



The effect of malting and fermentation on the nutritional and potential health-promoting properties of finger millet [*Eleusine coracana* (L.) Gaertn] grain

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BSc. (Hons) Applied Microbiology and Brewing

MSc. Food Science and Technology

A PhD thesis submitted for the Degree of Doctor of Philosophy in Agriculture (Food Science and Technology) at University of Venda, Thohoyandou 0950, Limpopo Province, South Africa

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August, 2018

Abstract

Finger millet (FM) [*Eleusine coracana*] is an underutilised cereal grain used as a food source in South Africa. Increased research interest in FM has span over the years owing to its unique nutritional and bioactive composition. Following the recent interest in natural curative substances over their synthetic counterparts in the treatment of food dependent diseases, FM has shown potential nutraceutical effects. Some important health effects like antidiabetic, antioxidative, anti-inflammatory and antimicrobial properties have been reported in recent trials with FM. In view of the increasing utilisation and application of FM in the region of Thulamela Municipality, Vhembe District of South Africa, two common indigenous FM varieties (brown and dark brown) were obtained and analysed for their physicochemical properties, levels of minerals, phytic acid, phenolic compounds and antioxidant activities. For this process, malted non-alcoholic beverages were produced and analysed for their physicochemical properties, levels of phenolic compounds, and total phenolics and antioxidant activities.

FM grains were soaked, germinated and kilned at an interval of 24 h for 96 h, using sorghum as an external reference. Mineral composition of the FM and sorghum samples were analysed using an inductively coupled plasma atomic emission spectroscopy (ICP-AES) and mass spectroscopy (ICP-MS), and atomic absorption spectrometer (AAS). Identification and quantification of phenolic compounds were performed using ultra-performance liquid chromatography mass spectrometer (UPLC-MS). All experiments were performed in triplicate except for the UPLC-MS analysis of the malted non-alcoholic beverages that was done in duplicate. Data were analysed by one way analysis of variance, and the mean values were separated by Duncan's multiple comparison test using SPSS version 24.0. Data showed that the FM varieties were rich in macro- and micro- or trace elements. The macro-elements calcium, magnesium, potassium, phosphorus and sulphur were found in *high amounts* ranging from 1597.37 mg/ kg – 6775.03 mg/ kg; iron, zinc, strontium and silicon were found in *significant amounts* in the range 21.47 mg/ kg – 55.67

mg/ kg, copper and boron were found in *low amounts* (2.2 mg/ kg – 7.7 mg/ kg), along with selenium and cobalt (0.02 mg/ kg – 0.05 mg/ kg). Heavy metals, barium and aluminium were found in the FM varieties. Varietal difference was found to play an important role in the mineral content of the grains during malting. Malting for 24 h reduced mineral content except for sodium. Beyond 48 h of malting, mineral content increased, particularly, for 96 h in FM grain malt. Significant ($p < 0.05$) increases in the mineral content of FM varieties were noted at 48 h and 96 h of malting. Increase occurred at 72 h of malting for potassium, iron and boron. Malting did not have any effect on the manganese content of the dark brown FM; however, it increased the manganese content at 48 h of malting for brown FM. Malting for 96 h significantly ($p < 0.05$) reduced sodium content. Consecutive decrease in phytic acid content of the grains was not recorded with durations in malting time. Although statistically significant differences ($p < 0.05$) were observed, malting did not result in too much change in the physicochemical properties of the grains. Several flavonoids, catechin, epicatechin, quercetin, taxifolin, and hesperitin were isolated, whilst protocatechuic acid was the only phenolic acid detected in the unmalted and malted FM. Increases in catechin, epicatechin and protocatechuic acid were observed for 72 and 96 h malt of brown FM with similar observations recorded for sorghum. Complete loss of taxifolin, catechin, and hesperitin were noted with malting time. FM grains exhibited 2, 2'-diphenyl-1-picrylhydrazyl (DPPH), 2, 2'-azinobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radical scavenging action and iron reducing activities. Increased iron reducing activity alongside ABTS radical scavenging activity was recorded with malting time. A fermentation-time dependent decrease in the pH of the non-alcoholic beverages, with a corresponding increase in sugar content were recorded. A similar decrease was also recorded for the viscosities of the beverages. The FM malt beverages were found to contain a higher amount of citric acid compared to the sorghum malt beverage. A decrease in the citric acid content with fermentation time was noted in the grain malt beverages fermented with *Lactobacillus fermentum*, particularly for the

FM beverage. The phenolic compounds detected in the FM malt beverages fermented with the grain microbial flora and *Lactobacillus fermentum* were protocatechuic acid, catechin and epicatechin. Taxifolin and kaempferol along with the earlier mentioned compounds were detected in the sorghum malt beverage. Catechin was found in higher amount compared to other phenolic compounds in the FM and sorghum malt beverages. FM malt beverages were found to contain a higher amount of total phenolics compared to the beverage prepared from sorghum malt. Fermentation with the grains' microbial flora and *L. fermentum* resulted in reduced total phenolics of FM and sorghum malt beverages, particularly after 24 h of fermentation. A fermentation-time dependent decrease in total phenolics of FM beverages fermented with *L. fermentum* was noted. Fermentation within 24 - 48 h using the grain microbial flora showed higher total individual phenolic compounds for the dark brown FM and sorghum, compared to other fermentation periods. Fermentation of the beverages for 24 h retained a higher amount of the total phenolics compared to other fermentation periods, especially for the *L. fermentum* beverages. Reduced total phenolic content and antioxidant activity of the beverages were noted at 24 h of fermentation for the two microbial sources. Significant ($p < 0.05$) increases in total phenolics were observed within 72 – 96 h of fermentation of the brown FM malt beverage with the grains' microbial flora. Fermentation for 72 h and 96 h with *L. fermentum* increased the total phenolic content of the brown FM. Increase in total flavonoid content (TFC) of brown FM malt beverage was noted at 72 h fermentation for both microbial sources. Unlike with *L. fermentum*, no significant ($p > 0.05$) change in TFC was observed for the dark brown FM beverage after 24 h fermentation with the grains' microbial flora. Beverages exhibited DPPH, ABTS radical scavenging action and iron reducing activities, which were significantly ($p < 0.05$) reduced at 96 h fermentation for both microbial sources. The 24 h fermented beverage retained a higher amount of total phenolic and flavonoid contents, and had higher antioxidant activity compared to other fermentation periods for both microbial sources. The study shows that FM is a rich source of essential minerals and

phenolic compounds, and demonstrates that 72 to 96 h of malting has a positive effect on minerals and certain phenolic compounds over the 48 h malting period widely used for preparation of FM malt. The presence of hesperitin in FM grain was established. A new method was developed for the production of FM non-alcoholic beverage with measurable amounts of health-promoting compounds. An ideal fermentation period (24 h) for FM malt non-alcoholic beverage production with enhanced health-promoting compounds, using *Lactobacillus fermentum* was demonstrated. Fermentation limit (96 h) for production of FM malt beverage using either the grain microbial flora or *L. fermentum* was confirmed. These findings provide a rationale for increased utilisation of FM as a functional food grain, and its use as malt in production of non-alcoholic beverage for health promotion and wellness.

Keywords: Millet, *Eleusine coracana*, malting, fermentation, non-alcoholic beverage, physicochemical properties, minerals, phytic acid, citric acid, phenolic compounds, antioxidant activity.

Declaration

I, Henry Okwudili Udeh student number 11626674, hereby declare that this thesis for the award of Doctor of Philosophy in Agriculture (Food Science and Technology) at the University of Venda, South Africa, has not been submitted previously for a degree at this or another university, and that it is my own work in design and in execution, and that all reference material contained therein has been duly acknowledged.

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Signature

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Date

Acknowledgment

I thank God the almighty, for the grace, knowledge and understanding to complete this doctoral study. May the glory of the Lord endure forever!

I want to thank my promoter, Professor Afam I. O. Jideani, for his inspiring support, insightful contributions, encouragement, discipline and guidance during the study. I am most grateful. My special thanks go to my co-promoter, Professor Kwaku G. Duodu, for his contributions, support, guidance and critical inputs, which has led to the completion of this thesis. Your critical input and words of encouragement saw me through this research. I want to thank my mother Mrs Teresa Udeh, my late dad Mr. Felix Udeh and my brothers and sisters; Ifeanyi, Ngozi, Chinyere, Ejike, and Chukwuemeka for their love, patience, prayers, and financial contributions all through the period of the study.

