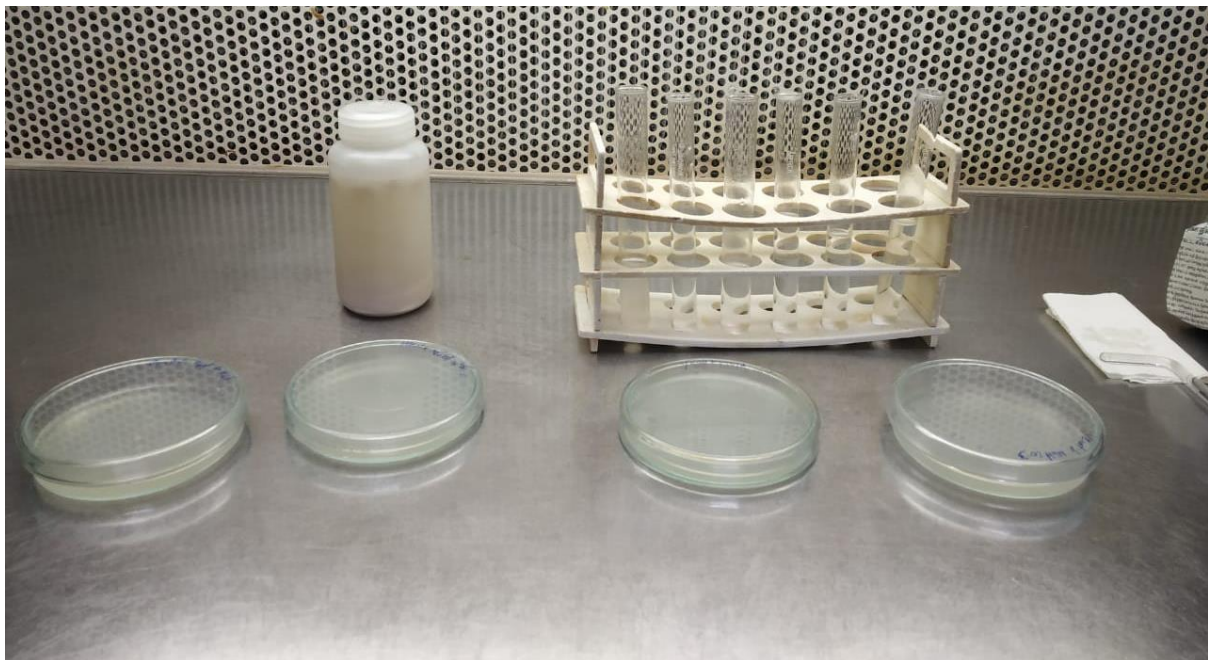


Figure 4.22 Serial dilution and spread plate for yeast and mold

4.2.10 ISOLATION OF FUNGI IN SLIDE CULTURE TECHNIQUE

To isolate the fungi, the colonies were differentiated based on the color and growth. Using a sterile blade cut out an agar block (7 x 7 mm) small enough to fit under a cover slip. Flip the barricade onto the surface of the agar plate. (Mpamah, 2017) Inoculate the four sides of the agar block with colony of the fungus to be grown. The coverslip which is flamed is centrally upon the agar block. Incubate the plate at 37°C until growth and sporulation have occurred. Remove the cover slip from the agar block. Apply a drop of 95% alcohol as a wetter. Gently lower the coverslip onto a little drop of Lactophenol cotton blue on a clean glass slide. Viewed under microscope for the morphological characteristics. (Haris JL., 1986)

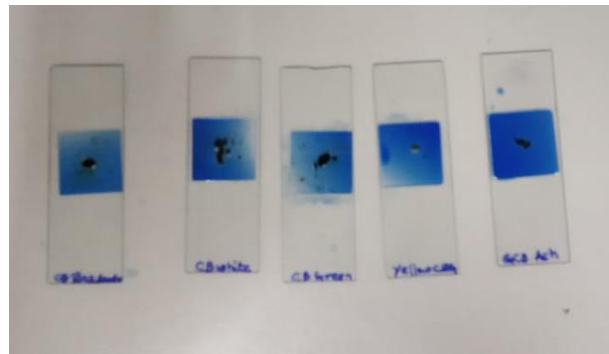


Figure 4.23 Stained using lactophenol cotton blue

CHAPTER 5 – RESULTS AND DISCUSSIONS

5.1 FORMULATION OF BREAD

5.1.1 FORMULATION OF STANDARD

According to material and methodology given for the standard bread baking is followed and the maida bread is baked as a standard. (Plessas,2005)



Figure 5.1 Standard bread



Figure 5.2 Standard bread sliced

5.1.2 FORMULATION OF CASSAVA BREAD

According to material and methodology given for the standard bread baking is followed. (Plessas,2005). By partially replacing the refined wheat flour by cassava flour the bread is baked.



Figure 5.3 Cassava bread



Figure 5.4 Ginger cassava bread and cassava bread

5.1.3 FORMULATION OF GINGER CASSAVA BREAD

The ginger cassava bread is also baked in the same way of standard protocol by partial replacement of refined wheat flour with cassava flour.



Figure 5.5 Ginger cassava bread

5.2 NUTRITIONAL ANALYSIS RESULT

5.2.1 CASSAVA BREAD PROXIMITY AND NUTRITIONAL ANALYSIS

Table 5.1 Proximity analysis result of cassava bread

Nutrients	Quantity (g)
Moisture	26
Ash	1.42
Protein	2.73
Fat	0.405
Crude fiber	1.35
Alcoholic acidity	1.18
Carbohydrates	68.095

5.2.2GINGER CASSAVA BREAD PROXIMITY AND NUTRITIONAL ANALYSIS

Table 5.2 Proximity analysis result of cassava bread

Nutrients	Quantity (g)
Moisture	27.3
Ash	1.34
Protein	2.57
Fat	0.476
Crude fiber	1.87
Alcoholic acidity	1.28
Carbohydrates	66.444

MOISTURE %

The loss of weight when a known sample is heated determines the moisture content of the sample from the table 5.1 and 5.2 the moisture content of cassava bread and ginger cassava bread is 26 and 27.3 respectively and it is found that the moisture content of cassava bread is less compared to ginger cassava bread.

