EFFECT OF PROCESSING METHODS ON SELECTED PHYTOCHEMICALS (TANNIN, OXALATE AND PHYTATE) OF FINGER MILLET (*Eleusine coracana* Gaertn.)

by

Rajeep Guragain

Department of Nutrition and Dietetics

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

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A dissertation submitted to the Department of Nutrition and Dietetics, Central Campus of Technology, Tribhuvan University, in partial fulfillment of the requirements for the degree of B.Sc. in Nutrition and Dietetics

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Tribhuvan University

Institute of Science and Technology

Department of Nutrition and Dietetics

Central Campus of Technology, Dharan

Approval Letter

This ***dissertation*** entitled ***Effect of Processing Methods on Selected Phytochemicals (Tannin, Oxalate and Phytate) of Finger Millet (Eleusine coracana*** **Gaertn.)** presented by **Rajeep Guragain** has been accepted as the partial fulfillment of the requirement for the **B.Sc. Degree in Nutrition and Dietetics.**

Dissertation Committee

1. **Head of the Department** __________________________________

   (Mr. Kabindra Bhattarai, Asst. Prof.)

2. **External Examiner** __________________________________

   (Mr. Dinesh Subedi, Food Research Officer, FTQCOB)

3. **Supervisor** __________________________________

   (Mr. Basanta Kumar Rai, Prof.)

4. **Internal Examiner** __________________________________

   (Mrs. Babita Adhikari, Assoc. Prof.)

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Mr. Rajeep Guragain

Abstract

Finger millet (*Eleusine coracana* L. Gaertn) is one of the most important crops found in Nepal. The main aim of present research work was to determine the effect of processing techniques (germination, soaking and roasting) on selected phytochemicals of finger millet. Finger millet sample were collected from Dharan Municipality, Sunsari district, Koshi province in the month of September 2021. The effect of different treatments, viz., germination for 2, 4, 6, 8 and 10 days, soaking for 6, 12, 18 and 24 h and roasting for 10 min at 125℃ on the antinutrients (tannin, phytate and oxalate) of finger millet were studied.

The mean values of tannin, oxalate and phytate in raw finger millet were found to be 356.49 mg/100 g, 52.33 mg/100 g and 131.49 mg/100 g, respectively on dry basis. The maximum reduction of antinutrients: tannin (56%), oxalate (48%) and phytate (58%) were found when the finger millet was germinated. The reduction by soaking was the least effective method compared to other methods to reduce tannin, oxalate and phytate. However, the processing methods, viz., germination, soaking and roasting reduced the antinutrients of finger millet significantly ($p<0.05$).

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List of Abbreviation

Abbreviations	Full form
ANF	Anti-Nutritional Factor
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
CCT	Central Campus of Technology
FAO	Food and Agriculture Organization
USDA	United States Department of Agriculture

Part I

Introduction

1.1 Background

Millet is an important group of small-seeded cereal crops and is a member of the grass family. A millet crop includes grasses like finger millet, *Eleusine coracana* (L.) Gaertn; pearl millet, *Pennisetum glaucm* (L.) R. Br; foxtail millet, *Setaria italica* (L.) P. Beauvois; kodo millet, *Paspalum scorbiculatum* L., bahiagrass, *Paspalum notatum* Flugge; little millet, *Panicum sumatrense* Roth ex Roem. & Schult., proso millet, *Panicum miliaceum* L., barnyard millet, *Echinochola crusgalli* (L.) P. Beauv; guinea grass, *Panicum maximum* Jacq; elephant grass, *Pennisetum purpurium* Schumach.) that belong to the family Poaceae of the monocotyledon group (Deosthale *et al.*, 2012).

Among the millets, finger millet is the most important crop in Nepal in terms of area and production followed by proso millet and foxtail millet. Besides, Sorghum, barnyard millet, pearl millet, little millet, and kodo millet are also grown in different parts of the country (Ghimire *et al.*, 2017). Finger millet cultivation is an indispensable part of farming system in the mountain terrain of Nepal where agricultural land is limited and food deficit is a problem (Subedi *et al.*, 2009). Among millet groups, finger millet is the most important crop and the fourth important cereal crop after rice, maize, wheat in Nepal. It was planted in the area of 263,261 ha in 2018/19 with average productivity of 0.90 MT/ha in 2019 which was 0.77 MT/ha a decade ago in 2008/09 (Gautam and Subedi, 2022). Recent official data of the Ministry of Agriculture and Livestock Development show that finger millet is grown in 70 districts except in 2 districts of high mountains (Manang and Mustang) and 4 districts of Terai namely, Kapilbastu, Banke, Bardiaya and Kanchanpur.

High diversity of finger millet exists in the mid hills of Nepal. Out of 15 species reported in the world, three of them namely *Eleusine coracana*, *E. indica* and *E. aegyptica* are found in Nepal (Kandel *et al.*, 2019b). In the climate change scenario, there is scope for increasing finger millet growing areas due to its adaptability and suitability for low, marginal lands and also for harsh weather conditions (Kandel *et al.*, 2019a). The major production districts of Nepal for this crop are Khotang, Sindhupalchok, Baglung, Syangja, Kaski, Gorkha, and Sindhuli and are considered very important crop for food and nutrition security in both mid-hill and mountain (Ghimire *et al.*, 2017). Finger millet is the number one crop in Humla and Mugu districts whereas it is the second important crop in Jumla, Khotang, and Sindhupalchok in terms of area and production. Baglung district of Gandaki Province has the largest area and production under finger millet in Nepal. Accroding to Gautam and Subedi (2022), the production area of finger millet in Nepal is decreasing, however, the production and productivity is increasing. Luitel *et al.* (2019) predicted that increase in temperature of mid mills area which is most potential area for millet production is one of the potential causes for plummet decrease of existing favorable finger millet areas in Nepal, whereas, Zomer *et al.* (2014) found that several climatic factors like moisture stress, changes in minimum and maximum temperature, increasing unpredictable trend of rainfall, and the shift of suitable ecological zones in different elevations in Nepal may play a role in the substantial decrease of a suitable area of finger millet. The increment in production and productivity of millet is due to increase in awareness about the health benefits, increase in demand and high price (Gautam and Subedi, 2022).

1.2 Statement of the problem

Finger millet is one of the most important and nutritious crops in Nepal. However, its utilization and acceptability are limited due to various reasons. One of the reason is the stigmatization of millet as marginalised crops, neglected crops or crops for the poor (Kodirekkala, 2024). Another reason for limited utilization of finger millet is the presence of various anti-nutritional components such as tannin, phytate, and oxalate. In developing countries like ours, people heavily rely on cereal grains to fulfill their energy demand, and millet is no exception. While different processing methods such as germination, soaking, and roasting can be used to lower the antinutrient levels, the comparative effectiveness of these methods is still subject matter of research. Also the research and development of finger millet is limited and there is a declining area in the last 3 decades (1991-2001) as per the national census of agriculture in Nepal (CBS, 2012; Gauchan, 2019; Gairhe *et al.*, 2021).

1.3 Objective

1.3.1 General objective

The general objective of this work was to study the nutrients and capability of different processing techniques (germination, soaking and roasting) to reduce anti-nutritional factors of finger millet with emphasis on choosing the best processing method.

1.3.2 Specific objectives

The specific objectives were as follows:

1. To determine tannin, phytate and oxalate content of finger millet.
2. To process finger millet with various treatments namely germination, soaking and roasting.
3. To determine the best processing method among three to reduce the maximum anti-nutrients.

1.4 Significance of the study

Carbohydrates in finger millet are absorbed and assimilated more slowly than those in other grains. Because of its high polyphenol and dietary fiber content, it has been shown to lower the risk of diabetes mellitus and gastrointestinal tract problems. Finger millet has a higher nutritional fiber content than many other grains (Opole, 2019). Delay in nutrient absorption, increased faecal volume, reduced blood lipids, protection of colon cancer, barrier to digestion, mobility of intestinal contents, extended faecal transit time, and fermentability features are all health advantages linked with finger millet (Devi *et al.*, 2014; Shrestha and Karki, 2019). However, the presence of anti-nutritional factors such as tannin, phytates, phenols, and enzyme inhibitors, reduces the nutrient digestibility and mineral absorption which may reduce the utilization of finger millet's nutrient potential (Samtiya *et al.*, 2020). Thus, this study specifically determines the content of antinutrient in finger millet and effect of various processing techniques to reduce those antinutrient. The results of this study might help in the establishment of the effective and optimized way for the use of finger millet in household level and industrial levels.

1.5 Limitations of the study

a. Antinutrients like trypsin inhibitors, hemagglutinin, lectins and saponins were not determined due to technical constraints.

Part II

Literature review

2.1 Introduction

Finger millet (*Eleusine coracana*) is an important cereal crop cultivated primarily in Africa and Asia. It is known for its rich nutritional profile, being a good source of minerals, vitamins, and dietary fiber (Chandra *et al.*, 2016). However, finger millet also contains several anti-nutritional factors that can interfere with the absorption of essential nutrients and potentially pose health risks. These anti-nutritional factors include tannins, phytate, and oxalate (Kumar *et al.*, 2016).

Tannins are polyphenolic compounds found in various plant-based foods, including finger millet. They have been reported to inhibit the absorption of proteins and minerals, such as iron and zinc, leading to micronutrient deficiencies. Phytate, also known as inositol hexaphosphate, is a phosphorus storage compound found in many cereals and legumes. It forms insoluble complexes with minerals, making them less bioavailable. Oxalate, on the other hand, is a plant-derived organic acid that can form insoluble calcium oxalate crystals, thereby reducing calcium bioavailability (Kumar *et al.*, 2016).

To mitigate the negative effects of these anti-nutritional factors, various processing methods, such as germination, soaking, and roasting, have been employed. Germination involves the controlled activation of enzymes, leading to biochemical changes that result in the breakdown of anti-nutritional factors (Abioye *et al.*, 2022). Soaking, a common traditional practice, can also reduce tannin, phytate, and oxalate contents by leaching them into the soaking water (Gupta *et al.*, 2015). Roasting, which involves subjecting the grains to dry heat, has been shown to enhance nutrient bioavailability and reduce the levels of anti-nutritional factors (Singh *et al.*, 2017).

Several studies have investigated the effect of these processing methods on the reduction of anti-nutritional factors in finger millet. For instance, Chauhan and Sarita (2018) reported a significant decrease in tannin content in germinated finger millet grains. They also found that roasting finger millet grains at high temperatures resulted in a substantial reduction in oxalate levels. Similarly, a study by Sheethal *et al.* (2022) demonstrated that soaking significantly reduced the phytate content of finger millet flour.