I thank my senior colleagues Drs. Adewale Omolola and Tonna Anyasi for their academic support during the course of the study. I thank every postgraduate student under the supervision of Professor Jideani: Olamiti Gbeminiyi, Oluwatoyin Onipe, Shonisani Ramashia and Udoro Elohor for their support, encouragement and contributions all through the period of my research. I thank every staff of the Department of Food Science and Technology, Moyo Barbara, Nethathe Bono, Mokhele Tabea, Mashau Mpho, for their assistance during the laboratory experiments.

I thank the Head of Department of Biochemistry, Professor A. Shonhai, for allowing me access to his laboratory equipment, and also the senior laboratory technicians of the Department of Soil Science Mr M. P. Tshidada, and Department of Chemistry Mr. F. B. Mutshaeni for their support during the laboratory analysis. My gratitude also goes to Mr. M. Taylor and Dr. M. Marietjie at Central Analytical Facility, LCMS division, Stellenbosch University for their assistance in analysing the phenolic compounds of the research samples. I thank the University of KwaZulu-Natal Centre of Excellence in Indigenous Knowledge Systems for their financial support during the study.

Dedication

This work is dedicated to all indigenous cereal grain farmers in Southern Africa.

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CHAPTER 1

INTRODUCTION

1.1. Background

Finger millet (*Eleusine coracana*) is an important minor cereal grain that could support the world's increasing demand for food and feed supply. It belongs to the *Eragrostideae* tribe of the grass family, *Poaceae*, formerly called *Gramineae*. The grain is known by several common names-: India: *ragi*, Ethiopia: *dagussa*, *tokuso*, *barankiya*, Swahili: *wimbi*, *ulezi*, Kenya: *wimbi*, *mugimbi*, Malawi: *mawere*, *lipoko*, *usanje*, *khakwe*, *mulimbi*, *lupodo*, *malesi*, *mawe*, Nepal: *kodo*, Sudan: *tailabon*, Tanzania: *mwimbi*, *mbege*, Uganda: *bulo*, Zambia: *kambale*, *lupoko*, *mawe*, *majolothi*, *amale*, *bule*, South Africa: *poho*, Zimbabwe: *rapoko*, *zviyo*, *njera*, *rukweza*, *mazhovole*, *uphoko*, *poho*, Nigeria: *tamba*, Afrikaans: *vogel gierst*, English: *finger millet*, *African millet*, *koracan*, France: *petit mil*, *eleusine cultivée*, *coracan*, *koracan*, German: *Fingerhirse* (NRC, 1996; Zvauya *et al.*, 1997; Jideani *et al.*, 2012).

Archaeological evidence suggests that finger millet (FM) originated in countries of Ethiopia and Sudan in Africa about 3,000 years ago, thereafter it was introduced to India in Asia about 1600 years ago (Seetharam *et al.*, 1986; Zarnkow, 2014; Chandra *et al.*, 2016). It is known to be a staple crop of the southern Africa region before maize. In parts of eastern and southern Africa, Asia and India, FM supports the diet of millions of people, especially the resource-poor. It is a principal cereal grain in Uganda as well as in north-eastern Zambia and Zimbabwe (NRC, 1996) and serves as an important famine food in parts of Mozambique. Considering its nutritional significance in providing essential minerals, calories, and protein, it is, therefore an ideal cereal crop for nutrition-agriculture research. Apart from the excellent nutraceutical value of the grain, its ability to tolerate various abiotic and biotic stresses, substantiates increased research on the crop, which would further strengthen its potential role in providing food security.

Up to the present, there is no documentation of FM production or use in any commercial or well-known local food or food product in South Africa. Finger millet has distinctive agronomic attributes, nutritional and nutraceutical advantages that are common to millets. Its fast maturation and all-season growth characteristics, and adaptation to a variety of conditions, make it a desirable crop for more intensive cropping systems; it could also be used as a relay crop, in combination with other crops that are slow in maturation. Despite these qualities, FM has been neglected, both scientifically and internationally. The main reason for this underutilisation has been its very small size, which makes handling of the grain, during harvest and processing difficult. Its minimal inclusion in the commercial food system, presumed nutritional insignificance, lack of research, and applicability in new product development have suppressed FM utilisation. This problem has been worsened by the various derogatory nicknames with which the grain is known, such as ‘poor person’s crop’, ‘lost crop’, ‘famine food’ and ‘birdseed’ (Gupta *et al.*, 2017). These terms, are used to indicate their overabundance in developed countries and their world production level, which however, is very low compared to other major food crops. Although neglected, the grain is significant by virtue of its contribution to biodiversity and being the means of livelihood of the poor in various parts of the world.

Promotion of crop varieties with enhanced nutraceutical value and improved stress tolerance has been one of the priority areas of research these days. FM has been drawing the attention of many researchers globally, particularly in India, as a potential crop against food insecurity and malnutrition. Recent research studies have shown that the FM grain has nutritional and health-promoting compounds that are comparable or superior to other major staple cereals like maize, rice, wheat and sorghum. The protein content of FM (7.3 – 9.8 %) is comparable to that of rice (7.5 - 7.9 %), maize (9.2 – 12.1 %) and sorghum (10.4 - 11 %) (Malleshi and Desikachar, 1986; Sripriya *et al.*, 1997; Shahidi and Chandrasekara, 2013; Devi

et al., 2014; Chandra *et al.*, 2016). Although considerable variation exists among varieties, at least one Indian-improved cultivar contains as much as 14 % protein (NRC, 1996). The main protein fraction of FM; eleusin, has a high biological value. The methionine level of FM, about 5 %, is of special benefit, notably for those who depend on plant protein (NRC, 1996). Moreover, it is rich in the essential amino acids, isoleucine, leucine, and phenylalanine, and B-vitamins, thiamine, and riboflavin (Chandra *et al.*, 2016). FM has a carbohydrate content of 72.6 – 81.5 % with fractions like free sugars (1 – 2 %), starch (70 – 76 %) and non-starch polysaccharides, mainly cellulose and hemicelluloses (Sripriya *et al.*, 1997; Obilana and Manyasa, 2002; Mathanghi and Sudha, 2012; Shahidi and Chandrasekara, 2013). Major sugars identified in the polysaccharides are arabinose, xylose, galactose, and glucose, with mannose and rhamnose being present in minute quantities. Studies have shown that FM contains dietary fibre in the range 18 - 22.0 %, which is more than the amount present in wheat (12.1 %), rice (3.7 %), maize (12.8 %) and sorghum (11.8 %) (Devi *et al.*, 2014; Chandra *et al.*, 2016; Udeh *et al.*, 2017). The work of Sridhar and Lakshminarayana (1994) shows a good lipid composition of FM compared to its counterparts, foxtail and proso millets. Predominant fatty acids of FM grain are oleic acid, linoleic and palmitic acids (Gull *et al.*, 2016).

Finger millet is a rich source of minerals and a variety of phytochemicals that are important for health; compared to other cereals, FM contains the highest amount of calcium (317.1 – 398 mg/ 100 g). In terms of the phytochemicals, studies have shown that FM contains a variety of phenolic compounds, which have been the main focus of many researchers. Phenolic extracts of FM have been shown to have several health-protective effects, such as antioxidative, antimicrobial, anti-diabetic and anti-inflammatory properties among others (Vishwanath *et al.*, 2009; Shahidi and Chandrasekara, 2013; Devi *et al.*, 2014). Finger millet is useful in the management of various physiological disorders such as diabetes mellitus, hypertension, vascular fragility, hypercholesterolemia, and prevention of oxidation of low-

density lipoproteins as well as in the improvement of gastrointestinal health (Scalbert *et al.*, 2005; Chandra *et al.*, 2016; Gupta *et al.*, 2017; Sharma *et al.*, 2017). A study by Pradhan *et al.* (2010), in India, on male and female diabetic patients, spread across different rural and urban areas, showed that glucose level was maintained after consumption of multigrain chapatti of finger millet and wheat in the ratio of 30:70. FM is a gluten-free grain and hence can be beneficial for patients suffering from celiac disease, as a strict gluten-free diet is currently the only treatment for the disease (Pagano, 2006; Gull *et al.*, 2016). The plant itself has been found to have diaphoretic, diuretic, and anthelmintic properties (Dida and Devos, 2006). It has been used as folk remedy for treatments, including leprosy, liver disease, measles, pleurisy, pneumonia and small pox (Dida and Devos, 2006).

