



**TO STUDY THE EFFECT OF NIXTAMALIZATION ON THE MICRO-  
STRUCTURE OF FLOURS AND ITS CHARACTERIZATION IN  
MILLETS**

DISSERTATION SUBMITTED TO

**ST. TERESA'S COLLEGE**

In Partial Fulfilment of the Requirements for the Degree of

**MASTER OF VOCATION**

IN

**FOOD PROCESSING TECHNOLOGY**

BY

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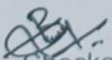


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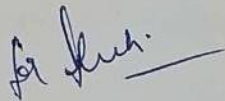
  
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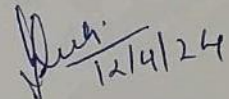
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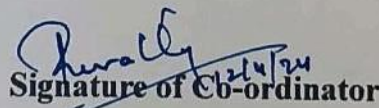
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## **DECLARATION**

I **Sneha T D**, do hereby declare that the dissertation titled **“To Study the Effect of Nixtamalization on the Micro-structure of Flours and its Characterization in Millets”** submitted in partial fulfilment of the award of the degree of Master of Vocation in Food Processing Technology from St Teresa’s College (Autonomous). It is an authentic record of studies and the research work carried out by me under the supervision and guidance of Dr. Shruti Joshi, Principal Scientist, Department of Grain Science Technology, CSIR-CFTRI, Mysore, Karnataka and no part of the work has been submitted in part or full to any other Degree and there is no plagiarism in the written thesis.

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## **ABSTRACT**

The study involved treating pearl millet, sorghum and finger millet with 2%  $\text{Ca(OH)}_2$ , followed by steeping in cooked liquid and dry milling to produce nixtamalized flours, which exhibited differences in physical, proximate, functional properties, and antioxidant profiles compared to their native flours. Notably, the nixtamalization process resulted in lesser bulk densities than tapped densities and higher true densities in physical properties. Nixtamalized samples had lower moisture content, along with increased ash, protein, and fat content in the proximate analysis, and higher levels of calcium, magnesium, and sodium but lower potassium content in mineral analysis. Additionally, nixtamalized samples displayed higher water absorption index (2.96-3.93) and lower water solubility index (2.43-3.3) than native samples, with both solubility and swelling power increasing with temperature. Color measurements showed nixtamalized samples to be less white but with higher yellowish tones, and differential scanning calorimetry (DSC) indicated higher onset, peak, and end temperatures for nixtamalized flours compared to the native grains. While both native and nixtamalized samples exhibited similar radical scavenging activity and total phenolic content (TPC), sorghum millet in its native form demonstrated the highest TPC. Overall, nixtamalization was found to be beneficial in enhancing various attributes and nutritional value of millet flours.

## **ACKNOWLEDGEMENT**

I would like to take this opportunity to express my gratitude to the people who have contributed towards the making of this project. I take this opportunity to spell out of my foremost gratitude to God Almighty for showering the blessings on me throughout this project.

I express my deep sense of gratitude to Dr. M.S. Meera (Chief Scientist), Head of the Department, for giving me an opportunity to pursue my dissertation work at the Department of Grain Science Technology, CSIR-CFTRI, Mysore.

I express my sincere gratitude and obligation to my guide Dr. Shruti Joshi., Principal Scientist, Department of Grain Science Technology for giving me the guidance and novel ideas provided on various aspects of the topic.

I express my heartfelt thanks to Dr. Sridevi Annapurna Singh, Director, CSIR-CFTRI, Mysore for giving me this opportunity and facilities to undertake this work. I am also grateful to CSIR-CFTRI for their contribution to the successful completion of my dissertation.

I express my sincere gratitude to Dr. Revathy Baskaran (Chief Scientist), Head of the HR Department, for his help in carrying out my work.

I am grateful to Ms. Naomi Vincent, CSIR-CFTRI student, Department of Grain Science Technology, for her support and valuable suggestions throughout my period of work.

I remember with gratitude the help and co-operation offered by the members of the Department of Grain Science Technology.

I would like to express my regards to Alphonsa Vijaya Joseph Principal, St. Teresa's College, Bhavya E P, Head of the Department of Food Processing Technology, Ms. Sandhra Santhosh, Lecturer, Department of Food Processing Technology, St Teresa's College and all other professors for their constant support and inspiration for my academic endeavors.

I am grateful to my family and my friends for their encouragement and inestimable help for the successful completion of my work.

**Sneha T D**



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## LIST OF ABBREVIATIONS.

Sl. No	Abbreviations	Full Form
1	AOAC	Association of Official Analytical Chemists
2	TPC	Total Phenolic Content
3	DSC	Differential Scanning Calorimeter
4	DPPH	2,2-diphenyl-1-picrylhydrazyl
5	FFA	Free Fatty Acid
6	TNP	Traditional Nixtamalization Process
7	FM	Finger Millet
8	PM	Pearl Millet
9	SM	Sorghum Millet
10	RO	Reverse Osmosis
11	g	Gram
12	mg	Milligram
13	RBF	Round Bottom Flask
14	NDF	Nixtamalized Dough Flour
15	hr. s	Hours
16	sec	Seconds
17	mins	Minutes
18	MP-AES	Microwave Plasma Atomic Emission Spectroscopy
19	ml	Millilitre
20	WAI	Water Absorption Index
21	WSI	Water Soluble Index



22	OAI	Oil Absorption Index
23	nm	Nanometer
24	μm	Micrometre
25	μl	Microlitre
26	FC	Folin-Ciocalteu
27	db	Dry Basis
28	aw	Water Activity
29	pH	Potential of Hydrogen
30	RH	Relative Humidity
31	SEM	Scanning Electron Microscopy

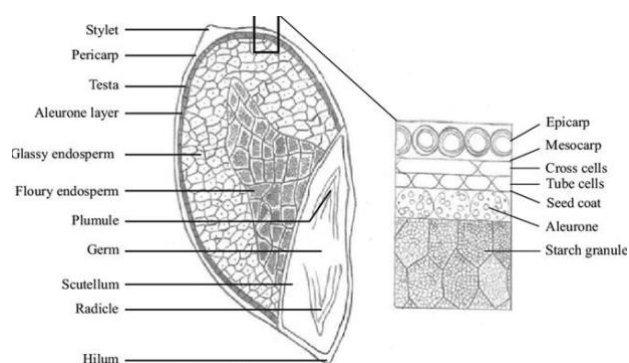
# INTRODUCTION

The name "millet" refers to a broad category of cereals, including seeds from multiple taxonomically distinct kinds of grass. Most of these grasses are cultivated in marginal agricultural areas and in agricultural settings where major cereals offer insufficient yields. Millet is one of the earliest crops to be cultivated, and it has been used as a staple crop in China, India, Central and Eastern Asia, Europe (primarily Russia), and some regions of Africa. Millions of people in Africa, on the other hand, primarily get their protein and energy from millet. Its capacity for growth in unfavorable environmental circumstances, such little rainfall, makes it a staple ingredient in many developing countries. Millet is a member of the Poaceae plant family, which also includes sorghum. The four-main species of millet are finger millet (*Eleusine coracana*), proso millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*), and pearl millet (*Pennisetum glaucum*), which accounts for 40% of global production. Many variants of millet exist. The most popular variety of millet used for human food is pearl millet, which yields the largest grains. Browntop millet (*Urochloa ramosa*), Pandan sumatrense (*Panicum sumatrense*), Barnyard millet (*Echinochloa spp.*), and Kodo millet (*Paspalum scrobiculatum*) are examples of minor millets. Many times, millets are also used to refer to fonio (*Digitaria exilis*) and teff (*Eragrostis tef*) (Amadou et al., 2011).

Millets (*Pennisetum gambiense*) are described as "the nutri-cereals of today, and yesterday's coarse grains." Millets are small-seeded grains, have a superior fatty acid profile and are a richer source of the amino acids' methionine and cysteine, which belong to protein and sulfur, than cereals. Considerable amounts of vitamins and minerals may be found in millets, making them a highly nutritious grain. Because millets are high in dietary fiber, slowly digesting starch, resistant starch, and energy, they promote appetite by releasing glucose gradually. Small cereal grains from the grass family are called millets. It was consumed in India as khichdi, idli, and chapati. Millets typically comprise 7-8% protein, 2-4% fat, 65-75% carbohydrates and 15-20% dietary fiber. Additionally, they contain a significant number of minerals, vitamins, and phenolic compounds. It includes bioactive compounds that have antimicrobial and antioxidant properties, among other possible health benefits. Millets are high-protein, starchy cereals. They are high in magnesium and phosphorus, and finger millet has the highest calcium content of any cereal. Millets are also frequently processed using various techniques. By processing the millet and improving its properties, it can be used to create new foods, which will increase food security in the area (Yousaf et al., 2021).



**Fig 1.1 Millets**



**Fig 1.2 Structure of Millet Grain**

Pearl millet, a C4 plant known for its exceptional photosynthetic efficiency and robust dry matter production, thrives in harsh agro-climatic conditions where other crops falter, exhibiting a notable adaptability to favorable environments due to its rapid growth and short developmental stages, rendering it ideal for short growing seasons with improved management practices. Cultivated across 30 million hectares in over 30 countries spanning five continents, including Asia, Africa, North America, South America, and Australia, pearl millet ranks as India's third most cultivated food crop, prized for its highly nutritious grains boasting abundant metabolizable energy, protein, iron, and zinc, as well as a superior amino acid profile compared to maize and sorghum, primarily utilized in various culinary forms such as flatbreads and porridges, while also serving as a valuable forage crop with lower hydrocyanic acid content than sorghum, providing rich protein and mineral fodder for livestock and finding applications in poultry and cattle feed as well as alcohol production. (Yadav et al., 2013).

*Pennisetum glaucum*, commonly known as Bajra, is a vital millet variety renowned for its drought tolerance within the Poaceae family. (Sandhu and Siroha, 2017). Thriving in challenging environments including low fertility, high temperatures, and saline soils, it

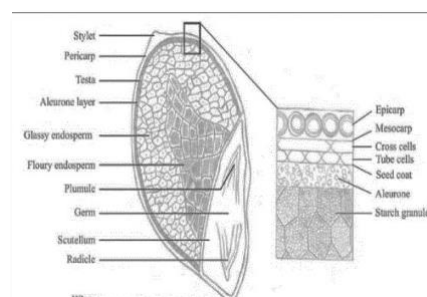


outperforms wheat, corn, and sorghum. Featuring elevated oil content compared to other cereals, Bajra is rich in protein, dietary fiber, starch, and essential phytochemicals and vitamins such as E, K, and B complex. It serves as a notable source of calcium, iron, zinc, and essential amino acids. However, its antinutritional factors like condensed tannins and phytates can hinder protein, carbohydrate, and mineral digestibility and bioavailability. (Gaytán-Martínez et al., 2017; Lestienne et al., 2007).

Pearl millet underwent manual cleaning to eliminate damaged seeds and debris before being stored in a clean, dry environment. Subsequently, these grains were cooked in lime solutions of varying concentrations (0.5%, 1.0%, 1.5%, and 2.0%) for 30 minutes and steeped for 2 hours, using a consistent grain-to-water ratio of 1:3 w/v. The cooking temperature was maintained uniformly at 95°C for all samples. Following steeping, the nixtamalized grains underwent thorough washing to eliminate excess lime and any extraneous pericarp material. (Pandey et al., 2022).



**Fig 1.3 Pearl millet**



**Fig 1.4 Structure of PM**

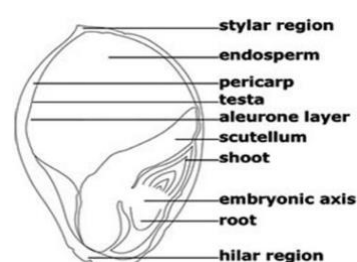
Sorghum (*Sorghum bicolor* L. Moench) is a crop that is competitively grown all over the world, due to its agronomic advantages in challenging areas. Because of its desirable qualities, which include its tolerance to drought and high yields, sorghum has gradually replaced other significant crops, primarily maize, in several countries, including South Asia and Africa (Cabrera-Ramírez et al., 2020). Sorghum ranks as the fifth most significant cereal produced worldwide. It can grow well and produce in the world's tropical regions and warmer temperatures. In Asia and Africa, sorghum is the most often consumed cereal grain. The United States is the world's top producer of sorghum, followed by Nigeria and Mexico, making it the fifth most significant crop globally. (Anglani, C., 1998).

Sorghum grains constitute a significant source of micro and macronutrients, comprising roughly 8-18% proteins, 1-4% fats, ~19% dietary fiber, and 70-80% carbs. However, sorghum, like other grains, has a notable lack of lysine and poor protein bio accessibility and digestibility when prepared in the traditional manner (Cabrera-Ramírez et al.,2020). In India sorghum is known as *jowar*, *cholam*, or *Jonna*. Sorghum is especially valued in hot and arid regions for its resistance to drought and heat. The grain is also used in making edible oil, starch, dextrose (sugar), paste, and alcoholic beverages (Britannica.,2023). Tropical regions produce infant foods by adding sugar to gruels made of sorghum and maize. Due to the low concentration of vital amino acids like lysine, tryptophan, and threonine, sorghum grain has low protein quality. While sorghum is difficult to digest for infants, it can be a good weaning diet when combined with foods high in lysine. Cooking reduces the digestibility of sorghum proteins. Like other cereals, sorghum is a good source of B vitamins, including niacin, biotin, thiamin, and vitamin B6; but refining results in the loss of all B vitamins. Sorghum's mineral composition has similarities to millets. The two main minerals found in sorghum grain are potassium and phosphorus, with relatively little calcium. Condensed tannins are a type of polyphenolic molecule that has antinutritional properties found in sorghum. Because they can bind to dietary proteins, digestive enzymes, minerals like iron, and B vitamins like thiamin and vitamin B6, condensed tannins reduce the nutritional value of sorghum grain. They are found in sorghums with pigmented testa and missing from white and colored sorghums (Anglani.,1998).

Nixtamalization of sorghum involves similar alkaline cooking methods used for maize, where sorghum grains are treated with calcium hydroxide or other alkaline solutions. Exploring sorghum nixtamalization offers opportunities to enhance the nutritional quality, culinary versatility, and sustainability of this important cereal grain. Research in this area contributes to broader efforts to promote food security, preserve cultural heritage, and foster innovation in the food industry.



**Fig 1.5 Sorghum millet**

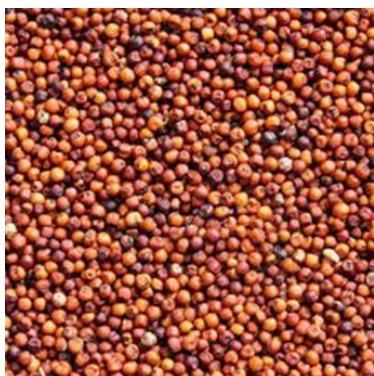


**Fig 1.6 Structure of SM**

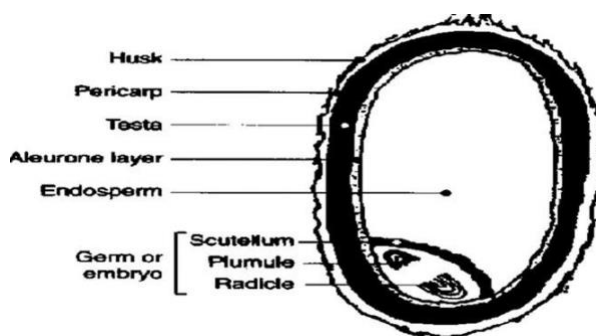
Sorghum and finger millet (also known as ragi) are both cereal grains that play significant roles in global food systems, particularly in regions with semi-arid and arid climates. While they belong to different botanical families (sorghum is in the Poaceae family, and finger millet is in the Poaceae subfamily Panicoideae), they share several similarities and connections.

