EFFECTS OF SOAKING, GERMINATION AND POPPING ON NUTRITIONAL AND ANTI-NUTRITIONAL FACTORS IN SORGHUM (*Sorghum bicolor L. Moench*) SEEDS

by

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A dissertation submitted to the Department of Nutrition and Dietetics, Central Campus of Technology, Tribhuvan University, in partial fulfilment of the requirements for a Bachelor's degree in Science in Nutrition and Dietetics

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Approval Letter

This ***dissertation*** entitled ***Effects of Soaking, Germination and Popping on Nutritional and Anti-nutritional Factor in Sorghum (Sorghum Bicolor) Seeds*** presented by **Susmita Adhikari** has been accepted as the partial fulfillment of the requirements for the **Bachelor's degree in Science in Nutrition and Dietetics.**

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Susmita Adhikari

Abstract

The main aim of the present research was to determine the effect of processing techniques on the nutritional and anti- nutritional factors of sorghum seeds (*Sorghum bicolor* L. Moench). The impact of different treatments—soaking for 12, 24, 36, and 48 h; germination for 24, 48, and 72 h; and popping at 200 to 240 °C for 1-2 min on protein content, DPPH radical scavenging activity, and anti-nutrients (oxalate, phytate, flavonoid, polyphenol, and tannin) in raw sorghum seeds was studied.

The mean values for protein, polyphenol, DPPH scavenging activity, tannin, phytate, flavonoid, and oxalate in raw sorghum seeds were found to be 12.42%, 698.11 mg GAE/100 g, 50.11%, 348.92 mg TAE/100 g, 127.53 mg/100 g, 58.22 mg QE/100 g, and 115.58 mg/100 g, respectively, on a dry basis. Among the three different treatments, germination showed a significant increase in protein, TPC, DPPH scavenging activity, and flavonoid content, followed by popping methods, while soaking showed a decrease. Phytate, tannin, and oxalate levels were significantly reduced by germination, followed by soaking and popping. The maximum increases in protein (28.65%), phenol content (49.85%), DPPH radical scavenging activity (69.36%), and flavonoid content (52.61%), as well as the reductions in phytate (51.38%), tannin (48.37%), and oxalate (58.58%), were observed when sorghum seeds were germinated for 72 hours. Overall, soaking, germination, and popping methods significantly ($p<0.05$) reduced the anti-nutrients in sorghum seeds and improved their functional and nutritional attributes.

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List of Abbreviations

Abbreviations	Full form
%	Percentage
°C	Degree Celsius
µg	Microgram
ANOVA	Analysis of Variance
CCT	Central campus of technology
d.f.	Degree of freedom
DB	Dry basis
DFTQC	Department of food technology and quality control
DPPH	2,2-diphenyl-1-picrylhydrazyl
FeCL3	Ferric chloride
fig.	Figure
G	Gram
GAE	Gallic acid equivalent
H	Hour (s)
Kcal	Kilocalorie
LSD	Least significant difference
MC	Moisture content
Min	Minute
Mg	Milligram
Ppm	Parts per million
QE	Quercetin equivalent
S	Second
TAE	Tannin acid equivalent
TFC	Total flavonoid content
TPC	Total phenolic content

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Part I
Introduction

1.1 General Introduction

Sorghum (*Sorghum bicolor* L. Moench) is a member of the Andropogoneae tribe within the Poaceae family (also known as *Gramineae*), commonly referred to as the grass family (Cabrera-Ramírez *et al.*, 2020; Pezzali *et al.*, 2020). In terms of production and area of plantation, sorghum grain ranks as the fifth most significant cereal, following barley, maize, wheat, and rice (Kamara *et al.*, 2011).

Sorghum serves as a staple food grain for nearly 500 million people worldwide, with global production reaching approximately 59 million tons in 2018 (Cabrera-Ramírez *et al.*, 2020; Pezzali *et al.*, 2020). Due to its drought endurance, high productivity, low nutritional requirements, low cost of production, and agronomic benefits in challenging areas, sorghum is a highly competitive crop globally, particularly in Africa and Asia (Adebowale *et al.*, 2020). Sorghum grass is cultivated for hay and fodder, while grain sorghum is used as food. Based on cultivation region and/or uses by different populations, sorghum has different names. Sorghum is referred to as giant millet, milo, durra, orshallu in Africa, gaoliang in China, and jowar, cholam, or jonna in India (Djameh *et al.*, 2015) , and *Junelo* in different parts of Nepal.

Sorghum grains are a valuable source of both micro and macronutrients, containing around 8-18% proteins, 1-5% fats, ~19% dietary fiber, and 70-80% carbs (Sergio O Serna-Saldivar and Espinosa-Ramírez, 2019). Despite to this, sorghum grains contain different antinutritional factors like tannins, phytic acids, and oxalate in relatively higher concentration as compared to other cereal crops (Ojha *et al.*, 2018). The anti-nutritional factors have a negative impact in human nutrition by hindering bioavailability through binding of important minerals (Fe, Ca, and Zn) and digestibility of proteins that interferes on growth, reproduction, and health of the general public and in particular children under ages of five (Popova and Mihaylova, 2019). Unfortunately, anti-nutritional substances that form complexes with dietary components and lower protein digestibility and mineral bioavailability are the reason sorghum has low nutritional value and inferior organoleptic properties (Derbew and Moges, 2017).

Soaking is the process of immersing biological material for a period of time (from several hours to days) at a specific temperature (4-80°C), preferably in an acidified water solution, to shorten the cooking time and improve the nutritional content of the final product. It's a common practice to soak grains, seeds, and beans (Fernandes *et al.*, 2010). In the food products business, soaking is one of the low-cost methods for improving raw material quality. During the soaking of sorghum grains in water, the antinutritional contents of soaked grains were decreased because the absorption of water activates the enzyme and breaks down food reserve material (Eltayeb *et al.*, 2017).

Germination is widely employed in both legumes and cereals to enhance their palatability and nutritional value. This process aids in breaking down certain antinutrients, such as phytate and protease inhibitors (Steiner *et al.*, 2007). In sorghum-producing regions, germination is a common practice. Grains are malted for the production of weaning foods, opaque beers, and other traditional dishes. Germination triggers enzymatic activity in sprouting grains, leading to the breakdown of proteins, carbohydrates, and lipids into simpler forms. This enzymatic process activates proteases that degrade proteins, thereby increasing nutrient bioavailability (Elmoneim and Bernhardt, 2010).

Popping is a simultaneous process of starch gelatinization and expansion, where grains undergo brief exposure to high temperatures for short duration. This rapid heating generates super-heated vapor within the grains, cooking the grain and causing the endosperm to expand suddenly, bursting through the outer skin (Mishra *et al.*, 2014). Popping is a traditional method employed to produce ready-to-eat products known for their crunchy and porous texture, high expansion, and low bulk density (Burgos and Armada, 2015; Dutta *et al.*, 2015). These puffed foods are favored for their appealing color, texture, flavor, and shape, enhancing consumer acceptance. Various cereals, including rice, wheat, maize, sorghum, and finger millet, are utilized for puffing (Mishra *et al.*, 2014). Additionally, the puffing process has been observed to reduce the levels of anti-nutritional factors present in cereal grains (Ramashia *et al.*, 2019).

1.2 Statement of the problems

The current food insecurity in numerous developing nations, along with the future challenge of feeding over nine billion people by 2050 (Makkar *et al.*, 2014), cannot be overstated. Humanity faces a major challenge in climate change, compounded by reliance on limited

crop species, hindering food security solutions. Expanding the use of underused, nutritious, and climate-resilient crops like sorghum is a potential strategy. Sorghum is vital for food security, especially in arid and semiarid regions. It provides substantial agronomic advantages in adverse conditions, including drought resilience, high yield potential, low nutritional requirements, adaptation to low soil fertility, and cost-effective production methods (Adebowale *et al.*, 2020).

Sorghum bicolor (Sorghum seeds) have been shown to have nutritional value, yet anti-nutritional factors frequently prevent people from using them. Reduced nutritional value and decreased organoleptic qualities of sorghum grain are caused by a variety of antinutritional substances that form complexes with many other dietary components (Ogbonna *et al.*, 2012). Processing strategies have been reported by various investigators to minimize the antinutrients found in cereal seeds, improving their nutritional quality and organoleptic acceptability. A few of the often-employed processing methods are steam blanching, autoclaving, sprouting, autoclaving, roasting, dehulling, soaking or boiling with water, alkaline or acidic solutions, and fermentation (Abioye *et al.*, 2022; Burgos and Armada, 2019; Chauhan and Sarita, 2018). Therefore, the aim of this study was to investigate the potential of soaking, germination, and popping as cost-effective home-based technologies to improve the functional and nutritional attributes of sorghum seeds.

1.3 Objectives of the study

1.3.1 General objective

The general objective of the dissertation work was to study effect of soaking duration, germination duration and popping technique on nutritional and anti-nutritional properties of sorghum seeds.

1.3.2 Specific objective

a. To assess the nutritional and anti-nutritional composition of raw sorghum seed.

b. To undertake processing of sorghum by soaking time, germination time and popping respectively.

c. To determine changes in nutritional composition and anti-nutritional parameters after soaking, germination and popping**.**

1.4 Significance of the work

Researchers reported that the nutritional value and the functional properties of sorghum can be improved while anti-nutritional content of sorghum can be reduced by using low-cost household practices (Derbew and Moges, 2017). Germination of most cereals and legumes has shown a positive effect on nutrients in the human diet compared to raw food grains (Adedeji *et al.*, 2014). Soaking and germination at different conditions and time significantly improved the physicochemical and functional properties of horse gram flour (Handa *et al.*, 2017).

Sorghum is eaten in various traditional forms in different geographical regions, such as Kisra (fermented flatbread), Aceda (thick porridge), Nasha (thin fermented gruel), Abreh, and Hulu-mur (non-alcoholic beverages (Hassani *et al.*, 2014). It was also used to make milk-related drinks, low dietary bulk weaning, and additional balanced foods and bakery products (Singh *et al.*, 2017).

Because of its antioxidant qualities, epidemiological research indicates that eating whole grains, such as sorghum, lowers the risk of dying from cardiovascular disease (Awika and Rooney, 2004). Soaking, germination, and popping methods would minimize the antinutrients present insorghum, increase protein digestibility and mineral bioavailability, and help in utilizing thenutritional values. Due to the availability of diverse sorghum food products in different geographical regions of different parts of the world. Therefore, the results of this study mighthelp in the establishment of an effective and optimized way for the use of underutilized sorghum seed at household and industrial levels to address food insecurity with nutritive value.

1.5 Limitations of the study

The study has the following limitations:

a. Analysis of vitamins and trace elements, amino acid and fatty acid compositionof the product could not be performed due to time constraints.

Part II

Literature review

1.6 Sorghum

Sorghum bicolor (L.) Moench, also known as broomcorn, common wild sorghum, chicken-corn, and so on, is a versatile crop that is often used for fiber, fuel, feed, and food. It is one among the top five cereal crops grown worldwide and is frequently planted (Ananda *et al.*, 2020). This annual C4 plant can withstand high temperatures and dry weather. It is the most significant food crop for the world's semi-arid and dry regions because, compared to maize and rice, it is far more efficient at utilizing high solar radiation energy in tropical latitudes (Sage and Zhu, 2011).

1.6.1 Origin and historical background

Moench distinguished the genus Sorghum from the genus Holcus in 1794. Person proposed naming Holcus sorghum (L.) as Sorghum vulgare in 1805. Clayton gathered all the sorghums under the name *Sorghum bicolor* (L.) Moench, which he suggested as the proper name for farmed sorghum in 1961 (Clayton, 1961), which is currently being used. The precise date and place of sorghum's domestication are unknown since there is a lack of reliable data. The majority of accounts, however, indicate that domesticating sorghum began in Africa about 5,000 years ago. In the Ethiopia-Sudan area of northeast Africa, domestication is said to have occurred (Doggett, 1988). The most verified location and time for sorghum domestication is Kassala in Northeastern Sudan between 3500 and 1500 BP, despite the fact that there are several stories relating these sites requirement (Mullet *et al.*, 2014).

1.6.2 Sorghum Taxonomy

Kingdom – *Plantae*

Sub-kingdom –*Tracheobionta*

Superdivision – *Spermatophyta*

Division – *Magnoliophyta*

Class – *Liliopsida*

Sub-class – *Commelinidae*

Order – *Cyperales*

Family – *Poaceae* (grass)

Tribe – *Andropogoneae*

Sub-tribe – *Sorghinae*

Genus – *Sorghum*

Species – Sorghum bicolor

Sub-species – Sorghum bicolor ssp. arundinaceum – common wild sorghum

Sub-species – *Sorghum bicolor ssp.* bicolor – grain sorghum

Sub-species – *Sorghum bicolor ssp. drummondii* – Sudan grass

Species – *Sorghum almum* – Columbus grass

Species – *Sorghum halepense* – Johnson grassSpecies – *Sorghum propinquum*

Source: Hariprasanna and Patil (2015)

1.6.3 Classification and nomenclature

Snowden (1955) created the most comprehensive categorization of sorghum. He described 31 cultivated species and 17 related wild species (Fig. 2.1).

Based on agronomics, plant, and fundamental applications Johnson and Peterson (1974) categorized the farmed sorghum into four main categories:

a. Grain sorghum is a small variety designed mostly for grain production.

b. Sweet/ forage sorghum has tall, juicy, sweet stalks used to produce sugar, syrup, and fodder.

c. Grassy sorghum has many tillers, tiny leaves, and slender stems that make them ideal forhay or cattle grazing.

d. Broom corns has lengthy panicle branches that are helpful for making brooms.

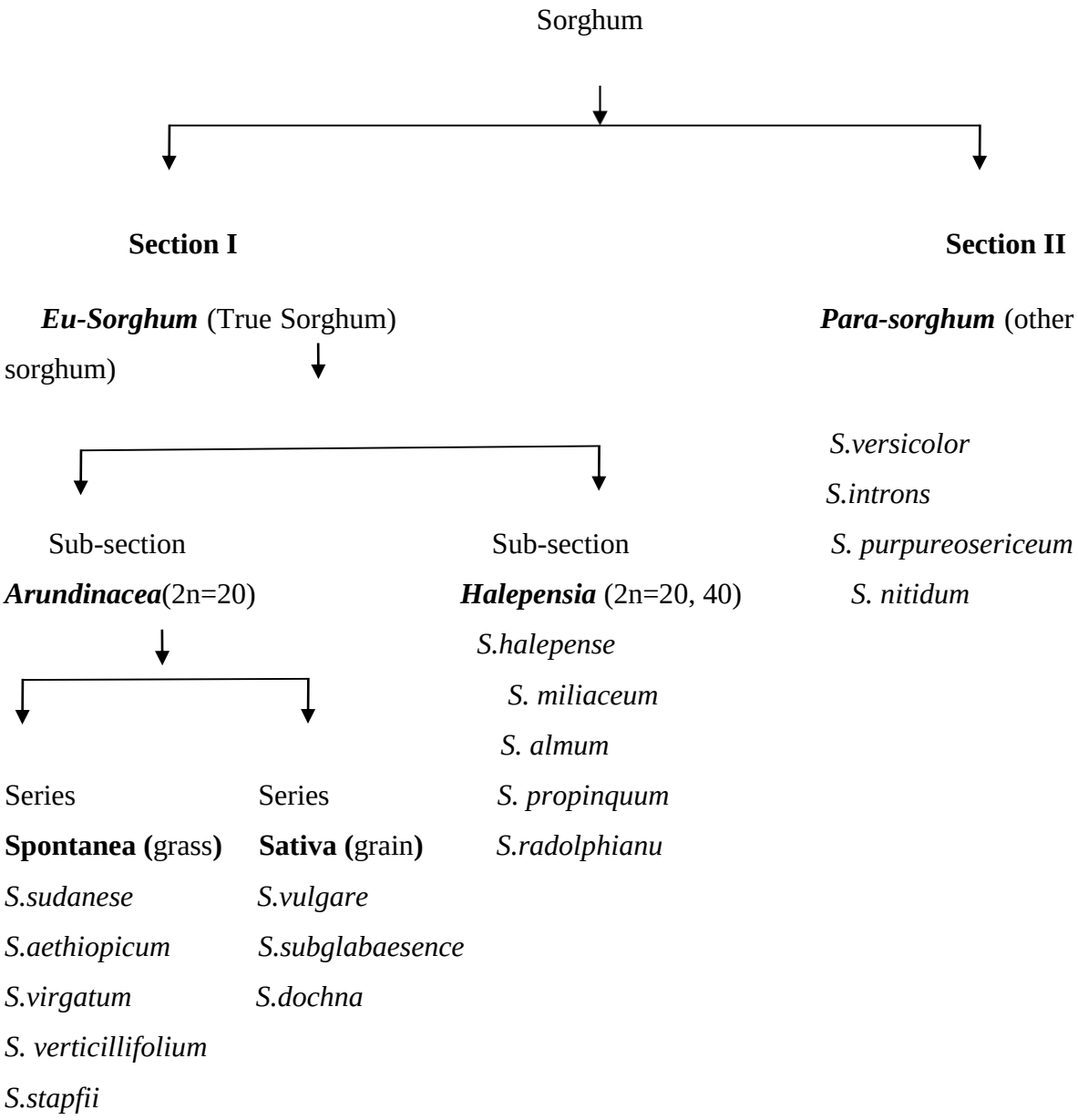

Fig. 0.1 Classification of Sorghum

Source: Hariprasanna and Patil (2015)

1.6.4 Morphology of Sorghum grain

For more than five thousand years, sorghum has been grown in Africa and represents the main source of carbohydrates for at least 20 million people. Grain sorghum will take from a tight-headed, round panicle to an open, droopy panicle that can be short or tall, in several shapes and sizes. Various types of sorghum are available including red, orange, bronze, brown, white, and black sorghum. The influence of various physical and biochemical

features of sorghum grain on the quality of traditional foods has been identified (Rami *et al.*, 1998). Sorghum is a strong grass that typically grows to a height of 0.6 to 2.4 m (2 to 8 feet), often reaching 4.6 m (15 feet) above sea level. Stalks and leaves are covered with white wax, and the stalks of some varieties are juicy and sweet in the pith or central part. The leaves are approximately 5 cm broad and 76 cm (2.5 feet) long. The small flowers are produced in panicles ranging from loose to dense; 800–3000 kernels are kept in each cluster of flowers. The seeds vary widely in color, shape, and size among different types, but they are smaller than wheat ones (Hassani *et al.*, 2014).