South Africa and most African countries have recognised the importance of non-communicable diseases, which are still on the increase owing to lifestyle disorders and increasing poverty rates. The major non-communicable diseases in South Africa are diabetes, obesity, cardiovascular diseases, cancer and chronic respiratory diseases (Mayosi *et al.*, 2009). South Africans are increasingly eating a typical Western diet accompanied with low fruit, vegetable and cereal intake. This dietary habit is key risk factor to the aforementioned health conditions with national surveillances suggesting that these patterns of unhealthy lifestyles are already present in South African children and youth; this may affect the health and productivity of the country in the future. It has been suggested that population-wide interventions that promote healthy living, through balanced diet, physical exercise and healthy environment, should be encouraged to reduce the disease burden of the country. The use of plant food that is rich in health-promoting compounds such as FM in food and beverage preparations, therefore, can afford its consumers potential health benefits.

Despite the well-documented health benefits of the grain, as an excellent source of nutrient and minerals, it is also high in antinutrients particularly phytates and polyphenols, that

negatively affects the grain's nutrient value by reducing digestibility of the nutrients and mineral absorption. The negative impact of these antinutrients, however, can be resolved by using common household food-processing techniques like decortications, milling, soaking, germination, malting, fermentation, popping and cooking. These methods can reduce some of the antinutrients, especially phytates and phenols, which in turn improves the digestibility and bioavailability of minerals (Shibairo *et al.*, 2014; Gull *et al.*, 2016; Gupta *et al.*, 2017). Food-processing methods have tremendous effect on the nutritional value of plant foods, either in a positive or negative way. Current research focus is on methods that best preserve both the food structure and the nutritional components of plant foods to avoid high energy-dense and nutritionally-poor food. Today, with relatively simple technology, it is possible to increase the level of health-promoting compounds of certain foods. These modifications allow for development of food products that could contribute to improving health conditions and wellness. Among food processing methods that enhances the nutritional quality of plant foods is malting - a simple and inexpensive method for production, preservation, and improving nutritional quality of plant foods (Fardet, 2014).

Malting is the process of soaking, germination and kilning of cereal grains under controlled condition for development of hydrolytic enzymes and flavour compounds (Briggs' *et al.*, 2004). This process, which is enhanced during germination, has a profound effect on the physiological properties of cereal grains, which consequently impact on its nutritional quality and digestibility. It is well known that germination of cereal grain results in modification of certain nutritional and bioactive components through increased enzymatic activities. Increases in vitamin and mineral content accompanied with reduced antinutrients have been reported (Bellaio *et al.*, 2013). Reduced phytic acid content in FM during germination was observed by Mbithi-Mwikya *et al.* (2000). Chethan *et al.* (2008a) have also reported a decrease in the total polyphenol content of FM during 96 hours of malting with a time-dependent increase in α -

alpha and β -amylase activity. In their study, the grain's crude phenolic extract and isolated phenolic compounds showed a non-competitive and uncompetitive mode of inhibition on digestive enzymes, respectively. This property has a potential application in the management of diabetic conditions and weight control. Malting has been shown to increase the amounts of bioaccessible minerals and phenolic compounds in FM (Platel *et al.*, 2010; Hithamani and Srinivasan, 2014).

Considering the increasing global population and anticipated increase in temperature, particularly in African and other tropical regions of the world, utilisation of FM has a great potential in addressing food insecurity, problem with nutrition and health as well as poverty alleviation, among other factors (Gupta *et al.*, 2017). The present study investigated the effect of malting period on the mineral composition, phytic acid content, some physicochemical properties, phenolic composition and antioxidant activity of FM. The study produced a non-alcoholic beverage by improving on the technique used for production of commercial sorghum beverage – *amahewu*; and examined the physicochemical properties, citric acid, phenolic compounds, total polyphenols and antioxidant activity of the beverage.

1.2. Problem statement

- i. Food security is a global concern particularly in developing economies. The increasing world population and consequently increasing demand for food, together with changing global climate, necessitate an increased agricultural production and food security, either by means of increasing yields on already cultivated lands or by changing current crop-consumption patterns (Licker *et al.*, 2010; Gupta *et al.*, 2017). Considering the agronomic advantages, nutritional and phytochemical properties of FM, it is logical to increase utilisation and research of the crop for nutritional and health security. In terms of cultivation, FM is not produced in South Africa, however, various healthy traditional

cereal grain and multi-grain substitutes for refined carbohydrates can be one important aspect of dietary modification and a means for further utilisation of locally-available cereal grains.

- ii. In southern Africa and most African countries, incidences of diabetes, obesity, heart disease and cancer are increasing, primarily due to lifestyle disorder and increasing poverty rate (Connor *et al.*, 2007; Mayosi *et al.*, 2009). These disease conditions are well associated with the choice of food among other factors, including genetic characteristics, lack of exercise, ageing, drug abuse, environmental pollution and diseases. High consumption of refined carbohydrates, such as maize meal, rice and pasta increases blood glucose level which complicates obesity and diabetic conditions. In addition, drug abuse which is becoming widespread increases chances of several degenerative health conditions like pulmonary and gastrointestinal cancer, as well as cardiovascular diseases.
- iii. Malting has been successfully utilised in the development of various health foods beside its wide application in the preparation of a variety of beverages. Most studies have focused on the screening and comparison of the antioxidant activities of FM and how they are affected by different processing methods (Krishnan *et al.*, 2009; Viswanath *et al.*, 2009; Chandrasekara *et al.*, 2012; Hithamanai and Srinivasan, 2014). There is, however, very little information on the effect of malting period on minerals, phenolic compounds, total polyphenols, and antioxidant potential of FM. Furthermore, there are no documented uses of FM as food or in food products in South Africa; additionally, relatively few or no studies have investigated the effect of fermentation on the physicochemical, phenolic compounds, total polyphenols and antioxidant activity of FM malt beverage.

1.3. Justification of research

- i. In South Africa, there are no established records of FM production for commercialisation. The majority of the varieties that are found in local markets in Thohoyandou, Limpopo Province of South Africa are grown in Zimbabwe, hence, the use of FM in South Africa as food or in food products is virtually non-existent. There is, therefore, a need for developmental approaches that will promote the grain's utilization is necessary.
- ii. South Africa is experiencing a burden of diseases with diabetes, obesity, cancer and cardiovascular diseases recorded at an increasing rate. In recent times, preventive nutrition has taken the scene of various research areas, especially in food science. Although not considered medicinal, a general consensus has been reached on the efficacy of cereal grains in offering immediate and lasting health effects. As part of the key developmental changes in reducing the risk of various metabolic diseases, healthy eating habits, and research into novel and nutritious food products are highly recommended, globally. Recent research findings have implicated FM with biologically active compounds that could find application in ameliorating several non-communicable diseases. In other words, FM food and its food products can be an ideal source of nutrients with potential positive impact on the incidence and progression of most of these degenerative health conditions.
- iii. The use of efficient and less expensive food-processing method in improving the nutritional value of foods is desirable. Malting and fermentation are recognised food processing methods that are used in improving the nutritional value of plant food particularly cereal grains. Soaking, germination, roasting and fermentation, are

recommended strategies through which bioactive substances of cereal products can be enhanced. There is, therefore, a need to investigate the effect of malting on availability of bioactive compounds of FM, their potential health effects and use in novel food production.

1.4. Hypotheses

- i. Mineral composition, phytic acid content and physicochemical properties of FM can be affected at varying malting periods.
- ii. Phenolic composition and antioxidant activity of FM can be improved by changing malting periods.
- iii. A non-alcoholic beverage with significant health-promoting compounds and desired physicochemical properties can be processed from FM grain malt.

1.5. Aim and objectives

To obtain a FM malt flour and non-alcoholic beverage with improved physicochemical and nutritional properties, bioactive compounds and antioxidant activity.

Objectives

- a. To determine the effect of malting on physicochemical properties, and minerals and phytic acid content of FM grain.
- b. To assess the impact of malting on the phenolic compounds and antioxidant activity of FM grain.

- c. To examine the effect of fermentation and microbial sources on the physicochemical properties, and citric acid, and phenolic content, and antioxidant activity of a FM malt non-alcoholic beverage.