2.2 Classification and nomenclature of finger millet

According to USDA, finger millet (*Eleusine coracana*) is a small-seeded cereal crop that belongs to the Poaceae family and is classified under the Chloridoideae subfamily. The scientific name *Eleusine coracana* reflects its botanical classification, with *Eleusine* referring to the genus and *coracana* representing the species. The classification of finger millet is given in Table 2.1.

Table 2.1 Scientific Classification of finger millet

Scientific Classification	
Kingdom	*Plantae* – Plants
Subkingdom	*Tracheobionta* – Vascular plants
Superdivision	*Spermatophyta* – Seed plants
Division	*Magnoliophyta* – Flowering plants
Class	*Liliopsida* – Monocotyledons
Subclass	*Commelinidae*
Order	*Cyperales*
Family	*Poaceae* Barnhart – Grass family
Genus	*Eleusine* Gaertn. – goosegrass
Species	*Eleusine coracana* (L.) Gaertn. – Finger Millet

Source: USDA (2016)

Finger millet has been cultivated for centuries and is well-adapted to diverse agro-climatic conditions. It is a hardy crop that can withstand drought, poor soil fertility, and high temperatures. Due to its resilience, finger millet has played a significant role in food security and nutrition in many regions, especially in marginal agricultural areas (Tadele, 2019).

2.3 Nutritional composition of finger millet

2.3.1 Carbohydrates

Finger millet is a major source of dietary carbohydrates and a good source of valuable micro nutrients along with major food components (Banusha and Vasantharuba, 2013). It is primarily composed of carbohydrates, making it a valuable source of energy. The carbohydrate content of finger millet varies between 70% and 75% of its total composition. It is predominantly made up of complex carbohydrates, including starch and dietary fiber. Starch is the major carbohydrate component in finger millet, constituting approximately 60% to 70% of the total carbohydrate content. It serves as a long-term energy reserve in the grain. The high starch content makes finger millet a good source of sustained energy release, providing a steady supply of glucose to the body (Abioye *et al.*, 2022).

Dietary fiber is another significant component of finger millet carbohydrates. It contributes to approximately 10% to 15% of the total carbohydrate content. Finger millet contains both soluble and insoluble fiber. Soluble fiber forms a gel-like substance in the digestive system and helps regulate blood sugar levels and lower cholesterol levels. Insoluble fiber adds bulk to the diet and promotes healthy digestion by preventing constipation (Wankhede *et al.*, 1979; Abioye *et al.*, 2022).

The high dietary fiber content of finger millet contributes to its low glycemic index (GI). Foods with a low GI release glucose more slowly into the bloodstream, preventing rapid spikes in blood sugar levels. This property makes finger millet an ideal food choice for individuals with diabetes or those aiming to manage their blood sugar levels effectively (Anitha *et al.*, 2021).

Furthermore, finger millet contains resistant starch, which is not digested in the small intestine and acts as a prebiotic. Resistant starch reaches the large intestine, where it is fermented by beneficial gut bacteria, promoting a healthy gut microbiome and providing additional health benefits (Tyagi *et al.*, 2018; Kaimal *et al.*, 2021).

2.3.2 Fat

While finger millet is not particularly high in fat compared to oilseeds and nuts, it still contributes to the overall nutrient profile of this grain. The fat content of finger millet typically ranges from 1% to 5%, depending on various factors such as the variety, growing conditions, and processing methods (Devi *et al.*, 2014; Abioye *et al.*, 2022).

The fats present in finger millet consist mainly of unsaturated fatty acids, which are considered beneficial for human health. Unsaturated fats, including monounsaturated and polyunsaturated fats, play a crucial role in maintaining cardiovascular health by lowering the levels of harmful cholesterol, i.e., low density lipoprotein (LDL) and promoting the levels of beneficial cholesterol, i.e., high density lipoprotein (HDL). They also support proper cell function, aid in the absorption of fat-soluble vitamins, and provide a source of energy (Gull *et al.*, 2014).

Polyunsaturated fatty acids (PUFAs), such as linoleic acid and alpha-linolenic acid, are essential fats that cannot be synthesized by the human body and must be obtained through the diet. These PUFAs have been associated with various health benefits, including reducing the risk of heart disease, inflammation, and certain chronic conditions like diabetes and obesity (Ander *et al.*, 2003).

The presence of unsaturated fats, particularly PUFAs, in finger millet makes it a valuable addition to a balanced diet. Incorporating finger millet into the diet can contribute to the intake of these essential fats and promote overall health and well-being (Devi *et al.*, 2014).

However, it is important to note that the fat content in finger millet can vary based on factors such as processing methods. Some processing techniques, such as dehulling and milling, may result in slight losses of fat due to removal of the outer bran layers. Nevertheless, finger millet remains a nutritious choice, providing a modest amount of healthy fats to support a well-rounded diet (Gull *et al.*, 2014; Abioye *et al.*, 2022).

2.3.3 Proteins

Proteins are essential macronutrients that play a crucial role in various physiological functions within the human body. Finger millet (*Eleusine coracana*) is a cereal crop that is known to possess a noteworthy protein content, making it a valuable dietary source for meeting protein requirements.

The protein content of finger millet typically ranges from 7% to 12%, depending on factors such as the variety, growing conditions, and processing methods (Amadou *et al.*, 2013; Hassan *et al.*, 2021). Finger millet stands out among other cereals due to its relatively higher protein content compared to staples like rice and maize. The proteins present in finger millet are characterized by a well-balanced amino acid composition. Finger millet is particularly rich in essential amino acids, including lysine, threonine, and valine, which are crucial for the body's growth, development, and overall maintenance. The presence of these essential amino acids in finger millet makes it a valuable protein source, especially for populations that rely heavily on plant-based diets (Barbeau and Hilu, 1993; Gebre, 2019).

Consuming finger millet as part of a balanced diet can contribute to meeting the recommended daily protein intake and support various bodily functions. Proteins are involved in tissue repair, muscle building, enzyme production, hormone synthesis, and immune function. They also provide a source of energy and help regulate blood sugar levels (Devi *et al.*, 2014).

Furthermore, finger millet proteins have been found to exhibit certain bioactive properties, such as antioxidant and antimicrobial activities. These bioactive proteins may contribute to the potential health benefits associated with the consumption of finger millet (Hassan *et al.*, 2021).

While the protein content of finger millet is significant, it is important to consider factors such as processing methods and dietary diversity to ensure optimal protein utilization. Processing techniques like malting and fermentation have been shown to enhance protein digestibility and bioavailability by reducing anti-nutrients and improving protein quality (Hejazi and Orsat, 2016).

2.3.4 Vitamins and minerals

Finger millet (*Eleusine coracana*) offers a range of essential vitamins and minerals, making it a valuable addition to a balanced diet. The presence of these micronutrients contributes to the overall nutritional quality of finger millet.

Finger millet is known to be a good source of several B vitamins, including thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), and vitamin B6. These vitamins play crucial roles in energy metabolism, neurological function, red blood cell production, and overall growth and development (Devi *et al.*, 2014; Chandra *et al.*, 2016). Incorporating finger millet into the diet can help fulfill the recommended daily intake of these essential B vitamins.

In addition to B vitamins, finger millet also contains important minerals such as calcium, iron, and magnesium. Calcium is essential for building and maintaining strong bones and teeth, while iron is vital for the production of hemoglobin and oxygen transportation within the body. Magnesium is involved in various enzymatic reactions and plays a role in nerve and muscle function (Makokha *et al.*, 2002).

The bioavailability of these vitamins and minerals in finger millet can be influenced by factors such as processing methods. Traditional processing techniques like soaking, germination, and fermentation have been found to enhance the bioavailability of certain vitamins and minerals by reducing anti-nutrients and promoting nutrient release (Sheethal *et al.*, 2022).

Including finger millet in the diet can help diversify nutrient intake and contribute to meeting daily vitamin and mineral requirements. However, it is important to note that the vitamin and mineral content may vary depending on factors such as the variety of finger millet, soil conditions, and processing methods used.

In conclusion, finger millet is a versatile and nutrient-dense cereal crop that offers a range of health benefits. Its rich composition of complex carbohydrates, including starch and dietary fiber, makes it an excellent choice for individuals seeking sustained energy release and the advantages associated with fiber intake. The high starch content provides a slow and steady release of energy, making finger millet a favorable option for maintaining blood sugar levels.

Moreover, the dietary fiber in finger millet contributes to its low glycemic index, aiding in weight management and promoting digestive health. Additionally, finger millet contains a moderate amount of fats, primarily consisting of beneficial unsaturated fatty acids, including polyunsaturated fatty acids (PUFAs). These fats contribute to the overall nutritional value of finger millet and have the potential to improve cardiovascular health and overall well-being (Devi *et al.*, 2014).

Furthermore, finger millet is a valuable source of proteins, offering a well-balanced amino acid composition and essential amino acids necessary for growth, repair, and various physiological functions. Incorporating finger millet into the diet can help meet protein requirements and support overall health. Also, finger millet provides essential vitamins and minerals, including B vitamins, calcium, iron, and magnesium. These micronutrients play vital roles in energy metabolism, bone health, blood production, and enzymatic reactions. Adding finger millet to the diet can aid in meeting daily nutrient needs and supporting overall well-being. In summary, incorporating finger millet into the diet can offer a nutrient-dense, fiber-rich carbohydrate source (Devi *et al.*, 2014). Finger millet grain is highly nutritious, being richer in protein, fat and minerals especially calcium and iron compared to rice (Pragya and Rita, 2012).

2.4 Health benefits of finger millet

The main constituents of the millet kernel are seed coat (testa), embryo and endosperm. Among several varieties of finger millets such as yellow, white, tan, red, brown, or violet colour, only the red-coloured are cultivated extensively throughout world. The presence of five layered testa in finger millet makes it unique compared to other millets such as foxtail millet, pearl millet, kodo millet and proso millet. This could be one of the possible reasons for the higher dietary fiber content in finger millet (Deosthale *et al.*, 2012).