Sorghum is one of the most important cereal crops in the world and a vital species for maintaining food security worldwide. The grain sorghum belongs to the grass family *Poaceae* (Gramineae). Within the family *Poaceae*, sorghum is classifed in the genus *Sorghum* and is native to Ethiopia in the Horn of Africa (Dillon *et al.*, 2007). The stalk of sorghum is thick, erect, 3–5 m high, and 2–5 cm in diameter, with supporting roots on the base section. The leaf sheath is glabrous or slightly whitely powdered; the ligule is hard and membranous, with a rounded apex and cilia at the margins; the leaf blade is linear to linear-lanceolate, 40–70 cm long, 3–8 cm wide, acuminate at the apex, dark green on the surface, and light green or white powder on the back, with a small sting and wider midribs (Xu, 2019). The caryopsis is convex on both sides, pale red to reddish-brown, slightly exerted at the top, 3.5–4 mm long, and 2.5–3 mm wide at ripening. The petiolate spikelet is approximately 3–5 mm in length, linear to lanceolate, brown to copper in coloring, and fruiting occurs from June to September (Xu, 2019). Furthermore, there are five distinct morphological races of sorghum: durra, kafir, bicolor, guinea, and caudatum .All ofthe sorghum races are different from one another, as shown by genetic tests (Taylor, 2019).All of the sorghum's domesticated races have emerged and spread independently. It is reported that the most primitive race of sorghum is bicolor, and all other races originated from it (Kimber, 2000).

1.6.5 Structure of the sorghum grain

Analyzing a microscopic perspective of a mature sorghum kernel enables the differentiation between its outer seed covering, known as the pericarp, the embryo or germ, and the endosperm (Wall and Blessin, 1969). The structure of sorghum is shown in Fig. 2.3

1.6.5.1 Pericarp

The pericarp accounts for 4.3% to 8.7% of the sorghum caryopsis (Waniska and Rooney, 2000). Its thickness ranges from 8 to 160 μm and varies within individual mature caryopses (Earp *et al.*, 2004). The pericarp is subdivided into three tissues: epicarp, mesocarp, andendocarp. The epicarp is typically pigmented and covered with a thin layer of wax. The sorghum mesocarp contains starch granules, which is a unique characteristic of sorghum (Serna-Saldivar *et al.*, 1994). The tube cells, integral to the pericarp, facilitate water conduction during germination, whereas the cross cells constitute a layer that restricts moisture loss. The pericarp comprises roughly 5% to 8% of the grain protein (Waniska and Rooney, 2000).

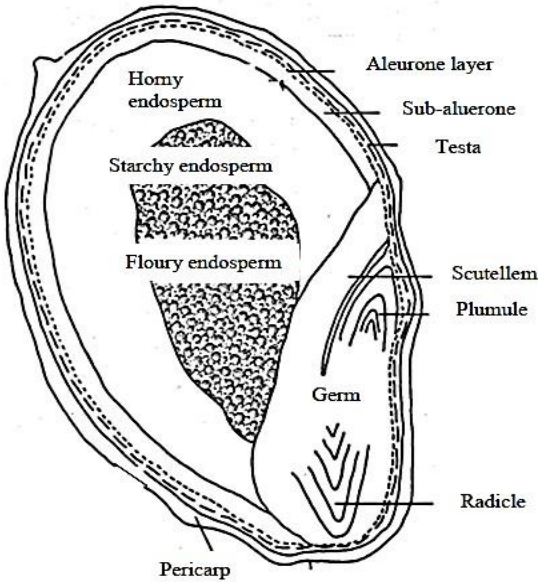

Fig. 0.2 Structure of Sorghum grain

Source: Sautier and O'Deye (1989)

1.6.5.2 Testa

Certain sorghum cultivars exhibit a pigmented sub-coat, known as the testa, situated between the pericarp and the endosperm as shown in Fig. 2.3 (Earp *et al.*, 2004) . The pigmented testa contains tannins (proanthocyanidins) (Waniska and Rooney, 2000). Tannins serve to shield the grain from insects, birds, and fungal threats; however, condensed tannins are linked to nutritional drawbacks and a decline in food quality (Serna-Saldivar and Rooney, 1995). The nutritional disadvantages of sorghum tannins lie primarily in their ability to form poorly digestible complexes with dietary protein (Butler *et al.*, 1984).

1.6.5.3 Aluerone layer

The endosperm comprises an outer single-cell layer of aleurone tissue. Aleurone cells are abundant in oil, protein, and ash (Wall and Blessin, 1970).

1.6.5.4 Endosperm

The endosperm constitutes 82% to 87% of the sorghum grain (Waniska and Rooney, 2000). It is composed of peripheral, and floury and corneous (horny, vitreous, glassy) areas as shown in Fig. 2.3 (Serna-Saldivar *et al.*, 1994). The peripheral region has several layers of dense cells containing more protein bodies and smaller starch granules than the corneous area. The peripheral and corneous areas affect processing and nutrient digestibility (Waniska and Rooney, 2000). In a review of the composition of the sorghum endosperm cells, (Taylor *et al.*, 2006) noted that both the floury and corneous endosperm cells are composed of starch granules, protein matrix, protein bodies and the cell walls are predominated by water insoluble glucuronoarabinoxylans (GAX). The endosperm contains approximately 81% of sorghum protein (Waniska and Rooney, 2000). In typical sorghum cultivars, the majority of proteins in the endosperm are prolamin, which are soluble in alcohol-water mixtures, along with limited amounts of glutelins, which are soluble in dilute acid and dilute alkali (Taylor and Schussler, 1986).

1.6.5.5 Germ

The portion of the sorghum grain that is alive is called the germ. It consists of two main parts: embryonic axis and scutellum as shown in Fig. 2.3. The embryonic axis contains the new plant. During germination and development, the radicle forms the primary roots while the plumule forms the shoot (Evers and Millar, 2002). The scutellum serves as the cotyledon and contains reserve nutrients, including a moderate quantity of oil, protein, enzymes, and minerals. It also functions as a link between the endosperm and the germ (Waniska and Rooney, 2000). The germ contains approximately 15% of the protein in sorghum. It is rich in albumin (water-soluble) and globulins (soluble in dilute salt solution) which are rich in lysine and other essential amino acids (Taylor and Schussler, 1986).

1.6.6 Production of sorghum

Global demand for sorghum increased dramatically between 2013 and 2015 when China began purchasing US sorghum crops to use as livestock feed as a substitute for domestically grown corn. Globally, sorghum production was estimated at 59.72 million metric tons in

2023/2024. TheUnited States of America leads in total production with 8.07 million metric tons (14%), followed by Nigeria, Sudan, Ethiopia, and India, as indicated in Table 2.1, which provides an overview of worldwide production estimates for the specified period.

Table 0.1 World sorghum production 2023/2024

Countries	% of Global Production	Total Production (Metric Tons)
United States	14%	8.07 million
Nigeria	11%	6.7 million
Sudan	8%	5 million
Ethiopia	7%	4.4 million
India	7%	4.4 million
Mexico	7%	4.35 million
Brazil	6%	3.6 million
China	5%	3 million
Argentina	4%	2.5 million
Australia	3%	2 million
Others	28%	15.7 million
Total	100%	59.72 million

Source: (USDA, 2023)

1.6.7 Physical properties of sorghum

Sorghum is characterized by its naked kernel, free from hull. Varieties of sorghum exhibit considerable variation in size and shape. On average, a sorghum caryopsis (grain) measures around 4 mm in length, 2 mm in width, and weighs approximately 25 to 35 mg (Haussmann *et al.*, 1999). The shape of sorghum kernels ranges from obovoid to ellipsoid,with the 1000-kernel weight varying from 20 to 80 g (Serna-Saldivar and Rooney, 1995).The mean particle density and bulk density of sorghum grain were determined to be 1.02g/cm³ and 568.5 g/cm³, respectively. It was observed that the particle density of sorghum grain decreased with increasing moisture within the moisture range of 8.89% to 16.50% wb (Simonyan *et al.*, 2007). The sphericity of sorghum grain is recorded as 0.67. Additionally, the 1000-kernel weight is 32.41 g, bulk density is 69.9 kg/HL, particle density (specific gravity) is 1.18 g/cm³, and porosity is 40.80% (Ndirika and Mohammed, 2005).

1.7 Chemical and nutritional composition of Sorghum

The chemical composition of sorghum grains shown in Table 2.2

Table 0.2 Chemical composition of sorghum grains

Components		Sorghum grains (100g)
	Water (g)	12.4
	Energy (kcal)	329
	Protein (g)	10.62
	Total lipid (fat) (g)	3.46
Macronutrients	Ash (g)	1.43
	Carbohydrate (g)	72.09
	Fiber, total dietary (g)	6.7
	Sugars, total including NLEA (g)	2.53
	Starch (g)	60
	Calcium (mg)	13
	Iron (mg)	3.36
	Magnesium (mg)	165
	Phosphorus (mg)	289
Minerals	Potassium (mg)	363
	Sodium (mg)	2
	Zinc (mg)	1.67
	Copper (mg)	0.284

	Manganese (mg)	1.605
	Selenium (μg)	12.2
	Total ascorbic acid (C) (mg)	0
	Thiamin (mg)	0.332
	Riboflavin (mg)	0.096
	Niacin (mg)	3.688
Vitamins	Pantothenic acid (mg)	0.367
	Vitamin B-6 (mg)	0.443
	Folate, total (μg)	20
	α-tocopherol (E) (mg)	0.5
	Phylloquinone (K) (μg)	0

Source: USDA (2018)

Sorghum is utilized for various purposes, with its grains, rice bran, and stalks being the primary parts used. From a nutritional standpoint, sorghum grains typically contain 4.4–21.1% protein, 2.1–7.6% fat, 1.0–3.4% crude fiber, 57.0–80.6% total carbohydrates, 55.6–75.2% starch, and 1.3–3.5% total minerals (ash). Sorghum provides approximately 350 Kcal energy per serving and serves as a source of calcium, phosphorus, potassium, carotene, and thiamin. Additionally, it contains antioxidants such as phenolics and various types of tannins (Ratnavathi and Komala, 2016). From a botanical perspective, the sorghum kernel is classified as a dry, indehiscent, single seeded fruit. The caryopsis, or grain, consists of three primary components: the outer covering known as the pericarp, the storage tissue called the endosperm, and the germ (Johnson and Peterson, 1974).

1.7.1 Protein

The protein content of sorghum grain typically ranges from 11 to 13 % (Dixit *et al.*, 2011). The protein content and composition of sorghum grain can vary significantly depending on

factors such as genotype, rainfall, soil fertility, temperature, and ecological conditions during grain growth. Kafirins are the predominant prolamins found in sorghum grain. They are stored in the endoplasmic reticulum and are classified into three main types: alpha-kafirins (comprising 66-84%), gamma-kafirins (comprising 9-21%), and beta-kafirins (comprising 8-13%) (Mesa-Stonestreet *et al.*, 2010). However, kafirins are resistant to proteases due to the formation of intermolecular disulfide bands reducing the protein digestibility. Kafirins form complexes with tannins, and thus protein digestibility is reduced up to 50 per cent in dark color varieties (Janet Taylor *et al.*, 2007). The interaction of kafirins with other grain components, including starch, fibers, phytic acid, and lipids, also contributes to lower digestibility (de Morais Cardoso *et al.*, 2017). Some processing methods such as germination and fermentation can be used to enhance digestibility. In terms of amino acids, glutamic acids and non-polar amino acids (proline, leucine and alanine) are the most important in sorghum, but lysine, methionine, cysteine, isoleucine, valine and threonine are deficient (Mesa-Stonestreet *et al.*, 2010).

1.7.2 Polysaccharides

Sorghum cereal is a great source of carbohydrates, which have a lot of industrial application potential and influence the nutritional, physical, and chemical characteristics of final foods. Pigments in the pericarp and endosperm of the grain have been connected to the pink color of sorghum starch. 81 to 96.5% of the mixture is made up of amylopectin, while 3.5 to 19% is made up of amylose (Hill *et al.*, 2012). The amylose and amylopectin ratio are highly affecting the rheological properties and starch digestibility. The complex formation between the starch granules and tannins is responsible for reduced starch digestibility (Barros *et al.*, 2012). Endosperm only includes 16.7-43.2 % of resistant starch, 30.0-66.2 % of slowly digested starch, and 15.3-26.6 % of rapidly digested starch. The two non-starch polysaccharides are insoluble (75.0-90.0 %) and soluble fibers (10.0-25.0 %) (Mkandawire *et al.*, 2013).

1.7.3 Lipids

The fat content in sorghum ranges from 3.20 to 3.90g per 100g, which is relatively high compared to other common cereals such as wheat, rice, and maize. Sorghum contains approximately 83-88 % unsaturated fatty acids. The various fatty acids present in sorghum include linolenic acids (1.4-2.8%), palmitic (12.4-16.0%), oleic (32.2-42.0%), and linoleic

(45.6-51.1%) (Hadbaoui *et al.*, 2010).

1.7.4 Vitamins and minerals

Regarding vitamins, sorghum is a great source of B complex vitamins (thiamine, riboflavin, and pyridoxine) and fat-soluble vitamins (D, E, and K), with the exception of B12 (de Morais Cardoso *et al.*, 2017). The decortication process, which involves removing the outer layers of the grain, can result in a reduction of vitamins stored in the aleurone layer and germ. However, sorghum remains rich in important minerals such as potassium, magnesium, zinc, copper, iron, and phosphorus (Shegro *et al.*, 2012). It has been observed that the polyphenols and phytates contained in sorghum possess an adverse effect on the bioavailability of minerals. Zinc availability varies from 9.7% to 17.1%, whereas iron availability varies from 6.6% to 15.7% (Kruger *et al.*, 2013).

1.7.5 Antioxidant activity

Compared to rice, wheat, barley, maize, rye, and oats, sorghum was shown to have higher levels of phenolic chemicals (Khan *et al.*, 2013). Almost all classes of phenolics are present in all varieties of sorghum, but the main classes are phenolic acids, flavonoids and tannins (Gaytán-Martínez *et al.*, 2017). The high antioxidant activity of phenolic compounds in sorghum grain is attributed to their capacity to eliminate free radicals. This antioxidant potency is directly linked to the concentration of phenolic compounds within a particular sorghum variety, which is influenced by both its genetic makeup and the conditions of its growth environment (Dykes *et al.*, 2005).

1.7.5.1 Phenolic acids

The dominant class of phenolics in sorghum are phenolic acids, tannins, and flavonoids (de Morais Cardoso *et al.*, 2017). Plants contain aromatic secondary metabolites called phenolic acids. The phenolic acid content of sorghum grain ranges from 135.5 to 479.40 mg/g (Chiremba *et al.*, 2012), categorized as hydroxybenzoic acid derivatives and hydroxycinnamic acid derivatives. Phenolic acids in sorghum are primarily bound to arabinoxylans chains or lignin and cannot be easily hydrolyzed by in vivo digestive enzymes, leading to reduced bioavailability. However, studies have demonstrated that fermentation with specific probiotics and certain cooking processes can enhance the bioavailability of phenolic acids (Barros *et al.*, 2013).

Colored sorghum grains, particularly brown and red varieties, produce foods that are richer in polyphenols, flavonoids (including anthocyanins), and tannins compared to wheat, barley, millet, and rye (Ragaee *et al.*, 2006). These grains have shown strong free radical scavenging and anti-inflammatory properties (Birhanu, 2021).

1.7.5.2 Flavonoids

The bioactive class of chemicals known as flavonoids has several benefits for health. Flavonoids are located in the outer layer providing the color to the grain and flavonoid amount along with nature is dependent on the thickness of the pericarp (Dykes *et al.*, 2009). Sorghum cereals contain three major classes of flavonoids, i.e. flavones, flavanones and anthocyanins. Sorghum flavanone is high in varieties with lemon-yellow pericarp (474 to 1,780 mg/g) but is less in white value (Dykes *et al.*, 2011). The aglycone forms of eriodictyol and naringenin are the most prevalent flavanones (Dykes *et al.*, 2011). The aglycone forms of flavones and flavanones are rapidly absorbed (Jiang *et al.*, 2016). Sorghum anthocyanin represents 79 % of the total flavonoids and belongs to the class of 3-deoxyanthocyanidins and is responsible for the color and antioxidant activity of grain Cultivars with pericarp and black testa contain about three-four times more 3-deoxyanthocyanidins (5.4-6.1 mg/g) than brown and red varieties (1.6-2.8 mg/g) (Awika *et al.*, 2004). Different types of anthocyanins like luteolinidin (0-282 mg/g), 5- methoxyluteolinidin (0-154 mg/g), 7-methoxyapigeninidin (0-137 mg/g) and apigeninidin (0- 166 mg/g) have been reported in sorghum kernels from different cultivars (Zhu, 2018).