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

Millet consists of six major-small grain cereal crops, namely, finger millet (*Eleusine coracana*), foxtail millet (*Setaria italica*), kodo millet (*Paspalum scrobiculatum*), proso millet (*Panicum miliaceum*), barnyard millet (*Echinochloa* spp.) and little millet (*Panicum sumatrense*); all of them are known for their unique traits and nutritional qualities (Kumar *et al.*, 2016a; Kumar *et al.*, 2016b; Gupta *et al.*, 2017). Millets need very little water for their production and can be cultivated under non-irrigated conditions or in very low-rainfall regimes (200 – 500 mm). Crops, such as rice and wheat do provide some form of food security, but their cost of production remains high. Millet accounts for manifold benefits, including being food, fodder, nutritious, healthy, environmentally-friendly and an inexpensive source of livelihood, making it an essential guardian of agricultural security (Gupta *et al.*, 2017).

Finger millet (FM) is an important millet in the world, serving as staple to millions of economically-disadvantaged people in African and Asian countries. The crop is an allopolyploid with 36 chromosomes which had evolved from a cross between two diploid species, *Eleusine indica* (AA) and *E. floccifolia* or *E. tristachya* as genome donors (Chandra *et al.*, 2016). *E. coracana* is the most common species of this millet cultivated for food use, while *E. indica* and *E. africana* are wild and semi-wild species (Gull *et al.*, 2016). FM grains are small-seeded, about 1 – 1.8 mm in diameter, spherical/ globular or oval in shape, with light brown or brick-red coloured seed coat with a thin membranous pericarp which is loosely attached, covering the entire seed that usually detaches during harvest or simple abrasion (Chethan and Malleshi, 2007; Shobana and Malleshi, 2007). Although dark brown, yellow, white, tan, violet and black-coloured seed varieties are plentiful, they are not so popular compared to the widely cultivated red-coloured type. The kernel does not have a true caryopsis

but a utricle. The endosperm of FM is white in colour and the seed coat contains five layers which are attached tightly to the endosperm (McDonough *et al.*, 1986). The endosperm is soft and fragile and is divided into three parts, namely, peripheral, corneous and floury. The endosperm comprises about 83 % of the whole grain and is mostly filled with starch granules. The shape of its starch granules may be spherical, polygonal and lenticular. Finger millet starch comprises amylose and amylopectin in the ratio of 25:75 (Gull *et al.*, 2016).

Finger millet can be grown on low fertility soils and is not dependent on the use of chemical fertilisers, hence, is appropriate for the vast arid and semi-arid regions (Gull *et al.*, 2014; Chandra *et al.*, 2016; Gull *et al.*, 2016). The different genotypes of FM have genes for early and vigorous growth, large panicle size, increased finger number and branching, as well as high density grains. Some of the genotypes are water-efficient with elevated carbon dioxide fixation rates and minimal leaf area, hence, can perform excellently, in semi-arid climates. The grain is also known to be one of the most efficient utilizer of nitrogen (Gupta *et al.*, 2017). FM seeds can resist storage infestation by insect and pests for as long as 10 years, ensuring all-round year food supply even during crop failure, which has earned it the popular name, ‘famine crop’ (Mgonja *et al.*, 2007; Gull *et al.*, 2016).

Research interest in FM has increased owing to its unique nutritional quality and abundance of health-promoting compounds. These bioactive compounds, including ferulic acid, quercetin, and ferulic-rich arabinoxylans or feraxans among others, have been shown to exhibit important health properties. Some important health effects such as antioxidative, anti-inflammatory and antimicrobial properties have been reported in recent trials with the millet (Sripriya *et al.* 1996; Anthony *et al.* 1998; Kumari and Sumathi 2002; Rajasekaran *et al.* 2004; Chethan and Malleshi 2007; Shobana and Malleshi 2007; Banerjee *et al.* 2012; Shahidi and Chandrasekara 2013). Medicinal use of the plant in various ailments has been reported (Dida and Devos, 2006) and information on FM its nutritional, nutraceutical and biodiversity value

is fast accumulating (Udeh *et al.*, 2017). Research on the bioactive composition, bioaccessibility and possible health benefits of the grain are, however limited. This review section highlights information on FM and its production, nutritional composition, and phenolic compounds with further discussion on the bioaccessibility and bioactivities of the grain.

2.2. Finger millet production and uses

Finger millet is an underutilised cereal grain owing to its minimal inclusion in the commercial food system, presumed nutritional irrelevance, lack of research, and applicability in novel-product development processes. Among millet grains in the world, FM ranks fourth in importance after pearl millet [*Pennisetum glaucum*], foxtail millet [*Setaria italic*], and proso millet [*Panicum miliaceum*] (FAOSTAT, 2011; Shahidi and Chandrasekara 2015; Annor *et al.*, 2017; Udeh *et al.*, 2017). FM is grown globally in more than 4 million ha, with < 4 million tonnes produced annually (Latha *et al.*, 2005; Rosentrater and Evers, 2018). The major FM producer in the world is India, which accounts for nearly 60 % of the total global production, at 1.92 million tonnes between 2011 and 2012 (NAAS, 2013; Sharma *et al.*, 2017). Nepal, Sri Lanka, East China, Bangladesh, Kenya, Tanzania and Uganda are the other main countries in which the grain is commonly grown (FAOSTAT 2004; Gull *et al.*, 2016). Table 1 shows the number of worldwide cultivated germplasm collection of FM preserved in international gene banks.

Finger millet is a resourceful grain that can be used in different types of foods. Its major uses include porridge, pasta, noodles, vermicelli, bread, malt, alcoholic and non-alcoholic beverage production. It is nutritious, easily digested, and is recommended particularly for infants and the elderly, as well as pregnant and lactating mothers (Gupta *et al.*, 2017). Popped products made from FM are widely enjoyed in India.

Table 1. Number of worldwide significant cultivated germplasm collection of finger millet preserved in international gene banks.

Country	Institutes	No. of accessions
Asia		
India	All India Coordinated Millet Project, UAS, Bangalore.	6257
	International Crop Research Institute for the Semiarid Tropics (ICRISAT), Patancheru.	6804
	National Bureau of Plant Genetic Resources (NBPGR), New Delhi.	9522
Japan	Department of Genetic Resources I, National Institute of Agrobiological Sciences (NIAS), Tsukuba-shi.	565
Nepal	Central Plant Breeding and Biotechnology Division, Nepal Agricultural Research Council (CPBBD), Khumaltar, Kathmandu.	869
Africa		
Ethiopia	Ethiopia Institute of Biodiversity Conservation, Addis Ababa	2156
Kenya	National Gene Bank of Kenya, Crop Plant Genetic Resources Centre, Muguga.	2875
Uganda	Serere Agricultural and Animal Production Research Insitute, Soroti.	1231
Zambia	SADC Plant Genetic Resources Centre, Lusaka	1037
America		
USA	National Center for Genetic Resources Preservation, Fort Collins, Colorado, USA	702
	Plant Genetic Resources Conservation Unit, USDA-ARS, Griffin, GA, USA	748

Source: Adapted from Gupta *et al.* (2017).

Much of the FM, in Africa is used as an adjunct for brewing beer and this potential is associated with the increased amylase activity of FM during malting which readily converts starch to sugar. FM has much higher saccharifying power than sorghum and maize except barley, which is the premium grain for beer production (Sastri, 1989; NRC, 1996; Reddy *et al.*, 2008). FM has been analysed in order to identify the varieties that are rich in amylase and low in tannins. Some of the varieties showed β -amylase levels similar to those of barley malt (Zarnkow, 2014). In Kenya, FM is used with corn to brew opaque beers and in other East African countries, FM malt is often combined with pearl millet malt to make other varieties of alcoholic beverages (Zarnkow, 2014). In Ethiopia, FM is use to make *arake* a strong distilled liquor (NRC, 1996) while *masvusvu* and *mangisi* are known traditional non-alcoholic beverage that are locally consumed for variety of purposes in Zimbabwe (Zvauya *et al.*, 1997). Other fermented products of FM are *roti* or *chapatti* (unleavened flat bread), *mudde* (dumpling), *ambali* (thin porridge) and *chang* which are mostly consumed in India (Malleshi and Hadimani, 1993; Sripriya *et al.*, 1997; Sharma *et al.*, 2017). Other notable uses of FM include, being fodder for animals. Finger millet straw makes good fodder better than pearl millet, wheat, and sorghum, with up to 60 - 61 % total digestible nutrients and a variety of phenolic compounds (NRC, 1996).

2.3. Nutritional composition of finger millet

Finger millet shows it has an excellent nutritional value. Table 2 shows the nutritional composition and phenolic content of FM in comparison with other minor and major cereal grains. Proximate composition of FM shows its superiority to wheat, maize, sorghum and rice with regard to dietary fibre, calcium and few micronutrients (Gull *et al.*, 2016; Sharma *et al.*, 2017). The protein content of FM ranges between 7.3 and 9.8 % depending on the variety (Chandra *et al.*, 2016; Gull *et al.*, 2016).