Finger millet is recognized for its health beneficial effects, such as anti-diabetic, anti-tumerogenic, anti-diarrheal, antiulcer, anti-inflammatory, atherosclerogenic effects, antioxidant and antimicrobial properties (Sripriya *et al.*, 1996; Chethan and Malleshi, 2007; Devi *et al.*, 2014). Finger millet is also useful in management of various physiological disorders such as diabetes mellitus, hypertension, vascular fragility, hypercholesterolemia, prevention of oxidation of low-density lipoproteins (LDLs) and also improves gastrointestinal health (Scalbert *et al.*, 2005). Millet flour is also used for anti-allergic, measles; ghamaura and bone fracture (Shrestha and Karki, 2019). *Eleusine coracana* is a rich source of primary metabolites and has the potentiality of use as a nonconventional food to supplement the nutritional needs of the under-nourished population (David *et al.*, 2014).

2.5 Anti-nutrients in finger millet

2.5.1 Tannins

Tannins are considered an important group of anti-oxidant polyphenols, usually present in food and beverages. After cellulose, hemicellulose, and lignin, tannins are considered the fourth most abundant constituent of the plant. Tannin is a polyphenolic biomolecule, having an astringent and bitter taste and a high molecular weight of 500-3000 Da (Jaiswal *et al.*, 2018). They are organic, nitrogenous substances and serve the plants' protection. However, according to Reddy and Pierson (1994) and Adeyemo and Onilude (2013), tannin negatively affect the nutritional value of food. The chemical structure of tannin is presented in Fig. 2.1.

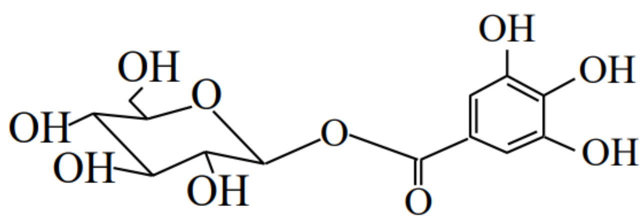

Fig. 2.1 Chemical structure of hydrolysed tannin

Source: Diouf *et al.* (2019)

In finger millet, tannins primarily belong to the proanthocyanidin group, which are condensed tannins. These compounds are responsible for the dark coloration of the grain and its astringent taste. The concentration of tannins in finger millet varies depending on factors such as the variety, growing conditions, and processing methods (Devi *et al.*, 2014; Abioye *et al.*, 2022).

Tannins have been found to have both beneficial and detrimental effects on human health. On one hand, tannins exhibit antioxidant properties and have been associated with potential health benefits, such as reducing the risk of certain chronic diseases, including cardiovascular disease and cancer (Devi *et al.*, 2014; Gull *et al.*, 2014). They can also contribute to the sensory attributes and stability of certain food products.

On the other hand, tannins can form complexes with proteins and other nutrients, impairing their bioavailability and digestibility. This can lead to decreased nutrient absorption and utilization, potentially impacting overall nutritional status (Chung *et al.*, 1998; Samtiya *et al.*, 2020; Ojo, 2022). Tannins also inhibit digestive enzymes like trypsin and chymotrypsin , further reducing protein digestibility (Kumar *et al.*, 2016). However, the impact of tannins on nutrient availability can be influenced by processing methods such as soaking, germination, and fermentation, which can help reduce tannin content and mitigate their anti-nutritional effects (Faizal *et al.*, 2023).

2.5.2 Phytate

Phytate, also known as inositol hexaphosphate or IP6, is a naturally occurring compound found in various plant-based foods, including finger millet (*Eleusine coracana*) (Reddy *et al.*, 2022). Phytate is considered an anti-nutrient due to its ability to bind to essential minerals, such as calcium, iron, zinc, and magnesium, forming insoluble complexes and reducing their bioavailability (Abdulwaliyu *et al.*, 2019). Phytate can inhibit the activity of enzymes like phytase, which are essential for breaking down phytate and releasing bound minerals for absorption. This inhibition can further reduce the bioavailability of minerals in finger millet (Woldemichael and Admasu, 2017). The chemical structure of phytate is presented in Fig. 2.2.

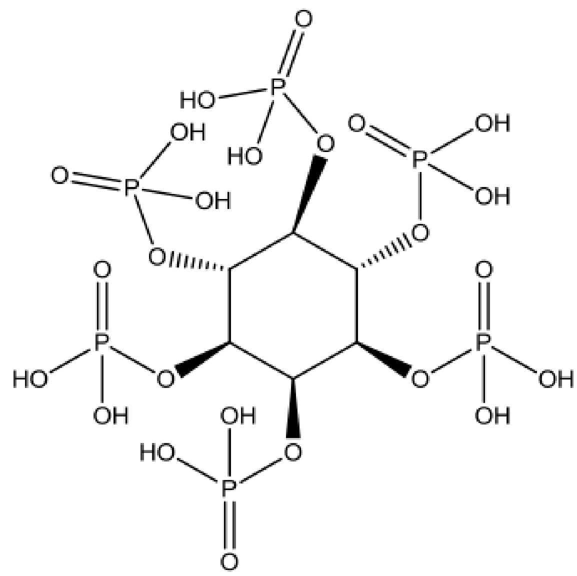

Fig. 2.2 Chemical structure of phytate

Source: Popova and Mihaylova (2019)

In finger millet, the concentration of phytate can vary depending on factors such as the variety, growing conditions, and processing methods (Makokha *et al.*, 2002). Phytate content in finger millet grains can range from moderate to high levels.

The presence of phytate in finger millet raises concerns regarding mineral absorption and utilization. However, it is important to note that phytate's impact can be mitigated through processing methods. Traditional techniques such as soaking, germination, and fermentation have been shown to significantly reduce the phytate content in finger millet, thereby improving the bioavailability of minerals (Makokha *et al.*, 2002; Coulibaly *et al.*, 2011).

Despite its anti-nutritional effects, phytate also possesses certain health benefits. It exhibits antioxidant properties, and studies have suggested its potential role in reducing the risk of chronic diseases, including certain types of cancer. Additionally, phytate has been associated with potential anticancer and antidiabetic activities, although further research is needed to fully understand its mechanisms of action and potential therapeutic applications (Abdulwaliyu *et al.*, 2019).

2.5.3 Oxalate

Oxalate, also known as oxalic acid, is a naturally occurring compound found in many plant-based foods, including finger millet (*Eleusine coracana*). Oxalate is considered an anti-nutrient due to its ability to bind with calcium, forming insoluble calcium oxalate crystals, which can interfere with calcium absorption and contribute to the development of kidney stones in susceptible individuals (Wafula *et al.*, 2018). The lower amount of soluble oxalate in finger millet limits the amount available to bind with calcium leading to reduced calcium bioavailability (Puranik *et al.*, 2017). The chemical structure of oxalate is presented in Fig. 2.3.

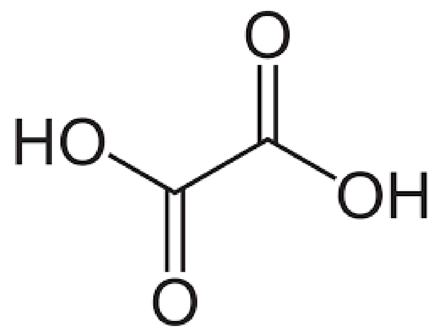

Fig. 2.3 Chemical structure of oxalate

Source: Popova and Mihaylova (2019)

The concentration of oxalate in finger millet can vary depending on factors such as the variety, growing conditions, and processing methods. Oxalate content in finger millet grains is also influenced by the levels present in the soil and the plant's ability to accumulate it (Ravindran, 1991; Amalraj and Pius, 2015).

The presence of oxalate in finger millet raises concerns regarding calcium bioavailability and the potential health risks associated with excessive oxalate intake. However, it is important to note that processing methods can influence oxalate levels. Studies have shown that traditional processing techniques such as soaking, germination, and fermentation can significantly reduce the oxalate content in finger millet, making it potentially safer for consumption (Puranik *et al.*, 2017; Chauhan and Sarita, 2018).

While oxalate is generally considered an anti-nutrient, it is worth noting that it also serves as a precursor for the synthesis of vitamin C and some other bioactive compounds by plants. Furthermore, the health effects of oxalate may vary among individuals, as some people are more susceptible to the formation of kidney stones due to oxalate intake (Mitchell *et al.*, 2019).

2.6 Processing methods

Processing methods play a crucial role in reducing the levels of anti-nutrients in finger millet and improving its nutritional quality. Various techniques, such as germination, soaking, and roasting, have been traditionally employed to enhance the nutritional value and reduce the anti-nutrient content of finger millet (Samtiya *et al.*, 2021).

2.6.1 Germination

Germination is a natural process that involves the sprouting of seeds under suitable conditions, such as moisture and optimal temperature. Germination plays a significant role in improving the nutritional composition and reducing the anti-nutritional factors in finger millet (*Eleusine coracana*) (Chauhan and Sarita, 2018).

During germination, various biochemical changes occur in the seeds, leading to the breakdown of complex compounds and the release of beneficial enzymes. These enzymes help break down anti-nutritional factors, such as phytate, tannins, and oxalate, thereby enhancing the nutritional quality of finger millet (Chauhan and Sarita, 2018; Nkhata *et al.*, 2018).

Germination has been found to have a positive impact on the carbohydrate content of finger millet. The process activates enzymes like amylase, which hydrolyzes starch into simpler sugars, making them more accessible for digestion and absorption. This increase in available carbohydrates contributes to the energy value of germinated finger millet (Budhwar *et al.*, 2020; Azeez *et al.*, 2022).

Moreover, germination has been shown to improve the protein quality and digestibility of finger millet. Enzymes released during germination break down complex proteins into smaller peptides and amino acids, making them more easily digestible and bioavailable (Hejazi and Orsat, 2016).

In addition to reducing anti-nutritional factors and improving nutrient availability, germination also enhances the content of bioactive compounds, such as antioxidants and phytochemicals, in finger millet. These compounds play a crucial role in protecting against oxidative stress and promoting overall health (Udeh *et al.*, 2017; Chauhan and Sarita, 2018).

Overall, germination is a natural and effective method for enhancing the nutritional value of finger millet. By reducing anti-nutritional factors, improving carbohydrate and protein digestibility, and increasing the content of bioactive compounds, germinated finger millet becomes a more nutrient-dense and beneficial food source.

2.6.2 Soaking

Soaking is a traditional pre-processing method that involves immersing finger millet (*Eleusine coracana*) grains in water for a certain period before consumption or further processing. Soaking is known to have a significant impact on the nutritional composition and anti-nutritional factors of finger millet (Abioye *et al.*, 2022).