1.8 Anti-nutritional factors

Anti-nutritional factors are substances produced in plants that inhibit nutrient absorption or have negative effects on metabolic pathways, thereby reducing nutrient availability and adversely affecting overall nutritional value (Chhikara *et al.*, 2018). Antinutritional components found in sorghum include tannin, phytic acid, protease and trypsin inhibitors, cyanogenic glucosides, and oxalates (Ojha *et al.*, 2018).

1.8.1 Phytic acids

Phytic acid, or phytate, is recognized as the primary form of phosphorus in cereals. It is considered a food inhibitor because it chelates micronutrients, thereby reducing their bioavailability. The phytate content in sorghum was found to be in the range of 556.52 to 606.07 mg/100 g dry matter of raw sorghum (Afify *et al.*, 2012c), and in sorghum flour, the

content was found to be 317.65 mg/100g (Mohammed *et al.*, 2011). Phytic acids are known to inhibit the absorption of zinc and iron. Research has indicated that in developing countries, where diets are predominantly composed of cereals rich in phytates, this contributes to poor iron and zinc status, particularly in pre-school children (El-Beltagi *et al.*, 2012).

1.8.2 Oxalates

Oxalates, also known as oxalic acids, and their salts, such as calcium and magnesium oxalates, are metabolic end products found in various plant tissues. The content of oxalates in sorghum flour was determined to be 1.12 mg/g (Ojha *et al.*, 2018). Oxalates combine with calcium and form a calcium oxalate complex in the intestinal lumen which makes calcium unavailable for absorption. Accumulation of calcium oxalates may cause kidney stones when the acid is excreted through urine.

1.8.3 Cyanogenic glucosides

Sorghum contains cyanogenic compounds known as dhurrin, which can produce a toxic substance, hydrogen cyanide (HCN), upon hydrolysis (Etuk *et al.*, 2012). The majority of dhurrin, the cyanogenic compound in sorghum, is stored in the aerial shoot of the plant. The concentration of hydrogen cyanide (HCN) in sorghum varies depending on growth conditions and cultivars but typically decreases with plant age. Oduguwa and Fafiolu (2004) reported a HCN content of 15.18 g/kg in Nigerian malted sorghum sprouts. In whole sorghum flour, the level of HCN was found to be 15.16 mg/100g (Ojha *et al.*, 2018). Cyanide toxicity may result in various neuropathies and amblyopia.

1.8.4 Protease inhibitors

Protease inhibitors are anti-metabolic proteins that disrupt protein digestibility and are synthesized in response to insect attacks. Trypsin and chymotrypsin inhibitors are the primary protease inhibitors found in sorghum. The mean trypsin and chymotrypsin inhibitors activity in six sorghum varieties was found to be 14.32 TIU/mg (trypsin inhibiter unit) protein and 4.46 CIU/mg proteins, respectively (El-latif, 2014). Another study showed that trypsin and protease inhibitors in sorghum are 0.366 and 1.750 mg/g, respectively (Adeyemo *et al.*, 2016).

1.8.5 Nitrates

In saliva, nitrate is changed to nitrite. When it enters the stomach's acidic environment, it

becomes nitrous acid and breaks down spontaneously to produce nitrogen oxides, which include nitric oxide (Hmelak and Cencic, 2013). The primary harmful effect of nitrite is its ability to react with hemoglobin, forming methemoglobin and nitrate. The oxygen distributed to tissues will be reduced because availability of hemoglobin responsible for oxygen transport is altered. However, nitrates are considered as essential nutrients for cardiovascular health by some individuals, as they enhance nitric oxide production (Bryan *et al.*, 2012).

1.8.6 Tannins

Tannins are the secondary metabolites distributed throughout the plants, serving a defensive purpose against predators and pathogens due to their protective mechanisms. The amount of tannins in dark grains tends to be higher compared to pale grains, and it is also influenced by environmental factors (Mkandawire *et al.*, 2013). The sorghum tannin ranges from 0.2 to 48.0 mg/g (Dykes *et al.*, 2014), and a huge variation in the tannin content might be due to cultivars effect, as well as environmental factors. Tannins found in sorghum come in a condensed form. Sorghum varieties containing these tannins exhibit greater antioxidant potential compared to those without tannins. This heightened capacity is attributed to their ability to effectively trap free radicals (Awika and Rooney, 2004).

Various genotypes of sorghum, each with diverse phenol profiles, have been identified as potent scavengers of free radicals (Sorour *et al.*, 2017). Tannins have the capacity to inhibit the activity of certain digestive enzymes, consequently impacting protein digestibility and cellulose hydrolysis negatively. Polymeric tannins exhibit a strong interaction with amylose granules, leading to the formation of resistant starch. Depolymerization of tannins results in an increase in the digestibility of starch, minerals, and proteins. Dry heat processing, such as heating at 121°C for 30 minutes or 95°C for 20 minutes, can depolymerize condensed tannins, thereby improving the nutritional bioavailability of sorghum (Barros *et al.*, 2012).

1.9 Adverse effect of anti -nutrients on health

Sorghum contains a wide range of antinutritional factors, including phytic acid, tannins, trypsin inhibitors, protease inhibitors, cyanogenic glucosides, and oxalates. These compounds have the potential to exert adverse effects on human health (Table 2.3) (Ojha *et al.*, 2018).

Table 0.3 Adverse effect of antinutrient on human body

Anti-nutrient	Effects on body
Phytates	Reduce Ca and Fe absorption, forms complexes with metal ions and inhibit their absorption, a key component of crops that causes zinc deficiency.
Oxalates	Reduce Ca absorption, encourage kidney stone formation.
Cyanide	Respiratory inhibitors.
Lectins (hemagglutinins)	Impaired growth, Hypertrophy and hyperplasia of pancreas, prevent absorption of digestive end products in the small intestine.
Protease inhibitors	Reduce protein digestion, retardation of growth.
Phenol compounds	Reduce bioavailability of some minerals (especially zinc), may negatively affect pH mechanism, reduce protein digestion, loss of appetite, breathing problems, cardiac complications.

Source: Gemede and Ratta (2014) and Muramoto (2017)

1.10 Ways to inactivation of anti-nutrients in sorghum seed

Various traditional methods and technological processing techniques, including milling, soaking, germination, popping, fermentation, cooking, and boiling, have been employed to reduce the levels of anti-nutritional components in foods.

1.10.1 Milling

Milling is indeed the most traditional method used to separate the bran layer from grains. It is the method used to grind grains into flour. The milling process eliminates anti-nutrients found in grain bran, such as lectins, tannins, and phytic acid, but it also removes vital minerals, which is a major drawback (Gupta *et al.,* 2015). Mahgoub and Elhag (1998) discovered that the milling process has the capability to decrease the levels of phytic acid in sorghum seeds from four Sudanese sorghum cultivars. However, they observed that this reduction was not as significant as that achieved through enzymatic methods such as fermentation and malting.

1.10.2 Soaking

Soaking treatment involves completely submerging the grains in water for a specific duration. This process results in the inactivation of enzymes, particularly endogenous phytase. It is often used for beans, grains and seeds. During soaking, water is absorbed by the cells of the grains, leading to changes in pH. The effects of the soaking process are influenced by factors such as duration, temperature, and the pH of the soaking water (Raes *et al.*, 2014). Soaking is also commonly required for fermentation, which can also be used to reduce the level of various anti-nutrients in foods (Gupta *et al.*, 2015).

Many of the anti-nutrients are water soluble in nature, which enhance their removal from foods through leaching. Soaking generally increases the hydration level of legumes and cereals, which make them soft and also activate an endogenous enzyme like phytase to enhance ease of further processing such as cooking or heating (Udensi *et al.*, 2010). Research carried out by Ayuba *et al.* (2020) showed that soaking white variety of sorghum for 14 hrs. reduced tannin content from 18.46 to 14.46 mg/100g, cyanide content from 0.027to 0.025 mg/100g, nitrate content from 34.69 to 20.82 mg/100g and phytate content from 160.16 to 84.08 mg/100g.

Similarly, Afify *et al.* (2012a) reported that soaking three white varieties of sorghum (Shandaweel-6, Dorado and Giza-15) for 20 hrs. decreased tannin content from 1.39, 1.99 and 21.79 to 1.29, 1.72 and 15.17 mg/100 g dry wt. respectively. The soaking of two sorghum cultivars Assuit 14 and Giza 15 grown in southern Egypt in (distilled water, KOH 2%, NH_4OH 30%, and NaOH 2%) had significant reduction of tannin and phytic acid content (Sorour *et al.*, 2017). Due to soaking, activity of phytase increased, which reduced the phytate component present in the grains. As a result of soaking and fermentation, phytochemicals are reduced due to leaching of water-soluble vitamins and minerals in grains and legumes (Kruger *et al.*, 2014; Ogbonna *et al.*, 2012).

1.10.3 Germination

A common process in legumes and grains, germination increases the nutritional content and palatability of the food, especially by breaking down specific antinutrients such phytic acid and protease inhibitors. Wheat grains are traditionally processed by germination and fermentation prior to consumption. The hydrolysis rate of the phytic acid varies according to the species and the variety, as well as the stage of germination, pH, moisture content,

temperature (optimum range 45–57 °C), solubility of phytic acid and the presence of certain inhibitors (Afify *et al.*, 2011). Germination is also widely regarded as a highly effective method for decreasing the presence of anti-nutrient compounds in plant-based foods (Nkhata *et al.*, 2018). Germination of seeds generally activates the enzyme phytase, which degrades phytate and leads to decreased phytic acid concentration in the samples. Germination commonly changes the nutritional level, biochemical property and physical features of the foods. For reduction of cereals anti-nutritional content, this method is most frequently used (Laxmi *et al.*, 2015; Oghbaei and Prakash, 2016). Germinated cereals showed enhanced activity of phytase-degrading enzyme while in non-germinated cereals the endogenous activity of phytase enzyme was observed in diminished amounts (Vashishth *et al.*, 2017).

Afify *et al.* (2011) conducted research on the impact of germination on phytate levels in sorghum grains. Their findings revealed that germinating sorghum seeds for 72 hours following a 20-hour soaking period led to a decrease in phytate content and an increase in the in vitro bioavailability of iron and zinc. The phytate content was notably reduced from 24.92% to 35.27% after undergoing the germination process. Liang *et al.* (2008) conducted a study focusing on the effects of germination to diminish the phytic acid content while maintaining adequate zinc levels, with the aim of enhancing its bioavailability. They discovered that extending germination durations at 30°C (12, 24, 36, 48, and 72 hours) resulted in an increase in phytic acid reduction ranging from 4% to 60% (12.9 ± 1.3, 11.4 ± 2.7, 7.6 ± 1.2, 6.0 ± 1.0, and 5.7 ± 0.9, respectively). Azeke *et al.* (2011) examined the phytic acid levels in various cereal grains, including rice, maize, millet, sorghum, and wheat. Their analysis revealed that subjecting the grains to a 10-day germination period resulted in a notable decrease in phytic acid content, with statistical significance ($P < 0.05$).

1.10.4 Popping

Popping is a traditional and cost-effective processing method that can be readily utilized to enhance the nutritional quality of grains (Hoseney *et al.*, 1983). Popping is a technique where kernels are subjected to heat until internal moisture expands, causing the kernel to burst through its outer shell (Mishra *et al.*, 2014). The pericarp and outer layers of the kernel play a direct role in the popping process by acting as a vessel that contains the endosperm. This method involves high temperature for a short duration (HTST), which sterilizes the product, gelatinizes its starch, enhances its aroma, and produces a ready-to-eat food at a minimal processing expense (Reddy *et al.*, 1991).

Popping contributes to enhanced starch digestibility by facilitating starch gelatinization and dietary fiber degradation (Nyman *et al.*, 1987). Popping methods can be achieved using various dry heat methods such as sand roasting, salt roasting, gun puffing, hot oil frying, or utilizing heat mediums like hot air or microwave radiation (Jaybhaye *et al.*, 2014). In India, as reported by Hoke *et al.* (2005) the most common method involves puffing in hot sand (with a temperature around 250°C) or in oil (ranging from 200-220°C).

The popping process serves to decrease antinutrients like phytates and tannins, enhance mineral bioavailability, impart a pleasing texture to the product, and aid in the digestion of proteins and carbohydrates (Piłat *et al.*, 2016). In additional research, popping sorghum resulted in a reduction of phytic acid content by 20–35%. This method offers an intriguing option as a standalone food or as an ingredient for creating other products like cereal bars (Liopart and Drago, 2016; Saravanabavan *et al.*, 2013)

1.10.5 Fermentation

Fermentation is a metabolic process in which carbohydrates are oxidized to release energy. It is a desirable process of biochemical modification of primary food matrix brought about by microorganisms and their enzymes and it is used to enhance the bioaccessibility and bioavailability of nutrients from different crops. In addition, it improves organoleptic properties as well as extending the shelf life (Nkhata *et al.*, 2018). Makokha *et al.* (2002) found that the fermentation at 96 h caused a decrease in phytic acid in sorghum. On the other hand, it decreased in finger millet after 72 and 96 h fermentation (54.3 and 72.3%, respectively).

The fermentation process can reduce the antinutritional factors present in sorghum, and thus enhance the quality of the cereal. The antinutritional factors in sorghum are also linked with low protein digestibility and mineral absorption. Ojha *et al.* (2018) reported that fermentation can reduce phytate, oxalate, tannins and hydrogen cyanide. Fermentation of the sorghum flour improves the protein solubility in acidic pH (pH 2-4) (Elkhalifa *et al.*, 2005). Fermentation is such an important process, which significantly lowers the content of anti-nutrients such as phytic acid, tannins, and polyphenols of cereals (Simwaka *et al.*, 2017). Fermentation also provides optimum pH conditions for enzymatic degradation of phytate, which is present in cereals in the form of complexes with polyvalent cations such as iron, zinc, calcium, magnesium and proteins. Such a reduction in phytate may increase the amount

of soluble iron, zinc, calcium several folds (Gupta *et al.*, 2015). A study by Samia *et al.* (2005) reported that fermentation and germination could enhance the nutritional level of cereals and legumes by altering the chemical composition and reduce the level of anti-nutritional factors.

1.10.6 Pressure cooking and autoclaving

Pressure cooking is the process of cooking food, which uses water or another cooking liquid, in a sealed container known as a pressure cooker. This type of heat treatment requires less cooking time and weight/volume ratio than boiling cooking (Burgos and Armada, 2019). Autoclave is an application, which is generally used for heat treatments. When this application is used on cereals and other plant-based foods, it activates the phytase enzyme as well as increases acidity (Ertop and Bektaş, 2018). Most of the foods showed health benefits when consumed after autoclaving. For example, boiling of food grains reduced anti-nutrients content, which improved their nutritional value (Rehman and Shah, 2005).

Food grains are generally cooked by boiling or by using a pressure cooker prior to consumption. Previous studies also reported that boiling or cooking of sorghum grains highly improved the nutritional value of foods by reducing their antinutritional as trypsin inhibitor and cyanogenic glucoside (hydrogen cyanide) contents (Boniface and Gladys, 2011). Another study by Wedad *et al.* (2008) reported that phytate and tannins concentration drastically decreased in sorghum grains when they were treated with fermentation followed by cooking. Most of the previous studies concluded that autoclaving is the best method to reduce levels of several anti-nutritional compounds when compared to other processing methods (Doss *et al.*, 2011; Shimelis and Rakshit, 2007; Vadivel *et al.*, 2008).

1.10.7 Boiling

Boiling is the act of cooking food in water that is bubbling vigorously at a normal atmospheric pressure (100 °C). However, there are some factors that influence the boiling point of water such as altitude of place. Thus, at higher altitude, lower boiling point of water (Nzewi and Egbuonu, 2011).

Boiling is also effective at eliminating the anti-nutritional factors in foods. The cooking time depends on variety of cereals (structure of grain, permeability for water), particle size and pretreatment of the cereal (soaked, pre-cooked) (Nzewi and Egbuonu, 2011). Towo *et*

al. (2006) studied the effect of boiling on phenolic groups of red sorghum and finger millet grains. This treatment reduced total phenolic levels between 79 and 40%, respectively. Other researchers reported a 54% reduction in the proanthocyanidin levels of sorghum grains (Bvochora *et al.*, 2005). Adebooye and Singh (2007) established that the losses of phenolic compounds by cooking can be due to outright destruction or breakdown or conversion of phenolics to other compounds during cooking, or it could also be attributed to possible labile nature of phenolics and its subsequent escape as vapour during cooking.

1.10.8 Roasting

Roasting is a simple and commonly used household technology for cooking food by dry heat for short periods of time. It uses an uncovered pan without water to produce a well-browned exterior and a moister-cooked interior. This treatment improves the edibility and digestibility of grains, reduces the anti-nutrients and the loss of nutritious components (Burgos and Armada, 2019). Kaur *et al.* (2012) reported a maximum degradation in phytic acid (52%), total phenolics (56%), oxalates (63%) and trypsin inhibitor activity (TIA) (80%) content at 110 °C for 25 min. Kaur *et al.* (2015) discovered that the decrease in phytic acid content observed during roasting treatments could be attributed in part to the sensitivity of phytic acid to heat and the formation of insoluble complexes between phytic acid and other constituents.

With respect to oxalate content, authors observed a maximum reduction (58.42%) when samples were subjected at 100 °C for 25 min. The oxalate content did not show significant reduction during the roasting process at higher temperatures. This may be due to concentration of oxalate in the bran due to faster rate of moisture loss from the brans. Also, roasting has a significant impact on trypsin inhibition (Kaur *et al.*, 2012).