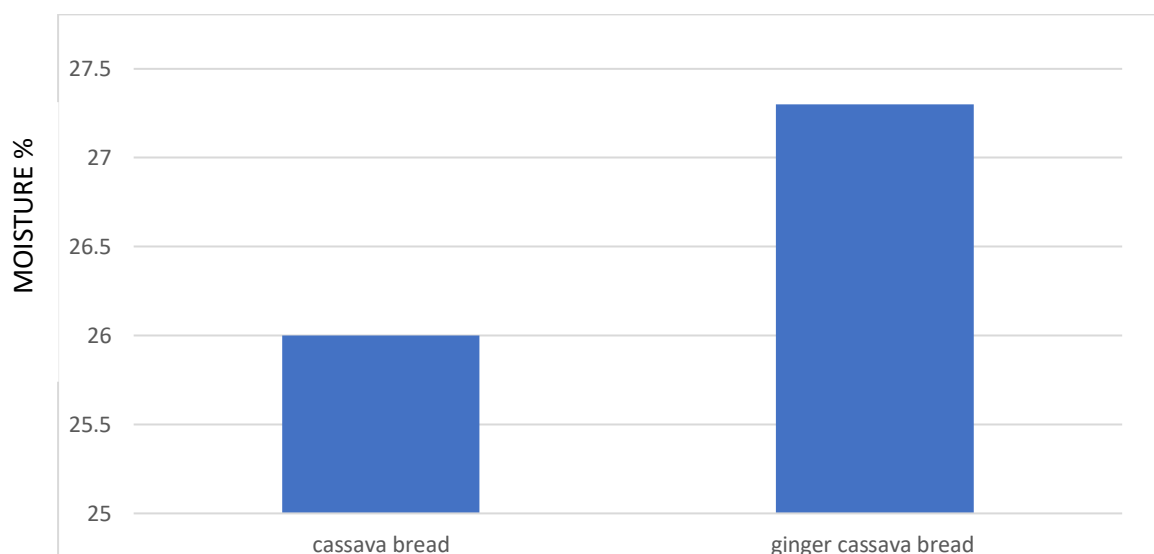


Figure 5.6 Graphical representation of moisture content of cassava bread and ginger cassava bread

ASH %

Ash content is found to be more in cassava bread than the ginger cassava bread.

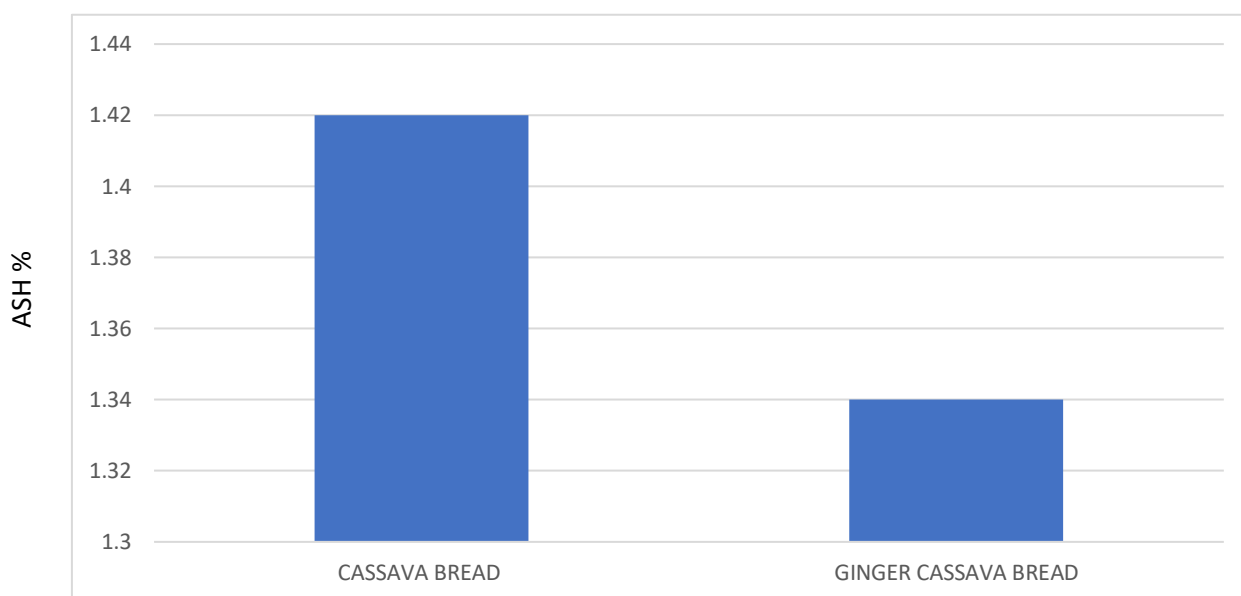


Figure 5.7 Graphical representation of Ash% of cassava bread and ginger cassava bread

PROTEIN %

Cassava bread has higher protein content compared to ginger cassava bread.