Table 2. Nutritional composition and phenolic content of finger millet and other minor, pseudo- and major cereal grains (per 100 g grains, DW).

Cereal grains (kcal)	Energy	Protein (g)	Fat (g)	Carbohydrate (g)	Starch (g)	Fibre (g)	Ash (g)	Total phenols (mg/ 100 g)
<i>Minor cereals</i>								
Finger millet	336.0	7.3 – 8.2	1.3 – 1.8	72.6 – 81.5	59.0	3.6	2.0 – 3.5	102.0
Pearl millet	363.0	11.8 – 14.5	5.1 – 4.8	67.0 – 67.5	60.5	2.0 – 2.3	2.0	51.4
Proso millet	364.0	11.0 – 12.5	3.5	63.8 – 70.4	56.1	5.2 – 9.0	3.6	-
Foxtail millet	351.0	11.7 – 11.2	3.9 – 4.0	60.9 - 63.2	59.1	6.7 – 7.0	3.0	106.0
Kodo millet	353.0	8.3 – 9.8	1.4 – 3.6	65.9 - 66.6	72.0	5.2 – 9.0	3.6	368.0
Little millet	329.0	7.7 – 9.7	4.7 – 5.2	60.9 - 67.0	60.9	7.6	6.9	21.2
Barnyard millet	-	6.2	4.8	65.5	60.3	13.6	4.0	26.7
Fonio	367	9.0	1.8	75	-	3.3	3.4	-
Teff	336	9.6	2.0	73	-	3.0	2.9	-
<i>Pseudo-cereals</i>								
Amaranth	-	15.2	8.0	59.2	67.3	4.1	3.2	2.71
Quinoa	-	13.3	7.5	69.0	69.0	3.8	2.6	2.8
Buckwheat	-	10.9	2.7	66.0	67.2	10.1	1.59	7.25
<i>Major cereals</i>								
Wheat	348.0	11.6 – 14.4	2.0 - 2.3	71.0 – 71.2	64.0	2.0 – 2.9	1.8 – 1.9	20.5
Rice	362.0	7.5 – 7.9	2.4 – 2.7	73.0 – 78.2	77.2	10.2	4.7	2.51
Maize	358.0	9.2 – 12.1	4.6	66.2 - 73.0	62.3	2.3 - 2.8	1.8	2.91
Barley	-	11.5	2.2	80.7	73.8	5.6	2.9	16.4
Sorghum	329.0	10.4 – 11	3.1 – 3.2	70.7 - 72.6	58.5	2.0 - 2.7	1.8	43.1
Oats	-	17.1	6.4	69.8	52.8	11.3	3.2	1.2
Rye	-	13.4	1.8	80.1	68.3	2.1	2.0	13.2

Adapted from NRC (1996); Malleshi and Desikachar (1986); Shahidi and Chandrasekara (2013); Devi *et al.* (2014); Chandra *et al.* (2016).

The prolamin content of the grain is about 35 – 50 %; albumins and globulins constitute 8 – 15 % of the total protein. It has a good amino acid composition of 2.5 % lysine, 13 % tryptophan, 2.9 % methionine, 3.1 % threonine and 4 % leucine and isoleucine (Gull *et al.*, 2016). Lipids of FM are mostly triglycerides and these are known to reduce the incidence of duodenal ulcer; FM has a lipid content of 1.5 %. 49 % oleic, 25 % linoleic and 25 % of palmitic acids and are the predominant fatty acids of the grain. About 72 % of the total lipids are present as neutral lipids, 13 % glycolipids and 6 % phospholipids (Mahadevappa and Raina, 1978).

Carbohydrates present in FM are slowly digested and assimilated, as a result of which there is delayed absorption of glucose, which ultimately controls blood glucose levels (Chethan and Malleshi, 2007; Sharma *et al.*, 2017). The dietary fibre consists mainly of non-starch polysaccharides (water-soluble and water-insoluble), hemicellulose A and B, cellulose, pectin, and lignin (Nirmala *et al.* 2000; Subba Rao and Muralikrishna 2001; Rao and Muralikrishna 2007; Amadou *et al.* 2013). The non-starch polysaccharides are largely made up of arabinoxylans, with a small amount of β -D-glucans, and both make up the main component of the soluble dietary fibre fraction of finger millet (Subba Rao and Muralikrishna 2001; Rao and Muralikrishna, 2006). Shobana and Malleshi (2007) reported 22.0 % total dietary fibre, 19.7 % insoluble dietary fibre and 2.5 % soluble dietary fibre. FM contains resistant starch (RS), which is a functional fibre. Resistant starch escapes enzymatic digestion and imparts various beneficial effects by preventing several intestinal disorders and also acts as a preventive mechanism for colon cancer by production of metabolites, such as butyrates that stabilise colon cell proliferation (Sharma *et al.*, 2017).

Finger millet contains both fat and water-soluble vitamins such as thiamine, riboflavin, niacin, ascorbic acid and tocopherols (Obilana and Manyasa, 2002). Water-soluble B-vitamins are concentrated in the aleuronic layer and germ, while lipid-soluble vitamins are mainly located in the germ. The grain has a mineral content of 2.0 – 3.5 % with the highest amount of

calcium among other cereal grains (Sripriya *et al.*, 1997; Chandra *et al.*, 2016; Gull *et al.*, 2016). Nutraceutical importance has been largely associated with its calcium content, phenolic compounds, as well as the dietary fibre.

2.4. Health properties of cereal grains and mechanisms of action

Health claims of cereal grains have been hypothetically-based in the past, however, with accumulating knowledge and recent technological breakthroughs, efforts have been targeted at, empirically describing the underlying mechanisms associated with whole-grain health properties. The health benefits of cereal grains have been attributed to the effect of the food structure (increases satiety, reduces gastric emptying or food transit time and glycaemic response), fibre content (improving faecal bulking and satiety, viscosity, short chain fatty acid production and glycaemic modulation), mineral content (better glycaemic homeostasis through increased insulin secretion especially by Mg) and the presence of various bioactive phenolics (Sreeramulu *et al.*, 2009; Fardet, 2010; Warwick *et al.*, 2012). Although the presence of citric acid in cereal grains is rarely reported, the acid has been linked with important health properties. It forms part of the Krebs cycle, where the acid acts as an intermediary substance in oxidative metabolism. Studies have shown that citric acid is an important antioxidative and anti-inflammatory agent, thus, can contribute to reduction of oxidative stress (Wyrzykowski *et al.*, 2011; Abdel-Salam *et al.*, 2014). Figure 1 shows the mechanisms of the health-protective effect of whole grain bioactive compounds alongside the physiological outcomes.

The health-promoting effect of phytochemicals is complex and multi-factorial, and particularly involves their antioxidant and anti-inflammatory properties since oxidative stress is the major factor in the aetiology of various non-communicable diseases, especially cancer.