1.11 Health benefits of Sorghum

The potential health advantages of sorghum are often linked to its abundance of phytochemicals, including phenolic acids, tannins, anthocyanins, phytosterols, and polycosanols. Sorghum exhibits higher antioxidant activity compared to other cereals, which may contribute to a decreased risk of certain cancers, cardiovascular diseases, diabetes, and obesity (de Morais Cardoso *et al.*, 2017).

1.11.1 Cardiovascular diseases

The effects of sorghum consumption on cardiovascular diseases are linked with the action of phytosterols, polycosanols and phenolic compounds which regulate synthesis, absorption and excretion of cholesterol. A cholesterol lowering was reported when a guinea pig is fed with 58 per cent low-tannin sorghum grains (Duodu *et al.,* 2003). This response was found to be great compared to wheat, rolled oats or pearl millet. Another study confirmed the hepatic and plasma cholesterol reduction when normolipidemic hamsters are fed with sorghum lipids (Hoi *et al.,* 2009). The cholesterol absorption is inhibited by the action of the bioactive phytosterols present in the sorghum lipid. These compounds inhibit the incorporation of cholesterol into the micelle causing a reduction in the amount of cholesterol and captured from the gut entrecote, thus lowering its absorption (Althwab *et al.,* 2015). Hoi *et al.* (2009) showed that cholesterol and its metabolites excretion can be enhanced in normolipidemic hamsters fed with sorghum lipids. It has been demonstrated that rats fed with freeze-dried extracts of phenolic compounds of sorghum (50 to 600 mg/kg for 14 days) result in a decrease in plasma cholesterol and triacylglycerol concentrations (El-Beltagi *et al.,* 2012).

The positive effects of sorghum on Cardiovascular diseases (CVDs) are not only limited on cholesterol management. It has been reported that the activity of the angiotensin I converting enzyme can be inhibited by sorghum a-kafirins in competitive and non-competitive ways (Kamath *et al.,* 2007). Hemoglobin-catalyzed oxidation of linoleic acid in cultured mullet fish can be inhibited by 63-97 % due to dietary tannin-sorghum distillery residues. This reduction is high compared to that of tannin from soybean (13 %) and rice bran (78 %) (Lee and Pan, 2003). The antioxidant activity of tannins and other polyphenols was associated with regulation of blood fluidity and prevention of RBC hemolysis (Awika *et al.,* 2004).

1.11.2 Cancer

Awika *et al.* (2005) found that sorghum tannins have the anti-carcinogenic activity against melanoma and melanogenic effects. The carcinogenic effect in humans is due to the activity of phase I (cytochrome P-450) and phase II enzymes (Bavei *et al.,* 2011). The phenolic compounds (3-deoxyanthocyanidins) present in sorghum modulate expression of phase II enzymes are responsible for regulation of the defense system by conversion of highly

reactive electrophilic species into non-toxic and excretable metabolites (de Morais Cardoso *et al.*, 2014). This effect is caused by an increase of nicotinamide adenine dinucleotide hydrogen: quinone oxyreductase (NQO) activity (Awika *et al.*, 2009). Black sorghum varieties have high 3-deoxyanthocyanidins which explains their high NQO activity. High consumption of sorghum has been correlated with low risk of esophageal cancer in many parts of the world, including Africa, Russia, India, China, Iran, etc. (Stefoska-Needham *et al.*, 2015). Another epidemiological study performed over six years on 21 communities from Sachxi Province in China confirmed that mortality from esophageal cancer in regions with high sorghum consumption was reduced (Duodu *et al.*, 2003).

1.11.3 Diabetes

Animal studies have shown that phenolic compounds from sorghum have strong effects on plasma insulin and glucose, resulting in hypoglycemic effect similar to glibenclamida (medication used in control group) (Chung *et al.*, 2011). Sorghum phenolic compounds can inhibit in vitro B. stearothermophilus a-glucosidase, as well as human pancreatic and salivary a-amylase enzymes which reduce the rate of digestion of glucose. Another study demonstrated that phenolic compounds from sorghum can increase insulin concentration in diabetic mice and shows better functioning of pancreatic b cells (Chung *et al.*, 2011). Recent research showed the effect of the sorghum grain extract on hepatic gluconeogenesis enzymes in streptozotocin-induced diabetic rats (Kim and Park, 2012). This effect is due toinhibition of expression of phosphoenolpyruvate carboxykinase and the phosphor p38 ratio,resulting in reduction of glucose concentration.

1.11.4 Obesity

Studies have demonstrated the effect of sorghum tannins on reduction of weight gain in rats, pigs, rabbits and poultry (Muriu *et al.*, 2002). Barros *et al.* (2013) reported that polymeric tannins from sorghum can react with amylose to form resistant starch which are not digested in small intestine and reach the large intestine giving health benefits of dietary fibers. In addition, sorghum tannins can inhibit saccharase and amylase enzymes, thus reducing starch digestibility (Mkandawire *et al.*, 2013).

1.11.5 Gastrointestinal tract

The natural microbial community present in the gastrointestinal tract gives important benefits for the host, such as protection against systemic diseases and infections, and health

professionals are recommending these for health betterment (Panghal *et al.*, 2018). Sorghum contains resistant starch and dietary fibers which have the ability to change the gut microbiota (Saballos *et al.*, 2012). Short-chain fatty acids produced during fermentation of resistant starch activate the chemo-protective enzyme and inhibit the growth of harmful bacteria in the colon (Niba and Hoffman, 2003). Studies have demonstrated that unabsorbed phenolic compounds and their metabolites can regulate gut microbial balance by stimulating the growth of good bacteria and inhibiting pathogenic bacteria with prebiotic like effects (Kumar *et al.*, 2012). A research study has been conducted to show the effect of sorghum lipid on gut microbiome. Supplementation of sorghum lipid increased the total bifidobacteria in hamsters, and this was linked with a rise in plasma HDL (Martínez *et al.*, 2009). Therefore, the results of this study suggested that the increase in HDL caused by sorghum lipids was due to alterations of gut microbiota.

1.11.6 Celiac disease

Celiac disease is an autoimmune disorder characterized by chronic inflammation of the small intestine due to gluten intolerance. In celiac patients, gliadin (subcomponent of gluten) produces toxicity due to transglutaminase enzyme reaction, leading to the activation of gluten-reactive T cells, and thus resulting in inflammatory reaction. So, gluten-free diet is suggested and is effective for celiac patients. The gluten level in sorghum flour was found to be less than 5 ppm which is low compared to the safe level (20 ppm) in celiac patients. Furthermore, Pontieri *et al.* (2013) demonstrated that the proteins similar to those present in wheat gliadins are absent in sorghum flour.

Part III

Materials and Methods

1.12 Materials

1.12.1 Sorghum

White sorghum was purchased from the local market of Dharan, Nepal.

1.12.2 Equipment and Chemicals

The following equipment and chemicals used were available in Central Campus of Technology (CCT). The list of chemicals and equipment used for the analysis is shown in Table 3.1 and Table 3.2 respectively.

Table 0.1 List of chemicals used

Chemical Specification	Supplier/Manufacturer	Other Specification
Boric acid	Merck (India) Limited	Amorphous
Hydrochloric acid (HCL)	Thermo Fisher Scientific IndiaPvt. Ltd.	36%, LR grade
Nitric acid	Thermo Fisher Scientific India Pvt. Ltd.	68-75% Assay
Potassium Permanganate	Avantor Performance Materials ltd.	99% Assay
Potassium thiocyanate	Thermo Fisher Scientific India Pvt. Ltd	97% Assay
Sodium Hydroxide (NaOH)	Thermo Fisher Scientific India Pvt. Ltd.	Pellets, AR grade, 98%
Sulphuric acid (H_2SO_4)	Thermo Fisher Scientific India Pvt. Ltd.	97%, LR grade
DPPH	Hi Media Laboratories Pvt. Ltd.	Analytical Reagent
Quercetin	Avarice Laboratories Pvt. Ltd	Analytical Reagent
Gallic acid	Avarice Laboratories Pvt. Ltd	Analytical Reagent

Table 0.2 List of equipment used

Physical Apparatus	**Specification**
Electric balance	Phoneix instruments, India
Spectrophotometer	Labtronics, India
Soxhlet apparatus	Y.P. scientific glass work, India
Hot air oven	Victolab, India
Incubator	Y.P. scientific glass work, India
Muffle furnace	Accumax, India
Cabinet dryer	AIset YDL-2000
Colorimeter	Jenway Ltd., UK
Centrifuge	Y.P. scientific glass work, India
Heating mantle	Y.P. scientific glass work, India

1.13 Methodology

The general outline for processing of sorghum seed is presented in Fig. 3.1.

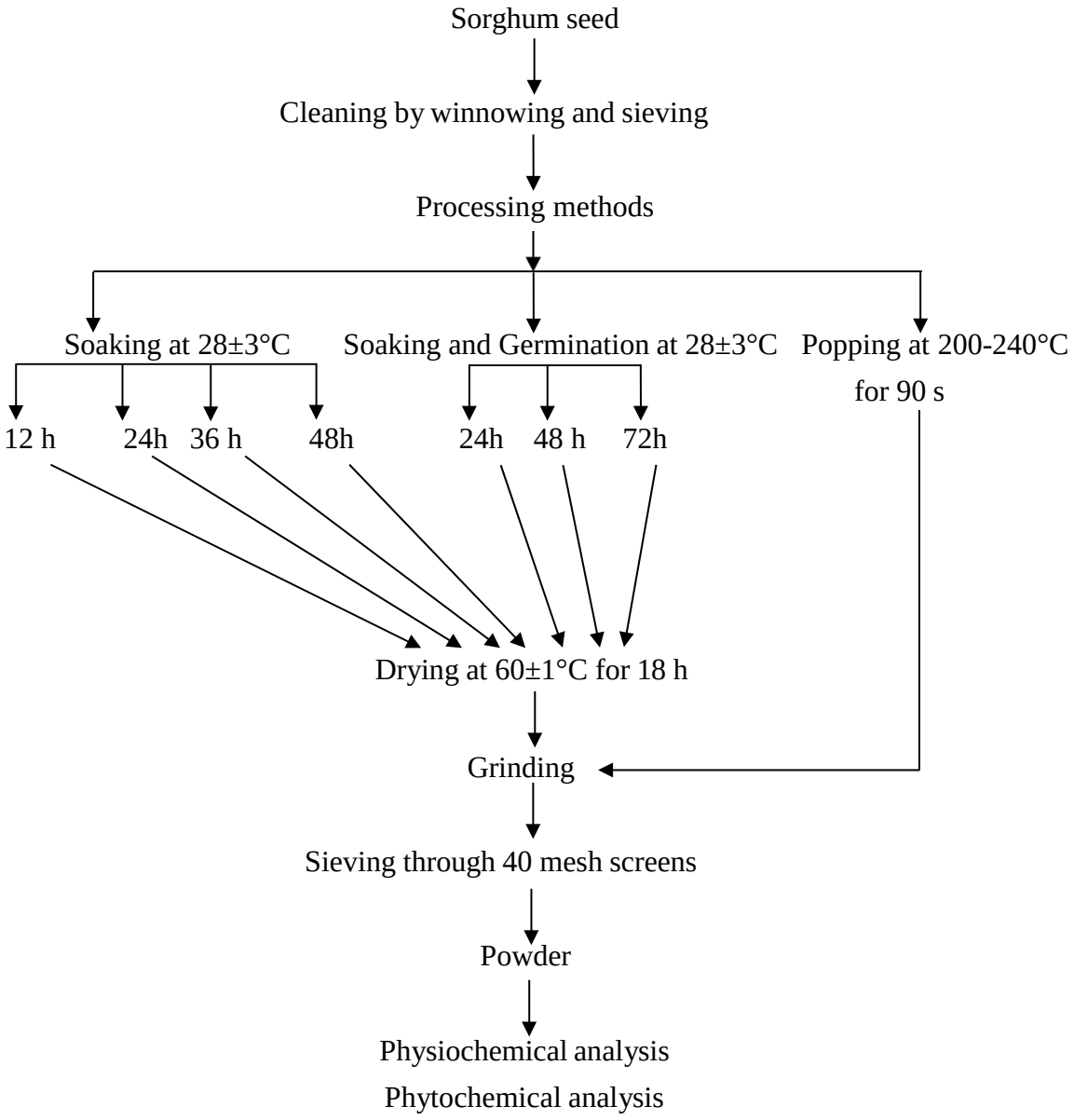

Fig. 3.1 General flowsheet for processing of sorghum seed

Source: (Valadez-Vega *et al.*, 2022)

1.14 Processing methods

1.14.1 Soaking

Sorghum seed were soaked according to the method described by Alvarenga *et al.* (2018) with slight modification. Sorghum seeds (500 g) were soaked in water for 12, 24, 36, and 48

h with a 1:3 ratio of seeds to water at room temperature (28 ± 3ºC). The soaked seeds were washed twice with ordinary water followed by rinsing with distilled water and then dried in an oven at 60±1°C for 18 h. Dry sorghum seeds samples were ground, and the flour was separated by particle size by passing through 40 mesh screens. The ground samples were stored packed in airtight plastic bags until further analysis.

1.14.2 Germination

Sorghum seeds were germinated according to the method of Derbew and Moges (2017) with slight modification. The cleaned sorghum grains were divided into two portions. The first portion, which was not subjected to germination, was milled and packed in airtight plastic bags and served as a control. The second sorghum grains portion were washed three times using tap water. Then, the cleaned and washed sorghum grains were soaked in a volume of water 3 times the weight of grains (3:1) for 24 h at room temperature (28 ± 3ºC) and drained to remove the excess water. Then it was dipped in KMS solution for 10 min to prevent the mold growth during germination. The soaked and washed sorghum grains were allowed to germinated for 24, 48 and 72 h. The treatment samples were covered with wet clean cloth and placed in a plastic sieve and watered two times a day to enhance the germination process. Finally, the sorghum sprouts were washed thoroughly to reduce the sour taste, and then the rootlets were removed manually. The sprouted samples were dried in a hot air oven at 60±1°C for 18 h and were milled to pass through a 40 mesh screens and were stored packed in airtight plastic bags until further analysis.

1.14.3 Popping

The traditional popping procedure with some modifications was adopted, whereby 200 g of dry sorghum seed was placed on the claypan and was heated between 200–240°C, on a gas cooker set to medium heat for about 90 second while stirring using a wooden ladle. Heating continued until the grain turned whitish, which typically required about 90 s (Valadez-Vega *et al.*, 2022). The popped samples were ground to pass through a 40 mesh screens and were stored packed in airtight plastic bags until further analysis.

1.15 Analytical Methods

1.15.1 Proximate analysis

1.15.1.1 Moisture content

A fine powered 5 g sample (W) was taken from a Petri dish of known weight. Petri dish containing sample (W1) was then placed in a hot air oven set to 110°C and dried until a constant weight was observed (W2). The difference in the sample weight was interpreted as the presence of water in the sample (Ranganna, 1986).

$$\text{Moisture content (\%)} = \frac{w_1 - w_2}{W}$$

1.15.1.2 Protein content

The protein content was determined using a 2 g sample. The total nitrogen content was measured employing the micro-Kjeldahl method (Ranganna, 1986). To convert the nitrogen content to crude protein, a conversion factor of 6.25 was applied.

$$\text{Nitrogen (\%, wt basis)} = \frac{(\text{Sample titer} - \text{Blank titer})\text{ml} * \text{N of HCL} * 14 * 100 * 100}{\text{Aliquot (ml)} * \text{wt. of sample (g)} * 1000}$$

1.15.1.3 Crude fat content

The crude fat content of the samples was determined by the solvent extraction method using a Soxhlet apparatus and petroleum ether solvent (Ranganna, 1986). 5 g sample (W) were taken in triplicate and placed in thimble. The thimble was covered using cotton wool. An empty, dry and clean round flask (W1) with a known weight was connected to the siphoning apparatus. The thimble containing the sample was placed in the siphoning apparatus and 200 ml of petroleum ether (with a boiling point 60-80°C) were added. Then the condenser was connected to the siphoning apparatus and the heater was switched on and extraction was applied for 4-5 h. After the extraction was completed the petroleum ether was evaporated from the round flask. The round flask containing the extracted fat (W2) was weighed and the fat content as percentage was calculated according to the following equation:

$$\text{Crude fat content (\%)} = \frac{w_2 - w_1}{W} * 100$$

1.15.1.4 Ash content

The total ash content of the five grams sample was determined using a muffle furnace (Ranganna, 1986). 2 g (W_2) of the sample was weighed by difference into a pre-dried, pre-weighed crucible (W_1). Then the sample was incinerated in a furnace at 525^{o}C for 4-6 h. The temperature of the furnace was decreased to180^{o}C and the crucibles were transferred into a desiccator and cooled for 15-30 min and weighed (W_3). The ash content was calculated by the following method.

$$\text{Ash } (\%) = \frac{W_3 - W_1}{W_2 - W_1} * 100$$

1.15.1.5 Crude fiber content

The crude fiber content of the samples was determined using the Chemical digestion method (Ranganna, 1986). Crude fiber was determined on three grams defatted 2 g dried sample (W), preferably from crude fat determination. Digestion was carried out by refluxing the sample for 30 min in 1.25% H_2SO_4 and acid digested residue was placed in filtering funnel containing muslin cloth, washed repeatedly with hot distill water till made acid free (the filtrate was tested with blue litmus paper, if blue litmus paper resists same color then residue was acid free). The acid digested residue was then subjected to 1.25% NaOH digestion for 30 min and after completion of digestion, alkali digested residue was transferred in filtering funnel containing muslin cloth, washed repeatedly with hot distill water till made alkali free (the filtrate was tested with red litmus paper, if same litmus paper resists same color, then residue was alkali free). Finally, alkali free residue was transferred carefully to a clean silica crucible which was dried in hot air oven at 100°C to bone-dryness and after cooling crucible along with residue in desiccator (W_2), weighed was taken. The same weighted sample were placed in muffle furnace at 450-500°C until all the carbonaceous materials are burnt out. This usually took about 30 min and cooled in a desiccator and then weighted the crucible along with ash (W_1).

$$\text{Crude fiber content } (\%) = \frac{W_2 - W_1}{W} * 100$$

1.15.1.6 Total carbohydrate content

Total carbohydrate content of the samples was determined by difference method (Ranganna, 1986).