Finger millet (*Eleusine coracana*), is a popular grain that can be eaten without de-hulled. India is the world's largest producer of finger millet, accounting for around 60% of production overall. It has been observed that finger millet variants contain 220-450 of calcium and 3–20% of iron (Gull et al.,2015). The cereal is cultivated for food throughout Africa and Southern Asia, primarily in Nepal and India's states of Uttar Pradesh, Bihar, Tamil Nadu, Karnataka, and Andhra Pradesh. The crop is mostly farmed in eastern Africa, specifically in Uganda, Kenya, Tanzania, and to a smaller amount in Ethiopia, Rwanda, Malawi, Sudan, Zambia, and Zimbabwe (Dida & Devos.,2006; FAO 1995). In the past, finger millet was processed in India using techniques like grinding, malting, and fermentation to make drinks, porridges, roti (unleavened flat bread), dosa (fermented pan cake), and idli (fermented steamed cake). One of India's oldest crops, finger millet, is known as "nrtta-kondaka" in ancient Indian Sanskrit literature. This means "Dancing grain," and it was also called "rajika" or "markataka" (Shobana et al., 2013).

Finger millet, also known as tamba, is consumed with its hull intact and serves as a staple food in specific areas of Africa and India. Despite being often overlooked and underutilized, this gluten-free grain offers nutritional and nutraceutical advantages due to its low glycemic index. The grains are rich in calcium, a macronutrient that is vital for developing children, expectant mothers, and the elderly. This is because calcium is necessary for healthy tissue growth, including the development of strong bones and teeth. (Jideani et al.,2018). As a subsistence food crop, finger millet has exceptional qualities and stands out from other cereals like barley, rye, and oats due to its higher nutritional content. Due to its high levels of calcium, phosphorus, iron, and zinc, finger millet (*Eleusine coracana L.*), one of the minor grains, is nutritionally notable. (Agarkar et al.,2020). Additionally, it has been noted that FM is high in essential amino acids including lysine, tryptophan, and methionine. Also, FM grains are a good supply of iron, magnesium, phosphorus, and carbohydrates. Grains are also a rich source of vitamin B complex nutrients like thiamine, riboflavin, folic acid, and niacin (Jideani, et al.,2018).



**Fig 1.7 Finger Millet**



**Fig 1.8 Structure of FM**

While nixtamalization is traditionally associated with maize (corn), it can also be applied to other grains, including finger millet (ragi). Nixtamalization involves cooking the grain in an alkaline solution, usually containing calcium hydroxide (lime) or wood ash, followed by soaking and washing to remove the outer hull or bran layer. This process brings about several changes in the grain's composition and properties, resulting in improved nutritional quality, flavor, and texture. Exploring the potential of nixtamalization for finger millet could open up new possibilities for utilizing this nutritious grain in diverse food products and improving food security in regions where it is a staple crop.

Nixtamalization is a traditional corn processing method involving cooking the grain in an alkaline solution, typically lime water, followed by soaking for 12-16 hours. This process removes the pericarp, making the grains easier to grind and enhancing their flavor, aroma, and nutritional value while reducing mycotoxin levels. After cooking, the grains are steeped in the cooking liquid, known as nejayote, which is later discarded due to its unpleasant taste. During cooking, the grains absorb the alkaline solution, hydrate, and undergo various chemical changes, including starch gelatinization and softening of the kernels' outer layers (Ocheme et al., 2010). The nixtamalized millet flours are used in making a variety of dishes such as flours, tortillas, pozole, tostadas, nachos, corn chips, munchies, tamales, etc.

Nixtamalization, also known as alkaline cooking, involves treating whole grains with alkaline solutions such as lime, wood-ash, or lye. This process induces various physical, chemical, and structural transformations in the grains. (Yadav et al., 2013). The changes include solubilization and dissolution of pericarp tissues, particularly at the endocarp layer, partial starch gelatinization in the endosperm, partial protein denaturation in the endosperm and the germ, hydration of starch and protein molecules, and calcium penetration and absorption into



the matrices of the endosperm and the germ are a few examples of these changes. These alterations result in swollen, softened kernels that are easily pulverized during processing using attrition milling (Owusu-Kwarteng & Akabanda.,2013). Nixtamalization enhance the calcium,bioavailability of niacin (Yadav et al., 2013) protein and reducing aflatoxin concentrations, (Owusu-Kwarteng & Akabanda.,2013).

The calcium added during nixtamalization interacts with grain constituents, impacting final product characteristics and nutritional value. Calcium in nixtamalized products is nutritionally significant, emphasizing its importance in diets. Various factors like cooking time, temperature, steeping duration, initial calcium hydroxide level, water content, pericarp state, grain type, and physical condition affect calcium content. Starch granules undergo complete gelatinization from outer endosperm layers, with partial gelatinization in inner layers, forming starch granule clusters. Steeping time shows a non-linear relationship with calcium content in instant grain flours, alongside changes in physical and chemical properties. Understanding these interactions is crucial for optimizing nixtamalization processes to enhance product quality and nutritional value. (Villegas et al.,2013).



**Fig 1.9 NDF**

# **REVIEW OF LITERATURE**

## 2.1 Millets

The name "millet" refers to a broad category of cereals, including seeds from multiple taxonomically distinct kinds of grass. Most of these grasses are cultivated in marginal agricultural areas and in agricultural settings where major cereals offer insufficient yields (FAO.,2008). Millet is one of the earliest crops to be cultivated, and it has been used as a staple crop in China, India, Central and Eastern Asia, Europe (primarily Russia), and some regions of Africa (Baltensperger.,1996). In contrast to other major cereals, millet has a short growing season, is more productive during droughts, and is resistant to pests and illness, (Devi et al., 2011). One kind of small-seeded cereal that is a member of the poaceae family is millets. For millions of people living in the semi-arid tropical regions, millet is an essential food source. It is mostly used for nourishment for humans. It continues to be an important source of calories and a crucial part of food security in the semiarid regions of the developing world (FAO.,1996). Millet has an efficient water user, and it is a short season crop. Therefore, they have grown in many areas of the world with limited rainfall (Bishop et al., 1982). Grinding millet and other cereals can help produce their distinctive qualities, including taste, aroma, texture, shelf life, and safety, through fermentation, which is facilitated by the raw materials' own metabolic activity and/or enzymes (Hammes, 1990).

There are various types of millet such as pearl millet (*Pennisetumglaucum*), foxtail millet (*Setaria italica*), finger millet (*Eleusine coracana*), proso millet (*Panicum miliaceum*), small millet (*Panicum sumatrense*), kodo millet (*Paspalum scrobiculatum*), and barnyard millet (*Echinochloa utilis*) (Saleh et al., 2013).

Millets are a staple grain as well as a component of many traditional foods and beverages such as porridges, snack meals, and fermented or unfermented breads (Chandrasekara and Shahidi 2011a; Chandrasekara et al., 2012).

Regarding their protein level, nutritional value, and macronutrient composition, millets are like regular cereals. Millets typically comprise 7-8% protein, 2-4% fat, 65-75% carbohydrates and 15-20% dietary fiber. Additionally, they contain a significant number of minerals, vitamins, and phenolic compounds (Hasan et al., 2019, Srilekha et al., 2019). Millets include bioactive compounds that have antimicrobial and antioxidant properties, among other possible health benefits (Singh & Sarita., 2016, Nazari et al., 2018). They make a substantial contribution to the diets of both humans and animals because of their large caloric content, as well as their high-quality proteins, lipids, calcium, iron, and zinc. Additionally,

they are a plentiful source of nutritional fiber and vitamins (**Hassan et al., 2021**). Previous research on the phytochemical content of millet showed that antioxidants such as tocopherols, phenolics, and carotenoids were present in high proportions. These findings have increased interest in using millet grains as a food source because they raise the way to the chances of using millet as a food source and producing nutritious value-added goods (**Liang et al., 2019**).

There are several potential health benefits associated with millet, including the prevention of cancer and cardiovascular illnesses, the reduction of tumor incidence, blood pressure reduction, heart disease risk reduction, cholesterol and fat absorption rate reduction, delayed gastric emptying, and provision of gastrointestinal bulk (**Truswell., 2002**).

Consuming millet as a supplemental food to improve livelihoods and nutritional security has been documented in several researches (**Cordelino et al., 2019, Durairaj et al., 2019, Marak et al., 2019**). But a comprehensive approach to addressing the current problems of hunger and malnutrition can be found in the careful development of millets and attention to their nutritional value (**Praveen & Tandon, 2016**). Millet can be consumed in various places in the form of Porridge or congee, pancake, chapati, khichdi, idli (**Jaybhaye et al., 2014, Embashu and Nantanga, 2019**).

## **2.2 Pearl Millet**

The pearl millet, or *Pennisetum glaucum*, is one of the most significant varieties of millet. It is sometimes referred to as bajra. It is a crop from the Poaceae family that can withstand drought (**Abdel-Hafez et al.2017; Adebisi et al.2016**). In South Africa and India's subcontinents, pearl millet is commonly cultivated (**Sandhu and Siroha, 2017**). Its ability to withstand drought and extreme temperatures makes it even more suitable for cultivation in areas where wheat, maize, and other cereal crops are not able to persist. Pearl millet is the largest producer of all the millet varieties, covering more than 29 million hectares; nevertheless, its distribution is restricted, mostly in Africa and Asia (**Rathore et al., 2016**). India is the world's largest producer of pearl millet, accounting for 9.8 million hectares of the overall production (**Rathore et al., 2016**), with over 95% of the crop coming from developing countries (**Basavaraj et al., 2010**).

Pearl millet is rich in protein, dietary fiber, carbohydrates, phytochemicals such as phytic acid, ferulic acid, tannins, and other phenolic compounds, as well as a variety of B complex vitamins including thiamine, riboflavin, niacin, vitamin B6, folate, and pantothenic acid (**Lestienne et al.2005; Jain and Bal, 1997**). It is a notable source of essential amino

acids, calcium, iron, and zinc (**Lestienne et al., 2007**). Condensed tannins, phytates, and other antinutritional substances found in pearl millet bind to proteins and minerals, decreasing their digestibility and bioavailability (**Gaytán-Martínez et al., 2017; Lestienne et al., 2007**). Proteins, minerals, and fiber are abundant in pearl millet (**Singh and Shurpalekar., 1989**). Pearl millet contains a high percentage of unsaturated fatty acids (75%) and linoleic acid (46.3%), it has a higher energy content than sorghum and is like brown rice (**Jaybhaye et al., 2014**). High levels of calcium, iron, and zinc (**Yadav et al., 2014; Sade, 2009; Lestienne et al., 2007**) along with superior protein quality measured by tryptophan and threonine content (**Elyas et al., 2002**) make this crop highly valuable to humans. Comparable to wheat, barley, and rice, pearl millet has an amino acid profile that is superior to that of sorghum and maize (**Ejeta et al., 1987**). The primary functions of pearl millet are used as food and dry feed. Compared to maize or sorghum, its grains exhibit a better balanced amino acid profile, high concentrations of iron and zinc, and high quantities of metabolizable energy and protein (**Rai et al., 2008**). For human consumption, grains are primarily utilized to make a variety of foods, including porridges and flat breads that are both leavened and unleavened. During the dry season of the year, a significant portion of the cattle food is made up of dry pearl millet stover (**Kelley et al., 1996**). Compared to sorghum, pearl millet has less hydrocyanic acid, making it a great grain for fodder. Its green fodder has safe amounts of oxalic acid and is high in protein, calcium, phosphorus, and other minerals (**Basavaraj et al., 2010**).

According to the information provided, pearl millet grains have a lot of promise as food because of some important nutritional qualities, such as their high protein content, dietary fiber, and minerals. They are also thought to be a cheap crop. Furthermore, because of its agronomic traits—such as its tolerance to high temperatures and low rainfall requirements—as well as the fact that its grains have a low incidence of mycotoxins and are not transgenic, millet is highly relevant for ensuring food safety. Therefore, there should be more encouragement for genetic improvement in order to introduce new cultivars with high grain yields. Additionally, consideration should be given to the potential uses and advantages of pearl millet in food, as this cereal has important implications for food safety and can be a good substitute for consumers looking for affordable, wholesome, and sustainable food options (**Dias-Martins et al., 2018**).

### 2.3 Sorghum Millet

Sorghum ranks as the fifth most significant cereal produced worldwide (**Serna-Saldivar et al., 1988**). It can grow well and produce in the world's tropical regions and warmer temperatures. In Asia and Africa, sorghum is the most often consumed cereal grain (**Obizoba., 1988**). The United States is the world's top producer of sorghum, followed by Nigeria and Mexico, making it the fifth most significant crop globally (**FAO, 2017**). Foods for both adults and children are prepared with it. Tropical regions produce infant foods by adding sugar to gruels made of sorghum and maize (**Obizoba., 1988**). Sorghum (*Sorghum bicolor L. Moench*) is a crop that is competitively grown all over the world because of its desirable qualities, which include its tolerance to drought and high yields, sorghum has gradually replaced other significant crops, primarily maize, in several countries, including South Asia and Africa (**Fracasso et al., 2016**).

Sorghum grains constitute a significant source of micro and macronutrients, comprising roughly 8-18% proteins, 1-4% fats, ~19% dietary fiber, and 70-80% carbs. However, sorghum, like other grains, has a notable lack of lysine and poor protein bio accessibility and digestibility when prepared in the traditional manner (**Serna-Saldivar and Espinosa-Ramírez., 2019**). Like other cereals, sorghum is a good source of B vitamins, including niacin, biotin, thiamin, and vitamin B6; but refining results in the loss of all B vitamins (**Hegedus et al., 1985**). The two main minerals found in sorghum grain are potassium and phosphorus, with relatively little calcium (**Khalil et al., 1984**). Due to the low concentration of vital amino acids like lysine, tryptophan, and threonine, sorghum grain has low protein quality (**Badi et al., 1990**). While sorghum is difficult to digest for infants (**MacLean et al., 1981**), it can be a good weaning diet when combined with foods high in lysine (**Eggum et al., 1990**). Cooking reduces the digestibility of sorghum proteins (**Kirleis et al., 1981, Monowar et al., 1983**).

Gaytán-Martínez et al. (2017) outlined a method for obtaining nixtamalized sorghum flours, which was followed in this study. In summary, a solution of Ca(OH)<sub>2</sub> (10 g Ca(OH)<sub>2</sub>/kg flour) was added to 1 kg of sorghum grains at a ratio of 1:3 (w/v). The mixture was then cooked at 94 °C for 30 min and allowed to stand for 12 h. Afterward, the nixtamalized grains were separated from the cooking water, washed to remove excess lime, and ground using a Nixtamatic® mill. Subsequently, both cooked and nixtamalized sorghum flours were dehydrated at 45 °C for 24 h using a convective food dehydrator, then processed

through a hammer mill and sieved to a particle size of 250 µm using a U.S. 60 mesh. Raw sorghum grains were also milled and sieved to the same particle size for comparison. All flours were stored in amber bottles at 4 °C until use. The cooking time and calcium concentration were chosen based on prior research to achieve the highest reduction in condensed tannins while maximizing the retention of polyphenolic compounds (PCs) and antioxidant capacity (Gaytán-Martínez et al., 2017). Samples were coded based on treatment (R: raw, C: cooked, N: nixtamalized), variety (W: white; R: red), and lime concentration [1: 10 g Ca(OH)<sub>2</sub>/kg flour]. Thus, the samples in this study were labeled as follows: RWS (Raw white sorghum) and RRS (Raw red sorghum); CWS (Cooked white sorghum) and CRS (Cooked red sorghum), NWS1 (Nixtamalized white sorghum, 10 g Ca(OH)<sub>2</sub>/kg flour), and NRS1 (Nixtamalized red sorghum, 10 g Ca(OH)<sub>2</sub>/kg flour) (Luzardo-Ocampo et al., 2020).

A variety of snacks can be made from nixtamalized maize and sorghum, and the masa can be cooked in water to make thick and thin porridges and drinks (Vivas et al. 1987). Local communities prefer sorghum varieties with a tan plant color and a white pericarp because they provide tortillas with acceptable color and flavor characteristics (Khan et al., 1980; Bedolla et al., 1983; Choto et al., 1985).