One of the main benefits of soaking finger millet is the reduction of anti-nutritional factors, such as phytate, tannins, and oxalate. These compounds are known to interfere with nutrient absorption and utilization. Soaking helps to break down and leach out these anti-nutrients, thereby enhancing the bioavailability of essential minerals and improving overall nutrient absorption (Gull *et al.*, 2014).

In addition to reducing anti-nutritional factors, soaking also improves the digestibility of finger millet. The soaking process initiates enzyme activity, leading to the breakdown of complex carbohydrates and proteins. This enzymatic activity results in the conversion of starch into simpler sugars and the hydrolysis of proteins into smaller peptides and amino acids, making them more easily digestible (Samtiya *et al.*, 2020).

Soaking has also been shown to enhance the sensory attributes of finger millet. It can help reduce the bitterness and astringency associated with certain compounds, making the soaked grains more palatable and enjoyable to consume (Gull *et al.*, 2014).

Overall, soaking is an effective pre-processing method for improving the nutritional quality of finger millet. By reducing anti-nutritional factors, enhancing nutrient bioavailability, and improving digestibility, soaked finger millet becomes a more nutritious and easily digestible food option.

2.6.3 Roasting

Roasting is a traditional processing method that involves subjecting finger millet (*Eleusine coracana*) grains to dry heat, typically over an open flame or in an oven. Roasting is known to have a significant impact on the nutritional composition and sensory characteristics of finger millet (Singh *et al.*, 2018).

One of the notable effects of roasting is the modification of carbohydrates in finger millet. During the roasting process, the heat causes starches in the grains to undergo gelatinization, leading to increased digestibility and availability of carbohydrates (Gull *et al.*, 2014; Abioye *et al.*, 2022). Roasting can also result in the formation of desirable flavors and aromas, enhancing the overall sensory appeal of roasted finger millet.

In terms of anti-nutritional factors, roasting has been shown to have a positive effect on reducing certain compounds. Studies have reported that roasting can effectively reduce the levels of phytate and tannins in finger millet (Udeh *et al.*, 2017). The heat applied during roasting helps to break down and degrade these anti-nutrients, improving the bioavailability of minerals and enhancing nutrient absorption.

Furthermore, roasting can have an impact on the lipid content of finger millet. The dry heat promotes the oxidation of fats, leading to changes in the fatty acid profile and the formation of desirable flavors. Roasting can result in the development of nutty and toasted flavors in finger millet, enhancing its sensory attributes (Abioye *et al.*, 2022; Gowda *et al.*, 2022).

Overall, roasting is a valuable processing method for finger millet that improves the digestibility of carbohydrates, reduces anti-nutritional factors, and enhances flavor profiles. It provides a convenient way to enhance the nutritional value and sensory appeal of finger millet, making it a more enjoyable and nutritious grain option. It is important to note that the effectiveness of these processing methods may vary depending on factors such as duration, temperature, and grain-to-water ratio. Optimal processing conditions need to be determined to achieve maximum reduction in anti-nutrients while preserving the nutritional composition of finger millet.

Part III

Materials and methods

3.1 Materials

All chemicals used were reagent grade unless specified otherwise and distilled water was used throughout the work.

3.1.1 Collection of finger millet

Finger millet (kabre kodo) was collected from local market in Dharan municipality at Sunsari district, Koshi Province, Nepal.

3.1.2 Equipment

All equipment required for the research were used from the laboratory of Central Campus of Technology. The list of equipment used for this work is shown in Table 3.1.

Table 3.1 List of equipment used

Physical Apparatus	
Heating arrangement (burner)	Thermometer
Electronic balance (AMPUT Electronic Balance Model No-457B, Sensitivity ±0.01)	Spectrophotometer (UV-VIS Single Beam Spectrophotometer MODEL NO-291)
Distillation set	Water bath (Intake Serological Wath Bath)
Titration apparatus	Desiccator
Soxhlet apparatus	Centrifuge
Hot air oven	Mortar and pestle
Glassware and utensils	Incubator

3.1.3 Chemicals

All chemicals required for this research were used from laboratory of Central campus of Technology. The list of chemicals used for this work is shown in Table 3.2.

Table 3.2 List of chemicals used

Chemicals	
Hydrochloric acid (20%)	Tannic acid solution (0.1 mg/ml)
Sulphuric acid (0.5 M)	Folin-ciocalteu reagent
Oxalic acid	Standard saponin solution
Sodium hydroxide solution	Magnesium carbonate solution
Saturated Sodium carbonate solution	Potassium permanganate solution (0.1 M)
Ammonium thiocynate solution (0.3%)	Calcium chloride solution (5%)
Iron chloride solution	Ammonium hydroxide solution (0.35 M)

3.2 Methodology

The general outline for processing of finger millet is presented in Fig. 3.1.

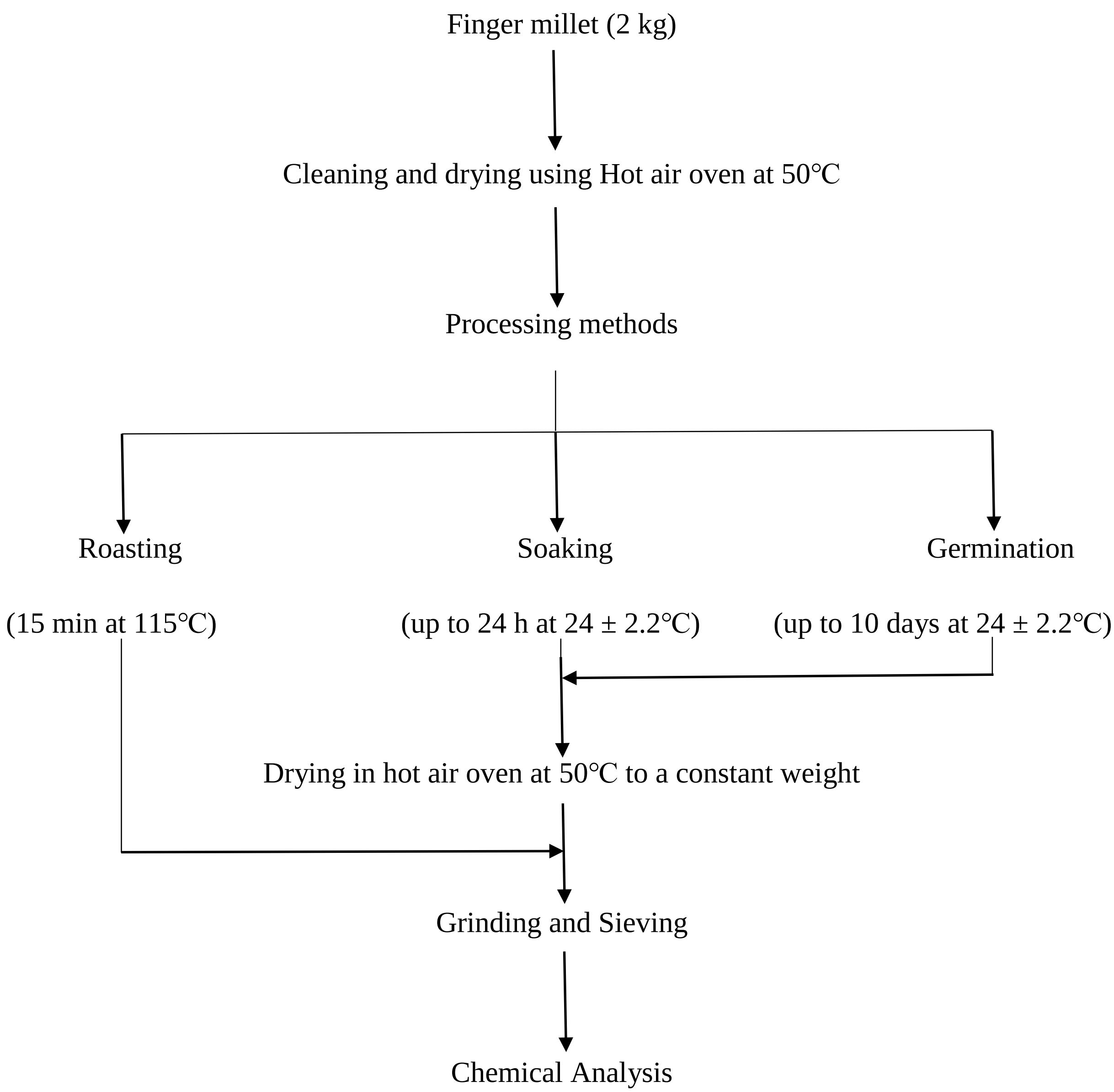

Fig. 3.1 General flow sheet for processing of finger millet

Initially, 2 kg of finger millet was cleaned and dried in a hot air oven at 50℃ as drying at 50°C helps remove moisture from the samples without causing significant changes to the nutrients or anti-nutrients (Abioye *et al.*, 2022). Following this, various processing methods were employed, including roasting for 15 min at 115℃, soaking for up to 24 h at 24 ± 2.2℃, and germination for up to 10 days at 24 ± 2.2℃. Subsequently, the millet was dried in a hot air oven at 50℃ until it reached a constant weight. The next steps involved grinding and sieving before conducting chemical analysis.

3.3 Experimental design

The independent and dependent variables used in the experiment were as follows:

1. Independent Variables: Processing techniques (germination, roasting, soaking), duration of soaking (6, 12, 18, and 24 h) and duration of germination (2, 4, 6, 8, 10 days).
2. Dependent Variables: Tannin content, oxalate level, and phytate concentration in finger millet.

The hypothesis of the experiment were as follows:

1. Null Hypothesis (H0): The processing techniques and their durations do not significantly affect the tannin content, oxalate level, and phytate concentration in finger millet.
2. Alternative Hypothesis (H1): The processing techniques and their durations significantly affect the tannin content, oxalate level, and phytate concentration in finger millet.

Factorial design was used to take into account the effects of multiple independent variables (processing tecnhiques at different levels (e.g., different durations of soaking and germination)). Finger millet samples were randomly assigned to different treatment groups to avoid any bias.

3.4 Processing methods to reduce antinutrients

3.4.1 Germination

Finger millet seeds were soaked overnight in fresh water for 12 h. After then, the seeds were rinsed and the water drained off. The seeds were then allowed to sprout in room temperature for about 10 days. Only the sprouted samples were taken and dried in a hot air oven at 50℃ to a constant weight, finely ground and stored in an air tight plastic container for the further analysis (Kavitha *et al.*, 2015).