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

1.15.1.7 Energy value

Energy value was expressed as Kcal/100g and was calculated by multiplying the values of crude protein, lipids and carbohydrates by recommended factors (4, 9 and 4 respectively) (Valdez-Solana *et al.*, 2015).

1.15.2 Ultimate analysis

1.15.2.1 Determination of iron

Iron in the sample was determined by converting all the iron into ferric form using oxidizing agents like potassium per sulphate or hydrogen per oxide and treating thereafterwith potassium thiocynate to form a red ferric thiocynate which was measured calorimetrically at 480 nm (Ranganna, 1986).

$$\text{Iron}\left(\frac{\text{mg}}{100\text{g}}\right) = \frac{\text{absorbance of sample} * 0.1 * \text{total vol. of ash solution} * 100}{\text{absrobance of standard} * 5 * \text{wt of sample taken for ashing}}$$

1.15.2.2 Determination of calcium

Calcium was precipitated as calcium oxalate. The precipitate was dissolved in hot dilute sulphuric and titrated with standard potassium permanganate (Ranganna, 1986).

$$\text{Calcium}\left(\frac{\text{mg}}{100\text{g}}\right) = \frac{\text{titer} * 0.2 * \text{total vol. of ash solution} * 100}{\text{vol taken for estimation} * \text{wt of sample taken for ashing}}$$

1.15.3 Physical analysis of sorghum seed

1.15.3.1 Thousand kernel weight

The 1000 kernel weight of sorghum seed was determined by measuring the weight of 1000 kernels of sorghum grains after selecting the appropriate sample size by quartering method (Imran *et al.*, 2016).

1.15.3.2 Bulk density

The bulk density was measured by pouring the seeds into the funnel-shaped hopper, the hopper was centered over the measuring bushel, the hopper valve was opened quickly, and the grains were allowed to flow freely into the measuring bushel. After the bushel was filled, the excess material was leveled off with gentle zigzag strokes using the standard seedburo

striking stick. The filled measuring bushel was then weighed, and the mass of grains in the bushel was determined by subtracting the mass of the measuring bushel itself (Clementson *et al.*, 2010).

$$\text{Bulk density} = \frac{\text{Mass of gram}}{\text{Volume of bushel}}$$

1.15.3.3 Sphericity

Sphericity of grain was determined as mentioned in Simonyan *et al.* (2007). Each kernel sample was measured for its length, breadth and thickness by using grain caliper and sphericity is calculated.

$$\text{Sphericity} = \frac{(\text{lbt})^{1/3}}{\text{l}}$$

where, l = length of grain, b = breadth of grain t = thickness of grain

1.15.4 Preparation of extract

Extracts were prepared according to the method described by Upadhyay *et al.* (2013) with slight modification.10 g of powdered samples were steeped in 80% methanol (100 ml) for 12 h at room temperature. They were then filtered using Whatman No.1 filter paper. Finally, extracts were transferred to brown colored glass bottles, sealed by using bottle caps and stored at 4 ± 2ºC until analysis. The extract concentration was determined by evaporating in rotary vacuum evaporator.

1.15.5 Qualitative analysis for Phytochemicals

The plant extracts were screened for the presence of the phytochemical classes by using the standard following methods (Jaradat *et al.*, 2015).

1.15.5.1 Test for protein

Ninhydrin test: Boil 2 ml of 0.2% Ninhydrin solution with the entire plant crude extract, appeared violet color indicate the presence of proteins and amino acids (Jaradat *et al.*, 2015).

1.15.5.2 Test for carbohydrates

Iodine test: 2 ml of iodine solution mixed with crude plant extract. Purple or dark blue colors prove the presence of the carbohydrate (Jaradat *et al.*, 2015).

1.15.5.3 Test for phenols and tannins

Two milliliters of 2% solution of $FeCl_3$ mixed with crude extract Black or blue-green color indicated the presence of tannins and phenols (Jaradat *et al.*, 2015).

1.15.5.4 Test for flavonoids

Alkaline reagent test: 2 ml of 2% NaOH solution was mixed with plant crude extract, intensive yellow color was formed, which turned into colorless when added 2 drops of diluted acid to solution, this result indicated the presence of flavonoids (Jaradat *et al.*, 2015).

1.15.6 Phytochemicals quantitative analysis

1.15.6.1 Determination of total phenolic content (TPC)

TPC was determined using the Folin–Ciocalteu method (Singleton *et al.*, 1999) with slight modifications. The reaction mixture was prepared by mixing 0.5 ml of plant extract solution, 2.5 ml of 10% Folin- Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of Na_2CO_3 aqueous solution. The samples were thereafter incubated in a thermostat at 45°C for 45 min. The absorbance was determined using spectrophotometer at wave length=765nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of gallic acid equivalent expressed in terms of (mg of GAE/ 100 g of dry sample).

1.15.6.2 Determination of total flavonoid content

Total flavonoid content was determined using a modified aluminum chloride assay method as described by Barek *et al.* (2015). 2 ml of solution was pipette out in a test tube in which

0.2 ml of 5% Sodium Nitrate ($NaNO_3$) was mixed and stand for 5 min. 0.2 ml of 5% Aluminum Chloride ($AlCl_3$) was pipetted out, mixed in the tube and allowed to stand for 5 minutes. This followed addition of 2 ml of 1N Sodium Hydroxide (NaOH) in the tube and finally volume was made up to 5 ml. The absorbance was measured after 15 min at 510 nm against a reagent blank. The total flavonoid content is expressed as mg QE/ 100g ofdry weight.

1.15.6.3 Determination of total tannin content

The tannins were determined by Folin-Ciocalteu method. About 0.1 ml of the sample extract

was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% Na_2CO_3 solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of TAE/100 g of extract (Ribarova *et al.*, 2005).

1.15.6.4 Determination of oxalate content

0.1 g of sample was weighed and mixed with 30 ml of 1 M HCL. Each mixture was then shaken in a water bath at 100^0 C for 30 min. To each mixture was added 0.5 ml of 5% $CaCl_2$ and thoroughly mixed to precipitate out calcium oxalate. The suspension was centrifuged at 3000 rpm for 15 min and the supernatant was separated. The pellet was washed twice with 2 ml of 0.35 M NH_4OH then dissolved on 0.5 M H_2SO_4. The solution was then titrated with standard solution of 0.1 M $KMnO_4$ with temperature (60^0C) to faint violet color that persisted for at least 15 s which is equivalent for 2.2 mg of oxalate (Patel and Dutta, 2018)

1.15.6.5 Determination of phytate content

The sample weighing 0.2 g was placed in a 250 ml conical flask. It was soaked in 100 ml of 20% concentrated HCl for 3 h, the sample was then filtered. 50 ml of the filtrate was placed in a 250 ml beaker and 100 ml distilled water was added to the sample. Then, 10 ml of 0.3% ammonium thiocyanate solution was added as indicator and titrated with standard iron (III) chloride solution which contained 0.00195 g iron per 1 ml (Emmanuel and Deborah, 2018).

$$\text{Phytic acid} = \frac{\text{user value} \times 0.00195 \times 1.19}{2}$$

1.15.6.6 Determination of DPPH free radical scavenging activity

Extract (100µL) were dissolved in 3.9 mL freshly prepared methanolic solution of DPPH (1 mM, 0.5 mL). The mixture was vortexed for 15 s and then left to stand at room temperature for 30 min in the dark. The absorbance of the resulting solution was read spectrophotometrically (UV/VI'S spectrometer) at 517 nm. The percentage inhibition of the radicals due to the antioxidant activity of extracts was calculated using the following formula (Vignoli *et al.*, 2011).

$$\%\ \text{scavenging activity} = {(A_c - A_s \times 100)}/{A_c}$$

Where A_c is the absorbance of control and A_s is the absorbance of test sample.

Finally, the IC_{50} (Efficient concentration) value, defined as the concentration of the sampleleading to 50% reduction of the initial DPPH concentration, was calculated from theseparate linear regression plots of the mean percentage of the antioxidant activity against concentration of the test extract (μg/ml).

Part IV

Results and Discussions

Sorghum bicolor (L.) was sourced from a local market in Dharan, Sunsari district, and subjected to various household processing methods, including soaking (for 12, 24, 36, and 48 h), germination (for 24, 36, and 48 h), and popping at temperatures between 200 and 240°C for 60 to 120 s. The processed samples were then analyzed to examine the effects of thesetechniques on their protein content, phytochemical properties (total phenolic content and DPPH free radical scavenging activity), and antinutrient levels, including tannin, phytate, flavonoid, and oxalate content.

1.16 Proximate composition of sorghum seed

The proximate analysis of sorghum seed is presented in Table 4.1.

Table 0.1 The proximate composition of sorghum seed

Parameters	**Values (%)**
Moisture content ,% (wb)	10.89±0.050
Crude protein ,% (db)	12.42±0.085
Crude fat ,% (db)	4.26±0.060
Crude fiber ,% (db)	2.01±0.092
Total ash ,% (db)	1.75±0.060
Carbohydrate ,% (db)	79.55±0.196

*The values in the table are the mean of the triplicate ± standard deviation

The moisture content was determined to be 10.89%, which aligns with the range reported by Mohammed *et al.* (2019) of 10.23–11.9% and is very close to the value noted by Tamilselvanand Kushwaha (2020) at 11.88%. However, it is higher than the values recorded by Karaye*et al.* (2023) at 5.90% and Keyata *et al.* (2021) at 8.7%.

The crude protein content was found to be 12.42%, which falls within the range reported by Tasie and Gebreyes (2020) of 8.20–16.48%. This value is slightly higher than those recorded by Tamilselvan and Kushwaha (2020) at 8.27%, Karaye *et al.* (2023) at 7.81%, Keyata *et al.* (2021) at 9.55%, and Mohammed *et al.* (2019) at 4.27–6.06%. However, it is lower than the range provided by Mawouma *et al.* (2022) of 19.62–23.78%.

The crude fat content was found to be 4.26%, which is comparable to the 3.68% reported by Keyata *et al.* (2021) and higher than the values noted by Tamilselvan and Kushwaha (2020) at 1.87% and Mawouma *et al.* (2022) at 2.74–3.62%. However, it is lower than the ranges reported by Mohammed *et al.* (2019) at 6.72–9.26% and Karaye *et al.* (2023) at 16.51%.

The crude fiber content was found to be 2.01%, which is consistent with the findings of Mohammed *et al.* (2019), who reported a range of 1.45 to 2.41%. This value is lower than those presented by Mawouma *et al.* (2022) at 2.56-4.70%, Tamilselvan and Kushwaha (2020) at 3.34%, Karaye *et al.* (2023) at 8.20%, and Keyata *et al.* (2021) at 2.96%.

The ash content was found to be 1.75%, which is similar to the findings of Tamilselvan and Kushwaha (2020) at 1.73% and Keyata *et al.* (2021) at 1.60%. It is slightly higher than the values reported by Mawouma *et al.* (2022) at 1.15–1.59% but lower than those recorded by Karaye *et al.* (2023) at 6.23%.

The carbohydrate content was determined to be 79.55%, which falls within the range reported by Sorour *et al.* (2017) at 76.43–80.51% and is similar to the finding of Karaye *et al.* (2023) at 79.64%. It is lower than the values reported by Tamilselvan and Kushwaha (2020) at 84.77% and Keyata *et al.* (2021) at 82.2%, but higher than those reported by Mawouma *et al.* (2022) at 67.28–72.71% and Mohammed *et al.* (2019) at 70.55–73.53%.

The energy content was found to be 406.26 Kcal/100 g, which is very close to the value reported by Keyata *et al.* (2021) at 400.2 Kcal/100 g. It is slightly higher than the results obtained by Tamilselvan and Kushwaha (2020) at 389.01 Kcal/100g, Karaye *et al.* (2023) at 396.90 Kcal/100g, and Mohammed *et al.* (2019) at 380.03–388.15 Kcal/100g.

The chemical composition and nutritional value of sorghum can be influenced by factors including its genotype, the climate, the type of soil, and the fertilization used (Ebadi *et al.*, 2005).

1.17 Phytochemical composition of sorghum seed

The phytochemical composition of sorghum seed is tabulated in Table 4.2.

Table 0.2 Phytochemical composition of sorghum seed

Parameters	Values (Dry basis)
Total Phenol content (TPC) (mg GAE/ 100g)	698.11±0.410
DPPH scavenging (%)	50.11±1.210
Tannin content (mg TAE/100 g)	348.92±0.540
Phytate content (mg/100 g)	127.53±0.890
Total flavonoid (mg QE/100 g)	58.22±1.890
Oxalate (mg/100 g)	115.58±2.100

*The values in the table are the mean of the triplicate ± standard deviation

The total phenolic content (TPC) was found to be 698.11 mg GAE/100g, which falls withinthe range reported by Sorour *et al.* (2017), who found phenolic content to be 178.28–825.36mg GAE/100g for two sorghum cultivars. This TPC value is similar to that reported by Arouna *et al.* (2020) at 6.62 mg GAE /g or 662 mg GAE/100 g. In comparison, Tamilselvan and Kushwaha (2020) reported a lower value of 92.62 mg GAE/100 g. Similarly, Sharma and Garg (2023) found a phenolic content of 180 mg GAE/100 g in sorghum.

Pasha *et al.* (2015) reported TPC values of 0.166- 0.362 mg GAE/g or 16.6–36.2 mg GAE/100g across seven different sorghum varieties, which are also lower than our result. The TPC data obtained by Mawouma *et al.* (2022) and Mohapatra *et al.* (2019) was higher than our result, which found 21.91–82.22 mg GAE/g or 2191–82222 mg GAE/100 g and 8.61 mg GAE/g or 861 mg GAE/100 g, respectively.

The DPPH free radical scavenging activity was measured at 50.11%, which closely resembles the finding of Mohapatra *et al.* (2019) at 49.46%. However, our study observed lower DPPH activity compared to Pasha *et al.* (2015) and Mawouma *et al.* (2022), who reported higher values ranging from 88.47% to 94.29% and 64.09% to 93.14%, respectively, across different sorghum varieties. In contrast, Sharma and Garg (2023) found a DPPH value of 41.11%, which was lower than our research findings. These variations highlight the influence of sorghum genotype, environmental factors, and methodologies used in different studies on DPPH antioxidant activity.

The tannin content was measured at 348.92 mg TAE/100 g, which closely aligns with Zubair *et al.* (2023), who reported 3.50 mg/g (or 350 mg/100 g) for white sorghum. It was

lower than the tannin content reported for red sorghum by Zubair *et al.* (2023), which was 4.82 mg/g (or 482 mg/100 g). In comparison, it was higher than the values reported by Anithasri *et al.* (2018) at 9.74–9.97 mg/100g, Tamilselvan and Kushwaha (2020) at 8.46 mg/100g, Derbew and Moges (2017) at 35.17–37.84 mg/100g, Sharma and Garg (2023) at 0.79 mg/g (or 79 mg/100g), Mohapatra *et al.* (2019) at 0.57 mg/g (or 57 mg/100g), and Keyata *et al.* (2021) at 55.81 mg/100g. These variations highlight the diversity in tannin content across different sorghum varieties and studies, influenced by factors such as genotype, processing methods, and analytical techniques used.

The phytate content was measured at 127.53 mg/100 g, falling within the range reported by Anithasri *et al.* (2018) at 125.8–132.21 mg/100 g. Derbew and Moges (2017) reported a higher phytate content of 416.08 mg/100 g in sorghum flour. Sharma and Garg(2023) reported 18.37 mg/g (or 1837 mg/100g). Zubair *et al.* (2023) reported phytate contents of 9.02 mg/g (or 902 mg/100g) for white sorghum and 9.06 mg/g (or 906 mg/100g)for red sorghum, both higher than our findings, indicating potential varietal differences influencing phytate levels in sorghum.

The total flavonoid content was measured at 58.22 mg QE/100 g, which closely matches the finding of Singh *et al.* (2019) at 57.68 mg QE/100 g. This value was higher than that reported by James *et al.* (2022) at 4.54 mg/100 g but lower than the value reported by Kewuyemi and Adebo (2024) at 3.75 mg QE/g (or 375 mg QE/100 g). These differences illustrate the variability in flavonoid content across different studies, influenced by factors such as sorghum variety, growing conditions, and analytical methods employed for flavonoid determination.

The oxalate content was measured at 115.58 mg/100 g. This value closely aligns with the findings reported by Aluge *et al.* (2016) at 1.16 mg/g (or 116 mg/100 g). It is lower than the oxalate content reported for white sorghum (0.88 mg/g or 88 mg/100g) and red sorghum (0.90 mg/g or 90 mg/100g) by Zubair *et al.* (2023), as well as lower than the value reported by Ojha *et al.* (2018) at 3.10 mg/g (or 310 mg/100g). However, it is higher than the oxalate content reported by Keyata *et al.* (2021) at 29.90 mg/100 g. These variations highlight the range of oxalate content in sorghum, influenced by factors such as sorghum variety, processing methods, and analytical techniques used in different studies.