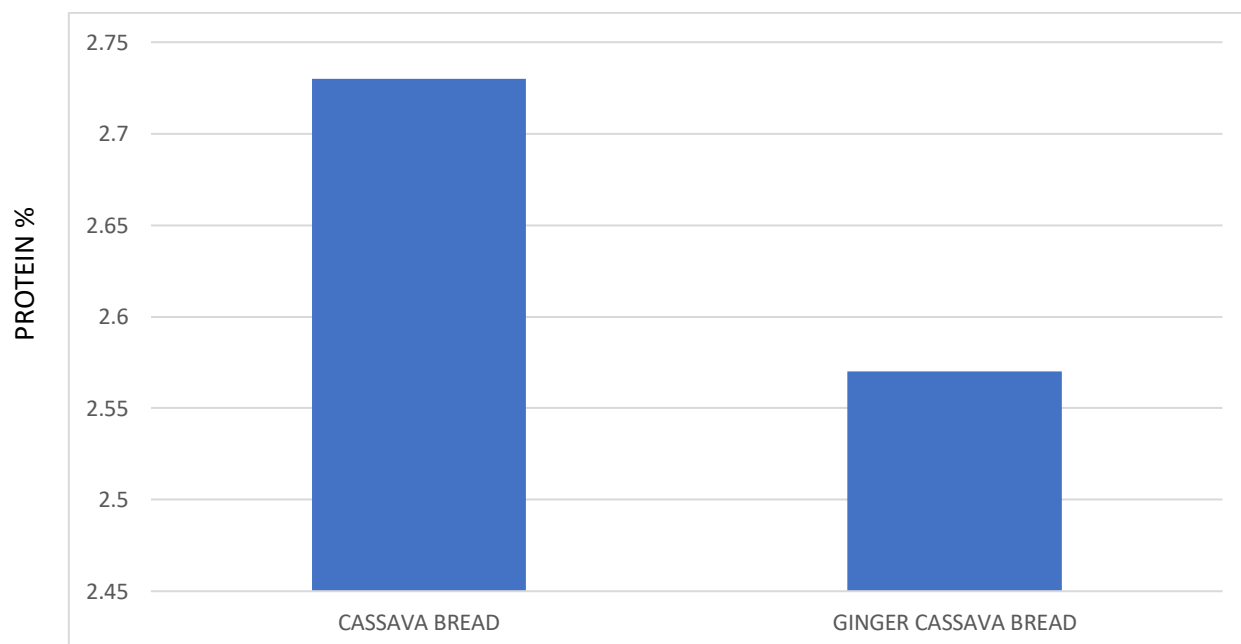


Figure 5.8 Graphical representation of protein content of cassava bread and ginger cassava bread

FAT%

The amount of fat content present in cassava bread and ginger cassava bread is 0.405 and 0.476 respectively.

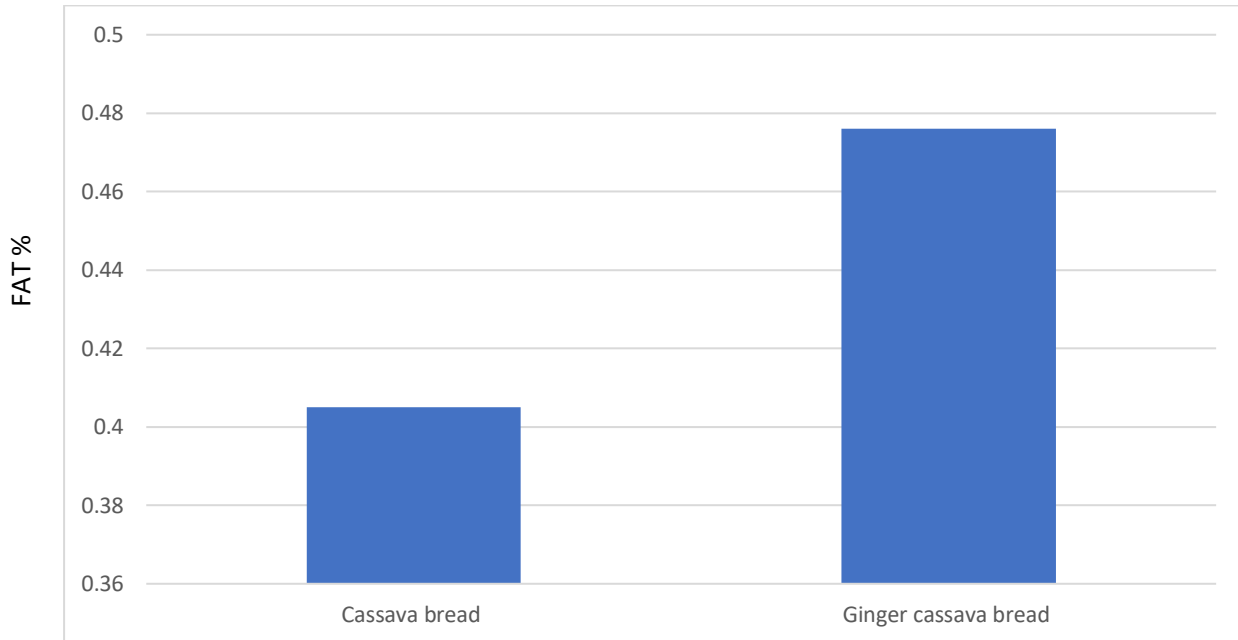


Figure 5.9 Graphical representation of Fat content of cassava bread and ginger cassava bread

CRUDE FIBER %

Crude fiber content is found to be high in ginger cassava bread when compared with the cassava bread.

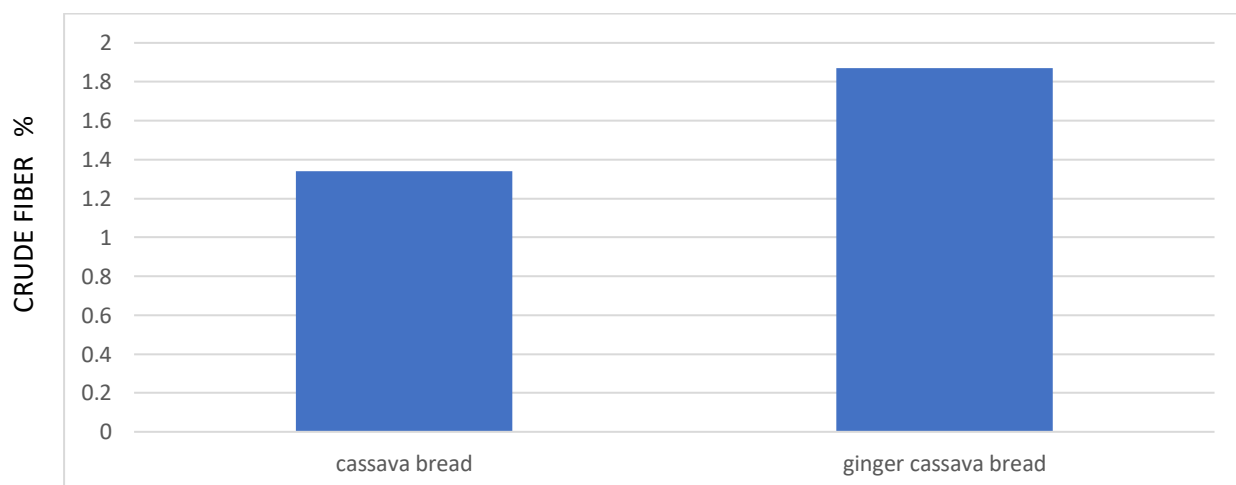


Figure 5.10 Graphical representation of crude fiber of cassava bread and ginger cassava bread

ALCOHOLIC ACIDITY %

Alcoholic acidity is hydrolysis of fats by lipase into free fatty acids and the acidity is due to the presence of certain acid salts. And it is found high in ginger cassava bread when compared with cassava bread.