3.4.2 Roasting

Finger millet seeds were roasted on pan at 115℃ for 15 min. Induction stove was used to accurately maintain the given temperature. The roasted seeds were dried at 50℃ to a constant weight, finely ground and stored in an air tight container for further analysis (Kavitha *et al.*, 2015; Oluwole *et al.*, 2019).

3.4.3 Soaking

Finger millet seeds were soaked in tap water at ratio 1:2 (w/v) at room temperature for 24 h. The soaked seeds were washed twice with ordinary water followed by rinsing with distilled water and then dried in an oven at 50℃ to a constant weight. Dried samples was ground, stored in an airtight plastic container for further analysis (Kavitha *et al.*, 2015).

3.5 Analytical methods

3.5.1 Proximate analysis of finger millet

3.5.1.1 Moisture content

The moisture content was determined by using hot air oven method. 5 g of raw sample was weighed and heated in an insulated oven at 110℃ to constant weight. The difference in weight was the water that has evaporated as mentioned by Ranganna (1986).

3.5.1.2 Protein content

Crude protein was determined by the Kjeldahl method, total protein was calculated by multiplying the nitrogen content by a factor of 6.25 (Ranganna, 1986).

3.5.1.3 Fat content

The fat content of the sample was determined by using Soxhlet apparatus as described in Ranganna (1986).

3.5.1.4 Ash content

The ash content was determined by incinerating the finger millet (5 g) in a muffle furnace at 525℃ for 4-6 h (Ranganna, 1986).

3.5.1.5 Crude fiber content

Crude fiber was determined by using chemical process, the sample was treated with boiling dilute sulphuric acid, boiling sodium hydroxide and then with alcohol as standard method of Ranganna (1986).

3.5.1.6 Carbohydrate content

Total carbohydrate content of the samples was determined by difference method.

Carbohydrate (%) = 100 – [sum of protein, total ash, crude fiber, moisture and fat]

3.5.2 Determination of tannin

Colorimetric estimation of tannins is based on the measurement of the blue color formed by the reduction of folin-ciocalteu reagent by tannin-like compounds in alkaline condition.

10 ml of standard tannic acid solution was pipetted into 100 ml volumetric flasks containing 75 ml water. Similarly, 10 ml of the sample was transferred into another 100 ml volumetric flask. Subsequently, 5 ml of Folin-Denis reagent and 10 ml Na_2CO_3 solution were added to each volumetric flask. The volume was adjusted to 100 ml with water, and the flasks were left to stand for 30 min. Absorbances of the standard tannic acid solution and the sample at 760 nm were measured. A graph of absorbance versus mg of tannic acid was plotted, and the amount (in mg) of tannic acid in the sample was determined (KC and Rai, 2007).

3.5.3 Determination of phytate

0.2 g of the sample was weighed into 250 ml conical flask and soaked in 100 ml of 20% concentrated HCl for 3 h. Afterward, the sample was filtered, and 50 ml of the filtrate was transferred into a 250 ml beaker. Subsequently, 100 ml distilled water was added to the sample. Then, 10 ml of 0.3% ammonium thiocyanate solution was introduced as an indicator, and titration was carried out with standard iron (III) chloride solution which containing 0.00195 g of iron per 1 ml (Emmanuel and Deborah, 2018).

3.6 Statistical analysis

For all chemical analysis, triplicates of the samples were used for determination of each constituent. Mean values with standard deviation were computed. Data on processing different techniques were subjected to analysis of variance (ANOVA) and considered at 95% confidence level using statistical software GenStat Twelfth Edition 12.1.

Part IV

Results and discussion

Finger millet (*Eleusine coracana)* was collected from market of Sunsari district and different processing techniques were carried out, i.e., germination, soaking and roasting. Then, thus obtained processed samples were analyzed to study the effect of different processing techniques on its antinutrients.

4.1 Proximate composition

The proximate composition of finger millet is given in Table 4.1.

Table 4.1 Proximate composition of raw finger millet (dry basis)

Parameters	Values (%)
Moisture	13.16 ± 0.12
Protein	7.13 ± 0.53
Fat	1.42 ± 0.62
Crude fiber	3.26 ± 0.37
Ash	3.88 ± 0.44
Carbohydrate	71.15 ± 0.11

[Values presented are the average of triplicate determination ± standard deviation.]

The moisture content in the finger millet was found to be 13.16% and similar data was there in DFTQC (2017) and Shrestha and Karki (2019), i.e., 13.1% and 13.2-14.5% whereas Banusha and Vasantharuba (2013) and David *et al.* (2014) found the moisture content of finger millet to be lower than the obtained value, i.e., 7.67% and 6.99%. The protein content in raw finger millet was found to be 7.13% which was similar to the value in DFTQC (2017) and Shrestha and Karki (2019), i.e., 7.3% and 6.8-7.3%. A slight decrease in protein content, i.e., 6.3% was found by Chauhan and Sarita (2018) whereas a slight increase in protein content, i.e., 10.66% and 10.28%

was observed by Banusha and Vasantharuba (2013) and David *et al.* (2014). It was found that the fat content of finger millet was 1.42% which is quite similar to the data in Banusha and Vasantharuba (2013), DFTQC (2017), and Shrestha and Karki (2019), i.e., 1.42%, 1.3% and 1.3-1.7%, respectively while David *et al.* (2014) found the fat content to be slightly lower, i.e., 0.83%. The crude fiber content of finger millet was found to be 3.26% which is quite similar to the results in Banusha and Vasantharuba (2013), David *et al.* (2014), DFTQC (2017), and Shrestha and Karki (2019), i.e., 3.94%, 3.10%, 3.6% and 2.8-3.3%, respectively. The ash content of finger millet obtained in the study was 3.88% which aligned with the range stated by Pragya and Rita (2012), i.e., 1.7-4.13%. Banusha and Vasantharuba (2013), David *et al.* (2014), and Shrestha and Karki (2019) found out the ash content to be in a slightly lower range, i.e., 2.84%, 2.37% and 1.9-2.2%, respectively. The carbohydrate content of raw finger millet was found to be 71.15% which is similar to the data of Pragya and Rita (2012), David *et al.* (2014), and DFTQC (2017), i.e., 76.43%, 72% and 72-79.5%, respectively, but the range obtained by Shrestha and Karki (2019) was very less, i.e., 59.8-60.3%.

4.2 Antinutrients present in raw finger millet

The mean values of different antinutrients determined are presented in the given Table 4.2.

Table 4.2 Distribution of anti-nutrients in raw finger millet (mg/100 g) (dry basis)

Anti-nutrients	Values (mg/100 g)
Tannin	356.49 ± 1.632
Oxalate	52.33 ± 1.553
Phytate	131.49 ± 1.339

[Values presented are the average of triplicated determination ± standard deviation.]

The tannin content in the raw finger millet was found 356.49 mg/100 g which is less than value obtained by Chauhan and Sarita (2018), i.e., 870.8 mg/100 g but it is greater then the value obtained by Abioye *et al.* (2018), i.e., 53.33 mg/100 g. Oxalate content in the finger millet was 52.33 mg/100 g which was greater than the oxalate content obtained by Chauhan and Sarita

(2018), i.e., 45.8 mg/100 g. Phytate content in the finger millet was found to be 131.49 mg/100 g which was greater than the result found by Abioye *et al.* (2018), i.e., 51.67 mg/100 g but it was less than the value obtained by Patel and Dutta (2018), i.e., 676.77 mg/100 g. According to different researches, it is concluded that antinutrients values of finger millet varies according to variety, climatic conditions, location, irrigation condition, types of soil etc. Luitel *et al.* (2020) also concluded with similar findings.

4.3 Effect of different processing method on tannin content of finger millet

The effect of soaking, germination and roasting on the tannin content of finger millet was studied. All the treatments significantly reduced ($p<0.05$) the tannin of the finger miller seed, but to the varying extent. Germination had most pronounced effect than other treatments in reduction of tannin content.

4.3.1 Effect of germination

The tannin content of finger millet was determined and the value obtained showed that there is significant reduction ($p<0.05$) in tannin content, which is reduced from 356.49 mg/100 g to 155 mg/100 g after germination, i.e., 56.52% reduction. This study shows that highest reduction of tannin in finger millet was seen in germinated sample. The effect of germination on tannin content is shown in Fig. 4.1.

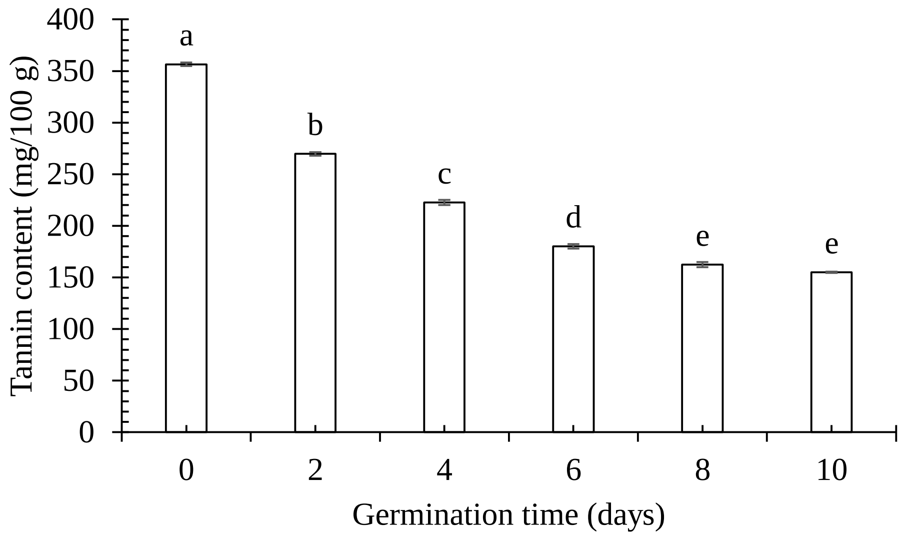

Fig. 4.1 Effect of germination on tannin content

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

Abioye *et al.* (2018) found that the reduction in tannin level due to germination of finger millet was about 50% which is similar to the data we obtained. Similarly, 58.6% reduction in tannin content after germination was found out by Chauhan and Sarita (2018) which is also similar to the data obtained in the present case. The reduction in tannin during germination is mainly due to leaching and also could be attributed to the increased activity of various enzymes like polyphenol oxidase and other catabolic enzymes (Patel and Dutta, 2018).

4.3.2 Effect of soaking

The tannin content of the finger millet was found to be significantly reduced ($p<0.05$) from 356.49 mg/100 g to 179.47 mg/100 g after soaking, i.e., 49.65% reduction. The effect of soaking on tannin content is shown in Fig. 4.2.