1.18 Mineral composition of sorghum seed

The iron content and calcium content in raw sorghum seeds were found to be 4.50 mg/100g and 28.11 mg/100g, respectively, which closely align with the findings reported by Anithasri *et al.* (2018) for iron (4.46-4.65 mg/100g) and calcium (27.95-28.25 mg/100g). Gerrano *et al.* (2016) also reported similar iron content (4.0–5.50 mg/100 g), confirming consistency with our results.

The mineral analysis value of sorghum seed is shown in Table 4.3.

Table 0.3 Mineral composition of sorghum seed

Minerals	**Values mg/100g (Dry basis)**
Iron (Fe)	4.50±0.210
Calcium (Ca)	28.11±0.380

*The values in the table are the mean of the triplicate ± standard deviation

In contrast, Mawouma *et al.* (2022) reported lower iron (2.75–3.28 mg/100 g) and calcium (10.81–12.91 mg/100 g) content in sorghum, which differs significantly from our findings. Similarly, Keyata *et al.* (2021) reported an iron content of 3 mg/100 g, and Gerrano *et al.* (2016) found a calcium content ranging from 11.0 to 13.0 mg/100 g, both lower than our results.

Conversely, Karaye *et al.* (2023) reported higher calcium content (116.64 mg/100 g) and iron content (23.06 mg/100 g) in sorghum compared to our findings. Similar higher values were reported by Keyata *et al.* (2021) for calcium (34.023 mg/100g), Sharma and Garg (2023) for calcium (31.09 mg/100g), and Sorour *et al.* (2017) for iron (20–27 mg/100g) and calcium (197.8–229.4 mg/100g) in two sorghum cultivars. The concentration of mineral elements in sorghum varies because of genotypic and environmental influences, as well as genotype-environment interactions Gerrano *et al.* (2016).

1.19 Physical properties of sorghum seed

The data presented in Table 4.4 shows that the sphericity, bulk density, and thousand kernel weight of sorghum were measured at 0.64, 82.05 kg/hl, and 30.20 g, respectively. These findings align closely with those reported by Paudel (2021), who documented sphericity of 0.66, bulk density of 82.53 kg/hl, and a thousand kernel weight of 30.81 g for sorghum. Similarly, Acharya (2021) also reported comparable values with sphericity at 0.65, bulk

density at 81.30 kg/hl, and thousand kernel weight at 29.89 g for sorghum. JambammaI and Kailappan (2011) investigated the physical properties of pearled sorghum grain variety (K 9) and reported a bulk density of 0.81 g/cm^2 (equivalent to 8.1 kg/hl), sphericity of 0.84, and a thousand kernel mass of 32.86 g. These values were generally higher than those obtained in our study, except for bulk density. Similarly, Dhadke *et al.* (2022) reported a thousand kernel weights of sorghum as 33.12±0.2g and a bulk density of 0.76±0.01 g/ml (equivalent to 7.6 ± 0.1 kg/hl).

Table 0.4 Physical properties of sorghum

Physical properties	**Sorghum seed**
Sphericity	0.64±0.070
Bulk density (kg/hl)	82.05±0.210
1000 kernel weight (g)	30.20±0.480

*The values in the table are the mean of the triplicate ± standard deviation

In contrast, Mishra *et al.* (2015) reported sphericity ranging from 0.99 to 1.20, bulk densityfrom 785 to 833.4 kg/m2 (equivalent to 78.5-83.34 kg/hl), and a thousand kernel masses from 19.4 to 30.1 g across four sorghum grain varieties, which were similar to our study's findings. These comparisons highlight variations in the physical properties of sorghum across different varieties and studies, influenced by factors such as grain processing, variety,and environmental conditions.

1.20 Effect of processing methods on the protein content of sorghum seed

The effect of soaking, germination and popping on the protein content of sorghum seed was studied. All the treatments significantly changed ($p<0.05$) the protein content of the sorghum seeds, but to the varying extent. Germination for 72 hours had the most pronounced effect in the increment of protein content whereas soaking for 48 hours had the most pronounced effect in the reduction of the protein content of sorghum seeds.

1.20.1 Effect of soaking on the protein content of sorghum seed

The analysis of variance indicated a significant difference ($p < 0.05$) in protein content across different soaking hours. The protein content decreased with progressive soaking times, from 12.42% to 9.86% over 48 hours. The total reduction in protein content was up to 20.61%. This result aligns with findings by Afify *et al.* (2012b), and Martinson*et al.* (2012). The

reduction in protein levels during soaking was attributed to hydrolysis and the breakdown of complex compounds into simpler ones (Kajihausa *et al.*, 2014; Kale *et al.*, 2015; Sousa *et al.*, 2020).

1.20.2 Effect of germination on the protein content of sorghum seed

The protein content of sorghum seeds after germination was determined , protein content increased with extended germination periods, with mean values of 13.49±0.09%, 14.72±0.13%, and 15.98±0.11% after 24, 48, and 72 hours of germination, respectively. The protein content increased up to 43.70% over 72 hours. The analysis of variance showed that there was significant difference between protein content in the different hours of germinated samples ($p < 0.05$).These results align with findings by Kumar *et al.* (2021), Derbew and Moges (2017), Sharma and Garg (2023) , Donkor *et al.* (2012) and Omoikhoje and Obasoyo (2018).

The increase in protein content may be attributed to the loss of dry matter, particularly carbohydrates, through respiration (Uppal and Bains, 2012). Bau *et al.* (1997) suggested that the increase was due to the synthesis of enzyme proteins, such as proteases, by the germinating seed or other constituents. Additionally, Nonogaki *et al.* (2010) explained that protein synthesis occurs during imbibition, with hormonal changes playing a crucial role in completing the germination process. Laetitia *et al.* (2005) suggested that protease enzymes break down peptide bonds in proteins, producing amino acids. The increase in protein content during germination is thus due to both the synthesis of new proteins and the reduction of dry matter during soaking.

In contrast, Elbaloula *et al.* (2014) ,Keyata *et al.* (2021) and Tamilselvan and Kushwaha (2020) observed a decrease in protein content after germination. The reduction in protein content after sprouting may be attributed to the loss of nitrogenous compounds during the rinsing and soaking of seeds (Chavan *et al.*, 1989). Additionally, the decreased protein content during malting could be due to the utilization of protein for the growth and development of the embryo (Nour *et al.*, 2015).

1.20.3 Effect of popping on the protein content of sorghum seed

The protein content of sorghum seeds after popping was determined, the protein content increased by 2.41%, with mean values rising from 12.42±0.09% to 12.72±0.18%. This

finding aligns with those reported by Sharma *et al.* (2015), Liopart and Drago (2016) and (Dhadke *et al.*, 2022) .During the popping process, starch becomes gelatinized, which contributes to a cooked flavor. Additionally, popping induces cell wall fragmentation, thereby improving the accessibility of the protein and starch reserves of the endosperm to digestive enzymes. The effect of different processing methods on the protein content of sorghum seed is shown in Table 4.5.

Table 0.5 Effect of processing methods on the protein content of sorghum seeds

Processing Method	**Protein Content (% ± SD)**	**Changes**
Soaking		
Raw*	12.42 ± 0.09e	
Soaking, 12 h*	11.88 ± 0.08d	-4.35%
Soaking, 24 h*	11.12 ± 0.09c	-10.48%
Soaking, 36 h*	10.54 ± 0.11b	-15.14%
Soaking, 48 h*	9.86 ± 0.09a	-20.59%
Germination		
Germination, 24 h*	13.49 ± 0.09b	+8.62%
Germination, 48 h*	14.72 ± 0.13c	+18.53%
Germination, 72 h*	15.98 ± 0.11d	+28.65%
Popping		
Popping, 90 s	12.72 ± 0.18	+2.42%

(*= significantly different)

1.21 Effects of processing methods on total phenolic content

The effect processing methods on total phenolic was studied, all the treatments significantly changed ($p<0.05$) the phenolic content of the sorghum seeds, but to the varying extent. Germination for 72 hours had the most pronounced effect in the increment of the total phenol content whereas soaking for 48 hours had the most pronounced effect in the reduction of the total phenol content of sorghum seeds.

1.21.1 Effect of soaking on total phenolic content

The mean TPC in raw sorghum was 698.11±0.41 mg GAE/100 g based on dry matter. Total

phenol content decreased with progressive soaking times, with mean values of 618.22±0.58 mg GAE/100g,588.81±0.67 mg GAE/100g, 558.21±0.57 mg GAE/100g, and 530.43±0.68 mg GAE/100g after 12, 24, 36, and 48 hours of soaking, respectively. Soaking significantly reduced TPC by up to 24.01%.

The analysis of variance showed that there was significant difference ($p < 0.05$) between TPC in the different hours of soaking samples. These findings align with previous studies from (Hejazi and Orsat, 2016; Panwar and Guha, 2014) , Sorour *et al.* (2017), Xiong *et al.* (2019) ,Afify *et al.* (2012a) and Singh *et al.* (2019) . The reduction in total phenol content during soaking is attributed to the leaching or solubilization of phenolic compounds into the soaking water (Lu *et al.*, 2007).

1.21.2 Effect of germination on total phenolic content

The mean total phenol content in sorghum soaked for 24 hours was 588.81±0.670 mg GAE/100 g on a dry matter basis. TPC increased with extended germination periods, with mean values of 833.41±0.66 mg GAE/100 g, 930.91±0.71 mg GAE/100 g, and 1046.31±0.56 mg GAE/100 g after 24, 48 and 72 hours of germination, respectively. This indicates a significant increase in TPC up to 49.85%. The analysis of variance (Appendix B) showed that there was significant difference between TPC in the different hours of germination samples ($p < 0.05$).

Our results demonstrated a substantial increase in TPC in seeds processed by germination, consistent with earlier studies from Singh *et al.* (2019) , Kewuyemi and Adebo (2024) ,Donkor *et al.* (2012). In contrast, Dicko *et al.* (2005) reported no detectable effect on TPC levels following sorghum germination. According to Owheruo *et al.* (2018), the increase in TPC after germination may be due to the enzymatic release of bound phenolic compounds. Phenols contribute to the antioxidative potential of grains and help extend the shelf life of cereal and millet products.

Conversely, Khoddami *et al.* (2017) ,Sharma and Garg (2023) ,Tamilselvan and Kushwaha (2020) and Arouna *et al.* (2020) found a decrease in the TPC of sorghum upon germination.The decrease in phenolics content after germination could be the water-soluble compounds that are found in the pericarp and testa leached out in the water (Awika and Rooney, 2004).

However, the effect of germination on the abundance of sorghum phenolic compounds can depend on several factors, including sorghum varieties (cultivar) and germination conditions such as temperature, germination time, and humidity, etc. (Dicko *etal.*, 2005; Garzón and Drago, 2018; Singh *et al.*, 2019).

1.21.3 Effect of popping on total phenolic content

The mean TPC in raw sorghum was 698.11±0.41 mg GAE/100 g on a dry matter basis. TPC increased by 19.33%, with mean values rising from 698.11±0.41 mg GAE/100 g to 832.81±0.59 mg GAE/100 g. Similarly, Xiong *et al.* (2019) observed a 20.58% increment in TPC when sorghum grain was roasted at 150 °C for 60 min, with levels rising from 0.34 mg GAE/g to 0.41 mg GAE/gon a dry basis.

In contrast, Tamilselvan and Kushwaha (2020) observed a 26.11% reduction in TPC when sorghum seeds were roasted for 2-3 min on a hot plate. Randhir *et al.* (2008) stated thatphenolic content is heat-labile, and high-temperature processing can cause oxidation and thermal degradation, leading to a reduction in TPC. The effect of different processing methods on the TPC of sorghum seed is shown in Table 4.6.

Table 0.6 Effect of processing methods on the total phenolic content of sorghum seeds

Processing Method	Phenol Content mg GAE/100 g	Changes
Soaking		
Raw*	698.11 ± 0.41e	
Soaking, 12 h*	618.22 ± 0.58d	-11.44%
Soaking, 24 h*	588.81 ± 0.67c	-15.65%
Soaking, 36 h*	558.21 ± 0.57b	-20.03%
Soaking, 48 h*	530.43 ± 0.68a	-24.01%
Germination		
Germination, 24 h*	833.41 ± 0.66b	+19.39%
Germination, 48 h*	930.91 ± 0.71c	+33.44%
Germination, 72 h*	1046.31 ± 0.56d	+49.85%
Popping		
Popping, 90 s	832.81±0.59	+ 19.33%

(*= significantly different)

1.22 Effects of processing methods on DPPH free radical scavenging activity content

The effect processing methods on DPPH free radical scavenging activity was studied, all the treatments significantly changed ($p<0.05$) the DPPH free radical scavenging activity of the sorghum seeds, but to the varying extent. Germination for 72 hours had the most pronounced effect in the increment of the DPPH free radical scavenging activity whereas soaking for 48 hours had the most pronounced effect in the reduction of the DPPH free radical scavenging activity of sorghum seeds.

1.22.1 Effect of soaking on DPPH free radical scavenging activity content

The mean DPPH free radical scavenging activity content in raw sorghum was 50.11±1.21% based on dry matter. DPPH activity decreased with progressive soaking times, with mean values of 48.71±1.45%, 47.33±1.35%, 45.21±0.98%, and 42.88±1.19% after 12, 24, 36, and 48 hours of soaking, respectively. This represents a decline in DPPH % up to 14.42%. The analysis of variance showed that there was significant difference between DPPH free radical scavenging activity in the different hours of soaking samples ($p < 0.05$). Similarly, Xiong *et*

al. (2019) and Afify *et al.* (2012a) and Singh *et al.* (2019) reported reduction in DPPH%. The reduction in antioxidant activity and capacity after soaking may be attributed to the leaching of total phenols, flavonoids, vitamin E, and β-carotene contents into the soaking water (Afify *et al.*, 2012a).

1.22.2 Effect of germination on DPPH free radical scavenging activity content

The mean DPPH free radical scavenging activity content in sorghum soaked for 24 hours was 47.33±1.35% based on dry matter. DPPH activity increased with progressive germination times, with mean values of 61.76±1.31%, 70.99±1.28%, and 80.19±1.48% after 24, 48, and 72 hours of germination, respectively. Germination significantly increased DPPH inhibition % by up to 69.36%. The analysis of variance showed that there was a significant difference between DPPH free radical scavenging activity in the different hours of germination samples ($p < 0.05$).

Our results demonstrated a substantial increase in DPPH free radical scavenging activity content in seeds processed by germination, consistent with earlier studies. Similarly, Singh et al. (2019), Sharma and Garg (2023) , Arouna et al. (2020) , Sorour et al. (2017) and Singh et al. (2019) also reported enhanced DPPH inhibition activity following sorghum germination. Numerous studies have established a strong correlation between TPC and the antioxidant activity of cereal grains (Donkor et al., 2012; Gabriele et al., 2018).

1.22.3 Effect of popping on DPPH free radical scavenging activity content

The mean DPPH free radical scavenging activity content in raw sorghum was 50.11±1.21% based on dry matter. The DPPH free radical scavenging activity increased by 5.30%, with average values rising from50.11±1.21% to 52.77±1.51%. Similarly, Xiong *et al.* (2019) reported a 4.92% increase in DPPH free radical scavenging activity when sorghum grain was roasted at 150 °C for 60 min, with levels increasing from 1.42 mg TE/g to 1.49 mg TE/g on a dry basis. The effect of different processing methods on the DPPH free radical scavenging activity of sorghum seed is shown in Table 4.7.

Table 0.7 Effect of processing methods on DPPH free radical scavenging activity of sorghum seeds

Processing Method	DPPH free radical scavenging activity (% dry matter)	Changes
Soaking		
Raw*	50.11 ± 1.210c	
Soaking, 12 h*	48.71 ± 1.45c	-2.80%
Soaking, 24 h*	47.33 ± 1.35bc	- 5.54%
Soaking, 36 h*	45.21 ± 0.98ab	- 9.78%
Soaking, 48 h*	42.88 ± 1.19a	- 14.42%
Germination		
Germination, 24 h*	61.76 ± 1.31b	+ 30.47%
Germination, 48 h*	70.99 ± 1.28c	+ 41.71%
Germination, 72 h*	80.19 ± 1.48d	+ 69.36%
Popping		
Popping, 90 s	52.77 ± 1.51	+ 5.30%

(*= significantly different)

1.23 Effects of processing methods on tannin content

The effect processing methods on tannin content was studied, all the treatments significantly changed ($p<0.05$) the tannin content of the sorghum seeds, but to the varying extent. Germination for 72 hours had the most pronounced effect in the reduction of the tannin content.

1.23.1 Effect of soaking on tannin content

The mean tannin content in raw sorghum was 348.92±0.540 mg TAE/100 g on a dry matter basis. The tannin content decreased progressively with longer soaking times, with average values dropping to311.11±0.79 mg TAE/100 g after 12 h, 288.23±0.63 mg TAE/100 g after 24 h, 261.21±0.91mg TAE/100 g after 36 h, and 230.41±0.80 mg TAE/100 g after 48 h. Soaking significantly reduced tannin by up to 33.96% over 48 h. The analysisof variance showed that there was significant difference b etween tannin content in the different hours

of soaking samples ($p < 0.05$).

Similarly, Zubair *et al.* (2023) , Keyata *et al.* (2021) , Afify *et al.* (2012a) , Xiong *et al.* (2019) and Ayuba *et al.* (2020) also reported a decrease in tannin content in sorghum after soaking . The decrease in tannin content can be attributed to the leaching of polyphenols into the water, as tannins are polyphenolic compounds that are soluble in water and primarily foundin the seed coat (Aluge *et al.*, 2016). Hemalatha *et al.* (2007) observed that steeping significantly reduced the tannin content in various food grains.