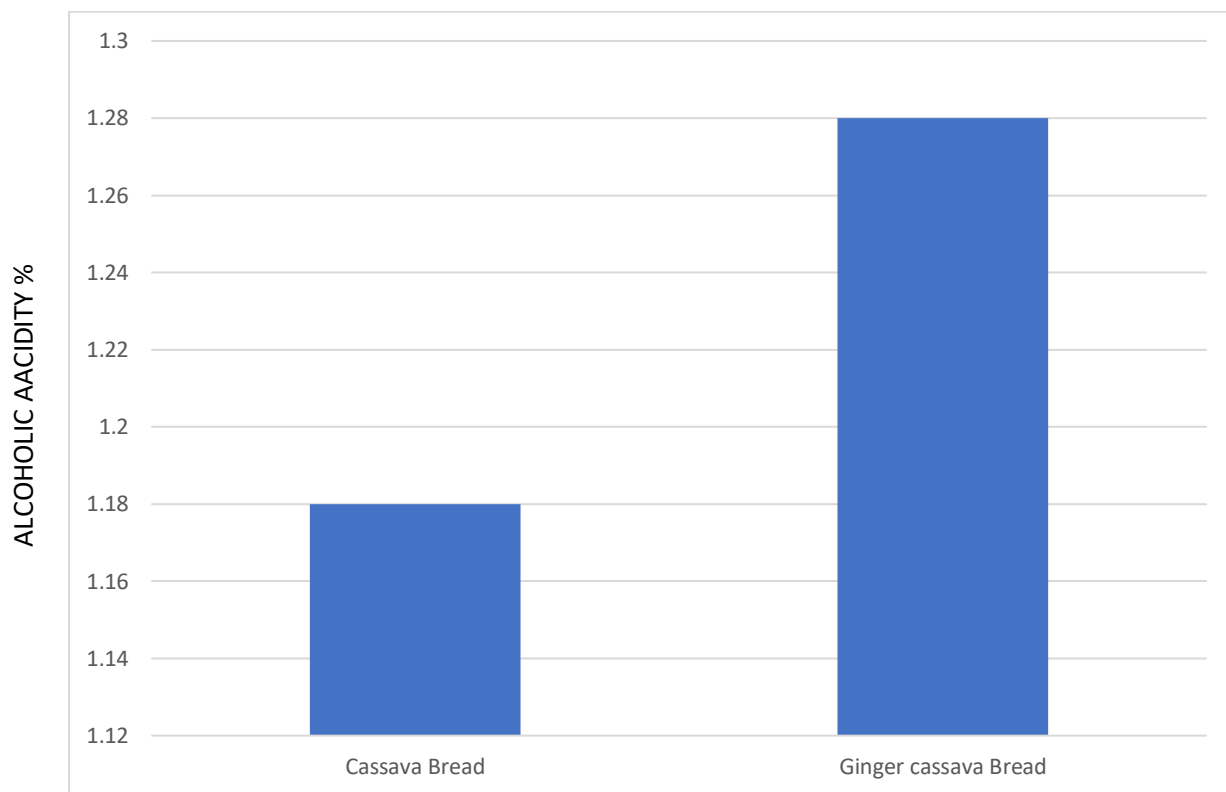


Figure 5.11 Graphical representation of alcoholic acidity of cassava bread and ginger cassava bread

CARBOHYDRATES %

Carbohydrate content is found in cassava bread higher when compared with ginger cassava bread.

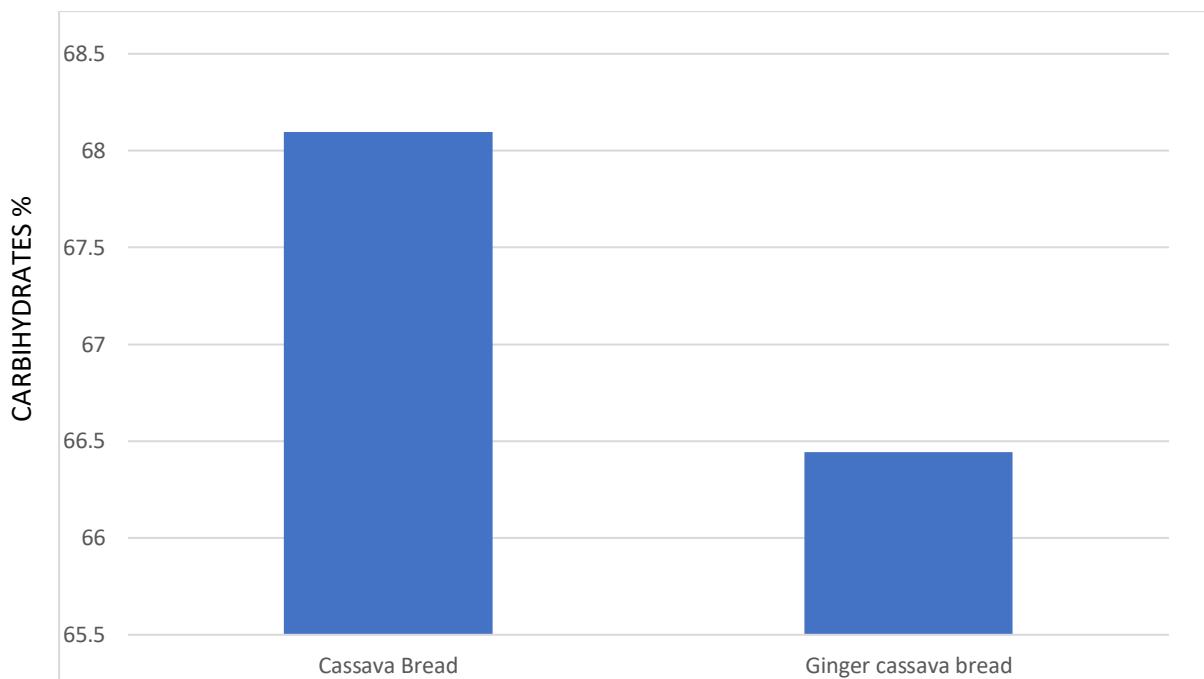


Figure 5.12 Graphical representation of crude fiber of cassava bread and ginger cassava bread