## 2.4 Finger Millet

In India, finger millet (*Eleusine coracana*), sometimes referred to as "ragi," is a popular grain that can be eaten without de-hulled. India is the world's largest producer of finger millet, accounting for around 60% of production overall (Shukla and Srivastava., 2014). It has been observed that finger millet variants contain 220-450 of calcium and 3–20% of iron (Balakrishna et al., 1973).

Globally and in many parts of India, finger millet is a widely grown crop. India is the world's largest producer of finger millet, accounting for around 60% of the crop (Kamini and Sarita., 2011). Finger millet, is cultivated for food throughout Africa and Southern Asia, primarily in Nepal and India's states of Uttar Pradesh, Bihar, Tamil Nadu, Karnataka, and Andhra Pradesh ((Klichowska 1984). After sorghum, PM, and foxtail millet in terms of production in semi-arid locations, FM comes in fourth position (Shiihii et al., 2011, Upadhyaya et al., 2011).

Africa is where finger millet originated and was domesticated. This millet was already being cultivated by farming groups in eastern Africa around 5,000 years ago, according to linguistic and archaeological data (Klichowska 1984). It grows more quickly between 100 and 130 days in regions with more rainfall (600–1,200 mm), especially in acidic soils. This millet is

highly productive among millets, and one of its main features is its capacity to adjust to various agroclimatic conditions (**Gopalan et al., 2002**).

Finger millet is eaten without being dehulled (**Gull et al., 2015**); it is also referred to as tamba (**Jideani et al., 1996**). In certain regions of Africa and India, the grains are a staple food (**Saleh et al., 2013, Siwela et al., 2010**). In the past, finger millet was processed in India using techniques like grinding, malting, and fermentation to make drinks, porridges, roti (unleavened flat bread), dosa (fermented pan cake), and idli (fermented steamed cake) (**Malathi & Nirmalakumari, 2007**). One of India's oldest crops, finger millet, is known as "nrtta-kondaka" in ancient Indian Sanskrit literature. This means "Dancing grain," and it was also called "rajika" or "markataka" (**Achaya, 2009**).

Ragi, also known as finger millet, is a gluten-free grain that is often overlooked and underused, despite its low glycemic index and numerous nutritional and nutraceutical advantages. (**Amadou et al., 2013, Jideani and Jideani, 2011**). Grains are also a rich source of vitamin B complex nutrients like thiamine, riboflavin, folic acid, and niacin (**Gull et al., 2015 and Saleh et al., 2013**). The grains are rich in calcium, and is high in essential amino acids including lysine, tryptophan, and methionine. Also, finger millet grains are a good supply of iron, magnesium, phosphorus, and carbohydrates (**Jideani, 2012**). Due to its high levels of calcium, phosphorus, iron, and zinc, finger millet, one of the minor grains, is nutritionally notable. Ten percent of the thirty million tons of millet produced worldwide is finger millet (**Dida et al., 2008**).

Untreated ragi flour displayed distinct rheological characteristics compared to wheat flour, but post-treatment, the samples showed consistent protein-related attributes (such as water absorption, stability, and mechanical weakening) and starch behavior (reflected in setback torque) in Mixolab analysis. Additionally, nutritional aspects like protein content, ash content, moisture level, total phenolic content (TPC), total flavonoid content (TFC), and water holding capacity (WHC) were largely unaffected by roasting, puffing, and germination processes. In summary, while replicating the unique rheological properties of wheat flour presents challenges, it's feasible to achieve comparable rheological behavior and enhanced functional attributes using roasted ragi flour as a substitute (**Mahajan et al., 2015**).

Consuming whole grains is linked to a lower risk of cardiovascular disease (CVD) and type 2 diabetes. Therefore, incorporating finger millet products made from whole meal into one's diet could be beneficial due to the protective properties of soluble dietary fiber (SCM),



offering a range of health advantages. Proper processing of finger millet products to reduce their glycemic index (GI), combined with accompanying dishes rich in vegetables and pulses, may aid in preventing or managing chronic diseases overall, with a focus on diabetes specifically (Shobana et al., 2013).

## **2.5 Nixtamalization**

Nixtamalization is a traditional method of steeping and boiling cereals, especially maize, with lime. It is widely used for many years throughout Mexico and Central America (Bressani et al., 1990; Serna-Saldivar et al., 1990; Martínez-Flores et al., 2002; 2006; Rendón-Villalobos et al., 2009). It is the process of pericarp removal from any grains by using alkaline solution. The whole grains are cooked in water with lime ( $\text{CaOH}_2$ ) and they are steeped for 12-16h. The steeped grains are called 'nixtamal' and the cooked steep liquid is called 'nejayote' which is rich in maize solids (Sahai et al., 2000). When it comes to food preparation, nixtamalized grains offer several advantages over unprocessed grains. Archaeological evidence indicates that the Mayas (wood ashes) used this procedure, but the Aztecs replaced the ashes with lime (Katz et al., 1974, Mariscal Moreno et al., 2015). Based on the greater pericarp removal, lime was used as a substitute of wood ashes. Lime improved the texture of the masa and tortillas by more effectively removing the seed coat from the grains. It is possible that the ancient populations experimented with this chemical, which may have contributed to the substitution of lime for ashes (Bressani., 1990).

The total amount of calcium added to the grain during the nixtamalization process is crucial because of the interactions it has with other grain constituents that are impacted by the physicochemical and sensory characteristics of the finished products. From a nutritional viewpoint, the calcium content in nixtamalized products is important and gives an importance of calcium in human diets. Calcium ions get incorporated into the millet grain because of hydration during the alkaline cooking process. The final product's physicochemical and textural characteristics are determined by the simultaneous hydration and partial gelling of the grain starches throughout these cooking and steeping processes, as well as the diffusion of calcium ions (Owusu-Kwarteng and Akabanda, 2013).

Cooking time, temperature, steeping time, initial calcium hydroxide level, water content, pericarp physical state, kind of grain, and grain physical state all affect the amount of calcium in the grain throughout the nixtamalization process (Cornejo-Villegas et al., 2010). Cooking and soaking times vary depending on the kind of food being made and local customs.

Several chemical reactions occur in the grains as they cook, including: the grain becomes hydrated and absorbs the alkali used in the cooking solution; the starches swell and gelatinize, some of which disperse into the liquid; the kernels soften and their pericarp loosen, causing the plant cell wall, including hemicelluloses and pectin, to become soluble (**Watcher, 2003**). During the first cooking stage, part of the pericarp's exterior waxy layer is destroyed, allowing the alkaline solution to diffuse inside the grain (**Gutiérrez-Cortez et al. 2016**). Small amounts of cellulose and other non-cellulosic components, including lignin, arabinoxylans, and ferulic acid, can be detached and lixiviated through further hydrolysis of ester bonds within the cellulose-hemicellulose-lignin structure (**Santiago-Ramos et al. 2018**). After steeping, dissolved components of the maize are contained in the alkaline liquid (nejayote), which are removed. The kernels are washed thoroughly and the remaining nejayote which has an unpleasant flavor (**Watcher, 2003**).

Alkaline cooking, or nixtamalization, is a useful technique for improving bio accessibility. It enhances cereal's amino acid profile (**Escalante-Aburto et al., 2019**). Tannin content is considerably reduced by nixtamalization (**Diaz Gonzalez et al., 2019**). Some protein fractions, including globulin, gliadin, and glutelin, which are lixiviated in the remaining cooking water, may also be lost during the nixtamalization process, which results in reductions in the protein level of sorghum (**Ramírez-Jiménez et al., 2019**). They're more readily ground, their nutritional value is raised, their flavor and aroma are enhanced, and their mycotoxin content is decreased (**Sefa-Dedeh et al., 2004**). Calcium oxide is integrated into the grain during nixtamalization, which raises the flour's ash content and, eventually, the product produced from the flour (**Salazar et al., 2014; Villada et al., 2017**). A reduction in the protein content because of starch gelatinization indicated the impact of nixtamalization on the protein content (**Obadina et al., 2016**). The heat treatment of the grain during alkaline cooking caused changes in the protein structure, which improved protein digestibility and made it easier to absorb, which is why the protein content decreased (**Gomez et al., 1989**). In the process of nixtamalization, high cooking temperatures cause the pericarp to be removed and the treated cereal's tannin content to become saturated (**Gaytán-Martínez et al., 2017**). An increase in the calcium content of the nixtamalized grains may also be the cause of the product's increased stiffness (**Lovera et al., 2014**). Better rheological qualities, such as viscoelasticity (**Torres et al., 1993; Véles-Medina et al., 2017**), and sensory qualities are among the numerous technological benefits of this procedure. The release of bound niacin, an improvement in protein quality (**Paredes López et al., 2009**), an increase in calcium content,

and a decrease in mycotoxin concentration are just a few of the well-known nutritional and microbiological improvements that should be mentioned. However, there are drawbacks to the Traditional nixtamalization process, including decreased levels of dietary fiber, total fat, and nutraceutical components (although there are no reported changes in the distribution of fatty acids) (Serna-Saldivar 2010, Wacher 2003). The impact of sorghum nixtamalization on the amount of phenolics and antinutrients has been the focus of most studies (Diaz Gonzalez et al., 2019, Gaytan-Martinez et al., 2017). Less attention has been received in the areas of the bioaccessibility and bioavailability of the nixtamalized flours. (Hernandez-Becerra et al., 2016, Johnson et al., 2010). The functional qualities (texture, color, crispness, flavor, and shelf life) of the finished nixtamalized product are influenced by the structural and chemical changes of the grain during processing. The microstructural alterations that occurred when grain was steeped and cooked in an alkaline condition (Paredes-Lopez and Saharopulos., 1982).

Globally, the tortilla and nixtamalized snack industries have been growing steadily. The nutritious value of the grain, which is used to make a variety of dishes such flours, tortillas, pozole, tostadas, nachos, corn chips, munchies, tamales, etc., must be improved by this process. (Santos et al., 2014).

This procedure produces a significant amount of highly alkaline and chemically oxygen-demanding polluting waste in the industrial setting. According to documentation, the nixtamalization process produces around 1500–2000 m<sup>3</sup> of wastewater on average per day in Mexico. This equates to 1.2 million m<sup>3</sup> per month and a total of 14.4 million m<sup>3</sup> per year (Castro-Muñoz et al., 2017). Figueroa et al. developed and patented ecological nixtamalization to lessen the contamination caused by the manufacturing of nejayote, a solution with high polluting residues (Figueroa et al., 2011). The pH of the residual solution is approximately 4.19–7.38, which is much lower than the value obtained by traditional nixtamalization (pH 12–14), even when the use of water is not lowered in this process. The source of calcium determines these variations in pH, and in this instance, calcium sulfate was an additive that significantly reduced pH. To achieve incomplete grain dehulling, calcium salts (such as sulfate, carbonate, and chloride) were utilized; this resulted in a product with a higher dietary fiber content and less formation of pollutants (Campechano et al., 2012). In conclusion, compared to products made by traditional nixtamalization, masa and tortillas produced with ecological nixtamalization had better sensory qualities and nutritional enhancement (Maya-Cortés et al., 2010, Mariscal-Moreno et al., 2017).

## **OBJECTIVES**

- ❖ To formulate dry millet nixtamalized micro structured flour, using alkaline process.
- ❖ To assess the physico-chemical, nutritional and functional properties of nixtamalized and native flour
- ❖ To compare the nutritional properties of the nixtamalized flours with the native flour.

# **METHODOLOGY**

## 4.1 RAW MATERIALS

The raw material sorghum millet, pearl millet and finger millet were purchased locally from the market, Mysuru. Then stored at 4°C in cold room packed in airtight bags to protect from any insects and pests. All the chemicals used for the proximations were of analytical grade (sigma) unless stated otherwise.

## 4.2. PREPARATION OF SAMPLE

- i. Pearl millet, sorghum and finger millet native samples are prepared by polishing and pulverizing.
- ii. Nixtamalized samples are prepared by following several steps including cooking (with 2% CaOH<sub>2</sub>), steeping, washing, drying, pulverizing and sieving.

### 4.2.1 Native Millet Flour

Purchasing Pearl Millet, Sorghum and Finger Millet



Precleaning of kernels to remove straw, dust, stones etc.



Polishing using mini rice mill



Pulverized using micro pulverizer



Sieved using 60 mesh sieves



**Fig 4.1 Pearl millet grain**



**Fig 4.2 Polished PM**



**Fig 4.3 Native PM**



**Fig 4.4 SM**



**Fig 4.5 Polished SM**



**Fig 4.6 Native SM**



**Fig 4.7 Finger millet grain**

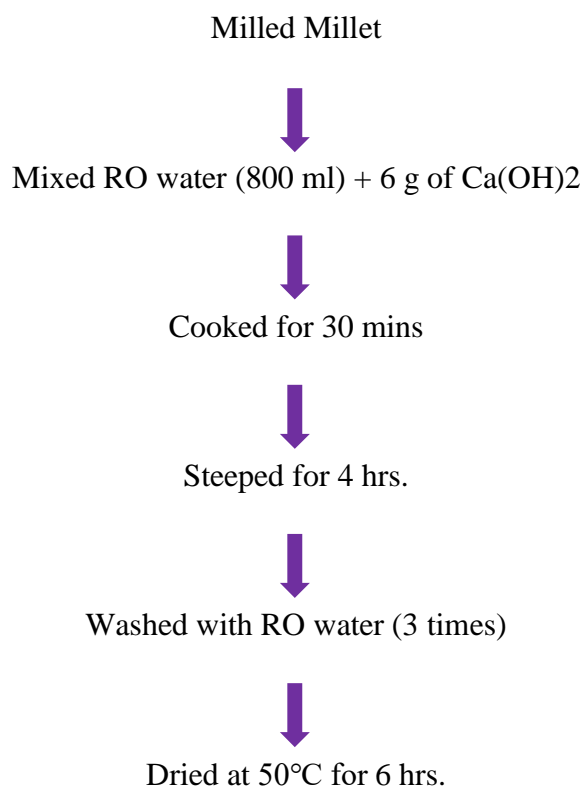


**Fig 4.8 Polished FM**



**Fig 4.9 Native FM**

#### **4.2.2 Dry Milled Nixtamalized Pearl Millet Flour**



↓  
Pulverized

↓  
Passed through 60 mesh sieves



**Fig 4.10 Cooked and steeped PM**



**Fig 4.11 Dried PM**



**Fig 4.12 Nixtamalized PM**

#### **4.2.3 Dry Milled Nixtamalized Sorghum Millet Flour**

Milled millet



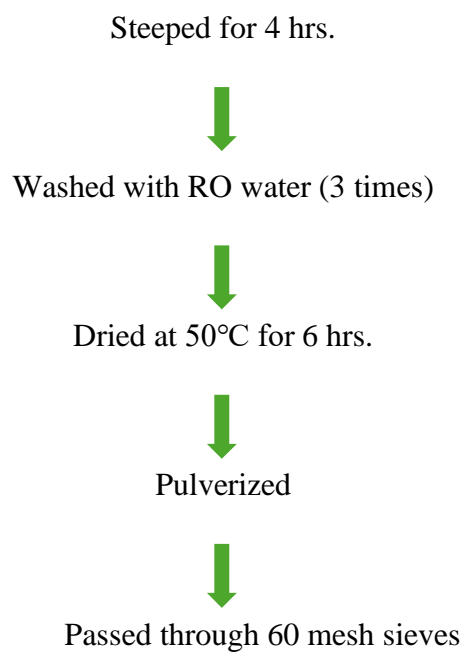
Mixed RO water (600 ml) + 6 g of  $\text{Ca}(\text{OH})_2$