1.23.2 Effect of germination on tannin content

The mean tannin content in sorghum soaked for 24 hours was 288.23±0.63 mg TAE/100 g on a dry matter basis. The tannin content decreased progressively with longer germination times, with average values dropping to 281.1±0.88 mg TAE/100 g after 24 h, 225.22±0.72 mg TAE/100 g after 48 h, and 180.22±0.67 mg TAE/100 g after 72 h. This reduction indicates that germination significantly reduced tannin content by up to 48.37% over 72 h. The analysis of variance showed that there was a significant difference between tannin content in the different hours of germination samples ($p < 0.05$).

These findings are comparable to those reported by Derbew and Moges (2017), Sharma and Garg (2023), Tamilselvan and Kushwaha (2020) Keyata et al. (2021) , Ojha et al. (2018) , Omoikhoje and Obasoyo (2018) , Kewuyemi and Adebo (2024) and (Choi et al., 1990; Elmaki et al., 1999; Osuntogum et al., 1989) , observing a decrease in tannin content in sorghum grains during germination.

Shimelis and Rakshit (2008) explained that the decrease in tannin content during germination might be due to the leaching of tannins into the sprouting medium and the decreased activity of polyphenol oxidase and other catabolic enzymes. Additionally, Hotz and Gibson (2007) stated that certain tannins and other polyphenols in legumes and sorghum may be reduced during germination due to the formation of polyphenol complexes with proteins and the gradual degradation of oligosaccharides. Such reductions in polyphenols can facilitate iron absorption. A decrease in tannin content during germination can be attributed to the leaching of polyphenols into soaking water and increased enzymatic action (Kumar et al., 2021).

1.23.3 Effect of popping on tannin content

Initially, the mean tannin content in raw sorghum was 348.92±0.540 mg TAE/100 g on a dry matter basis. When popped at temperatures between 200 and 240 °C for 90 s, the tannin content decreased by 17.71%, with average values dropping from 348.92±0.540 mg TAE/100 g to 287.11±0.99mg TAE/100 g. Similarly, Anithasri *et al.* (2018) also reported a 29.26% decrease in tannin content of the K2 variety sorghum after popping at 230°C for 2.5 min in a continuous popping machine.

Tannin content was significantly decreased during both the germination and popping processes (Hussain *et al.*, 2011). This reduction in tannin content during germination and popping processes has been validated by other studies (Sade, 2009). Results indicate that anti-nutrients such as tannin, phytic acid, oxalic acid, and trypsin inhibitor activity content decreased significantly during both germination and popping. However, the maximum reduction was observed during germination compared to popping (Chauhan and Sarita, 2018). The effect of different processing methods on the tannin content of sorghum seed is shown in Table 4.8.

Table 0.8 Effect of processing methods on the tannin content of sorghum seeds

Processing Method	Tannin Content (mg TAE/100 g)	Changes
Soaking		
Raw*	348.92 ± 0.540e	
Soaking, 12 h*	311.11 ± 0.79d	- 10.83%
Soaking, 24 h*	288.23 ± 0.63c	- 17.40%
Soaking, 36 h*	261.21 ± 0.91b	- 25.14%
Soaking, 48 h*	230.41 ± 0.80a	- 33.96%
Germination		
Germination, 24 h*	281.1 ± 0.88c	-19.40%
Germination, 48 h*	225.22 ± 0.72b	-35.51%
Germination, 72 h*	180.22 ± 0.67a	-48.37%
Popping		
Popping, 90 s	287.11 ± 0.99	-17.71%

(*= significantly different)

1.24 Effects of processing methods on the phytate content

The effect processing methods on phytate content was studied, all the treatments significantly changed ($p<0.05$) the phytate content of the sorghum seeds, but to the varying extent. Germination for 72 hours had the most pronounced effect in the reduction of the phytate content of sorghum seeds.

1.24.1 Effect of soaking on phytate content

The mean phytate content in raw sorghum was 127.53±0.890 mg/100 g on a dry matter basis. The phytate content decreased progressively with longer soaking times, with average values dropping to 107.74±0.82 mg/100 g, 100.21±0.88 mg/100 g, 91.55±1.21 mg/100 g and 82.52±0.98 mg/100 g after 12, 24, 36, and 48 hours of soaking, respectively. This decreases from indicates that soaking significantly reduced phytate by up to 35.27% over 48 h. The analysis of variance showed that there was significant difference between phytate content in the different hours of soaking samples ($p < 0.05$). Zubair *et al.* (2023) ,(Afify *et al.*, 2011) , Keyata *et al.* (2021) , Sorour *et al.* (2017) , Omoikhoje and Obasoyo (2018) (Lestienne *et al.*, 2005) and Ayuba *et al.* (2020) observed a decline in phytate content after soaking of sorghum seeds. Soaking has been recognized as the most effective method for reducing phytic acid content (Gupta *et al.*, 2015). This reduction is likely due to the leaching of tannins into the soaking water, as tannins are concentrated in the seed coats. The decrease in phytate content during soaking is attributed to the water solubilization of certain phytate salts (Eltayeb *et al.*, 2017).

1.24.2 Effect of germination on phytate content

The mean phytate content sorghum soaked for 24 hours was 100.21±0.88 mg/100 g on a dry matter basis.The phytate content decreased progressively with longer germination times, with average values dropping to 89.98±1.11 mg/100 g after 24 h, 78.76±1.2 mg/100 g after 48 h, and 61.98±0.91mg/100 g after 72 h. Germination significantly reduced phytate content by up to 51.38% over 72 h. The analysis of variance showed that there was a significant difference between phytate content in the different hours of germination samples ($p < 0.05$).

These findings are consistent with results obtained by Derbew and Moges (2017), Omoikhoje and Obasoyo (2018) , Azeke *et al.* (2011) , (Afify*et al.*, 2011) , Sharma and Garg (2023) , Keyata *et al.* (2021) , Tizazu *et al.* (2011) Sorour *et al.* (2017) and Ojha *et al.* (2018) reporting the reduction of phytate content after germination.

The reason for the reduction of phytate content after germination may be due to enhancing the phytase activity that hydrolysis of phytate phosphorus into inositol monophosphate (Yenasew and Urga, 2022). The decrease in phytate content during germination can be due to leaching during hydration and the activation of phytase post- germination.

1.24.3 Effect of popping on phytate content

The mean phytate content in raw sorghum was 127.53±0.890 mg/100 g on a dry matter basis. When popped at temperatures between 200 and 240 °C for 90 s, the phytate content decreased by 20.54%, with average values dropping from 127.53±0.890 mg/100 g to 101.31±1.14 mg/100 g. Liopart and Drago (2016), Saravanabavan *et al.* (2013) and Anithasri *et al.* (2018) also reported a decrease in phytate content after popping of sorghum seeds. Phytate formation occurs during the maturation of plant seeds, and variations in phytate content in cereal grains can be attributed to several factors, including differences in the degree of maturation at harvest, genetics, environmentalfluctuations, location, irrigation conditions, soil type, year, and fertilizer application (Wu *etal.*, 2009). The effect of different processing methods on the phytate content of sorghum seed is shown in Table 4.9.

Table 0.9 Effect of processing methods on the phytate content of sorghum seeds

Processing Method	Phytate Content (mg/100 g)	Changes
Soaking		
Raw*	127.53±0.89e	
Soaking, 12 h*	107.74±0.82d	-15.53%
Soaking, 24 h*	100.21±0.88c	-21.42%
Soaking, 36 h*	91.55±1.21b	-28.19%
Soaking, 48 h*	82.52±0.98a	-35.27%
Germination		
Germination, 24 h*	89.98±1.11c	-29.42%
Germination, 48 h*	78.76±1.20b	-38.25%
Germination, 72 h*	61.98±0.91a	-51.38%
Popping		
Popping, 90 s	101.31±1.14	-20.54%

(*= significantly different)

1.25 Effects of processing methods on the flavonoid content

The effect processing methods on flavonoid content was studied, all the treatments significantly changed ($p<0.05$) the flavonoid content of the sorghum seeds, but to the varying extent. Germination for 72 hours had the most pronounced effect in the increment of the flavonoid content of sorghum seeds and soaking for 48 hours had the most pronounced effect in the reduction of the flavonoid content.

1.25.1 Effect of soaking on total flavonoid content

The mean flavonoid content in raw sorghum was 58.22±1.890 mg QE/100 g on a dry matter basis. The flavonoid content decreased progressively with longer soaking times, with average values dropping to 52.03±1.98 mg QE/100 g after 12 h, 48.82±1.74 mg QE/100 g after 24 h, 42.51±1.80 mg QE/100 g after 36 h, and 35.77±1.91 mg QE/100 g after 48 h. Soaking significantly reduced flavonoid by up to 38.69% after 48 h. The analysis of variance showed that there was significant difference between flavonoid content in the different hours of soaking samples ($p < 0.05$).

Similarly, Singh *et al.* (2019), Afify *et al.* (2012a) reported a reduction in total flavonoids after soaking. This reduction may be attributed to the leaching of phenols into the soaking medium. The lower levels of total phenols and total flavonoids after soaking may result from the release of phenolic compounds into the soaking water. This phenomenon is likely enhanced with longer soaking durations, allowing more phenolics to diffuse out (Akillioglu and Karakaya,2010).

1.25.2 Effect of germination on flavonoid content

The average TFC in sorghum soaked for 24 hours was 48.82±1.74 mg QE/100 g based on dry weight. TFC showed a gradual increase with longer germination periods, measuring 69.10±1.91 mg QE/100 g after 24 h, 83.55±1.96 mg QE/100 g after 48 h, and 88.75±1.83 mg QE/100 g after 72 h. The total flavonoid content increased significantly up to 52.61% due to germination over 72 h. The analysis of variance (Appendix B) showed that there was a significant difference between flavonoid content in the different hours of germination samples ($p < 0.05$). The findings were consistent with findings from earlier studies, Singh *et al.* (2019) also observed a significant increase in TFC.

1.25.3 Effect of popping on flavonoid content

The average TFC in raw sorghum was 58.22±1.890 mg QE/100 g on a dry weight basis. When popped at temperatures ranging from 200 to 240 °C for 90 s, the TFC increased by 22.55%, with the average values rising from 58.22±1.890 mg QE/100 g to 71.35±1.99 mg QE/100 g.

The results demonstrated a significant increase in TFC in seeds processed by popping aligning with findings from previous studies. Xiong *et al.* (2019) reported a 32.39% increase in TFC when sorghum grain was roasted at 150 °C for 60 min, with levels increasing from 0.71 mg CAE/g to 0.94 mg CAE/g on a dry basis.The effect of different processing methods on the flavonoid content of sorghum seed is shown in Table 4.10.

Table 0.10 Effect of processing methods on the total flavonoid content of sorghum seeds

Processing Method	TFC (mg QE/100 g)	Changes
Soaking		
Raw*	58.22±1.890d	
Soaking, 12 h*	52.03±1.98c	-10.64%
Soaking, 24 h*	48.82±1.74c	-16.12%
Soaking, 36 h*	42.51±1.80b	-26.98%
Soaking, 48 h*	35.77±1.91a	-38.69%
Germination		
Germination, 24 h*	69.10±1.91b	+18.68%
Germination, 48 h*	83.55±1.96c	+43.45%
Germination, 72 h*	88.75±1.83d	+52.61%
Popping		
Popping, 90 s	71.35±1.99	+22.55%

(*= significantly different)

1.26 Effects of processing methods on the oxalate content

The effect processing methods on oxalate content was studied, all the treatments significantly changed ($p<0.05$) the oxalate content of the sorghum seeds, but to the varying extent. Germination for 72 hours had the most pronounced effect in the reduction of the

oxalate content of sorghum seeds.

1.26.1 Effect of soaking on oxalate content

The mean oxalate content in raw sorghum was 115.58±2.100 mg /100 g on a dry matter basis. The oxalate content decreased progressively with longer soaking times, with average values dropping to 89.33±2.18 mg /100 g after 12 h, 82.11±1.88 mg /100 g after 24 h, 74.15±2.21 mg /100 g after 36 h, and 67.31±2.25 mg /100 g after 48 h. Soaking significantly reduced oxalate by up to 41.74% over 48 h. The analysis of variance (Appendix B) showed that there was significant difference between oxalate content in the different hours of soaking samples ($p < 0.05$).

The results demonstrated a substantial decrease in oxalate content in seeds processed by soaking, consistent with earlier studies. Similarly, Zubair *et al.* (2023), Omoikhoje and Obasoyo (2018) ,Keyata *etal.* (2021) also observed a decline in oxalate content after soaking the sorghum seeds.

This reduction might be due to the leaching of oxalate compounds into the water during combined processing techniques such as washing and soaking. Similar findings wereereported for the reduction of oxalate content in soaked and malted barley grains by Brudzyński and Salamon (2011).

1.26.2 Effect of germination on oxalate content

The mean oxalate content in sorghum soaked for 24 hours was 82.11±1.88 mg /100 g on a dry matter basis. The oxalate content decreased progressively with longer germination times, with average values dropping to 70.21±1.94 mg /100 g after 24 h, 59.73±2.11 mg /100 g after 48 h, and 47.85±2.19 mg /100 g after 72 h. Germination significantly reduced oxalate by up to 58.58% over 72 h. The analysis of variance (Appendix B) showed that there was a significantdifference between oxalate content in the different hours of germination samples ($p < 0.05$).

The results demonstrated a substantial decrease in oxalate content in seeds processed by germination, consistent with earlier studies from Omoikhoje and Obasoyo (2018), Keyata *et al.* (2021) and Ojha *et al.* (2018). During germination, the decrease in oxalate content is attributed to the activation of oxalate oxidase, which breaks down oxalic acid into carbon dioxide and hydrogen peroxide, consequently releasing calcium. This mechanism hasbeen

previously investigated by Murugkar *et al.* (2013) and Pal *et al.* (2016).

1.26.3 Effect of popping on oxalate content

The mean oxalate content in raw sorghum was 115.58±2.10 mg/100 g on a dry matter basis. When popped at temperatures ranging from 200 to 240 °C for 90 s, the oxalate content decreased by 21.93%, with average values dropping from 115.58±2.10 mg/100 g to 90.22±2.12 mg/100 g.

The results demonstrated a significant decrease in oxalate content in seeds processed by popping, consistent with findings from previous studies. Indu *et al.* (2020) reported a decrease in oxalate content for finger millet, pearl millet, and little millet after popping using the sand roasting method in an iron pan at 250°C ± 10°C for 3–4 min.

The reduction of anti-nutritional factors during popping occurs because these components are mainly found in the outer layers of cereal grains and seed coats, which are affected by the popping process (Rao and Deosthale, 1983).The effect of different processing methods on the oxalate content of sorghum seed is shown in Table 4.11.

Table 0.11 Effect of processing methods on the oxalate content of sorghum seeds

Processing Method	Oxalate Content (mg/100 g	Changes
Soaking		
Raw*	115.58±2.10e	
Soaking, 12 h*	89.33±2.18d	-22.69%
Soaking, 24 h*	82.11±1.88c	-28.94%
Soaking, 36 h*	74.15±2.21b	-35.87%
Soaking, 48 h*	67.31±2.25a	-41.74%
Germination		
Germination, 24 h*	70.21±1.94c	-39.23%
Germination, 48 h*	59.73±2.11b	-48.20%
Germination, 72 h*	47.85±2.19a	-58.58%
Popping		
Popping, 90 s	90.22±2.12	-21.93%

(*= significantly different)

Part V

Conclusion and recommendations

1.27 Conclusions

The study on the processing of sorghum seeds demonstrated that a variety of methods, including soaking and germination for different durations and popping can significantly affect ($p<0.05$) the levels of phytochemicals in sorghum seeds. Following conclusions can be drawn from this study:

i. Out of all the processing methods, germination was the most effective method for increasing protein content, with a 28.65% increase observed after 72 hours of germination. Additionally, the study also revealed that germination for 72 h was the most effective method for increasing phenol content, DPPH scavenging activity, and flavonoid content, with increases of 49.85%, 69.36%, and 52.61%, respectively.

ii. Out of all processing methods, germination for 72 h was the most effective method to reduce the phytate, tannin, and oxalate content of sorghum seeds for resulting in reductions of 51.38%, 48.37%, and 58.58%, respectively. This was followed by soaking for 48 h, which resulted in reductions of 35.27%, 33.96%, and 41.74%, respectively

iii. Popping of the sorghum seeds at 200 to 240 °C for 90 s also caused an increase in protein, phenol content, DPPPH scavenging activity and flavonoid content by 2.42%,19.33%,5.30% and 22.55%, respectively. Additionally, the study revealed that popping also resulted in reduction of phytate, tannin, and oxalate content of sorghum seeds by 20.54%, 17.71% and 21.93%, respectively.

iv. Overall, the best processing method was found to be germination for 72 h as it not only significantly reduced phytate, tannin and oxalate content but also enhanced protein, phenol content, DPPH scavenging activity and flavonoid content which exhibit antioxidant properties along with several other health benefits.

1.28 Recommendations

i. The effects of different combined treatments (germination and popping, soaking and popping) on anti-nutritional factors can be studied.

ii. Temperature and soaking solutions (such as salt solutions like 2% $NaHCO_3$ and

alkalis like 2% CaO, 2% K2CO3, 2% NaOH, and 2% HCHO) can be varied in different processing methods.

iii. Popping methods such as sand roasting, salt roasting, gun puffing, hot oil frying, or using heat mediums like hot air or microwave radiation can be further studied.

iv. The effect of processing methods on reducing other antinutrients like trypsin inhibitor, hemagglutinin, lectin, etc., present in sorghum seeds can be studied.