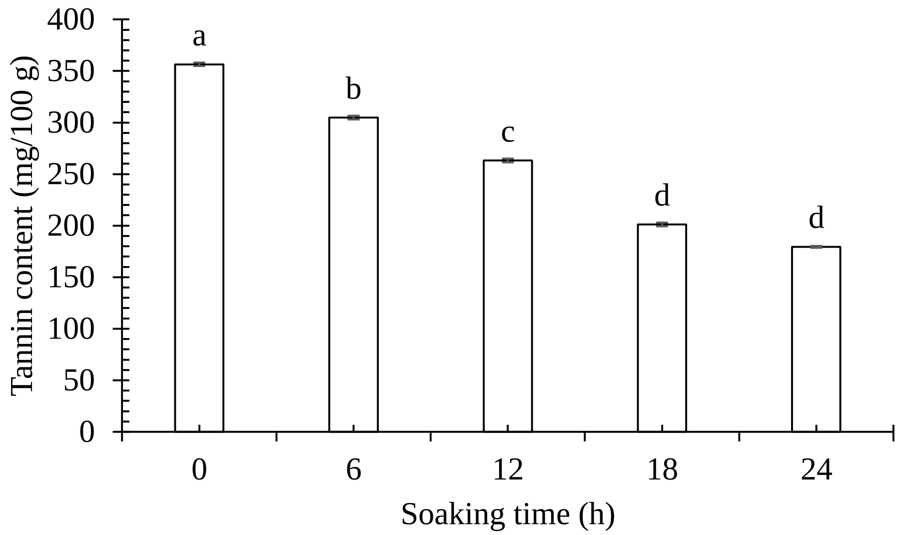

Fig. 4.2 Effect of soaking on tannin content

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

The result obtained agree with result obtained by Bhuvaneshwari *et al.* (2020). They reported a reduction of 41% after 24 h of soaking of finger millet. In the present case, there was slightly higher reduction. Similarly, Khandelwal *et al.* (2010) also concluded that there is significant reduction in oxalate content of various legumes after soaking. The reduction of tannin content during soaking is mainly due to leaching (Ogunlakin *et al.*, 2012).

4.3.3 Effect of roasting

The effect of roasting on finger millet was studied. The value obtained showed that there was significant reduction ($p<0.05$) in tannin content, which was reduced from 356.49 mg/100 g to 171.80 mg/100 g, i.e., 51.80% reduction. The effect of roasting on tannin content is shown in Fig. 4.3.

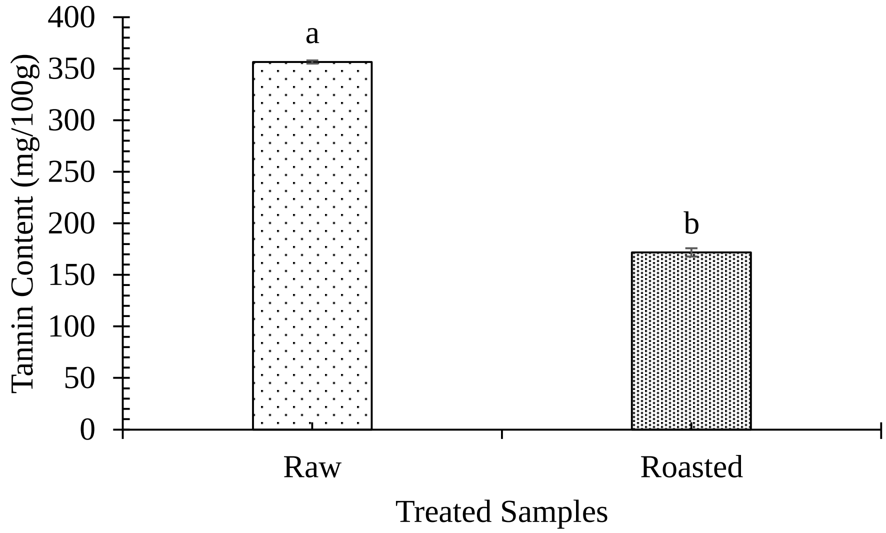

Fig. 4.3 Effect of roasting on tannin content

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

Attou *et al.* (2020) found a reduction of 41% in tannin content after heat treatment of different lentils which is lower than the obtained result. Yadav and Bhatnagar (2017) also concluded that tannin were significantly reduced after roasting, i.e., 57%. In the present case, there was slightly lower reduction. This reduction might be because of denaturation of tannin due to high temperature by roasting as tannin are heat labile (Ndidi *et al.*, 2014).

The effect of different processing methods on tannin content are shown in Fig. 4.4.

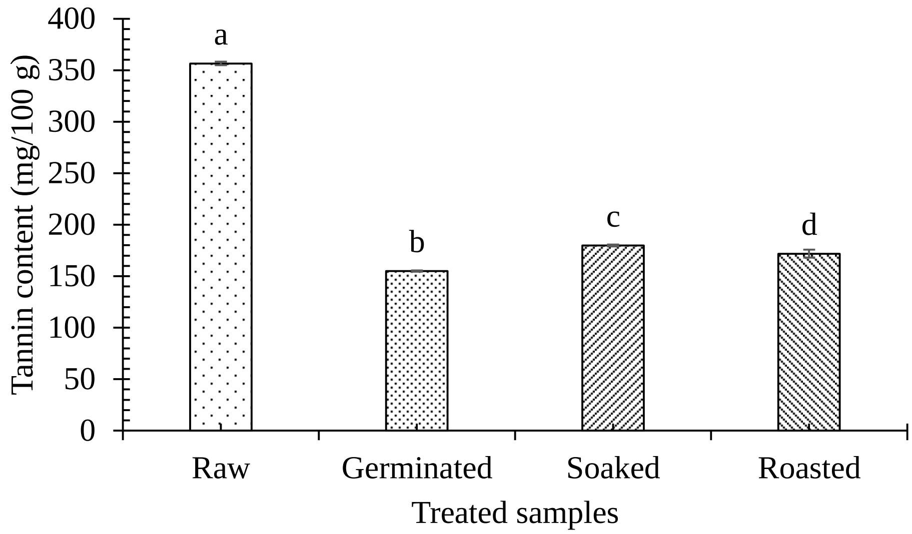

Fig. 4.4 Effect of different processing methods on tannin content

[Plotted values are means of triplicates. Values on top of the plot bearing similar superscript are not significantly different (p<0.05) at 5% level of significance. Vertical error bars represent ± standard deviations.]

4.4 Effect of different processing method on oxalate content of finger millet

The effect of soaking, germination and roasting on the oxalate content of finger millet was studied. All the treatments significantly reduced ($p<0.05$) the oxalate of the finger miller seed, but to the varying extent. Germination had most pronounced effect than other treatments in reduction of oxalate content.

4.4.1 Effect of germination

The oxalate content of finger millet was determined and the value obtained showed that there is significant reduction ($p<0.05$) in oxalate content, which is reduced from 52.33 mg/100 g to 26.9 mg/100 g after germination, i.e., 48.59% reduction. The present study shows that highest reduction of oxalate in finger millet was seen in germinated sample. The effect of germination time on oxalate content is shown in Fig. 4.5.

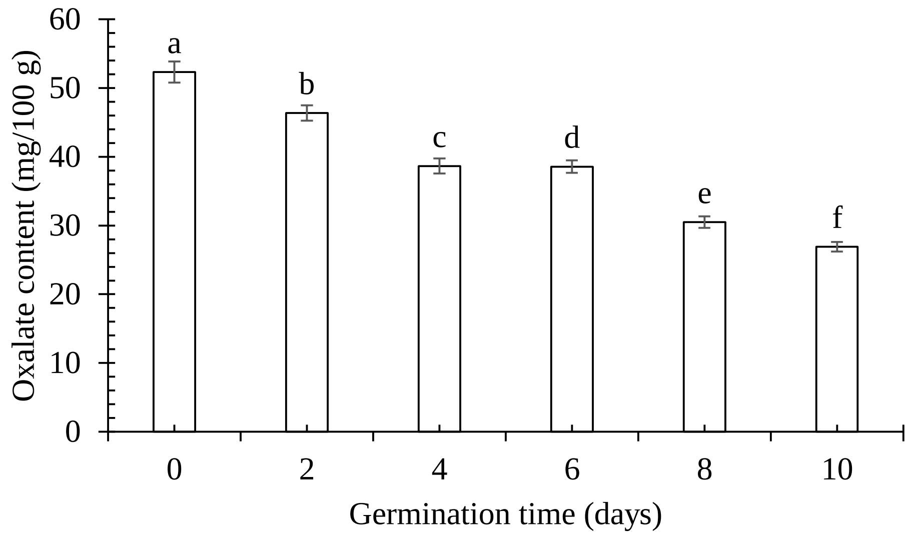

Fig. 4.5 Effect of germination on oxalate content

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

The result tally in line with result obtained by Patel and Dutta (2018), they found significant reduction ($p<0.05$) in oxalate during germination of finger millet (54.36%). Chauhan and Sarita (2018) found a significant reduction in oxalate content of germinated finger millet by 35%, which was lower than the result obtained in this study. The reduction in oxalic acid during germination and soaking may be due to leaching of oxalate oxidase and oxalate decarboxylase (Patel and Dutta, 2018). Similar result for reduction in oxalic acid content of germinated grains were reported by Brudzyński and Salamon (2011).

4.4.2 Effect of soaking

The oxalate content of the finger millet was found to be significantly reduced ($p<0.05$) from 52.33 mg/100 g to 41.06 mg/100 g after soaking, i.e., 21.52% reduction. The effect of soaking time on oxalate content is shown in Fig. 4.6.