Cooked for 30 mins

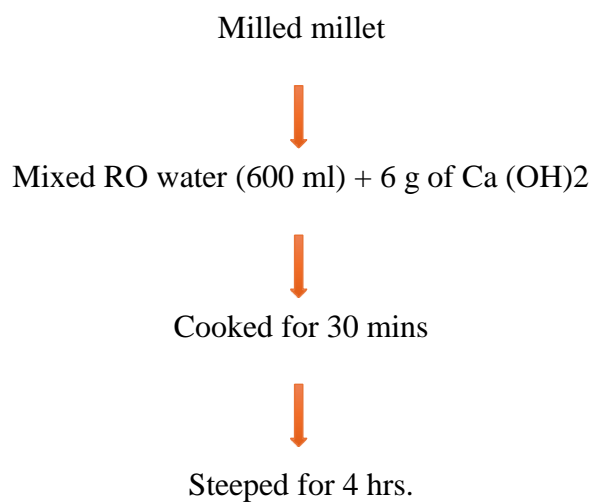


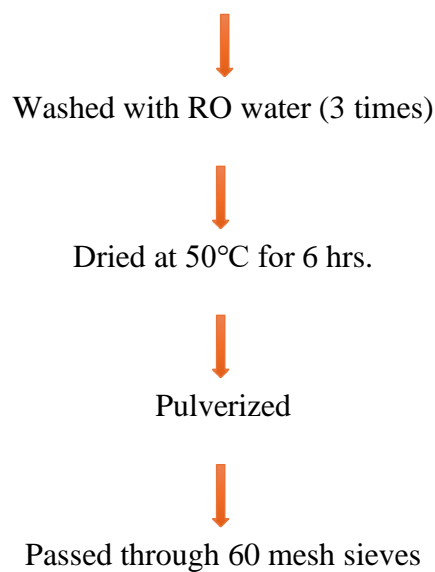




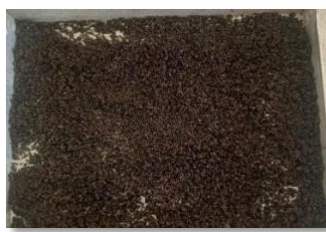
**Fig 4.13 Nixtamalized SM**

#### **4.2.4 Dry Milled Nixtamalized Finger Millet Flour**





**Fig 4.14 Cooked and Steeped FM**



**Fig 4.15 Dried FM**



**Fig 4.16 Nixtamalized FM**

## **4.3 METHODS**

### **4.3.1 Machines Used**

#### **a) Rice Polisher**

The primary purpose of the rice polishing machine is to get rid of the bran powder that has stuck to the white rice surface. After the rice surface has been polished, a waxy layer forms to provide protection. This layer not only keeps the rice clean and stops the rice bran from sticking to or falling off during production, storage, transportation and sales, but it also raises the finished rice quality and market value is ideal for rice storage since it keeps rice fresher longer and makes it more palatable. A specialized tool called a rice polisher machine is used to buff rice kernels in order to alter its flavor, look and texture. Additionally, it can be used to turn brown rice into

white rice. This kind of abrasive machine uses talc or some other fine dust to buff the rice outer surface. It is chosen over other comparable items on the market due to its superior build quality and consistent performance.



**Fig 4.17 Rice polisher**

#### **b) Mini Rice Mill**

The mini rice mill consists of a paddy-cleaner, sheller, separator and a polisher. The separator is a compact unit designed on the densimetric classification principle. The polisher could be either a vertical cone polisher or a horizontal rotor polisher. Even a huller used for milling could serve as a polisher though there may be more breakage of rice. The most important feature of the mill is that the shelling and polishing are kept separate. Because of the low capacity, a centrifugal sheller is most commonly employed. Different units could be used as polisher. For maximum advantage, it is necessary to use a paddy separator, whereby need of a high polish can be avoided.



**Fig 4.18 Mini rice mill**

### c) Micro pulverizer

The micro pulverizer has a rotor assembly that rotates at a high speed and is equipped with hammers. At the point of mill discharge, a retaining screen and a cover with several deflector liners are installed in the grinding chamber. Feed material is frequently introduced into the grinding chamber using a screw mechanism. Three fundamental factors, including the kind of hammers used, the speed of the rotor, and the size and form of the screen opening, all have an impact on the grinding process. The “stirrup,” which is advised when a tiny particle size is wanted, and the “bar,” which produces a coarser grind with the least number of fines, are the two different types of hammers. Both versions have different abrasion-resistant metals topped at the wear edge. The fineness of the grind is influenced by the rotor speed in the following way: low speed generates a coarse grind, while high speeds provide a fine grind. The product is also impacted by the size and shape of the retaining screen openings. A finer screen opening will often produce a finer grind. Except for coarse granulations, the retaining screen does not serve as a sifting screen.



**Fig 4.19 Micro pulverizer**

## **4.4 PHYSICAL PROPERTIES**

### **4.4.1 Bulk Density**

#### **Principle**

Bulk density is the mass of a substance per unit volume, which includes the volume filled by the voids or empty spaces between its individual particles. It gauges how firmly the material particles are packed into a certain specific volume.

### **Materials Required**

Samples, measuring cylinder and weighing balance.

### **Procedure**

A measuring cylinder with a known volume was used and filled with a known mass of flour sample to measure the bulk density. The volume occupied by the flour was noted, and the bulk density of the sample was determined after that.

### **Calculation**

$$\rho \text{ (g/ml)} = m / V$$

Where,  $\rho$ - Density (g/cm<sup>3</sup>), m- Mass (g), V- Volume (ml)

## **4.4.2 True Density**

### **Principle**

The term “true density” describes a density of substance when no voids or air spaces are taken into consideration. True density is a physical characteristic that tells us about how tightly packed and arranged the particles are inside a substance.

### **Materials Required**

Samples, measuring cylinders, weighing balance, toluene

### **Procedure**

Using measuring cylinder with a known volume, a known volume of toluene was added to measure the true density. A known quantity of flour was then added, and the total volume was recorded to determine the true density.

### **Calculation**

$$\rho \text{ (g/cm}^3\text{)} = \frac{M}{V_2 - V_1}$$

Where,  $\rho$ - Density (g/cm<sup>3</sup>), m- Mass (g), V1- Volume of toluene (ml), V2- Final volume of flour with toluene (ml)

### 4.4.3 Porosity

#### Principle

Porosity, the volume of voids or air gaps within flour particles, offers insights into the structure and density of the flour and is occasionally expressed as a percentage. Various factors such as flour type, processing methods, and particle size distribution contribute to differing porosity levels. Porosity plays a significant role in determining the behavior of flour in both industrial and culinary settings.

#### Calculation

$$\text{Porosity } (\Phi) = 1 - \frac{\text{Bulk Density}}{\text{True Density}} \times 100$$

### 4.4.4 Tap Density

#### Principle

The density attained after tapping or mechanical compression is referred to as the tap density of a product, especially flour. Usually, it is expressed in gram per milliliter (g/ml) or other suitable units. However, depending on the type of flour, moisture content, particle size distribution, and compaction circumstances, the tap density of flour may change. The tap density of a material, such as flour, describes how tightly its particles can be compressed or tapped together under mechanical tapping. It is a measurement of the maximum bulk density that the substance can reach under tapping forces.

#### Materials Required

Samples, measuring cylinder and weighing balance

## Procedure

To determine the tap density of a substance, a measuring cylinder of known volume was filled with a specified mass of flour and tapped 25 times. The volume occupied by the flour after tapping was then recorded.

## Calculation

$$\rho = m / V$$

Where,  $\rho$ - Density ( $\text{g}/\text{cm}^3$ ), m- Mass (g), V- Volume (ml)

## 4.4.5 pH

### Principle

pH meters are wholly based on the ion exchange between the sample and the glass electrode's inner solution, which generates electrical voltage. The result of the principle of pH meters is based on the hydrogen ion concentration and the relation between electric voltage and the pH readings wholly based on the ion exchange between the sample and the glass electrode's inner solution, which generates electrical voltage.



**Fig 4.20 pH meter**

## 4.4.6 Water Activity

### Principle

Water activity is defined as the availability of free water in a sample. Only this component takes an active part in the exchange with the ambient humidity and can possibly from the ideal medium for microbiological growth on the surface. Above the sample, the humidity is

measured immediately after reaching the humidity balance. The relative humidity is measured in % RH and converted to  $a_w$  electronically. The net amount of water that is transferred decreases steadily as the water exchange between the free available water of samples and humidity moves of air towards equilibrium. As a result, the change in air humidity and water activity over time now provides information on the setting an equilibrium humidity level.



**Fig 4.21 Water activity meter**

## **4.5 PROXIMATE ANALYSIS**

### **4.5.1 Moisture Content**

#### **Principle**

It is estimated by loss on drying, in which the sample is heated and the weight loss due to evaporation of moisture is recorded.

#### **Materials Required**

Hot air oven, aluminum moisture cup, weighing balance and desiccator

#### **Procedure**

The produced flour samples were placed in aluminum moisture cup and placed in a hot air oven for two hours at a temperature between 120-130°C. Each sample weighed two grams. Once it has cooled to room temperature, the weight change is calculated as a percentage. The following formula was used to determine the moisture content.



### Calculation

$$\text{Moisture Content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where, W<sub>1</sub> – Weight of empty plate

W<sub>2</sub> – Weight of empty plate + sample before drying

W<sub>3</sub> – Weight of empty plate + sample after drying

## 4.5.2 Total Fat Content

### Principle

In order to determine fat, Soxhlet is used. The continuous extraction method used by the fat extractor is the reflux and siphon concept with a pure solvent (petroleum ether). The fundamental idea of Soxhlet extraction method is the repetitive cycling of the solvent, which enables effective component extraction from solid samples. The method makes use of the target component's capacity to dissolve in the solvent, the solvent cycling between the solid sample and boiling flask, and the component's gradual concentration in the boiling flask as the extraction advances. This method is particularly useful for extracting components that have low solubility in the solvent or components that are difficult to extract using other methods.

### Materials Required

Sample, RBF, material required, petroleum ether (60-80°C), thimbles, soxhlet

### Procedure

2g of sample weighed and packed in the thimbles and labeled. In the extraction tube, thimbles were then inserted. A weighed round bottom flask (RBF) was filled with petroleum ether. 6-7 hrs. are spent operating after it is turned on. The solvent was entirely dried from the flasks after the run time, and the final weight of the flasks was determined. Consequently, the following formula was used to determine the fat content.

### Calculation

$$\text{Fat Content (\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Weight of sample}} \times 100$$



**Fig 4.22 Soxhlet Apparatus**

### **4.5.3 Total Protein Content**

#### **Principle**

The quantifications of proteins using the kjeldahl method entails three steps: digestion, distillation, and titration. Utilizing concentrated  $\text{H}_2\text{SO}_4$ , heat,  $\text{K}_2\text{SO}_4$  (to raise the boiling point), and a catalyst (such as selenium) to speed up the reaction, organic material can be digested. Any nitrogen in the sample is converted into ammonium sulphate during this procedure. The digestate then neutralized by adding  $\text{NaOH}$ , which changes the ammonium sulphate into ammonia. This ammonia is then distilled out and collected in a receiving flask of surplus boric acid to create ammonium borate. To determine the sample's total nitrogen concentration, the leftover boric acid is titrated with a standard acid using a suitable end-point indicator. The measured nitrogen content must be converted to the crude protein content using a specified conversion factor after the total nitrogen has been determined.

#### **Reagents:**

- Digestion Mixture: Powdered potassium sulphate, copper sulphate and selenium dioxide were mixed in a ratio of 5:2:1.
- Sodium Hydroxide solution (40%): 40% of sodium hydroxide was dissolved in distilled water and made up to 100ml.
- Boric Acid (2%): 2g of boric acid is dissolved in warm water, cooled and made up to 100ml.
- Mixed Indicator: 0.1% sodium each of bromocresol green and methyl red was prepared in absolute alcohol and then five parts of the first one was mixed with one part of the second.

- N/70 Hydrochloric Acid: approximately 1.3cc of AR hydrochloric acid was dissolved in 1 liter of water.
- Standard Ammonium Sulphate: 0.942g of AR ammonium sulphate was dissolved in distilled water and made up to 1 liter. It may be noted that 5ml of this solution is equivalent to 1mg of nitrogen.

### Procedure

Approximately 2g of sample was weighed into the Kjeldahl flask.

- Then, 5g of digestion mixture, 20ml of concentrated H<sub>2</sub>SO<sub>4</sub> and 2-3 glass beads were added into the tubes and they were kept for digestion at 350°C for 6-8hrs.
- The flask was kept for digestion, removed when the liquid was clear, indicating completion of digestion process, then cooled and transferred to a 100ml standard volumetric flask and was made up to the mark using distilled water.
- 5ml of that solution was pipetted out into the distilled flask and about 25ml of distillate that contained ammonia was collected in the flask having 5ml of 2% boric acid and 3 drops of mixed indicator.
- It is steam distilled with sodium hydroxide (NaOH) and about 25ml of distillate is collected.
- The ammonia was converted into ammonium metaborate and was titrated with standardized N/70 hydrochloric acid.
- And a blank value was determined using the same procedure.

### Calculation

The percentage of protein was calculated using the following formula:

$$\text{Protein (\%)} = \frac{A-B}{C} \times \frac{D}{E} \times \frac{\text{Multiplication Factor (6.25)}}{F \times 1000} \times 100$$

Where, A- HCL consumed for sample

B- HCL consumed for blank (ml)

C- Volume of sample for standard for ammonium sulphate (ml)

D- Volume of sample made after digestion (ml)

E- Volume of sample solution taken for distillation (ml)

F- Weight of the sample taken for digestion (g)

#### **4.5.4 Total Ash Estimation**

##### **Principle**

The residue that is left over after a substance has been entirely burned at a high temperature is referred to as its total ash content. It represents the inorganic minerals found in the initial sample.

##### **Materials Required**

Silica crucibles, samples, nitric acid, heating mantle, muffle furnace, desiccator.

##### **Procedure**

2g of each sample were weighed in a pre-weighed and labeled silica crucible. The crucible is heated on the heating mantle and the material is charred after the smoke subsides the crucible are then placed in a muffle furnace for ignition at 400-500°C for 3-4hrs. The ash should be white in appearance after ignition but in case they are still black, 2-3 drops of nitric acid are added and ignited again. Then the crucibles were taken out and cooled in a desiccator and weighed.

##### **Calculation**

$$\text{Ash Content (\%)} = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Weight of sample}} \times 100$$

Where,

Final weight = weight of the silica crucible with sample after incineration (g)

Initial weight = weight of the silica crucible before incineration without sample i.e., empty weight (g)

### 4.5.5 Mineral Estimation

**Minerals Estimated:** Calcium, sodium, iron, potassium, magnesium.

#### Principle

To identify the element, present in the sample, a high-temperature plasma is created using a microwave, and the produced light from the plasma is then examined. A small quantity of sample is introduced, and the high temperature of plasma excites the element's atoms to a highly excited state before returning to the ground state, causing them to emit light with a distinctive wavelength. In order to detect and measure the elements contained in the sample, the emitted light is then directed to a spectrophotometer.

#### Materials Required

Silica crucibles, conc. HCl, triple distilled water, water bath, volumetric flask, pipettes, Whatman no. 41 filter paper, microwave plasma atomic emission spectrophotometer.

#### Procedure

1ml of triple distilled water and 4ml of conc. HCl is added in silica crucibles with the ash samples and is evaporated to dryness on the water bath. 5ml of conc. HCl is added and evaporated again then, 4ml conc. HCl and 1ml triple distilled water are added and boiled for 5 sec. Then the prepared solution was filtered with Whatman no. 41 filter paper in a 50ml volumetric flask and the volume is made up of triple distilled water. Then this prepared sample is used for mineral estimation in MP-AES.



**Fig 4.23 MP-AES**

### **4.5.6 Carbohydrates**

#### **Principle**

Total carbohydrate content of a food must be calculated by subtractions of the sums of the protein, total fat, moisture and ash. Along with fats and proteins, carbohydrates are one of the three major macronutrients included in food. Organic substances known as carbohydrates are composed of carbon, hydrogen and oxygen atoms, often in the ratios of 1:2:1. The main purpose of carbohydrates in the diet is to give the body a source of energy.