5.2.3 MINERAL ANALYSIS RESULT FOR CASSAVA BREAD

Table5.3 Mineral quantity of cassava bread

S.NO	MINERALS	Ppm/kg
1	Sodium	55.6
2	Magnesium	1.5
3	Iron	0.50
4	Calcium	6.8
5	Zinc	0.15

5.2.4MINERAL ANALYSIS RESULT FOR GINGER-CASSAVA BREAD

Table5.4 Mineral quantity of Ginger -Cassava bread

S.NO	MINERALS	Ppm/kg
1	Sodium	66.8
2	Magnesium	3.7
3	Iron	0.50
4	Calcium	11.7
5	Zinc	0.53

5.3 SHELF- LIFE ANALYSIS

Fungal growth was not observed on the first two days but the fungal growth is observed from the 4th day and the growth and the fungal count increased with days. ((Nirmala Ravimannan.*et al.*,2016)

The bread when handled at different environments like wet bread in dark area, room temperature, airtight bag and refrigerator. From the day 2 fungal growth was observed in the wet bread in the room temperature, bread at room temperature shows the fungal growth from the day 4 and the bread in airtight bag and refrigerator shows no fungal growth till day 5. (Unachukwu.*et al.*, 2015)

5.3.1 SHELF LIFE AT ROOM TEMPERATURE

Cassava Bread – 8 Days

Ginger Cassava bread – 12 Days

Shelf life of the cassava bread and ginger cassava bread was assessed when they were kept at room temperature in an airtight glass container, with the ambient temperature around 28-30°C and 50 percent relative humidity. Cassava bread had mold growth on the 8th day, and the ginger cassava bread on the 12th day leading to the spoilage of bread.



Figure 5.13 Ginger cassava bread
at room temperature



Figure 5.14 Cassava bread at room
temperature

5.3.2 SHELF LIFE ON REFRIGERATION

Cassava bread - 25 days

Ginger cassava bread – 30 days

Shelf life of the cassava bread and ginger cassava bread, kept in a refrigerator at a temperature of 4°C and 65 percent relative humidity to check the growth of mold was assessed. Cassava bread showed growth on the day 25th day, while ginger cassava bread, the growth was observed on the 30th day.



Figure 5.15 Ginger cassava bread on refrigeration

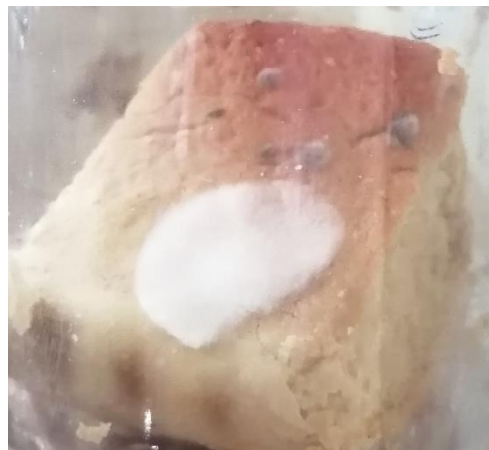


Figure 5.16 Cassava bread on refrigeration

5.3.3 SHELF LIFE AT STABILITY CHAMBER

Cassava bread -4 days

Ginger cassava bread – 6 days

To check the shelf life at different temperature and humidity, bread samples were packed in zip lock covers and placed in stability chamber at 40°C and 80 percent relative humidity. Cassava bread had mold formation on the 4th day and ginger cassava bread on the 6th day.



Figure 5.17 Ginger cassava bread at stability chamber
 5.18 Cassava bread at stability chamber

5.4 MICROBIAL ANALYSIS

5.4.1 TOTAL PLATE COUNT OF CASSAVA BREAD AT ROOM TEMPERATURE

Table 5.5 Colonies counted on cassava bread sample diluted plates

DAY	10^{-4}	10^{-6}	10^{-8}
DAY 1	18	12	6
DAY 2	26	20	10
DAY 3	38	27	18
DAY 4	45	38	21
DAY 5	52	44	35
DAY 6	61	55	49
DAY 7	83	66	52

5.4.2 TOTAL PLATE COUNT OF GINGER - CASSAVA BREAD AT ROOM TEMPERATURE

Table 5.6 Colonies counted on Ginger- cassava bread sample diluted plates

DAY	10^{-4}	10^{-6}	10^{-8}
DAY 1	22	18	12
DAY 2	25	20	15
DAY 3	33	24	18
DAY 4	39	28	21
DAY 5	49	39	30
DAY 6	56	50	39
DAY 7	78	59	46