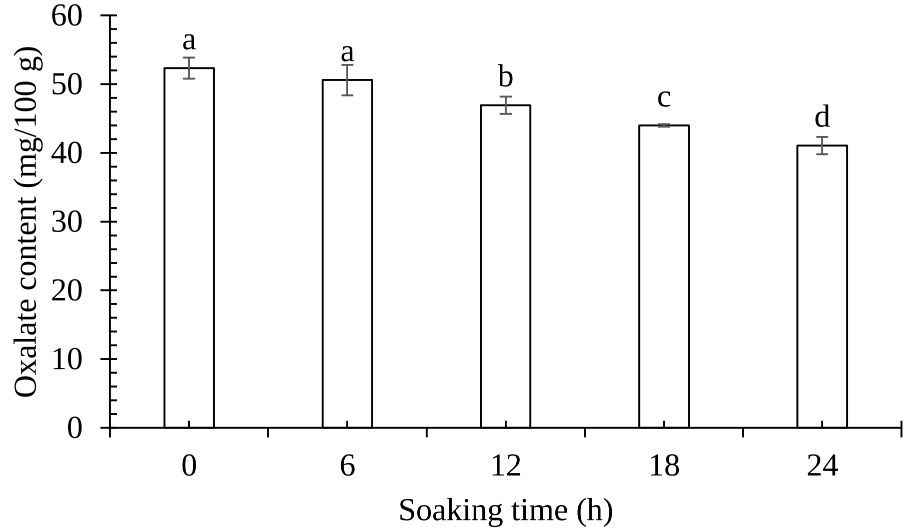

Fig. 4.6 Effect of soaking on oxalate content

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

A significant reduction of 18.52% in oxalate content of finger millet by Patel and Dutta (2018), which is only slightly lower than the reduction obtained by the data. They also stated that the decrease in oxalate content during germination and soaking is maybe due to the leaching of oxalate oxidase and oxalate decarboxylase. Similar result for reduction in oxalic acid content of soaked grains were reported by Brudzyński and Salamon (2011).

4.4.3 Effect of roasting

The effect of roasting on finger millet was studied. The value obtained showed that there was significant reduction ($p<0.05$) in oxalate content, which was reduced from 52.33 mg/100 g to 37.4 mg/100 g, i.e., 28.53% reduction. The effect of roasting on oxalate content is shown in Fig. 4.7.

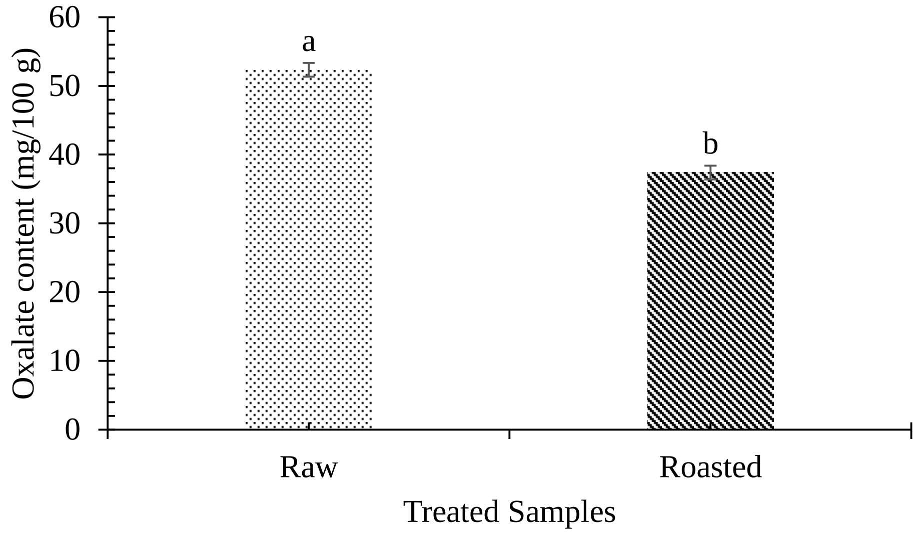

Fig. 4.7 Effect of roasing on oxalate content

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

This reduction might be because of denaturation of oxalate due to high temperature by roasting as oxalic acid are heat labile (Ndidi *et al.*, 2014).

The effect of different processing methods on oxalate content are shown in Fig. 4.8.

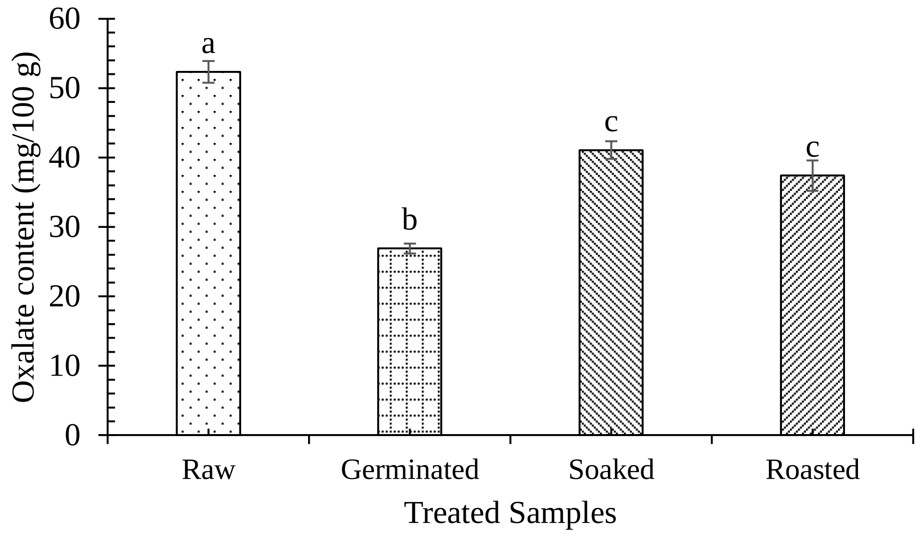

Fig. 4.8 Effect of different processing methods on oxalate content

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

4.5 Effect of different processing method on phytate content of finger millet

The effect of soaking, germination and roasting on the phytate content of finger millet was studied. All the treatments significantly reduced ($p<0.05$) the phytate of the finger miller seed, but to the varying extent. Germination had most pronounced effect than other treatments in reduction of phytate content.

4.5.1 Effect of germination

The phytate content of finger millet was determined and the value obtained showed that there is significant reduction ($p<0.05$) in phytate content, which is reduced from 131.49 mg/100 g to 55 mg/100 g after germination, i.e., 58.17% reduction. This study shows that highest reduction of phytate in finger millet was seen in germinated sample. The effect of germination time on phytate content is shown in Fig. 4.9.

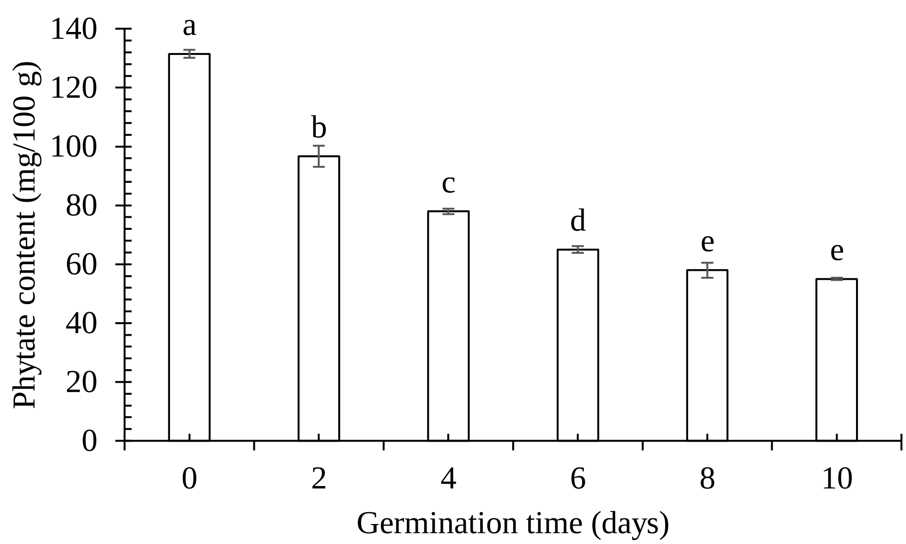

Fig. 4.9 Effect of germination on phytate content

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

This result agrees with the data obtained by Patel and Dutta (2018), they also found that the phytate content content in germinated sample of finger millet was reduced by 65%. Chauhan and Sarita (2018) reported that the reduction of phytate was 72% after germination which is quite higher than the data obtained in this study. According to various researchers, the decrease in level of phytate during germination is due to leaching out and phytase activity in sprouting grains (Larsson and Sandberg, 1992; Tizazu *et al.*, 2010). Similarly, Ghavidel and Prakash (2007) also reported that there was significant reduction of phytate content after germination of legume seeds.

4.5.2 Effect of soaking

The phytate content of the finger millet was found to be significantly reduced ($p<0.05$) from 131.49 mg/100 g to 104.42 mg/100 g after soaking, i.e., 20.59% reduction. The effect of soaking time on phytate content is shown in Fig. 4.10.

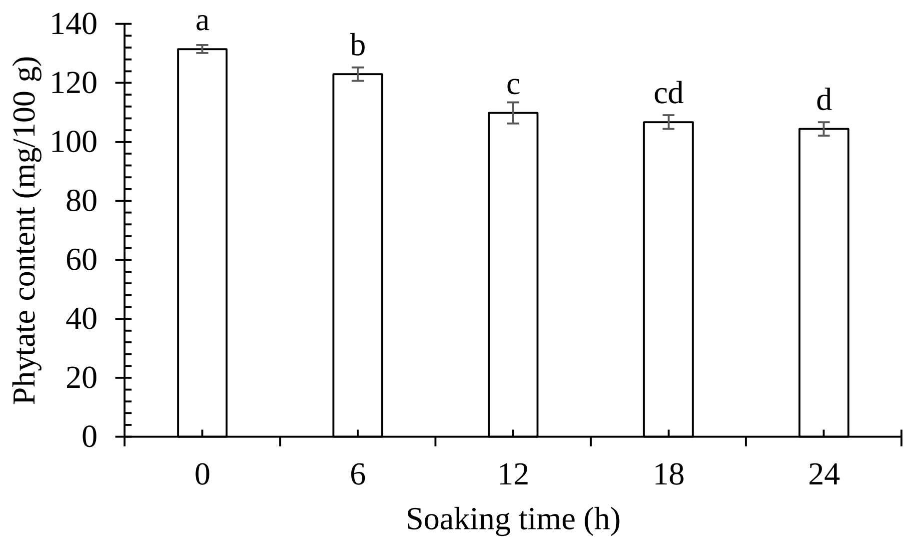

Fig. 4.10 Effect of soaking on phytate content

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

From the research done by Patel and Dutta (2018), the reduction in phytate content of finger millet after soaking was found to be 13.22% which was slightly lower reduction than the data obtained by the present study. The reduction of phytate during soaking could be attributed to leaching out during hydration (Tizazu *et al.*, 2010) .