#### **Calculation**

$$\text{Carbohydrate \%} = 100 - (\% \text{moisture} + \% \text{fat} + \% \text{protein} + \% \text{ash})$$

## **4.6 FUNCTIONAL PROPERTIES**

### **4.6.1 Water Absorption and Soluble Index**

#### **Principle**

A food product's ability to hold water is measured using the water absorption index (WAI), which is especially useful for starchy foods like grains, flours, and cereals. It gives details regarding the product's capacity to absorb and hold water, which might be important for a variety of food preparation and processing tasks. In order to understand how various ingredients and processing techniques affect the texture and behavior of food items in the presence of water, the WAI frequently employed in the field of food science and engineering.

#### **Materials Required**

Sample, distilled water, centrifuge tube (50ml), petri dish, centrifuge, water bath, vortex and measuring cylinder.

#### **Procedure**

One gram of the sample was mixed with 10 mL of distilled water in a pre-weighed 50 mL centrifuge tube and vigorously stirred (vortexed) for 1 minute. Subsequently, the mixture was allowed to settle at room temperature for 30 minutes before being centrifuged at 3000 rpm for 10 minutes. The resulting supernatant was collected in a pre-weighed petri dish, and the residue was weighed. The supernatant in the petri dish was then evaporated.

### Calculation

$$\text{WAI} = \frac{\text{Weight of centrifuge tube with pellet} - \text{Empty weight of centrifuge tube}}{\text{Weight of the sample taken}}$$

$$\text{WSI} = \frac{\text{Weight of the dry petri dish} - \text{Empty weight of petri dish}}{\text{Weight of the sample taken}} \times 100$$

## 4.6.2 Oil Absorption Index

### Principle

An indicator used to determine how much oil a substance, often a powder or solid material, can absorb is called the oil absorption index (OAI). For commodities like flours, starches, and other powders that are used in cooking, baking and food processing, this index is particularly relevant to the food industry. The result, which is given as percentage, shows how much more weight the sample gained up as a result of absorbing oil. A sample's ability to hold more oil is indicated by a higher OAI score.

### Materials Required

Sample, vegetable oil, centrifuge tube (50ml), centrifuge, water bath, vortex and measuring cylinder.

### Procedure

1g of the sample was suspended in 10ml of vegetable oil in a pre-weighed 50ml centrifuge tube and energetically stirred (vortex) for 1 min. Then the sample was left undisturbed at room temperature for 30 mins and then centrifuged at 3000 rpm for 10 mins. The supernatant was collected in a pre-weighed petri dish and the residue was weighed. The supernatant in the petri dish was evaporated on the water.

### Calculation

$$\text{OAI} = \frac{\text{Weight of the centrifuge tube with pellet} - \text{Empty weight of centrifuge tube}}{\text{Weight of sample taken}}$$

### 4.6.3 Swelling and Solubility Power

#### Principle

The ability of flour to absorb and hold water when combined with a liquid, usually water, is referred to as its swelling power. As it impacts the texture, viscosity and functioning of flour-based products, this feature is significant in a variety of food and industrial applications. Starch, protein and the type of flour used are the main factors that affect the swelling power of flour.

The ability of flour to dissolve or disperse in a liquid, usually water, is referred to as its solubility power. Starch and protein are two of the main factors that affect the solubility of flour, together with the kind of flour that is utilized. Proteins, carbs and minerals are just a few of the many ingredients found in flour, and they all dissolve differently in water. The solubility of the ingredients in the flour affects the dough's capacity to come together cohesively, as well as the texture, flavor and nutritional value.

#### Materials Required

Samples, centrifuge tubes (50ml), petri dish, centrifuge, water bath.

#### Procedure

About 500mg (on a dry weight basis) of the sample was combined with 20ml of distilled water in a pre-weighed centrifuge tube. The sample underwent cooking at 30°C, 50°C, 70°C, and 90°C for 30 minutes each. After centrifugation, the supernatant was collected, while the residue was weighed for swelling power. The supernatant was transferred to a pre-weighed petri dish for evaporation on a water bath. Upon complete evaporation, the petri dish was cooled and weighed.

#### Calculation

$$\text{Swelling Power} = \frac{\text{Weight of the wet residue (mg)}}{\text{Weight of dry sample (mg)} - \text{Weight of dry petri dish (mg)}}$$

$$\text{Solubility (\%)} = \frac{\text{Weight of the petri dish (mg)} \times 2.5}{\text{Weight of the sample (mg)}} \times 100$$



## 4.6.4 Color Measurement

### Principle

Hunter Lab colorimetry is a color space and color measurement technique used to quantitatively describe and study the color of objects and samples. It is often referred to as Hunter Lab color measurement or the Hunter Lab color scale. For quality control and color matching purposes, it is widely used in variety of sectors, including those involving food, textiles, plastics, paints, cosmetics and more. The Hunter Lab methods offers a standardized approach to express and communicate color information and is based on the fundamentals of human vision and color perception.

The colorimeter CM 5 is a tool for determining a sample's color depending on how much light it absorbs. The Beer-Lambert law, which states that the amount of light absorbed by a sample is proportional to the concentration of the absorbing species in the sample, forms the basis for the operation of this system.

### Materials Required

Samples, petri dishes and colorimeter CM 5 (Konica Minolta)

### Procedure

The colorimeter CM 5 is calibrated with a non-absorbing blank sample before analyzing a sample. It measures the amount of light transmitted through the sample. The color is characterized by three tristimulus values: L (Lightness), a (red-Green Axis), and b (Yellow-Blue Axis). L\* represents the perceived lightness, ranging from 0 for black to 100 for white. Positive a\* values indicate a shift towards red, while negative values suggest a shift towards green. Similarly, positive b\* values signify a shift towards yellow, while negative values indicate a shift towards blue. The difference between two colors is assessed as  $\Delta E$ .



**Fig 4.24 Colorimeter**

## 4.6.5 Particle Size Analysis

### Principle

An equipment used to assess the size and distribution of particles in a sample is referred to as a particle size analyzer, particle size distribution analyzer, or particle size measurement instrument. The Microtrac Bluewave is a particle characterization tool that uses the light diffracted by suspended particles to derive their size distribution. The Bluewave is able to measure the particles in the size range 50 nm to 2800  $\mu\text{m}$ . The system suspends the particles in a liquid and circulates the suspension through an optical cell where lasers of three different wavelengths strike the sample. The light is diffracted by an ensemble of particles in the sample, and the maxima and minima of the combined diffraction pattern are recorded by the detectors. An inversion of algorithm is used to separate the patterns created by each particle in the ensemble, and the system software calculates the particle size distribution based on these diffraction patterns.



**Fig 4.25 Particle size distribution analyser**

## 4.6.6 Differential Scanning Calorimetry (DSC)

### Principle

DSC works by heating or cooling a sample and a reference material at the same rate while measuring the temperature difference between them. Any heat absorbed or released by the sample compared to the reference is recorded, providing information about phase transitions, reactions and purity.

### Procedure

Take sample (usually a few mg) in a small aluminum pans. Fill an identical empty pan with an inert material, such as an empty aluminum pan or an empty pan containing the same

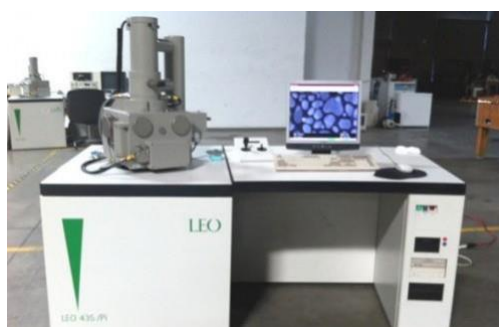
material as sample but without the component of interest. Calibrate the DSC instrument using standard reference material to ensure accurate temperature and enthalpy measurements. Place the sample pan and the reference pan in the DSC instrument. Typically, the sample pan is placed on top of the reference pan. Start the experiment by heating or cooling the pans at a controlled rate (usually constant) while recording the heat flow between the sample and reference pans as a function of temperature.

#### **4.6.7 Scanning Electron Microscopy (SEM)**

Scanning electron microscopy (SEM), which is also recognized as SEM analysis or SEM technique, has been used worldwide in many disciplines. It can be regarded as an effective method in analysis of organic and inorganic materials on a nanometer to micrometer ( $\mu\text{m}$ ) scale. SEM works at a high magnification reaches to 300,000x and even 1000000 (in some modern models) in producing images very precisely of wide range of materials.

##### **Working Principle**

A beam of electrons is formed by the Electron Source and accelerated toward the specimen using a positive electrical potential. The electron beam is confined and focused using metal apertures and magnetic lenses into a thin, focused, monochromatic beam. Electrons in the beam interact with the atoms of the specimen, producing signals that contain information about its surface topography, composition and other electrical properties. These interactions and effects are detected and transformed into an image.



**Fig 4.26 Scanning electron microscopy**

## 4.7 ANTIOXIDANT PROFILE

### 4.7.1 Radical Scavenging Activity

#### Principle

Radical scavenging DPPH (2,2-diphenyl-1-picrylhydrazyl) activity assay is a widely used method in antioxidant research to evaluate the ability of compounds or samples to neutralize free radicals. A synthetic stable free radical called DPPH is visible as a purple solution. It is highly reactive due to the unpaired electron it possesses, and it can take on an electron or a hydrogen atom to become stable. An antioxidant can give the DPPH radical an electron or a hydrogen atom when it is introduced to a solution containing the radical, neutralizing it. The reduction of DPPH to a non-radical state happens as a result of this electron or hydrogen atom transfer. A switch from purple to yellow occurs along with a decrease in DPPH. By measuring the absorbance at a particular wavelength (often about 517 nm), this color shift can be assessed spectrophotometrically. The color shift is more noticeable and the absorbance is lower as antioxidant activity increases.

#### Materials Required

Samples, 70% ethanol, methanol, DPPH (2,2-diphenyl-1-picrylhydrazyl), conical flask, centrifuge tube, shaker, water bath, centrifuge, beakers, test tubes, UV- spectrophotometer.

#### Procedure

The extraction of the sample is to be done with 70% ethanol. Adding sample and 70% ethanol in a conical flask and kept for shaking for 3 hrs and centrifuged at 3000 rpm for 15 mins. The supernatant is collected and evaporated to 1ml. This extract is now used for evaluation. 40 µl of extract and 2.9ml of DPPH solution is added to a test tube and incubated in the dark for 30 mins. Then the color change is studied under UV- spectrophotometer at 517 nm.

#### Calculation

$$\text{DPPH Scavenging Effect (\%)} = \frac{A_o - A}{A_o} \times 100$$

Where,  $A_o$  – The absorbance of control

A<sub>1</sub> – The absorbance of sample

#### **4.7.2 Total Phenolic Content (TPC)**

##### **Principle**

A common technique for calculating the number of phenolic compounds in a sample is to use the Folin-Ciocalteu (FC) reagent to determine the total phenolic content (TPC) of sample. The oxidation of phenolic compounds by the reagent under alkaline conditions, which results in the production of blue-colored complex, is the basis of TPC assay utilizing the FC reagent. The degree of blue color intensity is directly related to the number of phenolic compounds present in the sample.

##### **Materials Required**

Sample extracts, FC reagent, 20% Na<sub>2</sub>CO<sub>3</sub>, test tubes, micropipettes, vortex, UV-spectrophotometer.

##### **Procedure**

The sample extract is diluted 5 times and then 0.2ml of this solution is added with 6ml of distilled water in a test tube. 0.5 ml of FC reagent and 15ml of 20% Na<sub>2</sub>CO<sub>3</sub> is added and the volume was made up to 10ml. Then the solution was vortexed and incubated in the dark for 15 mins and the changes was observed under a UV-spectrophotometer at 760 nm.

# **RESULT AND DISCUSSION**

## 5.1 OUTCOME AFTER MILLING

Samples	Grains taken (g)	Flour after milling (g)	Loss (%)
PM	300	290	3.33
SM	300	287	4.3
FM	300	294	2

**Table 5.1 Outcome of the native samples**

Samples	Wt of grains taken (g)	Wt after cooking (g)	Wt after steeping (g)	Wt after washing (g)	Wt after drying (g)	Wt after sieving (g) (-60)
PM	300	475	933	611	236	230
SM	300	430	694	509	267	257
FM	300	461	733	528	254	241

**Table 5.2 Outcome of Milled Nixtamalized flour samples**

## 5.2 PHYSICAL PROPERTIES

### 5.2.1 Bulk Density, Tapped Density, True Density and Porosity

Samples	Bulk Density (g/ml)	Tapped Density (g/ml)	True Density (g/ml)	Porosity ( $\Phi$ )
PM (Native)	0.58±0.11	0.65±0.13	1.47±0.06	60.5
PM (Nixtamal)	0.59±0.12	0.71±0.11	1.48±0.07	60.2
SM (Native)	0.6±0.13	0.64±0.11	1.47±0.06	59.25
SM (Nixtamal)	0.59±0.12	0.63±0.11	1.48±0.07	60.15
FM (Native)	0.68±0.14	0.85±0.15	1.48±0.07	54.3
FM (Nixtamal)	0.74±0.16	0.79±0.1	1.61±0.07	53.45

**Table 5.3 Physical properties**

When compared to bulk and tapped densities, the bulk densities of all flour samples are lesser than tapped densities. The bulk density of all flour samples are ranges from 0.58 g/ml to 0.74 g/ml, while the tapped density ranges from 0.63 g/ml to 0.85 g/ml. While flours with higher bulk and tapped density are better for storage, shipping, and packaging, lower density flours can also improve the texture of food, particularly in complementary foods and certain culinary applications. Plaami (1997) states that the arrangement of starch polymers affects the bulk density A decreased bulk density may arise from polymers being arranged less densely. This phenomenon becomes evident after nixtamalization, wherein starch polymers are digested into smaller segments, consequently reducing the density of the resulting nixtamalized flour. (Gopika & Joshi., 2024).

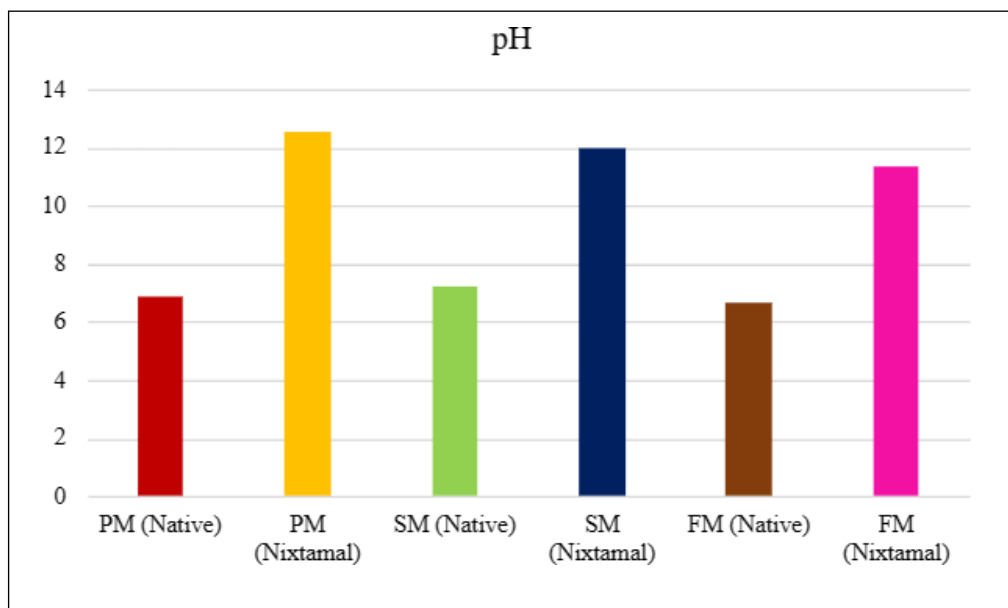
### 5.2.2 pH

Samples	pH
PM (Native)	6.86
PM (Nixtamal)	12.57
SM (Native)	7.21
SM (Nixtamal)	12.00
FM (Native)	6.67
FM (Nixtamal)	11.32

**Table 5.4 pH**

Nixtamalized flour samples have highest pH values varied from 11.32 to 12.57 as compared to native samples. The pH values of control samples are ranges from 6.67 to 7.21. Nixtamalized samples have more alkaline character because of it cooked with  $\text{Ca(OH)}_2$ . The immersion of flour in lime solution led to a notable rise in pH levels, statistically significant at  $p < 0.05$ . The pH of food items holds significance as it impacts both flavor and longevity. The elevation in pH observed in millet flour treated with lime likely stems from the absorption and retention of lime within the flour. Consequently, this suggests that the flour could be more susceptible to spoilage under comparable storage conditions due to its decreased acidity (Ocheme et al., 2010).