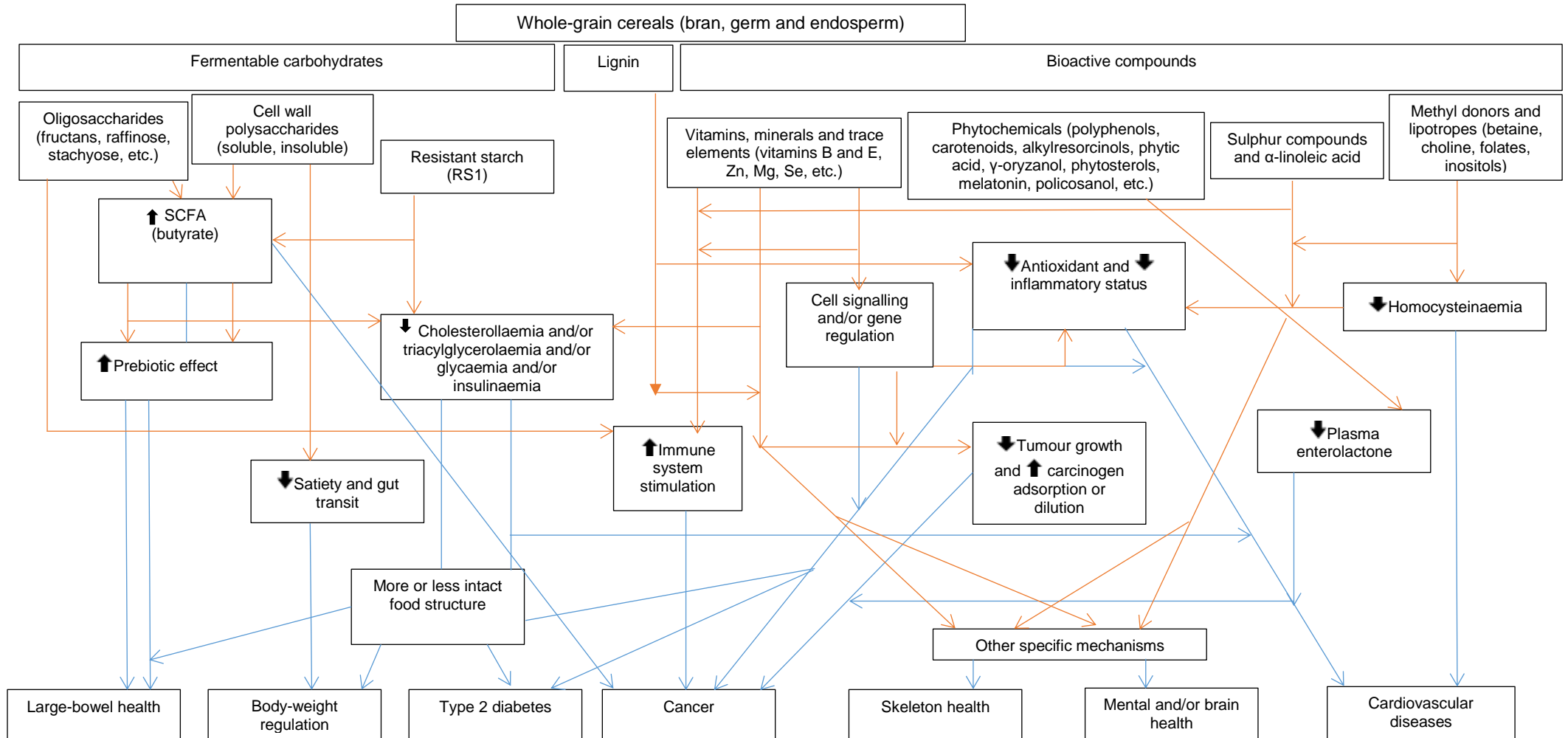


Figure 1. Proposed physiological mechanism of action of the protective effect of whole grain bioactive phytochemicals. Brown arrow lines (→) indicate whole grain bioactive compounds and protective physiological mechanism of action, while the blue arrow lines (→) indicate relationship of the mechanism and health outcomes. Source: Fardet (2010).

Bioactive phenolics are reported to modulate cellular oxidative status and prevent biologically critical molecules, such as DNA, proteins and membrane lipids from oxidative damage (Yu *et al.*, 2002); they also participate in the regulation of cell-signalling pathways, thus modifying gene regulation and/ or cell redox status (Maggi-Capeyron *et al.*, 2001; Yun *et al.*, 2008). Epidemiological and *in vitro* studies have shown that dietary ferulic acids may be important in the prevention of certain chronic diseases (Slavin, 2000; Kikuzaki *et al.*, 2002; Shahidi and Chandrasekara, 2013). It has been suggested that the health-protective effect of cereal bioactive compounds may be more complex than those perceived from the simple-absorption mechanistic view (Manach *et al.*, 2004; Whent *et al.*, 2012). In the case of free phenolic compounds, which show rapid absorption and metabolic turnover in the gastrointestinal tract, it has been proposed that cell-wall bound phenolics can terminate free radicals more effectively during their slow and continuous release in the intestinal lumen, unlike the free phenolics (Vitaglione *et al.*, 2008; Wang *et al.*, 2014).

It is becoming increasingly evident and conclusive that the health effects of whole-cereal grains' bioactive compounds are a result of the synergistic and / or additive effects of dietary fibre, phenolic compounds, and other bioactive components of the grain and not as a consequence of their isolated species (Liu, 2004; Liu, 2007; Fardet, 2010; Jideani *et al.*, 2014; Wang *et al.*, 2014). This assertion is based on the fact that their individual content in whole grains is too low to have any significant or lasting physiological effect.

Studies have shown that health conditions associated with obesity/ poor body-weight regulation, cardiovascular diseases, type 2 diabetes as well as cancer, gut, mental/ nervous system and skeletal health are potentially prevented by at least 10, 34, 17, 32, 10, 26 and 16 different bioactive phytochemicals that act in concert (Healthgrainforum, 2011). The work of Tan *et al.* (2010) allude to this hypothesis of the complex mixture effect of bioactive compounds, as candidate phytochemicals fail to induce phase II enzymes in an *in vitro* study with human lung-cell lines. Some crucial health effects such as antidiabetic, antioxidative, anti-inflammatory and antimicrobial properties have been reported in recent trials with FM grain (Kumari and Sumathi,

2002; Rajasekaran *et al.*, 2004; Hedge *et al.*, 2005; Banerjee *et al.*, 2012). The following section focuses on the phenolic compounds of FM and their properties and further discusses available evidence on their bioaccessibility and bioactivity.

2.5. Bioactive compounds of finger millet

The nutritional and phytochemical components of FM have been increasingly investigated over the years. Studies carried out on the grain have reported certain groups of biologically active compounds that are also nutritionally important (Subba Rao and Muralikrishna, 2001; Chethan *et al.*, 2008a; Platel *et al.*, 2010; Krishnan *et al.*, 2012; Devi *et al.*, 2014). These compounds which are clustered around plant nutrients as dietary fibre, vitamins, minerals and polyphenols exhibit potential nutraceutical effects through their participation in several biological systems. Table 3 shows a list of the dietary fibre fractions, mineral and vitamin contents of FM.

2.5.1. Phenolic compounds of finger millet

Phenolic compounds are among the most highly diversified groups of phytochemicals found in plant foods and as such, constitute an important part of the human diet. They represent several groups of compounds that include, hydroxybenzoic acids, hydroxycinnamic acids, flavonoids, stilbenes, and lignans (Naczki and Shahidi 2004; Chandrasekara and Shahidi 2012). Several studies have shown that FM contains a wide variety of phenolic compounds. The widely studied phenolic compounds in the grain are the phenolic acids and flavonoids. Table 4 shows the amounts of identified phenolic compounds in FM. These values might vary on the basis of experimental conditions, assay methods and grain type studied.

Studies have shown that the brown type of FM contains a high proportion of phenolic content compared to the white type; this is found to concentrate in the seed coat rather than in the flour fraction (Chethan and Malleshi 2007). Sripriya *et al.* (1996) indicate that brown finger millet has a higher (0.1 %) polyphenol content than the white counterpart (0.003%).

Table 3. Dietary fibre fractions, minerals and vitamin content of finger millet.

Component	Amount	References
<i>Dietary fibre fractions (%)</i>		
Total dietary fibre	18 - 22.0	Kamat & Belavady (1980); Shobana & Malleshi (2007)
Soluble dietary fibre	2.5	Ramulu & Rao (1997); Shobana & Malleshi (2007)
Insoluble dietary fibre	19.7	Ramulu & Rao (1997); Shobana & Malleshi (2007)
Hemicellulose A	1.4	Rao & Muralikrishna (2001)
Hemicellulose B	1.9	Rao & Muralikrishna (2001)
<i>Minerals (mg/ 100 g)</i>		
Calcium	317.1 – 398.0	Shobana & Malleshi (2007); Shahidi & Chandrasekara (2013)
Copper	0.2 – 0.47	Platel <i>et al.</i> (2010); Muthamilarasan <i>et al.</i> (2016)
Iron	2.12 – 3.9	Platel <i>et al.</i> (2010); Muthamilarasan <i>et al.</i> (2016)
Manganese	3.73 – 5.98	Sripriya <i>et al.</i> (1996); Muthamilarasan <i>et al.</i> (2016)
Zinc	1.44 – 2.31	Sripriya <i>et al.</i> (1996); Shashi <i>et al.</i> (2007)
Magnesium	5.49 – 137	Chandra <i>et al.</i> (2016); Muthamilarasan <i>et al.</i> (2016)
Phosphorous	211.0 – 320.0	Shobana & Malleshi (2007); Muthamilarasan <i>et al.</i> (2016)
Potassium	294.0 – 1070.0	Chandra <i>et al.</i> (2016); Shashi <i>et al.</i> (2007)
Sodium	0.6 – 11	Chandra <i>et al.</i> (2016); Shashi <i>et al.</i> (2007)
<i>Vitamins (mg/ 100 g)</i>		
Thiamine	0.24 - 0.42	NRC (1996); Anthony <i>et al.</i> (1996); Chandra <i>et al.</i> (2016)
Riboflavin	0.11 - 0.19	NRC (1996); Chandra <i>et al.</i> (2016)
Niacin	1.0 - 1.1	NRC (1996); Chandra <i>et al.</i> (2016)

Table 4. Phenolic composition of finger millet.