4.5.3 Effect of roasting

The effect of roasting on finger millet was studied. The value obtained showed that there was significant reduction ($p<0.05$) in phytate content, which was reduced from 131.49 mg/100 g to 94.43 mg/100 g, i.e., 28.18% reduction. The effect of roasting on phytate content is shown in Fig. 4.11.

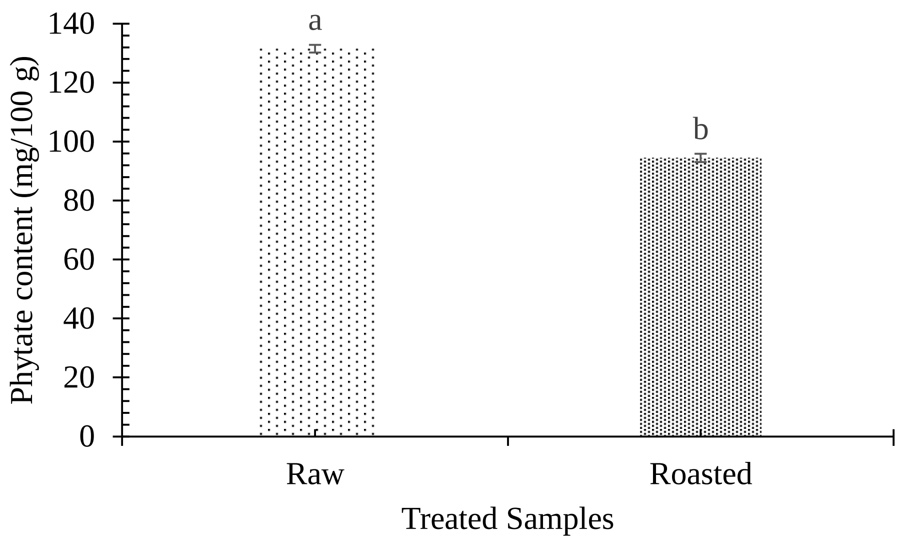

Fig. 4.11 Effect of roasting on phytate content

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

A significant decrease of phytate was recorded for roasted pearl millet, i.e., reduction upto 27.61% (Chauhan *et al.*, 2015) which agrees with the data obtained in the present study. Several studies shows that the rise in temperature causes a degradation of phytates, Tiwari *et al.* (2014) reported that roasting reduced the phytate content of grain flour to about 43.68%. A reduction of 19.31% to 34.89% of phytate was seen after roasting of millet. It should also be noted that it is necessary to exceed 100 °C to have a significant degradation of phytates (Salif Sow *et al.*, 2019).

The effect of different processing methods on phytate content are shown in Fig. 4.12.

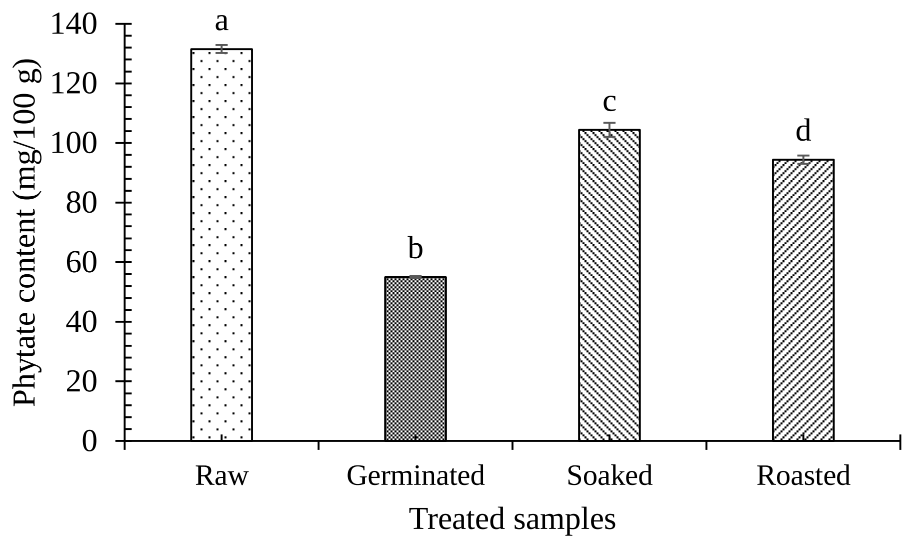

Fig. 4.12 Effect of different processing methods on phytate content

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

Part V

Conclusions and recommendations

5.1 Conclusions

In this research, raw finger millet was processed with several treatments and following conclusions can be drawn.

1. The nutrient composition, i.e., protein, carbohydrate, fat, crude fiber, ash and moisture content of raw finger millet was found to be 7.13%, 71.15%, 1.42%, 3.26%, 3.88% and 13.16%, respectively.
2. Germination, soaking and roasting of finger millet decrease the anti-nutrients significantly.
3. The mean value of tannin, oxalate and phytate in raw finger millet was found to be 356.49 mg/100 g, 52.33 mg/100 g and 131.49 mg/100 g on dry basis.
4. Based on the percentage reduction, the most effective method for reducing all three antinutrients in finger millet was germination, with a 56.52% reduction in tannins, a 48.59% reduction in oxalates, and a 58.17% reduction in phytates.

5.2 Recommendations

Based on the above findings the following recommendations are made for further study:

1. The effect of processing methods to reduce other anti-nutrients can be studied.
2. With the variation of time and temperature of different processing methods, optimum time and temperature can be determined which provides maximum reduction of anti-nutritional factors of finger millet.
3. Effect of different combined treatments on anti-nutritional factors can be studied.

Part VI

Summary

Finger millet (*Eleusine coracana)* is an important cereal crop cultivated primarily in Africa and Asia better known for its rich nutritional profile, being a good source of minerals, vitamins, and dietary fiber. Finger millet have been recognized to offer several health benefits such as anti-diabetic, protection from diet related chronic diseases, hypocholesterolaemic, antioxidant, and antimicrobial effects to its regular consumers. Moreover, it is likewise rich in carbohydrate, energy and nutrition, making finger millet an important ingredient of dietary and nutritional balanced foods.The regular use of finger millet as a nutrient and its products helps in managing different disorders of body by maintaining blood glucose homeostasis.

To evaluate the lowering of anti-nutrient levels in finger millet, three different proceessing were used in this research. The processing methods include soaking, germination and roasting. The antinutrients seen were tannin, oxalate and phytate. Tannin was determined by spectrophotometric methods however phytate and oxalate were determined by titration using iron chloride solution and potassium permagnate solution, respectively.

The mean value of tannin, oxalate and phytate of raw finger millet were 356.49 mg/100 g, 52.33 mg/100 g and 131.49 mg/100 g, respectively. All the processing methods reduced ($p<0.05$) significantly the antinutrients of finger millet whereas germination was seen as the best treatment for reduction than other processing methods. The reduction in tannin by germination after 8 days and by soaking after 18 h were not significantly ($p>0.05$) different. The reduction in oxalate by soaking during 0 days and 6 days were not significantly ($p>0.05$) different. The reduction in phytate by germination after 8 days and by soaking after 18 h were not significantly ($p>0.05$) different. The comparision graph concluded that the best method for reduction of all three antinutrients, i.e., tannin, oxalate and phytate were seen during germination followed by roasting.

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Appendices

Appendix A

A.1 Effect of germination on anti-nutritional factors

Table A.1. 1 One way ANOVA table for tannin content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	5	90408.51	18081.70	491.44	<.001
Residual	12	441.52	36.79		
Total	17	90850.03			

Table A.1. 2 One way ANOVA table for phytate content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	5	12796.435	2559.287	524.81	<.001
Residual	12	58.520	4.877		
Total	17	12854.955			

Table A.1. 3 One way ANOVA table for oxalate content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	5	1381.091	276.218	242.53	<.001
Residual	12	13.667	1.139		
Total	17	1394.758			

A.2 Effect of soaking on anti-nutritional factors

Table A.2. 1 One way ANOVA for tannin content

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Treatments	4	63324.0	15831.0	104.75	<.001
Residual	10	1511.3	151.1		
Total	14	64835.4			

Table A.2. 2 One way ANOVA for phytate content

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Treatments	4	1627.621	406.905	66.68	<.001
Residual	10	61.027	6.103		
Total	14	1688.648			

Table A.2. 3 One way ANOVA for oxalate content

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Treatments	4	256.837	64.209	30.52	<.001
Residual	10	21.040	2.104		
Total	14	277.877			

Appendix B

Table B. 1 Standard curve data for tannin as tannic acid

Tannic acid concentration (µg/ml)	Absorbance
0	0
20	0.154
40	0.173
60	0.195
80	0.272
100	0.334

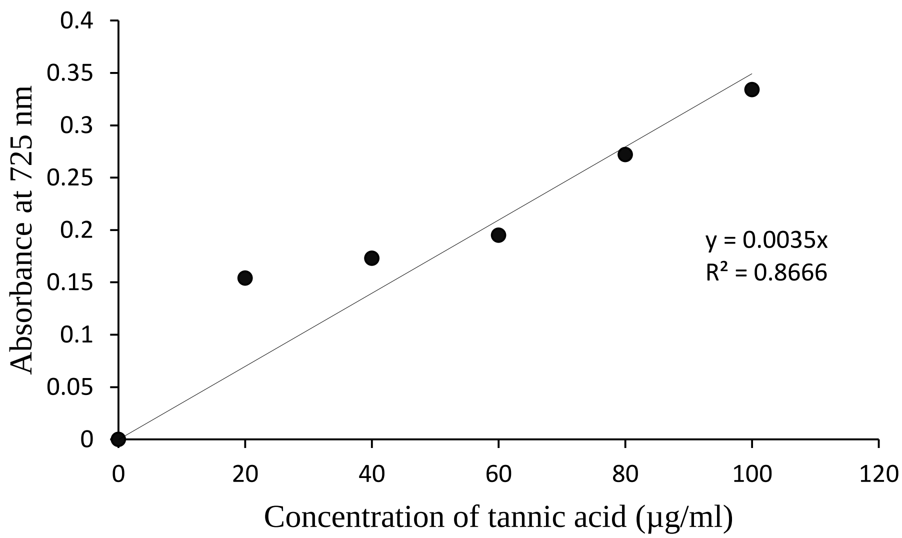

Figure B. 1 Standard curve for tannin determination

Appendix C

Fig C.1: Finger millet seeds after cleaning

Fig C.2: Germinated and Dried Finger millet seeds

Fig C.3: Finger millet seeds after roasting

Fig C.4: During lab analysis for Antinutrients determination