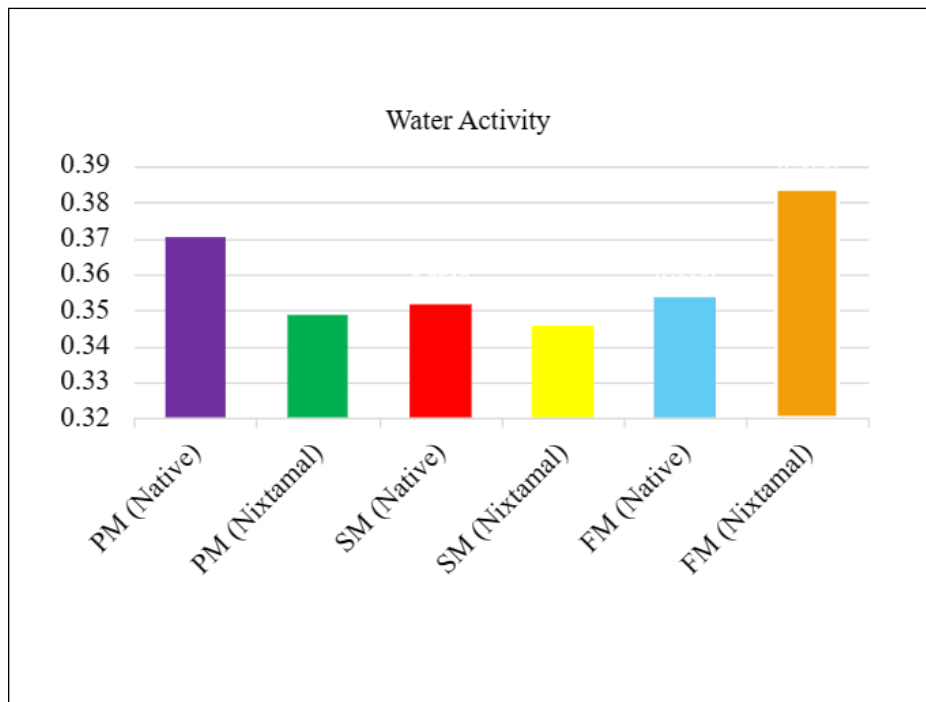
**Fig 5.1 pH**

### 5.2.3 Water Activity (aw)

Samples	Water Activity
PM (Native)	0.37
PM (Nixtamal)	0.34
SM (Native)	0.35
SM (Nixtamal)	0.34
FM (Native)	0.35
FM (Nixtamal)	0.38

**Table 5.5 Water activity**

The water activity of all samples has no significant difference between each other. However, FM (Nixtamal) (0.38) have comparatively highest aw than other flour samples. Relatively high-water activity levels can result in the food being prone to spoilage by microorganisms (Ocheme et al., 2010).



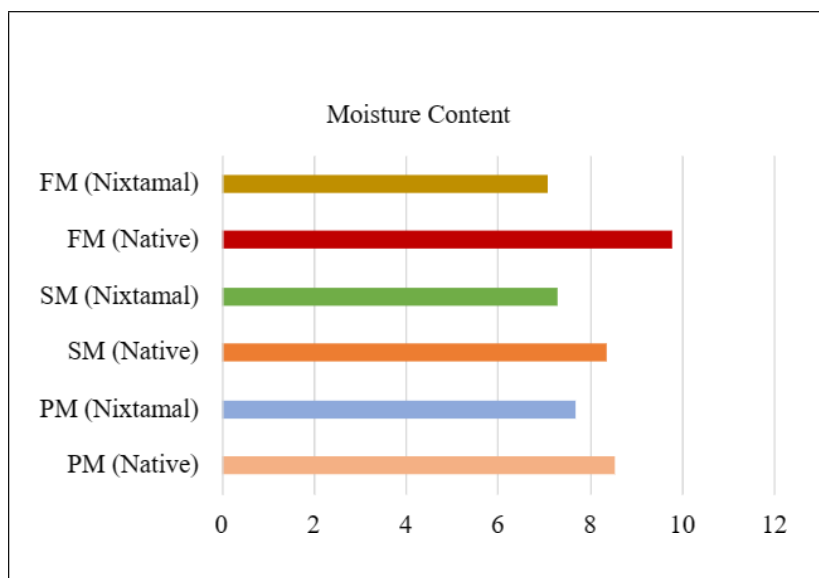
**Fig 5.2 Water activity (aw)**

### 5.3 PROXIMATE ANALYSIS

Samples	Proximate Analysis				
	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Carbohydrates (%)
PM (Native)	8.55±0.21	4.65	9.66±0.23	1.23±0.27	75.95
PM (Nixtamal)	7.7±0.14	5.56	9.93±0.05	2.19±0.14	74.61
SM (Native)	8.35±0.07	3.65	10.56±0.23	1.08±0.28	76.42
SM (Nixtamal)	7.3±0.84	4.69	10.83±0.05	1.93±0.007	75.23
FM (Native)	9.8±0.14	3.78	8.46±0.23	1.76±0.19	76.13
FM (Nixtamal)	7.1±0.28	4.03	8.73±0.05	3.61±0.25	76.51

**Table 5.6 Proximate Analysis**

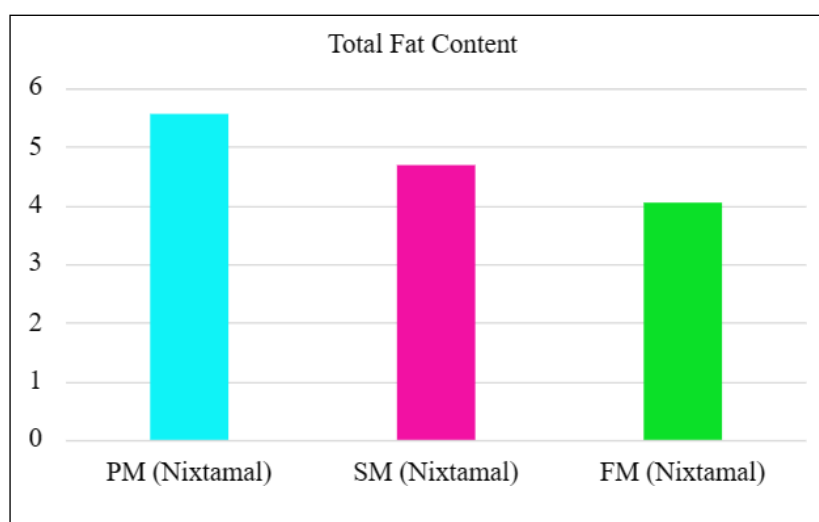
### 5.3.1 Moisture Content



**Fig 5.3 Moisture content**

Fig 5.3 shows the moisture content of native and nixtamalized flour samples. When compared to native and nixtamalized flour samples, the moisture content of nixtamalized samples are lesser than native samples. The moisture content of control samples ranges from 8.35% to 9.8%. While nixtamalized flour samples ranges from 7.1% to 7.7%. During nixtamalization process, it may reduce the moisture content of the grains by allowing water to penetrate the grain structure more effectively and facilitating moisture loss during cooking.

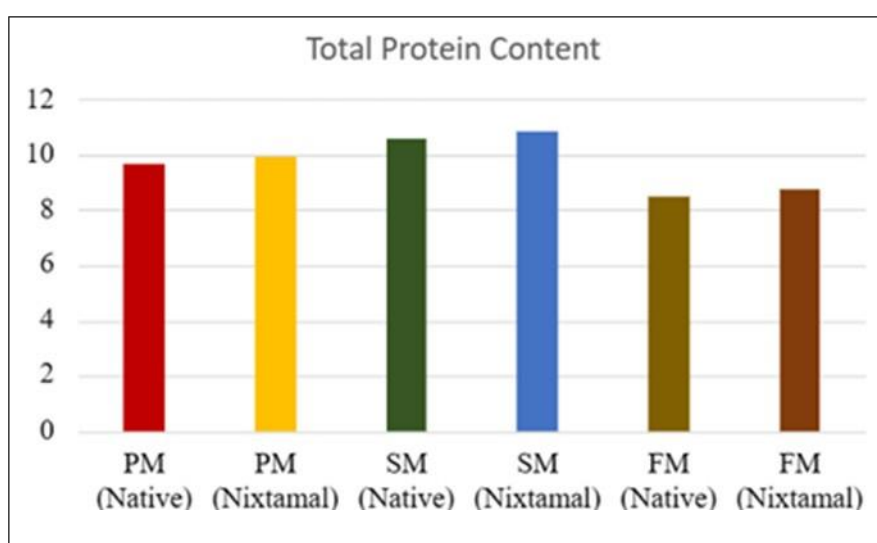
### 5.3.2 Total Fat Content



**Fig 5.4 Total fat content**

In these three samples, PM (nixtamal) has highest fat content (5.56%) than SM (nixtamal) and FM (nixtamal). According to Gopika & Joshi., 2024, the fat content varied between 4.14% and 4.97%, with nixtamalized flour showing lower levels compared to untreated flour. In Afify et al., 2012, the fat content in raw sorghum ranged from 3.58% to 3.91%. The fat content of raw finger millet shows 3.84% (Owheruo et al., 2019). Then the fat content of nixtamal samples had greater fat contents as compared to native flour samples. Fat content decreased due to fat oxidation and degradation when exposed to high temperature alkaline cooking with the presence of calcium ions (Martínez Flores et al., 2006).

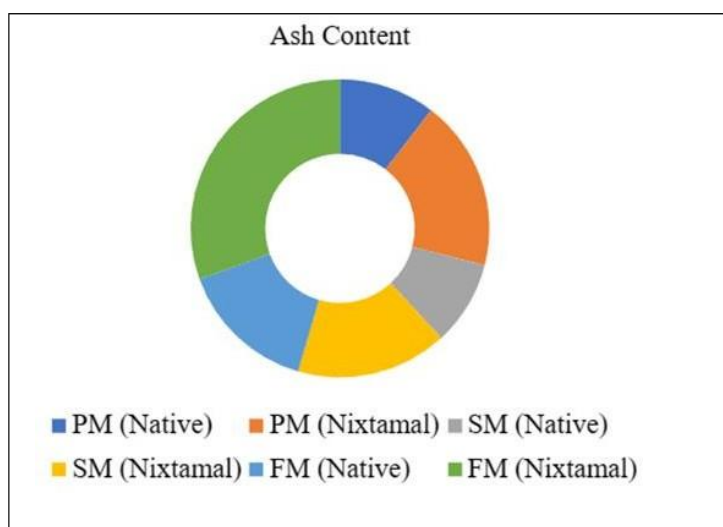
### 5.3.3 Total Protein Content



**Fig 5.5 Total protein content**

As compared to native flour samples, nixtamal flour samples had greatest protein content ranges from 8.73% to 10.83%. While, the protein content of native samples ranges from 8.46% to 10.56%. Lime cooked millet grains led to a notable increase in the protein content of the flour caused by a different concentration of lime (Ocheme et al., 2010). In contrast to other studies, observed as a decrease in protein content during nixtamalization due to starch gelatinization. The protein content of untreated grains estimated at 9.73g/100g, while treated samples ranged from 7.29 to 9.1g/100g. This reduction in protein content results from the heat treatment during alkaline cooking, leading to alterations in protein structure that enhance digestibility and absorption. (Obadina et al. 2016; Gomez et al.1989).

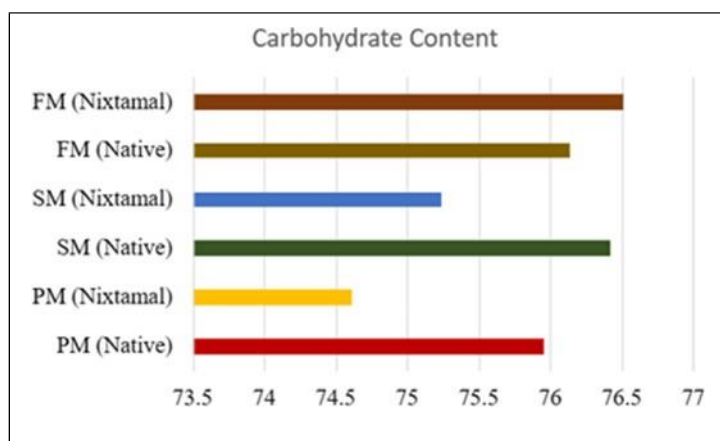
### 5.3.4 Total Ash Content



**Fig 5.6 Total ash content**

The ash content of native samples ranges from 1.08% to 1.76% while nixtamalized flour samples ranges from 1.93% - 3.61% as seen in table 5.6. When compared to native and nixtamalized flour samples, finger millet nixtamalized flour showed a higher ash content. According to a study, the ash content of the flour varied from 1.12% to 3.23%, with flour made from untreated millet exhibiting the lowest value and flour cooked in lime exhibiting the highest value. The amount of ash in flour was significantly ( $p < 0.05$ ) enhanced by lime heating. The absorption of calcium ions ( $\text{Ca}^{2+}$ ) from the cooking medium could be the cause of this rise. This indicates that eating meals made from millet cooked in lime is beneficial for the mineral content (Ocheme et al., 2010).

### 5.3.5 Carbohydrates



**Fig 5.7 Carbohydrate content**

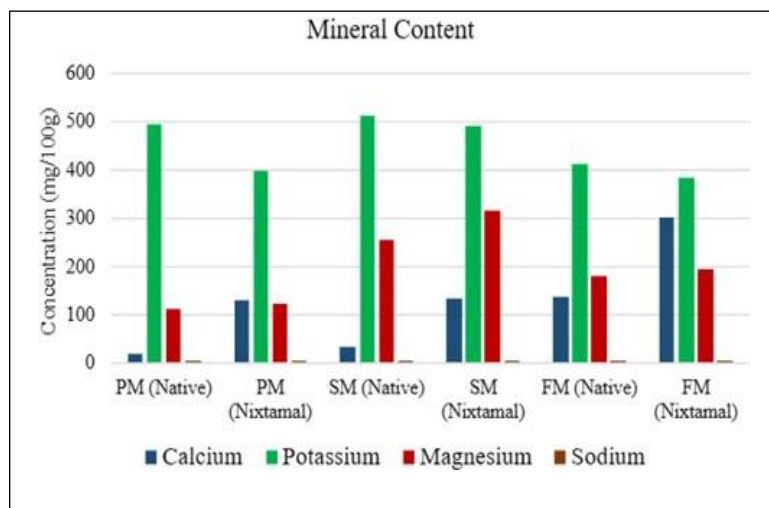
The carbohydrate content of native and nixtamal flour samples ranged from 75.23% to 76.61% as seen in table 5.6. Significant changes were not found between the nixtamalized grains and native grains. As seen from the above tables the carbohydrate contents of nixtamalized sorghum and pearl millet grain decreased due to elevated levels of protein and ash.