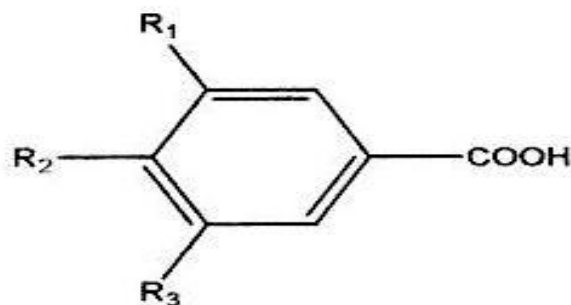
Phenolic group	Phenolic compounds	Quantity (µg/ g)	References
Phenolic acids	<u>Hydroxybenzoic acids</u>		
	Gentisic	4.5	Hithamani & Srinivasan (2014)
	Vanillic	20.0	Chethan <i>et al.</i> (2008a)
	Gallic	3.91 - 30.0	Chethan <i>et al.</i> (2008a), Hithamani & Srinivasan (2014)
	Syringic	10.0 - 60.0	Dykes & Rooney (2007); Hithamani & Srinivasan (2014)
	Salicylic	5.12 - 413.0	Hithamani & Srinivasan (2014)
	Protocatechuic	119.8 - 405.0	Chethan <i>et al.</i> (2008a); Hithamani & Srinivasan (2014)
	<i>p</i> -Hydroxybenzoic	6.3 - 370.0	Dykes & Rooney (2007); Chethan <i>et al.</i> (2008); Hithamani & Srinivasan (2014)
	<u>Hydroxycinnamic acids</u>		
	Chlorogenic	-	Shahidi & Chandrasekara (2013)
	Caffeic	5.9 - 10.4	Hithamani & Srinivasan (2014)
	Sinapic	11.0 - 24.8	Hithamani & Srinivasan (2014)
	<i>p</i> -coumaric	1.81 - 41.1	Chethan <i>et al.</i> (2008a), Shahidi & Chandrasekara (2013)
	<i>Trans</i> -cinnamic	35 - 100.0	Chethan <i>et al.</i> (2008a), Hithamani & Srinivasan (2014)
	<i>Trans</i> -ferulic	41 - 405.0	Shahidi & Chandrasekara (2013)
Flavonoids	Quercetin	3	Chethan <i>et al.</i> (2008a)
	Catechin Gallocatechin, epicatechin, epigallocatechin, taxifolin, vitexin, tricetin, luteolin, myricetin, apigenin, kempherol, narigenin, diadzein, procyanidin B1, orientin, isoorientin, isovitexin, saponarin, violanthin, lucenin-1, saponarin, violanthin	-	Rao & Muralikrishna (2002), Shobana <i>et al.</i> (2009), Viswanath <i>et al.</i> (2009), Chandrasekara & Shahidi (2011), Banerjee <i>et al.</i> (2012), Shahidi & Chandrasekara (2013).

In addition, Ramachandra *et al.* (1977) reported that brown FM contains a higher proportion (about 0.12 – 3.47%) of proanthocyanins than the white variety (0.04 – 0.06%). Most of the studies have focused on the two major finger millet types (brown and white), with the dark brown and black type rarely reported upon. It is important to investigate these varieties to further identify, quantify and characterise of their phenolic compounds. Like other cereal grains, the phenolic compounds of FM occur in free, conjugated and bound forms (Chethan and Malleshi 2007; Chethan *et al.*, 2008a; Chandrasekara and Shahidi 2012). Reported amounts of the free, esterified, etherified and bound-phenolic compounds of FM are 1970, 216, 81, and 536 µg/g, respectively, with the total phenolic content ranging from 265 to 373.15 mg/ 100 g (Shobana and Malleshi 2007; Sreeramulu *et al.*, 2009; Shahidi and Chandrasekara 2013). The content of the soluble (free, esterified, and etherified) and insoluble fractions is the sum of the derivatives of hydroxybenzoic and hydroxycinnamic acids and flavonoids as determined in the report of Chandrasekara and Shahidi (2011).

2.5.1.1. Phenolic acids

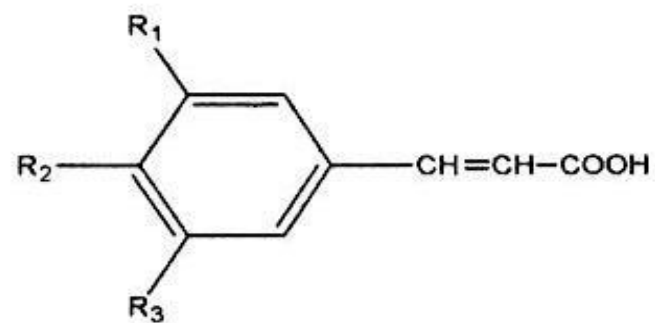
Phenolic acids are derivatives of benzoic and cinnamic acids and are found in all cereals, with sorghum and millets containing the widest variety (Dykes and Rooney, 2007; Liu, 2007). There exist two classes of phenolic acids: hydroxybenzoic acids which are derived from benzoic acid and hydroxycinnamic acids which are derived from cinnamic acid (Figure 2). The hydroxybenzoic acids form the major free phenolic fraction of FM and constitute about 70 – 71 % of the phenolic acids of the grain. They include gallic acid, protocatechuic, *p*-hydroxybenzoic, vanillic, gentisic and syringic acids, with protocatechuic acid identified as the main free phenolic acid [45 mg/ 100 g] (Subba Rao and Muralikrishna 2002; Chandrasekara *et al.* 2012; Hithamani and Srinivasan 2014). Minute amounts of hydroxycinnamic acids (ferulic, caffeic, and *p*-coumaric acids) are also reported in the free phenolic fraction.

(a) Benzoic acid



Benzoic acid	Substitutions		
Derivatives	R ₁	R ₂	R ₃
<i>p</i> -Hydroxybenzoic	H	OH	H
Protocatechiric	H	OH	OH
Vannilic	CH ₃ O	OH	H
Syringic	CH ₃ O	OH	CH ₃ O
Gallic	OH	OH	OH

(b) Cinnamic acid



Cinnamic acid	Substitutions		
Derivatives	R ₁	R ₂	R ₃
<i>p</i> -Coumaric	H	OH	H
Caffeic	OH	OH	H
Ferulic	CH ₃ O	OH	H
Sinapic	CH ₃ O	OH	CH ₃ O

Figure 2. General structure of phenolic acids: (a) benzoic acid and derivatives and (b) cinnamic acid and derivatives (Liu, 2004).

The hydroxycinnamic acids form the majority of the bound phenolic acids of FM and include caffeic, chlorogenic, sinapic, cinnamic (*trans*-cinnamic), coumaric (*p*-coumaric), and ferulic (*trans*-ferulic) acids (Subba Rao and Muralikrishna, 2001; Subba Rao and Muralikrishna 2002; Chethan and Malleshi 2007; Dykes and Rooney 2007; Chandrasekara *et al.* 2012; Shahidi and Chandrasekara 2013; Hithamani and Srivivasan 2014). Hydroxycinnamic acids make up about 30% of the phenolic acids of the grain. Ferulic acid is the most abundant bound-phenolic acid in millet, followed by caffeic and coumaric acids, with their occurrence in the order of 18.6, 1.64, and 1.20–1.25 mg/ 100 g flour, respectively (Subba Rao and Muralikrishna 2001, 2002; Dykes and Rooney 2006). The hydroxybenzoic acids, protocatechuic and syringic acids are also found in trace amounts in the bound fraction.