5.4.3. YEAST MOLD METHOD (CASSAVA BREAD)

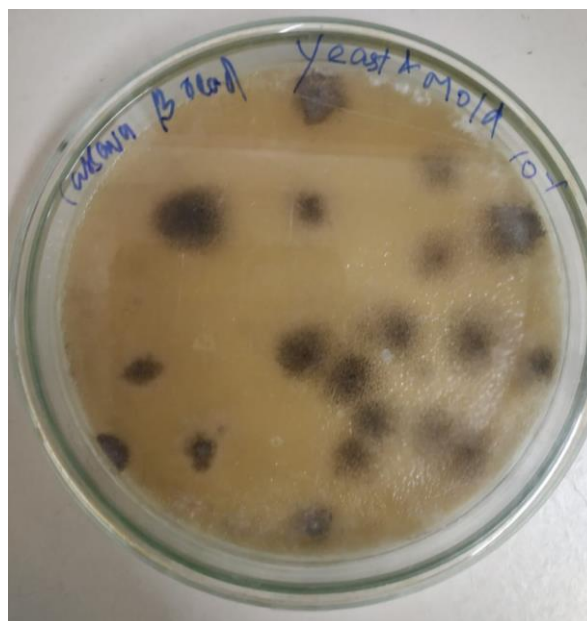


figure 5.19 yeast and mold 10¹dilution-cassava bread

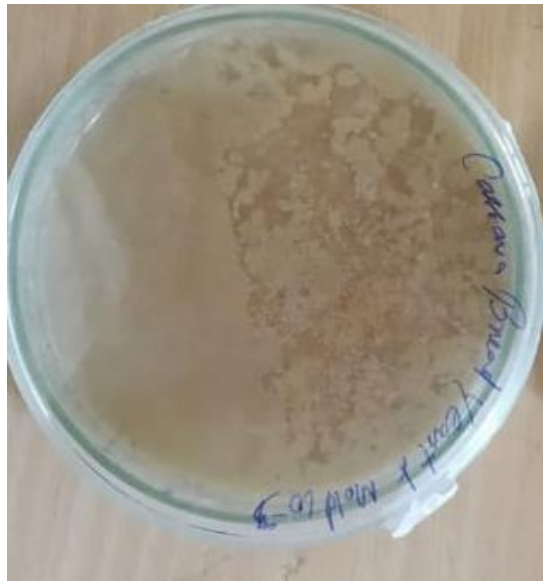


figure 5.20 yeast and mold 10^3 dilution- cassava bread

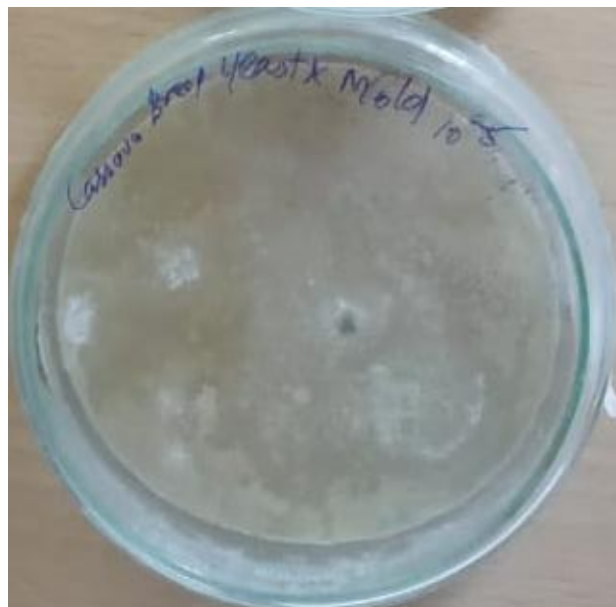


figure 5.21 yeast and mold 10^5 dilution- cassava bread

These were the colonies observed in the cassava bread sample after 4 days on incubation.

5.4.4. YEAST MOLD METHOD (GINGER-CASSAVA BREAD)

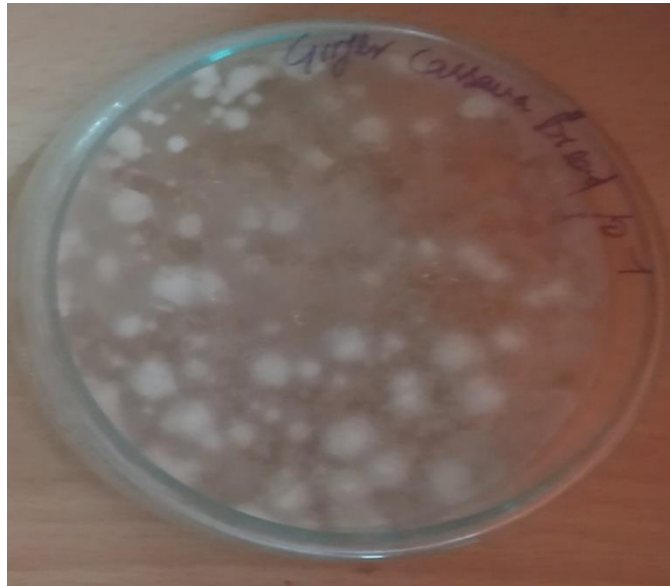


figure 5.22 yeast and mold 10¹dilution- ginger cassava bread



figure 5.23 yeast and mold 10³dilution- ginger cassava bread



figure 5.24 yeast and mold 10^5 dilution- ginger cassava bread

These were the colonies observed in the ginger- cassava bread sample after the 4 days of incubation period