Part VI

Summary

Sorghum (*Sorghum bicolor L*. Moench) is a highly competitive crop globally, especially in Africa and Asia, and ranks as the fifth most significant cereal after barley, maize, wheat, and rice. It serves as a staple food grain worldwide and is a valuable source of both micro and macronutrients, including proteins, fats, dietary fiber, and carbohydrates. Sorghum has antioxidant, strong free radical scavenging, anti-carcinogenic, antidiabetic, and anti-inflammatory properties. It is used in many sorghum-based food products such as ready-to-eat breakfast cereals (porridge, flaked breakfast biscuits, muesli, bakery products (flatbread, baked sweet biscuits, sorghum flour and meal, snack foods (biscuit-style bars, popcorn, ready-to-eat meals (pasta, noodles), and beverages (beer and baijiu, a Chinese colorless spirit). Consequently, its use is increasing worldwide. The potential for sorghum to be used as a nutraceutical and functional food is very promising.

In the present study, three processing methods were used to investigate their effects on protein content, DPPH radical scavenging activity, and anti-nutrients (flavonoid, oxalate, phytate, polyphenol, and tannin) in sorghum seeds. The processing methods included soaking for 12, 24, 36, and 48 hours; germination for 24, 48, and 72 hours; and popping at 200 to 240 °C for 1-2 minutes. Additionally, the minerals iron and calcium were analyzed. DPPH free radical scavenging activity, tannin, polyphenol, and flavonoid contents were measured spectrophotometrically, while oxalate content was determined by titration with potassium permanganate, phytate content was assessed using ammonium thiocyanate, and protein content was evaluated using the micro-Kjeldahl method.

The mean values for protein, polyphenol, DPPH scavenging activity, tannin, phytate, flavonoid, and oxalate in raw sorghum seeds were found to be 12.42%, 698.11 mg GAE/100 g, 50.11%, 348.92 mg TAE/100 g, 127.53 mg/100 g, 58.22 mg QE/100 g, and 115.58 mg/100 g, respectively, on a dry basis. Protein, phenol, DPPH scavenging activity, and flavonoid content increased with germination and popping methods but decreased with soaking. Phytate, tannin, and oxalate levels were significantly reduced by all three treatments. Among the three processing methods, germination was the most effective in increasing protein, phenol, DPPH scavenging activity, and flavonoid content, as well as reducing phytate, tannin, and oxalate levels.

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Appendices

Appendix A

1. Standard curve for total phenol content

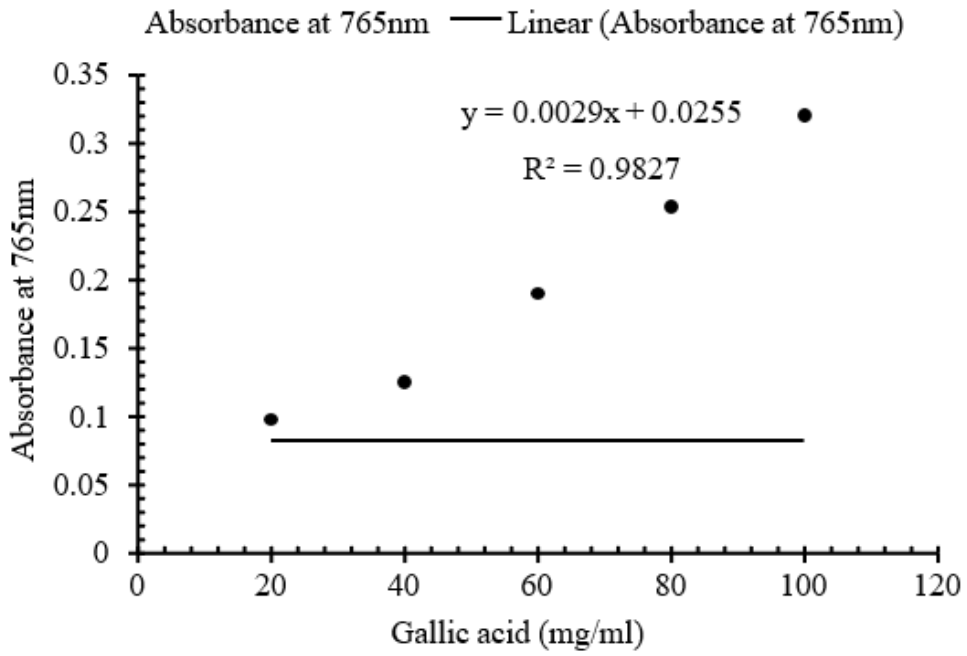

Fig. A.1 Standard curve of gallic acid for total phenol content

2. Standard curve for tannin content

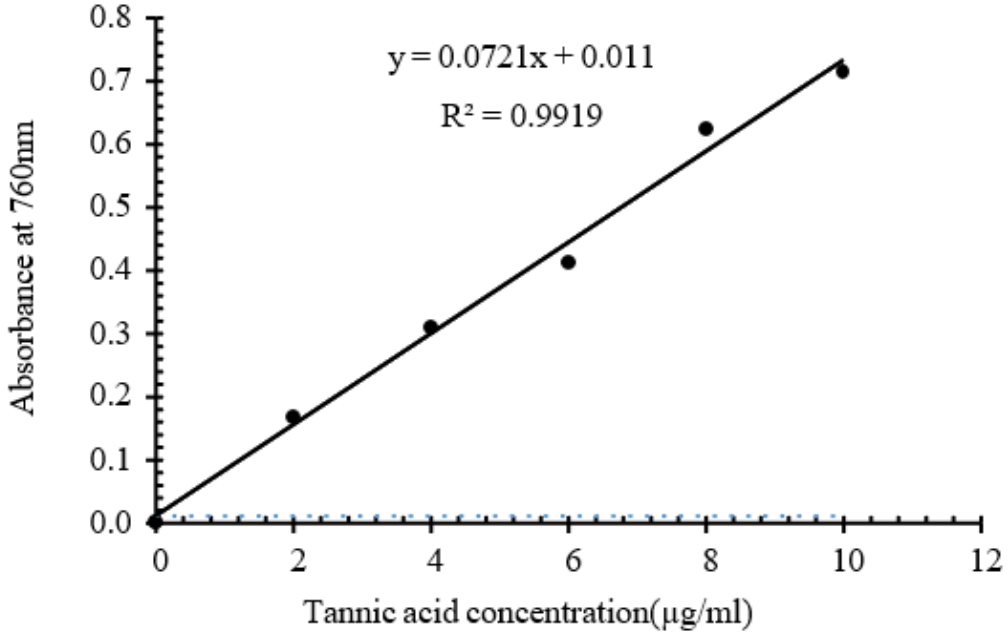

Fig. A.2 Standard curve of tannic acid for tannin content

3. Standard curve for total flavonoid content

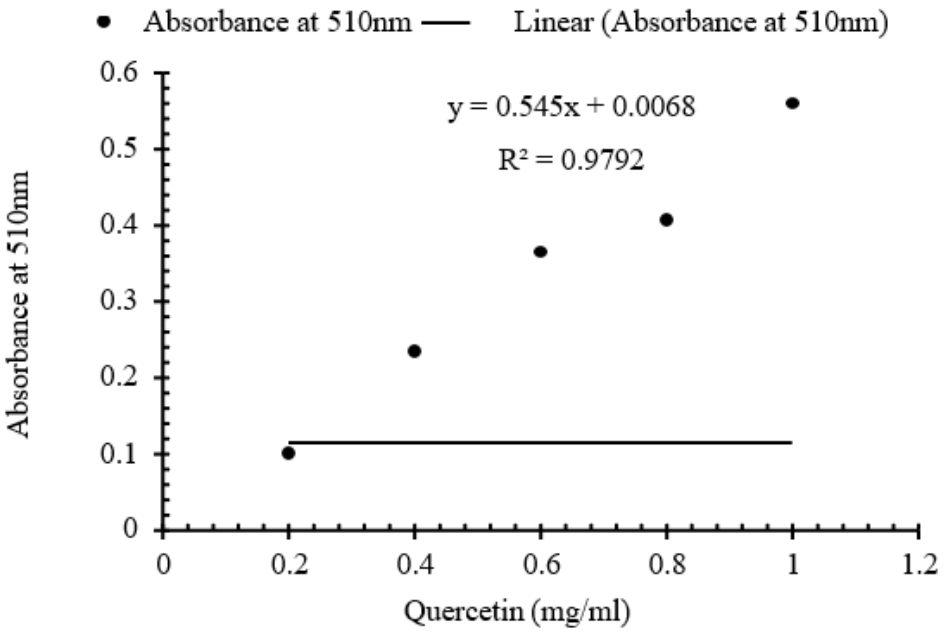

Fig. A.3 Standard curve of quercetin for total flavonoid content

Appendix B

Table B.1 ANOVA for effect of soaking on protein content

Source of Variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
Soaking hours	4	12.54576	3.13644	366.41	<.001
Residual	10	0.0856	0.00856		
Total	14	12.63136			

Since p< 0.05, there is a significant difference between the samples in different soaking hours so LSD testing is necessary.

Table B.2 LSD of means effect of soaking on protein content

No of hours	Mean	Column A	l. s. d	d. f
48*	9.86±0.09	a	0.1683	10
36*	10.54±0.11	b		
24*	11.12±0.09	c		
12*	11.88±0.08	d		
0 (Raw*)	12.42±0.09	e		

(*= significantly different)

Table B.3 ANOVA for effect of germination on protein content

Source of variation	Degree of Freedom	Sum of square	Mean square	Variance ratio	F probability ratio
Germination hours	3	21.3068	7.10227	628.52	<.001
Residual	8	0.0904	0.0113		
Total	11	21.3972			

Since p< 0.05, there is a significant difference between the samples in different germinationhours so LSD testing is necessary.

Table B.4 LSD of means effect of germination on protein content

No of hours	Mean	Column A	l. s. d	d. f
0 (24h soaked*)	11.12±0.09	a	0.2001	8
24*	13.49±0.09	b		
48*	14.72±0.13	c		
72*	15.98±0.11	d		

Table B.5 ANOVA for effect of soaking on TPC

Source of variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
Soaking hours	4	49984.2	12496.1	35893.8	<.001
Residual	10	3.4814	0.3481		
Total	14	49987.7			

Since $p < 0.05$, there is a significant difference between the samples in different soaking hours so LSD testing is necessary.

Table B.6 LSD of means effect of soaking on TPC

No of hours	Mean	Column A	l. s. d	d. f
48*	530.43±0.68	a	1.073	10
36*	558.21±0.57	b		
24*	588.81±0.670	c		
12*	618.22±0.58	d		
0 (Raw*)	698.11±0.41	e		

(*= significantly different)

Table B.7 ANOVA for effect of germination on TPC

Source of Variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
Germination hours	3	1.96E+05	6.55E+04	1.84E+05	<.001
Residual	8	2.84E+00	3.55E-01		
Total	11	1.96E+05			

Since p< 0.05, there is a significant difference between the samples in different germinationhours so LSD testing is necessary.

Table B.8 LSD of means effect of germination on TPC

No of hours	Mean	Column A	l. s. d	d. f
0 (24h soaked*)	588.81±0.670	a	1.122	8
24*	833.41±0.66	b		
48*	930.91±0.71	c		
72*	1046.31±0.56	d		

Table B.9 ANOVA for effect of soaking on DPPH free radical scavenging activity content

Source of Variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
Soaking hours	4	98.304	24.576	15.82	<.001
Residual	10	15.531	1.553		
Total	14	113.835			

Since p< 0.05, there is a significant difference between the samples in different soaking hours so LSD testing is necessary.

Table B.10 LSD of means effect of soaking on DPPH free radical scavenging activity content

No of hours	Mean	Column A	l. s. d	d. f
48*	42.88±1.19	a	2.267	10
36	45.21±0.98	ab		
24	47.33±1.35	bc		
12	48.71±1.45	c		
0 (Raw)	50.11±1.210	c		

(*= significantly different)

Table B.11 ANOVA for effect of germination on DPPH free radical scavenging activitycontent

Source of Variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
Germination hours	3	1489.501	496.5	283.35	<.001
Residual	8	14.018	1.752		
Total	11	1503.519			

Since p< 0.05, there is a significant difference between the samples in different germinationhours so LSD testing is necessary.

Table B.12 LSD of means effect of germination on DPPH free radical scavenging activity content

No of hours	Mean	Column A	l. s. d	d. f
0 (24h soaked*)	47.33±1.35	a	2.492	8
24*	61.76±1.31	b		
48*	70.99±1.28	c		
72*	80.19±1.48	d		

(*= significantly different)

Table B.13 ANOVA for effect of soaking on tannin content

Source of Variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
Soaking hours	4	24839.0422	6209.7605	11165.82	<.001
Residual	10	5.5614	0.5561		
Total	14	24844.6036			

Since p< 0.05, there is a significant difference between the samples in different soaking hours so LSD testing is necessary.

Table B.14 LSD of means effect of soaking on tannin content

No of hours	Mean	Column A	l. s. d	d. f
48*	230.41±0.80	a	1.357	10
36*	261.21±0.91	b		
24*	288.23±0.63	c		
12*	311.11±0.79	d		
0 (Raw*)	348.92±0.54	e		

(*= significantly different)

Table B.15 ANOVA for effect of germination on tannin content

Source of variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
Germination hours	3	47763.9609	15921.32	31321.14	<.001
Residual	8	4.0666	0.5083		
Total	11	47768.0275			

Since p< 0.05, there is a significant difference between the samples in different germinationhours so LSD testing is necessary.

Table B.16 LSD of means effect of germination on tannin content

No of hours	Mean	Column A	l. s. d	d. f
72*	180.22±0.67	a	1.342	8
48*	225.22±0.72	b		
24*	281.1±0.88	c		
0 (24h soaked*)	288.23±0.63	d		

(*= significantly different)

Table B.17 ANOVA for effect of soaking on phytate content

Source of variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
Soaking hours	4	3529.695	882.4237	946.12	<.001
Residual	10	9.3268	0.9327		
Total	14	3539.0218			

Since $p < 0.05$, there is a significant difference between the samples in different soaking hours so LSD testing is necessary.

Table B.18 LSD of means effect of soaking on phytate content

No of hours	Mean	Column A	l. s. d	d. f
48*	82.52±0.98	a	1.757	10
36*	91.55±1.21	b		
24*	100.21±0.88	c		
12*	107.74±0.82	d		
0 (Raw*)	127.53±0.89	e		

(*= significantly different)

Table B.19 ANOVA for effect of germination on phytate content

Source of variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
Germination hours	3	6957.581	2319.194	2161.26	<.001
Residual	8	8.585	1.073		
Total	11	6966.166			

Since p< 0.05, there is a significant difference between the samples in different germinationhours so LSD testing is necessary.

Table B.20 LSD of means effect of germination on phytate content

No of hours	Mean	Column A	l. s. d	d. f
72*	61.98±0.91	a	1.95	8
48*	78.76±1.20	b		
24*	89.98±1.11	c		
0 (24h soaked*)	100.21±0.88	d		

(*= significantly different)

Table B.21 ANOVA for effect of soaking on oxalate content

Source of variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
Soaking hours	4	4171.422	1042.855	230.28	<.001
Residual	10	45.287	4.529		
Total	14	4216.709			

Since p< 0.05, there is a significant difference between the samples in different soaking hours so LSD testing is necessary.

Table B.22 LSD of means effect of soaking on oxalate content

No of hours	Mean	Column A	l. s. d	d. f
48*	67.31±2.25	a	3.872	10
36*	74.15±2.21	b		
24*	82.11±1.88	c		
12*	89.33±2.18	d		
0 (Raw*)	115.58±2.10	e		

(*= significantly different)

Table B.23 ANOVA for effect of germination on oxalate content

Source of variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
Germination hours	3	7886.96	2628.987	603.61	<.001
Residual	8	34.844	4.355		
Total	11	7921.804			

Since p< 0.05, there is a significant difference between the samples in different germinationhours so LSD testing is necessary.

Table B.24 LSD of means effect of germination on oxalate content

No of hours	Mean	Column A	l. s. d	d. f
72*	47.85±2.19	a	3.929	8
48*	59.73±2.11	b		
24*	70.21±1.94	c		
0 (24h soaked*)	82.11±1.88	d		

(*= significantly different)

Table B.25 ANOVA for effect of soaking on flavonoid content

Source of Variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
Soaking hours	4	899.011	224.753	64.55	<.001
Residual	10	34.816	3.482		
Total	14	933.827			

Since p< 0.05, there is a significant difference between the samples in different soaking hours so LSD testing is necessary.

Table B.26 LSD of means effect of soaking on flavonoid content

No of hours	Mean	Column A	l. s. d	d. f
48*	35.77±1.91	a	3.395	10
36*	42.51±1.80	b		
24	48.82±1.74	c		
12	52.03±1.98	c		
0 (Raw*)	58.22±1.890	d		

(*= significantly different)

Table B.27 ANOVA for effect of germination on flavonoid content

Source of variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
Germination hours	3	1735.522	578.507	160.58	<.001
Residual	8	28.821	3.603		
Total	11	1764.343			

Since p< 0.05, there is a significant difference between the samples in different germinationhours so LSD testing is necessary.

Table B.28 LSD of means effect of germination on flavonoid content

No of hours	**Mean**	**Column A**	**l. s. d**	**d. f**
0 (24h soaked*)	48.82±1.74	a	3.574	8
24*	69.10±1.91	b		
48*	83.55±1.96	c		
72*	88.75±1.83	d		

(*= significantly different)

List of Plates

P1 Raw sorghum grains

P2 Germinated sorghum grains

P3 Germinated sample kept for

P4 Distillation in Kjeldahl's distillation set digestion for analysis of protein