### 5.3.6 Mineral Estimation

Samples	Calcium (mg/100g)	Potassium (mg/100g)	Magnesium (mg/100g)	Sodium (mg/100g)
PM (Native)	17.91	493.1	109.72	4.17
PM (Nixtamal)	129.17	397.6	119.9	4.4
SM (Native)	32.25	509.7	253.45	3.89
SM (Nixtamal)	133.3	489.82	314.15	4.44
FM (Native)	134.93	411.25	178.7	4.21
FM (Nixtamal)	301.43	380.88	191.9	4.71

**Table 5.7 Mineral estimation**

When compared to native and nixtamalized flour samples, nixtamalized flour samples have more calcium, magnesium and sodium content and less potassium content than native samples. Calcium, potassium, magnesium and sodium content of nixtamal samples varied from 129.17 mg/100g to 301.43 mg/100g, 380.88 mg/100g to 489.82 mg/100g, 119.9 mg/100g to 314.15 mg/100g and 4.4 mg/100g to 4.71 mg/100g respectively. During the lime cooking and steeping, the grains will absorb  $\text{Ca}^{+}$  ions it was led to increase in calcium content.



**Fig 5.8 Mineral Analysis**

## 5.4 FUNCTIONAL PROPERTIES

### 5.4.1 Water absorption index, oil absorption index and water solubility index

Samples	WAI (g/g)	WSI (%)	OAI (g/g)
PM (Native)	2.28±0.19	7.3±0.28	2.02±0.03
PM (Nixtamal)	3.93±0.14	3.35±0.21	2.22±0.13
SM (Native)	2.68±0.21	5.5±0.42	2.21±0.009
SM (Nixtamal)	2.89±0.13	2.48±0.13	2.21±0.17
FM (Native)	2.55±0.0007	3.37±0.0007	2.29±0.01
FM (Nixtamal)	2.96±0.04	2.43±0.07	2.15±0.16

**Table 5.8 WAI, WSI and OAI**

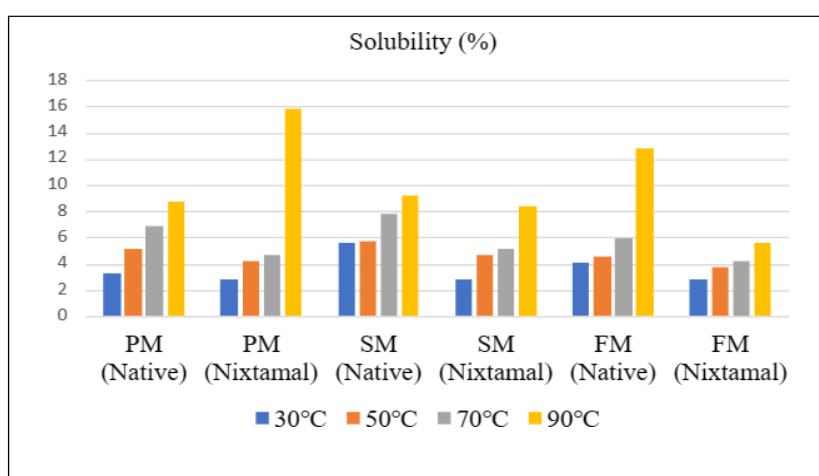
When compared to native and nixtamalized samples, nixtamalized samples have high WAI and less WSI than native samples. WAI and WSI of nixtamalized samples varied from 2.89g/g to 3.93 g/g and 2.43% to 3.35%, respectively, while native flour samples varied from 2.28g/g to 2.68 g/g and 3.37% to 7.3%, respectively. In the case of OAI, all samples have no significant difference each other. However, FM (CO) (2.29g/g) has the highest OAI than others. Nixtamalized flours with high WAI should be used to make sausage and baked goods. The solubility and leaching of amylose increase with WAI, further contributing to the degradation of starch crystalline structure (Gopika & Joshi., 2024).

### 5.4.2 Solubility (%)

Samples	30°C	50°C	70°C	90°C
PM (Native)	3.22	5.07	6.91	8.76
PM (Nixtamal)	2.78	4.17	4.63	15.76
SM (Native)	5.54	5.68	7.84	9.23
SM (Nixtamal)	2.78	4.63	5.09	8.34
FM (Native)	4.09	4.54	5.91	12.73
FM (Nixtamal)	2.79	3.72	4.19	5.59

**Table 5.9 Solubility**

Solubility of all samples are increased with increase in temperature from 30°C to 90°C. The solubility of PM (Native), PM (Nixtamal), SM (Native), SM (Nixtamal), FM (Native) and FM (Nixtamal) flour samples are ranges from 3.22% to 8.76%, 2.78% - 15.76%, 5.54% - 9.23%, 2.78% - 8.34%, 4.09% - 12.73% and 2.79% - 5.59%, respectively. The alkaline solution aids in the breakdown of hemicellulose in grains, rendering them more water-soluble. Additionally, it partially converts starches into more soluble forms such as dextrin. By removing the pericarp, the inner components of the grains are exposed to the alkaline solution and cooking, further enhancing solubility (Gopika & Joshi., 2024).



**Fig 5.9 Solubility**

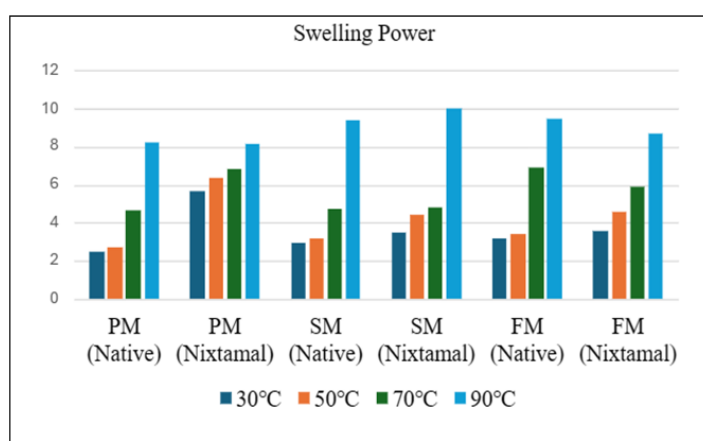


### 5.4.3 Swelling Power

Samples	30°C	50°C	70°C	90°C
PM (Native)	2.52	2.74	4.62	8.23
PM (Nixtamal)	5.65	6.37	6.82	8.09
SM (Native)	2.94	3.19	4.69	9.34
SM (Nixtamal)	3.48	4.42	4.83	9.98
FM (Native)	3.15	3.40	6.9	9.44
FM (Nixtamal)	3.57	4.55	5.91	8.69

**Table 5.10 Swelling power**

The swelling power of all samples are increased with increase in temperature from 30°C to 90°C. Swelling power of native flour samples ranges from 2.52 to 9.44, while nixtamalized flour samples varied from 3.48 to 9.98. According to Ochame et al., (2010), all treatments led to a notable increase ( $p < 0.05$ ) in the swelling power of the flours, with lime cooking showing the most significant enhancement. This could be attributed to the lower fat content of the flour, as noted by Zobel (1984), who suggested that fats might hinder swelling by forming complexes with starch, alongside its high-water absorption capacity. Lime treatments resulted in a significant decrease ( $p < 0.05$ ) in hydrogen cyanide content. This reduction in cyanide content could be attributed to the soaking and cooking process, which likely led to the loss of hydrogen cyanide during these stages.



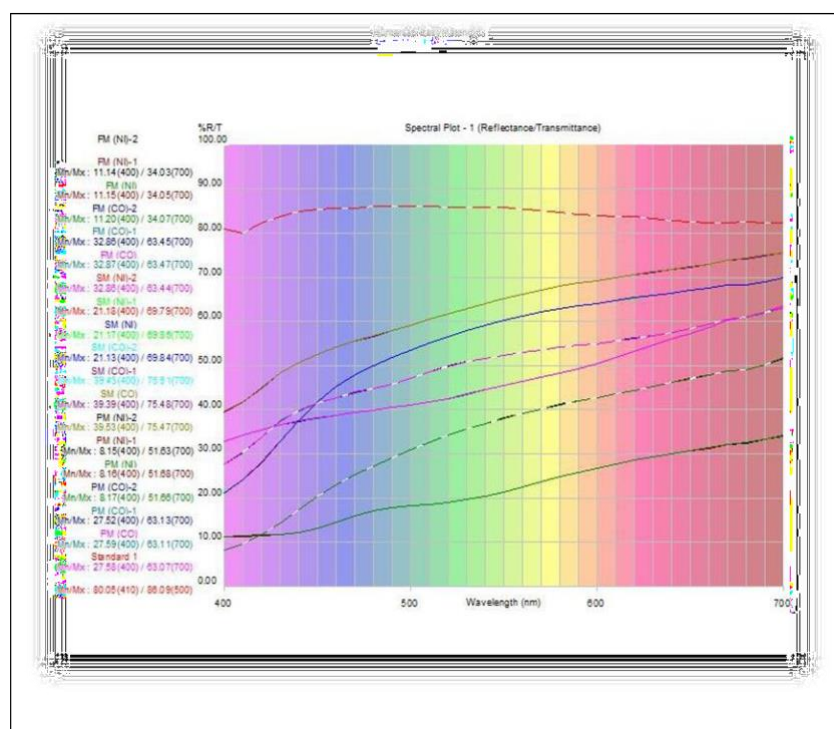
**Fig 5.10 Swelling power**

#### 5.4.4 Color Measurement

Samples	L*	a*	b*	dE
PM (Native)	77.04±0.007	0.49±0.007	11.94	20.67
PM (Nixtamal)	67.44	0.85±0.007	26.01±0.007	37.07
SM (Native)	84.24±0.007	1.2±0.014	11.84±0.014	15.37
SM (Nixtamal)	81.36	-1.04±0.007	19.1	22.7±0.007
FM (Native)	73.45	4.53±0.007	9.28±0.01	23.2
FM(Nixtamal)	54.04±0.01	5.43±0.007	17.35±0.024	43.97±0.024

**Table 5.11 Colour measurement**

Comparatively, native samples have the highest L\* values that is whiter than nixtamalized samples. All the samples except nixtamalized SM had positive a\* values indicating redness. But in the case of nixtamalized SM, it has negative a\* value indicating greenness. All the samples have the positive b\* values which denotes yellowish color. Here, nixtamalized samples has the highest b\* values than native samples.



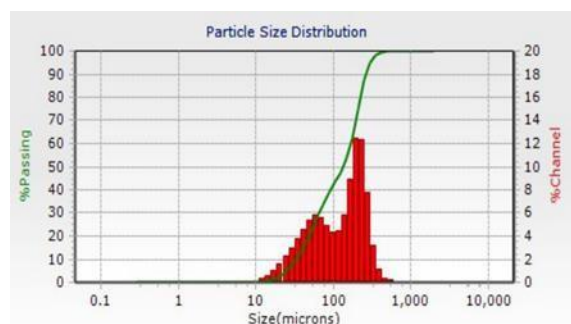
**Fig 5.11 Colour measurement**

### 5.4.5 Particle Size Distribution

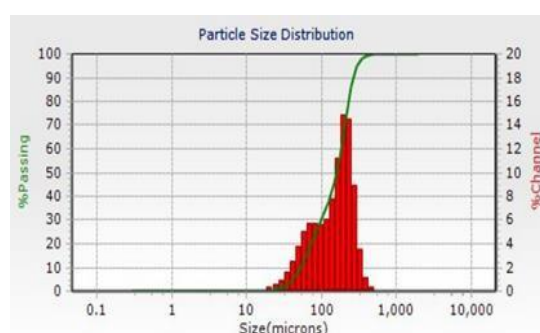
Samples	Diameter ( $\mu\text{m}$ )	Volume (%)	Width
PM (Native)	194.4	61.2	133.9
PM (Nixtamal)	159.8	100	177.4
SM (Native)	197.4	59.7	149.7
SM (Nixtamal)	107.3	100	185
FM (Native)	174.1	36.6	106
FM (Nixtamal)	201.7	66.4	137.6

**Table 5.12 Particle size distribution**

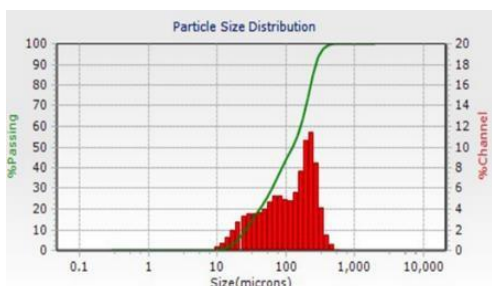
When compared to native and nixtamalized flour samples, nixtamalized flour samples had highest volume and width ranges from 66.4% to 100% and 137.6 to 185, respectively. In the case of FM, diameter of nixtamal FM (201.7  $\mu\text{m}$ ) is greater than native FM (174.1  $\mu\text{m}$ ). In order to PM and SM, nixtamal samples have lowest diameter than native samples. Reducing particle size enhances the texture of substances, which is advantageous in numerous food items like cakes, pastries, and batters where a consistent and smooth texture is favored. Smaller particles possess a greater surface area compared to their volume, influencing processes like hydration and mixing by providing more contact points for interaction with other components or liquids. Smaller flour particles facilitate even mixing with other ingredients, ensuring better dispersion and integration into the end product, thus enhancing overall consistency and quality (Gopika & Joshi., 2024).



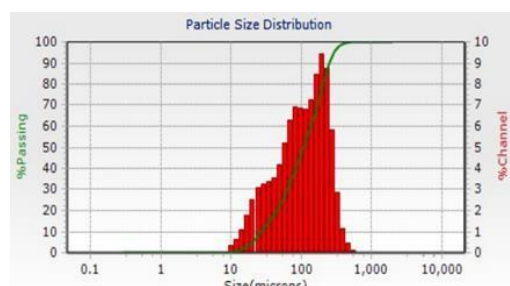
**Fig 5.12 Native PM particle size**



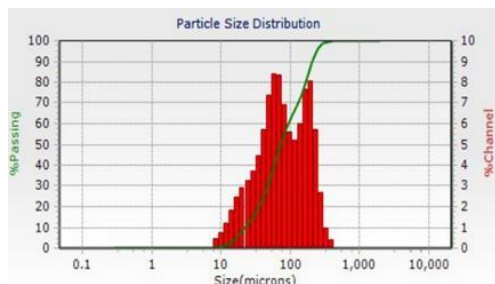
**Fig 5.13 Nixtamal PM particle size**



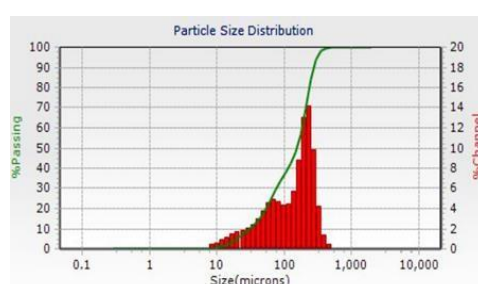
**Fig 5.14 Native SM particle size**



**Fig 5.15 Nixtamal SM particle size**



**Fig 5.16 Native FM particle size**



**Fig 5.17 Nixtamal FM particle size**

#### 5.4.6 Differential Scanning Calorimetry (DSC)

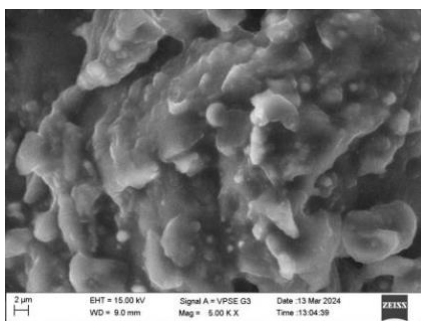
Samples	Onset Temperature ( $T_o$ ) (°C)	Peak Temperature ( $T_p$ ) (°C)	End Temperature ( $T_c$ ) (°C)	Delta H (J/g)	( $T_c - T_o$ ) (°C)
PM (Native)	69.49	73.59	77.98	6.85	8.49
PM (Nixtamal)	79.01	80.36	87.79	2.48	8.78
SM (Native)	72.08	75.30	78.73	6.25	6.65
SM (Nixtamal)	77.37	80.67	85.00	14.24	7.63
FM (Native)	71.80	75.27	79.16	9.26	7.36
FM (Nixtamal)	77.05	80.68	89.89	4.1537	11.84

**Table 5.13 DSC**

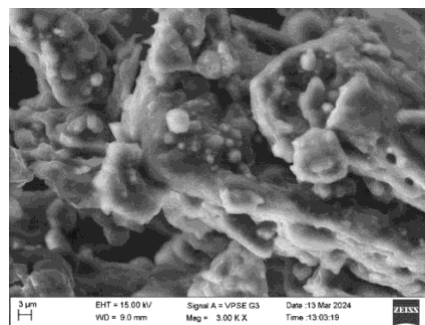
The onset, peak and end temperatures of nixtamalized flour samples had higher values as compared to native samples. The onset, peak and end temperatures of nixtamalized samples ranges from 77.05°C to 79.01°C, 80.36°C to 80.68°C and 85°C to 89.89°C, respectively. While native samples range from 69.49°C to 72.08°C, 73.59°C to 75.30°C and 77.98°C to 79.16°C, respectively. A decrease in the enthalpy of of the nixtamalized flour was observed because of this partial gelatinization (**Santiago et al., 2015**). A possible explanation for increase in the enthalpy of the nixtamalized can be attributed to the structural changes that occur during the nixtamalization process. The increase in enthalpy change ( $\Delta H$ ) in the nixtamalized samples suggests greater energy absorption during gelatinization, further supporting the notion of enhanced starch gelatinization due to nixtamalization.