5.4.5 MICROSCOPIC VIEW OF FUNGI PRESENT IN CASSAVA BREAD



figure 5.25 microscopic view of mucor spp.in cassava bread

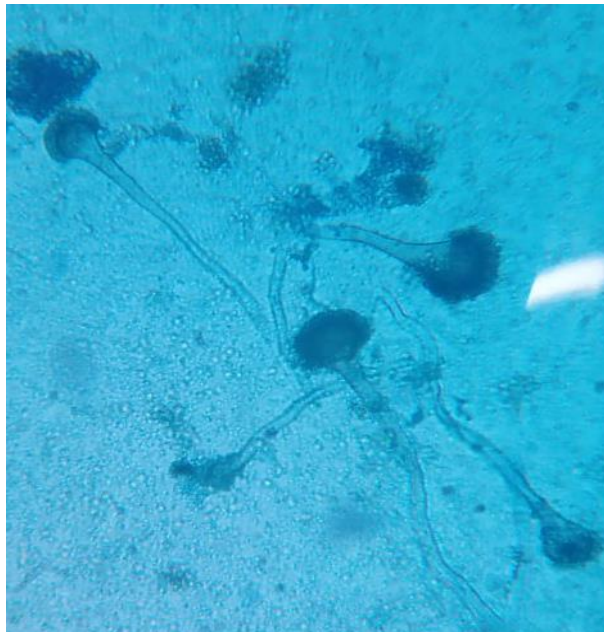


figure 5.26 microscopic view of rhizopus spp. in cassava bread

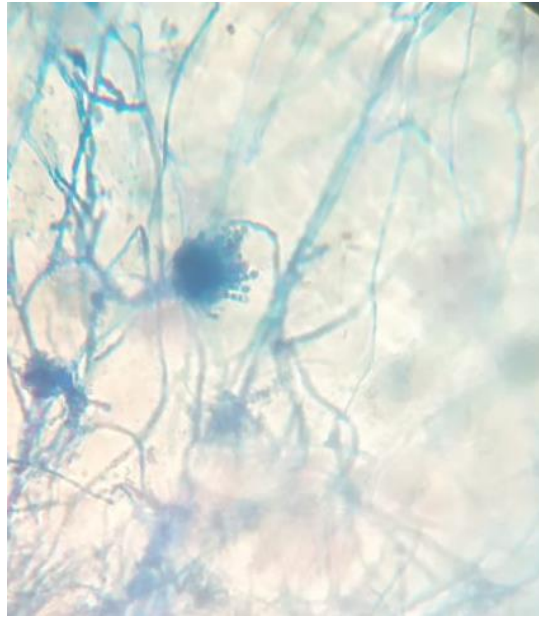


figure 5.27 microscopic view of aspergillus in cassava bread



figure 5.28 microscopic view of phoma spp. in cassava bread

The microscopic view of the colonies observed using a light microscope in 40X magnification lens.

5.4.6 MICROSCOPIC VIEW OF FUNGI PRESENT IN GINGER CASSAVA BREAD

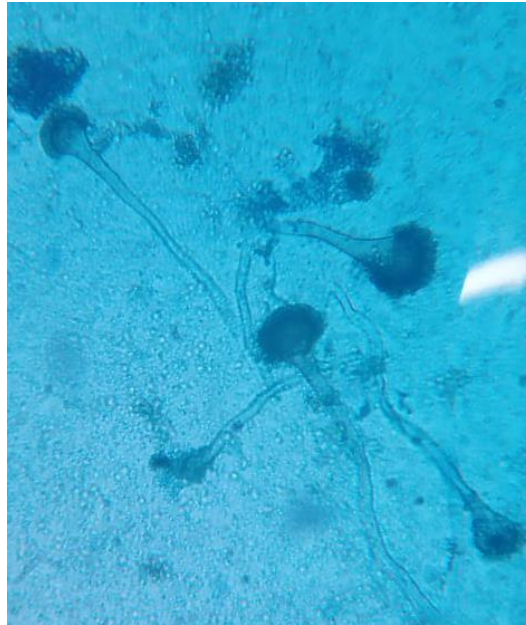


figure 5.29 microscopic view of rhizopus spp. in ginger cassava bread

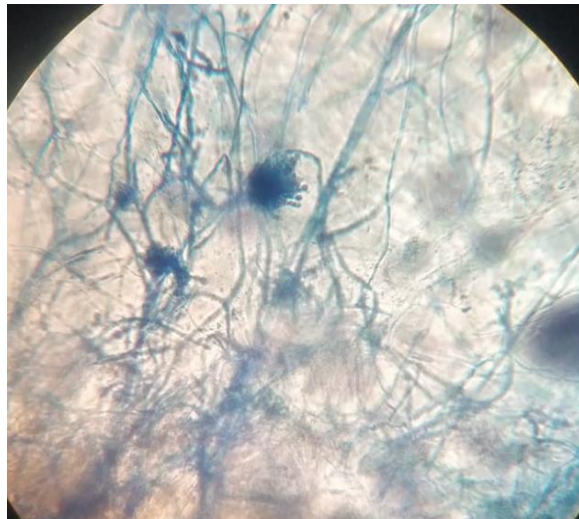


figure 5.30 microscopic view of aspergillus spp.in ginger cassava bread

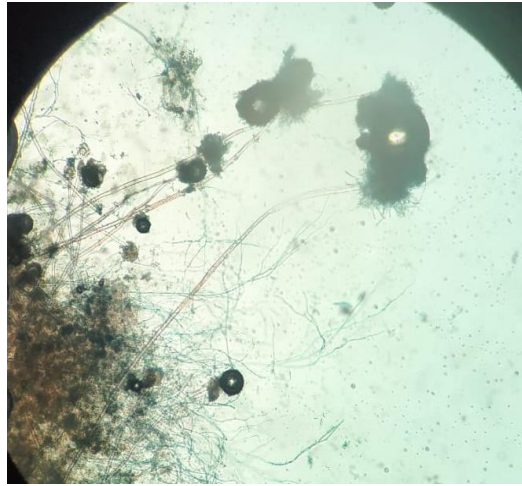


figure 5.31 microscopic view of phoma spp.in ginger cassava bread

The microscopic view of the colonies were observed using a light microscope in 40X magnification lens

Table 5.7 Colony and morphological characteristics of Cassava bread

Fungal isolates	Cultural characteristics	morphological characteristics
Mucor spp.	White colonies turns to black	It has broad hyphae and sporangiophores. The sporangiophores are long branched and round spores filled with sporangia.
Rhizopus spp.	White cottony colonies	Hyphae are broad, rhizoid and stolon are present. sporangiophores are tufts sporangia are ovoid.
Aspergillus spp.	Green colonies with distinct margin	Vesicle is present in the top of the conidiospore. conidia are found in chains.
Phoma spp.	White colonies turns to olive green color	They produce sub- hyaline to hyaline, septate hyphae. Conidia are unicellular, hyaline, and oval-shaped. Each conidium typically has two oil droplets inside

Table 5.8 Colony and morphological characteristics of Ginger- Cassava bread

Fungal isolates	Cultural characteristics	morphological characteristics
Rhizopus spp.	White cottony colonies	Hyphae are broad, rhizoid and stolon are present. sporangiophores are tufts sporangia are ovoid.
Aspergillus spp.	Green colonies with distinct margin	Vesicle is present in the top of the conidiospore. conidia are found in chains.
Phoma spp.	White colonies turns to olive green color	They produce sub- hyaline to hyaline, septate hyphae. Conidia are unicellular, hyaline, and oval-shaped. Each conidium typically has two oil droplets inside

After the five days of incubation fungal growth was observed in bread and based on the colony and morphological characteristics they were identified as Mucor spp. Rhizopus spp., Aspergillus spp. and Penicillium spp. (Nirmala Ravimannan,2016).

Based on the colonies observed (Table 5.7 and table 58) in the plates and those colonies were isolated and using slide culture technique pure culture can be obtained and the morphological characteristics were seen in 40x magnification lens in a light microscope. Mucor spp. Rhizopus spp., Aspergillus spp., Phoma spp. are found to present in cassava bread. Rhizopus spp., Aspergillus spp., Phoma spp. are found to present in ginger cassava bread.

CHAPTER 6 – SUMMARY AND CONCLUSION

Bread is one of the most important food in the traditional dietary. Owing to lifestyle changes, people are more attracted by the ready to eat foods and they are aware of eating a nutritious food product with proper dietary supplements. In current scenario, vitamin-C and immunity boosting foods are more important.

Ginger is one of the best sources as immunity booster and cassava is best for the diet foods. The main objective of the study is to identify and compare the fungi growth and presence in the cassava bread and ginger cassava bread. Ginger not only the immunity booster it also has antifungal property in it.

The conclusion drawn from the result of this work is that *Mucor* spp. is not found to grow in presence of Ginger. *Phoma* spp. is found present in the bread made with cassava flour. The common bread fungi like *Aspergillus*, *Rhizopus*, *Mucor*, are found in normal cassava bread the *Mucor* spp. is not found in ginger cassava bread. According to proximate principles determined from the moisture, ash, protein, fiber, alcoholic acidity and fat they were not much difference in it. From the mineral analysis using atomic adsorption spectrophotometer, ginger cassava bread has more minerals when compared to cassava bread. The shelf life of the ginger cassava bread is found to be excess of 4-5 days when compared to the cassava bread in all types of climatic conditions.

REFERENCE

1. Ali, A., Shehzad, A., *et al.*, Yeast, its types and role in fermentation during bread making process-A. Pakistan Journal of Food Sciences, 22(3), 2012, Pp 171-179.
2. An K., Zhao, D., *et al.*, Comparison of different drying methods on Chinese ginger (*Zingiber officinale* Roscoe): Changes in volatiles, chemical profile, antioxidant properties, and microstructure. Food chemistry, 197, 2016, Pp 1292-1300.
3. Chiwona-Karlun, T. Tyllieskar, J. Mkumbira, M. Gebre-Medhin, H. Rosling, L. Low dietary cyanogen exposure from frequent consumption of potentially toxic cassava in Malawi. International journal of food sciences and nutrition, 51(1), 2000, Pp 33-43.
4. De Hoog, G. S., *et al.*, "Phoma Species" Atlas of Clinical Fungi, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, 2nd ed, vol. 1. 2000.
5. Dietary guidelines of America, 2016.
(<http://www.dietaryguidelines.gov/>)
6. Domsch, K. H., Gams, W., & Anderson, T. H. Compendium of soil fungi. Academic Press (London) Ltd, Volume 1, 1980.
7. Fauquet, C., & Fargette, D. African cassava mosaic virus: etiology, epidemiology and control. Plant Dis, 74(6), 1990, Pp 404-411.
8. Fengfeng Wang. Inhibition of secretion by asexual reproduction in *Aspergillus niger*. 2014.
9. Finney, K. F. An optimized, straight-dough, bread-making method after 44 years. Cereal Chemistry, 61(1), 1984, 20-27.

10. Food and Agriculture Organization of United Nations, 1995.
11. Fundukian, L.J., Memorial Sloan Kettering Cancer Center: "About Herbs: Ginger",
The Gale Encyclopedia of Alternative Medicine, third edition, 2009
12. Jillian Levy, CHHC, "Cassava flour: The best grain- free baking alternative?"
Dr. Axe nutritional article, 2019.
13. Joe Leech, M S, "11 Proven health benefits of ginger", 2021.
14. Kew science article, "plant of the world online" , 2017.
15. Larone, D. H., & Larone, D. H. Medically important fungi: a guide to
identification New York: Elsevier. (Vol. 189) ,1987.
16. Master class articles, how long does bread last? 4 stages of the bread
freshness, December 2020.
17. Mpamah Ihuoma Chaste., Bread Fungi: Phytochemical Constituents and
Antimicrobial activity of isolates. (Journal of Applied & Environmental
Microbiology, 7(1), 2019, Pp 20-24.
18. Noor Arida Fauzana .et al "isolation, identification and selection of cellulolytic
fungi from banana waste (Musa paradisiaca)",2011.
19. Nancy Keller. *Aspergillus flavus*. September 2011.
20. Ravimannan, N., Sevel, P., & Saarutharshan, S. Study on fungi associated
with spoilage of bread. Int. J. Adv. Res. Biol. Sci, 3(4), 2016, Pp 165-167.

21. Pena, R.J., "Wheat for bread and other foods. Food And Agriculture Organisation", Retrieved 1 October 2016.
22. Plessas, S., Pherson, L., Bekatorou, A., Nigam, P., & Koutinas, A. A. Bread making using kefir grains as baker's yeast. Food chemistry, 93(4), 2005, Pp 585-589.
23. Ponte, J.G. and Tsen, C.C. Bakery products In: Food and Beverage mycology (ed. L. R. Beuchat), AVI Publishing Co., Westport, CT, 1978, Pp. 191-223.
24. Saranraj, P., & Sivasakthivelan, P. Microorganisms involved in spoilage of bread and its control measures. Bread and its fortification Nutrition and Health Benefits, Edition, 13, 2015, 132-149.
25. Singh, D. I. N. K. E. R., & Kumar, S. W. A. S. H. A. N. K. H. Effect of ginger and mint extract on microflora of yoghurt cultures. Trends Biosci, 6, 2013, Pp 80-81.
26. Susan Grey June 28, 201.
(How Long does Bread last? The Complete Guide – Grow Your Pantry)
27. Tobias M Hohl & Feldmesser, M. Aspergillus fumigatus: principles of pathogenesis and host defense. Eukaryotic cell, 6(11), 2007, Pp 1953-1963.
28. Tewe, Olumide, The Global Cassava Development Strategy, U.N. Food and Agriculture Organization, 2014.
29. Unachukwu, M. N., & Nwakanma, C. The fungi associated with the spoilage of bread in Enugu state. International Journal of Current Microbiology and Applied Sciences, 4(1), 2018, Pp 989-995.
30. Zingiber officinale Roscoe, "Kew Science, Plants of the World Online. Royal Botanic Gardens, Kew. 2017, Retrieved 25 November 2017.