#### **5.4.7 Scanning Electron Microscopy (SEM)**

The SEM image provides a highly magnified view of the surface structure of the nixtamalized pearl millet, showing the detailed texture and morphology at a microscopic level. The magnification level is 3.00 KX (3000 times), and the image was taken with a voltage of 15.00 kV. The scale bar in the bottom left indicates a length of 3 micrometers. The image is monochromatic because SEM images are typically in grayscale, showing the topography and composition of the sample rather than its color. The surface texture of nixtamalized pearl millet grains may appear smoother or more uniform compared to raw pearl millet. This is because nixtamalization involves cooking the grains in an alkaline solution, which can soften the outer layers and potentially alter surface features. Nixtamalization can lead to changes in the size and distribution of starch granules, as well as other structural components. In Fig 5.19 we observe fine details such as grooves, ridges, pits, and other irregularities on the surface of the grains. Nixtamalization causes structural changes within the grains, including the breakdown of cell walls and the release of starch granules. This breakdown of cell walls can contribute to the overall softening of the grains and may result in changes in texture and appearance as seen in fig 5.18. The internal microstructure of nixtamalized pearl millet grains may show signs of gelatinization or swelling as seen in Fig 5.18. The alkaline solution used in nixtamalization facilitates the hydration of the grains, allowing water to penetrate the internal structure of the grains more effectively. This hydration, combined with the gelatinization of starch, can lead to expansion and swelling of the grains, altering their size, shape, and internal microstructure. This could result in a more porous or swollen appearance compared to raw pearl millet.

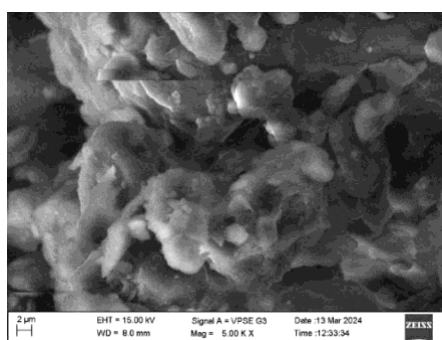


**Fig 5.18 Nixtamal PM**



**Fig 5.19 Native PM**

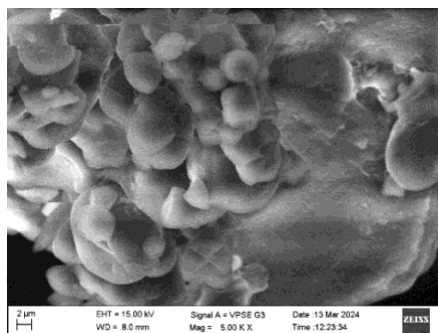
The SEM image provides a highly magnified view of the surface structure of the nixtamalized pearl millet, showing the detailed texture and morphology at a microscopic level. The magnification level is 3.00 KX (3000 times), and the image was taken with a voltage of 15.00 kV. The scale bar in the bottom left indicates a length of 3 micrometers. The image is monochromatic because SEM images are typically in grayscale, showing the topography and composition of the sample rather than its color. Figure 3 shows a rough and irregular surface texture of the nixtamalized finger millet grains. This texture is a result of the physical and chemical changes that occur during nixtamalization, such as the breakdown of outer layers and modification of the grain's components. The alkaline treatment during nixtamalization can lead to the gelatinization of starch granules, which results in an increase in volume and the formation of void spaces within the grain matrix. The presence of pores and voids in nixtamalized millet may influence its texture, making it softer and more digestible compared to untreated millet.



**Fig 5.20 Nixtamal FM**

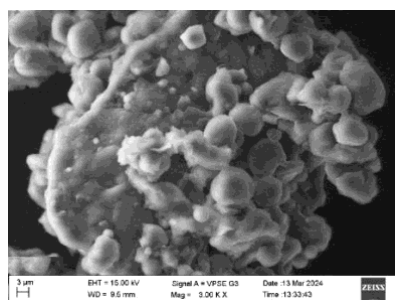
The surface of the finger millet is uneven with various structures that appear to be starch granules and possibly protein bodies or other cellular components. The rounded structures that are prominent in the image are likely starch granules, which are a common feature in cereal grains. They vary in size and shape, indicating the natural diversity found within the

seed's endosperm. Some are larger and more prominent, while others are smaller. This variation in size could be related to the developmental stages of the starch grains or the presence of different types of starch within the seed. The granules have a smooth texture, which is typical for starch. The matrix in which these granules are embedded appears fibrous or flaky, which could be the remnants of the cell walls or other cellular material.



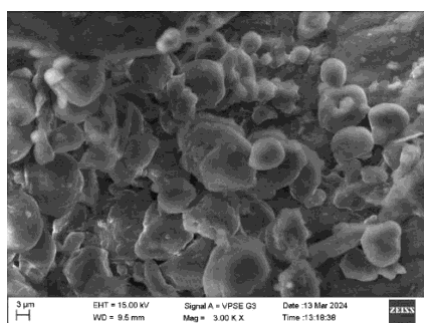
**Fig 5.21 Native FM**

The surface of the nixtamalized sorghum shows a heterogeneous texture with various sizes and shapes of particles. This is typical for nixtamalized grains, as the process involves soaking and cooking in an alkaline solution, usually lime (calcium hydroxide), which can lead to partial gelatinization and alteration of the starch granules. The particles exhibit a range of morphologies, including some that are more rounded and others that are irregularly shaped. This could be indicative of the different components of the sorghum grain, such as the starch granules and protein matrix, undergoing changes during the nixtamalization process. There appear to be some pores or cavities on the surface, which might be a result of the chemical and physical changes that occur during nixtamalization, such as the dissolution of certain grain components. Some of the particles seem to be clumped together, which could be due to the gelatinization of starch and the formation of a sticky matrix that causes particles to adhere to one another.



**Fig 5.22 Nixtamal SM**

The image magnification level is indicated as 3.00 KX (3000 times magnification), and the scale bar represents 3 micrometers ( $\mu\text{m}$ ). The most prominent features in the image are the rounded, lobe-shaped structures that are likely starch granules. These granules are characteristic of cereal grains and are the primary storage form of carbohydrates in sorghum. The smoothness of the granules can be related to their crystalline nature, as starch is semi-crystalline. The starch granules in sorghum vary in size and shape, with some appearing almost spherical while others are more irregular. This diversity is typical of the granule morphology found in different cereal grains. Some of the starch granules appear to be clustered together, which might be due to the way they are packed within the seed's endosperm or the result of the sample preparation process for SEM imaging.



**Fig 5.23 Native SM**

## **5.5 ANTIOXIDANT PROFILE**

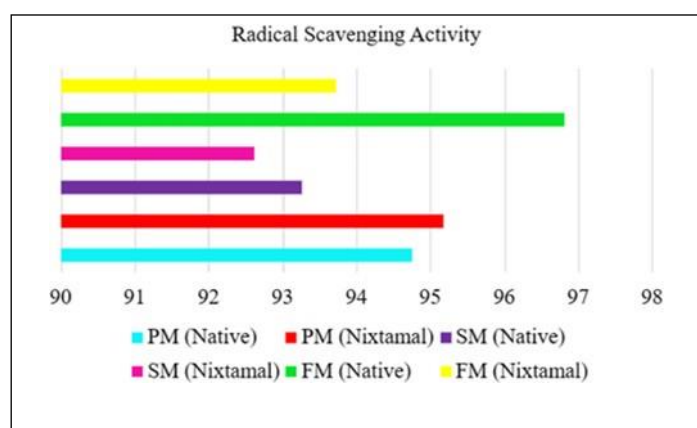
### **5.5.1 Radical Scavenging Activity (DPPH)**

<b>Samples</b>	<b>Antioxidant (%)</b>
PM (Native)	94.74 $\pm$ 0.62
PM (Nixtamal)	95.18 $\pm$ 1.28
SM (Native)	93.26 $\pm$ 1.7
SM (Nixtamal)	92.61 $\pm$ 0.5
FM (Native)	96.81 $\pm$ 0.09
FM (Nixtamal)	93.72 $\pm$ 1.51

**Table 5.14 Radical scavenging activity**



The radical scavenging activity of both native and nixtamalized flour samples had no significant difference between each other. However, FM (native) (96.81%) has higher DPPH (radical scavenging activity) as compared to other flour samples. The flour blends demonstrated their antioxidant qualities by scavenging free radicals and lowering iron levels. The human body naturally produces free radicals through metabolic processes, which are extremely reactive and powerful enough to destroy cells. Similarly, in one of the research articles, it was found that the nixtamalization process led to the most notable decrease in phenolic contents and antioxidant activity. This reduction ranged from 0.5% to 14.5% due to the transfer of these components into the boiling liquid. The losses were exacerbated by the thermal and alkaline conditions utilized during nixtamalization. Although there was a decrease in DPPH (radical scavenging activity) in the treated samples compared to the original flour, this decrease was not statistically significant (Kamau et al., 2020).



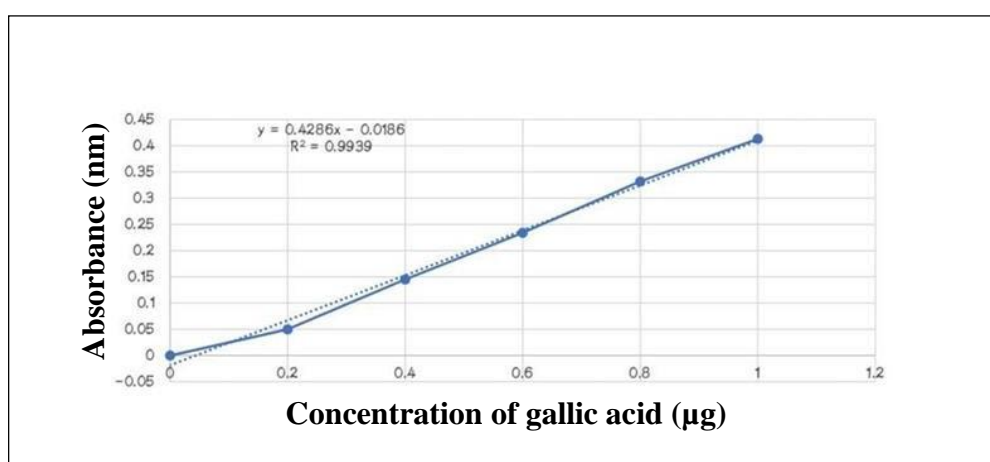
**Fig 5.24 Radical scavenging activity**

### 5.5.2 Total Phenolic Content (TPC)

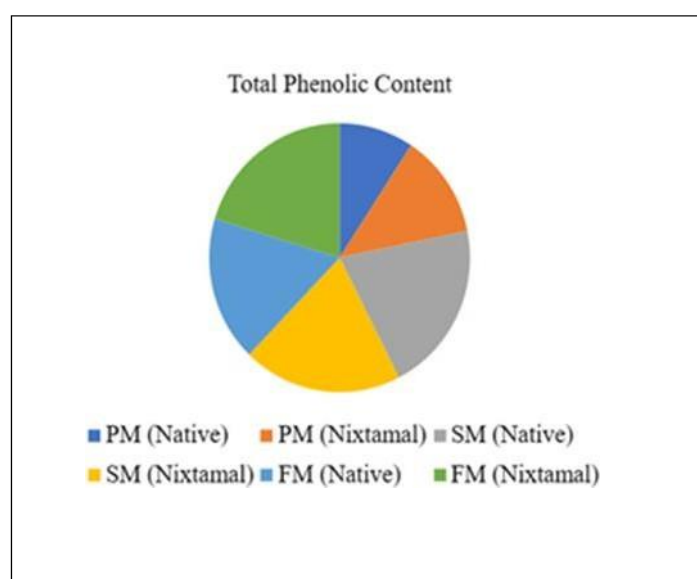
Samples	TPC (mg/100g)
PM (Native)	2.48
PM (Nixtamal)	3.36±0.9
SM (Native)	5.54±0.14
SM (Nixtamal)	5.28±3.97
FM (Native)	4.69
FM (Nixtamal)	5.44±3.86

**Table 5.15 Total phenolic content**

The TPC of native samples ranges from 2.48 mg/100g to 5.54 mg/100g. While the TPC of nixtamalized flour samples ranges from 3.36 mg/100g to 5.44 mg/100g. When compared to both types of samples, SM (native) showed greater TPC. In a research paper, both cooking and nixtamalization reduced the TPC content when compared to raw samples. The colored sorghum grains contain around 60 g/kg of the total phenolic contents in free forms, which are quickly lost when cooking is done. Lower phenolic contents values in the processed samples can be explained by the washing step of the nixtamalization process, which results in pericarp losses and phenolic decrease (Luzardo-Ocampo et al., 2020). Physical losses of the pericarp and phenolic leaching into the cooking liquor, together with the combined effects of alkaline and heat processing during nixtamalization, are generally the cause of these significant decreases. (Mora-Rochin et al., 2010).



**Fig 5.25 Standard graph of gallic acid**



**Fig 5.26 Total phenolic content**

# **SUMMARY AND CONCLUSION**

In this study, pearl millet, sorghum millet and finger millet were cooked with 2%  $\text{Ca(OH)}_2$  and steeped in cooked liquid. Then the grains had undergone dry milling to form nixtamalized flours. Physicochemical, functional properties and antioxidant profile were carried out for the nixtamalized and native flours. When compared to bulk and tapped densities, the bulk densities of all flour samples a lesser than tapped densities. In proximate analysis, nixtamalized flours had highest ash, protein and fat content, lowest moisture content than native flours. Mineral analysis revealed that the nixtamal samples had more calcium (129.17 mg/100g to 301.43 mg/100g), magnesium (119.9 mg/100g to 314.15 mg/100g) and sodium (4.4 mg/100g to 4.71 mg/100g) content and less potassium (380.88 mg/100g to 489.82 mg/100g) content than native samples. According to functional properties, nixtamalized samples have high WAI and less WSI than native samples. WAI and WSI of nixtamalized samples varied from 2.89g/g to 3.93 g/g and 2.43% to 3.35%, respectively, while native flour samples varied from 2.28 g/g to 2.68 g/g and 3.37% to 7.3%, respectively. Solubility and swelling power of all samples are increased with increase in temperature from 30°C to 90°C. Thermal properties were analyzed for all the flours. it revealed the gelatinization temperature increased with the process of nixtamalization. The radical scavenging activity of both native and nixtamalized flour samples had no significant difference between each other. The TPC of native samples ranges from 2.48 mg/100g to 5.54 mg/100g. While the TPC of nixtamalized flour samples ranges from 3.36 mg/100g to 5.44 mg/100g. When compared to both types of samples, SM (native) showed greater TPC. Generally, the pre-treatment of millet via nixtamalization was found to be advantageous in improving the attributes and nutritional value of the flour.

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