2.5.1.2. Flavonoids

Finger millet shows the highest total flavonoid content (Shahidi and Chandrasekara 2013). A variety of the compounds has been identified both from the grain and the leaves of the millet, however little has been done to quantify their respective amounts. FM flavonoids are mainly present in the soluble form and have been shown to be higher at the amount of 2100 µg/g followed by kodo and foxtail millet (Chandrasekara *et al.* 2012). Esterified forms of flavonoids are also reported in FM which are markedly different from flavonoids of other millets, such as kodo millet (Shahidi and Chandrasekara 2015). The major flavonoids reported in millet are quercetin, catechin, gallocatechin, epicatechin, and epigallocatechin. In addition, proanthocyanidins or condensed tannins, which are oligomeric or polymeric flavonoids, are found in significant amounts in the grain with procyanidin B1 and B2 reported to be the major dimers (Chandrasekara and Shahidi 2012; Shahidi and Chandrasekara 2015).

Phenolic compounds are recognised for their antioxidative, anti-proliferative, anti-allergic and anti-inflammatory properties (Dykes and Rooney, 2007; Fardet *et al.*, 2008;

Shahidi and Chandrasekara, 2013). There are, however, no structural representations of these compounds peculiar to FM, instead, they are presumed to adopt similar structural identity and activity of the compounds in themselves. There is a need, therefore to characterise the structural forms of these compounds which will make them relevant in predicting the bioactivities of the grain, with respect to the phenolic compound(s) involved.

2.6. Bioaccessibility of finger millet bioactive compounds

Bioaccessibility of plant food nutrients, especially of cereal grains, is a novel scientific area of study, owing to its arbitral description of the essential nature of food nutrients and health. Although controversies still linger on a working definition, recent studies have adopted a paraphrase of the term's definition to be 'the fraction or amount of food substance from the food matrix that is soluble in the gastrointestinal environment and is available for absorption' (Cardoso *et al.*, 2015). The release and subsequent activity of bioactive compounds of foods are dependent on their accessibility to an enzymatic attack either from the food or the gastrointestinal tract. The gastrointestinal release of cereal bioactive compounds is very low due to their association with other food components like dietary fibre, polyphenols, and other antinutritional factors, such as phytic acid (Plate *et al.*, 2010; Krishnan *et al.*, 2012; Cardoso *et al.*, 2015).

Relatively few emerging studies have reported on the bioaccessible compounds of FM, especially for the minerals and phenolic compounds (Tatala *et al.*, 2007; Platel *et al.*, 2010; Chandrasekara and Shahidi 2012; Hithamani and Srinivasan 2014; Chitindingu *et al.*, 2015; Hithamani and Srinivasan, 2017). The effect of malting on the availability and bioaccessibility of iron, zinc, calcium, copper, and manganese in FM was evaluated by Platel *et al.* (2010). The report indicated a modest decrease in the mineral contents of FM after malting, however, an increase in bioaccessible iron and calcium was recorded (Platel *et al.*, 2010). The malting

process is known to reduce antinutritional factors, especially phytates, by activating endogenous enzymes such as phytase, which results in the breakdown and subsequent reduction. It is suggested that the decrease in mineral content was a result of the effect of malting on phytates and other antinutritional factors that form complexes with the minerals (Platel *et al.*, 2010). In addition, their release may have resulted from the ionisation reaction of the minerals during hydrolysis of the phytate -mineral complex. In another study carried out by Tatala *et al.* (2007), a similar increase in bioaccessible/bioavailable iron (0.75 to 1.25 mg/100 g) was observed after the germination of FM grain. The increase in bioaccessibility of iron could result from the activation of endogenous enzymes, such as esterases and phytase which consequently acts on polyphenols or phytate-mineral complexes, in turn resulting in the release of the mineral. The physiological impact was recorded as increases in the haemoglobin and serum ferritin levels in children fed the germinated-grain diet, for a period of 6 months.

The processing method is an important determinant of the bioaccessibility of cereal bioactive compounds. Some of the reported processing methods, such as milling, sprouting, roasting, enzymatic digestion and fermentation are effective in the release of these compounds by increasing their surface area ratio, inducing the activity of endogenous enzymes and bioconversion of the bioactive compounds to more active compounds. The effect of sprouting, roasting, pressure cooking, open-pan boiling, and microwave heating on the FM phenolic profile and their bioaccessibility was evaluated by Hithamani and Srinivasan (2014). Except for roasting, other processing methods brought about a reduction in the total phenolic acids and flavonoid contents. The reduction observed in the sprouted grains was attributed to the loss of phenolic compounds during soaking. In the process of germination, endogenous phenolic enzymes are activated, resulting in their hydrolysis and consequent loss during soaking. Sprouting and roasting were found to have a positive impact on the bioaccessibility of phenolic compounds in the grain, with an increase of 67% after sprouting. The effect of the processing

methods on the food matrix and transformation of the compounds into more active forms could account for the observed increase in bioaccessible phenolics (Hithamani and Srinivasan 2014).

The effects of simulated gastrointestinal pH conditions, gastric and gastrointestinal digestion, and colonic fermentation on the bioaccessibility of phenolic compounds of FM were investigated by Chandrasekara and Shahidi (2012). In all the treatments, higher bioaccessible total phenolics was observed for the gastric and gastrointestinal digest, compared to the pH treatment and colonic fermentation. Digestion of proteins and the subsequent release of their bound phenolics present in the grain were suggested to account for the increase in total bioaccessible phenolic compounds. The activity of endogenous enzymes, such as esterases could also play a role in the release of the phenolic compounds from the cell wall matrix during digestion.

2.7. Bioactivity of finger millet grain

Interest in natural curative substances over their synthetic counterparts has fostered investigations into several food materials, with potential health benefits. Studies on FM millet have indicated some therapeutic effect that could have relevance in some food-dependent diseases, such as diabetes mellitus, obesity, and gastrointestinal tract disorders (Gopalan, 1981; Geetha and Parvathi 1990; Tovey, 1994; Shobana *et al.*, 2010). Diabetes mellitus is a complex metabolic disorder characterised by high systemic glucose levels, resulting from an impaired insulin secretion, with alterations in carbohydrate, protein, and lipid metabolism (Kumari and Sumathi 2002; Devi *et al.*, 2014). In the treatment of diabetes mellitus, one preventive approach is to decrease the postprandial hyperglycaemia or glucose surge by blocking or reducing the action of carbohydrate hydrolysing enzymes. α -amylase and α -glucosidase are essential catalytic enzymes that convert carbohydrates into glucose for absorption in the human gut, and their modulation can be relevant in some metabolic conditions, especially diabetes.

It has been hypothesised in recent studies that the regular consumption of FM is associated with a reduced risk of diabetes and this property has been attributed to the high polyphenol and dietary fibre content of the grain (Gopalan, 1981; Devi *et al.*, 2014). This proposition is based on the role of bioactive compounds in the metabolism of food materials, especially carbohydrates. Phenolic compounds of FM have been shown to have a potential modulatory effect on the breakdown of carbohydrates, either by reacting with proteins/enzymes or altering properties of biopolymers. High dietary fibre is implicated in lower postprandial glucose and insulin responses owing to its participation/ formation of unabsorbable complexes with available carbohydrates (Devi *et al.*, 2014; Lafiandra, *et al.*, 2014). These processes often affect carbohydrate digestibility and result in the delay of the systemic absorption of glucose, which ultimately controls the postprandial blood glucose surge.

In a report of Chethan *et al.* (2008b), FM seed-coat phenolics were found to reversibly inhibit aldose reductase, in a non-competitive mode (Chethan and Malleshi 2007). Aldose reductase is the key enzyme implicated in diabetes-induced cataractogenesis (Chethan *et al.*, 2008b; Devi *et al.*, 2014). It is suggested that the mechanism of the inhibition of millet polyphenols on aldose reductase is by preventing the enzymatic conversion of glyceraldehyde to glycerol and glucose to sorbitol. The structure-activity relationship reveals that the hydroxyl group at position 4 and the neighbouring *O*-methyl group of polyphenols were responsible for the inhibition of the enzyme. In another study by Chethan *et al.* (2008a), FM phenolic extracts were found to exert a mixed non-competitive mode of inhibition on the malt amylases, whereas the individual phenolic compounds showed an uncompetitive inhibition. Among the phenolic compounds evaluated, *trans*-cinnamic acid was found to exhibit a higher degree of inhibition of 79.2 % with syringic acid showing a weaker inhibition of ~ 56 %; the finding of Shobana *et al.* (2009) was also consistent with this observation. In their study, FM seed-coat phenolics were found to exhibit a non-competitive inhibition against pancreatic amylase and intestinal α -

glucosidase in a dose-dependent manner. The mode of inhibition was suggested to be dependent on the concentration of phenolic compounds as well as the number and position of hydroxyl groups (Rohn *et al.*, 2002; Chethan *et al.*, 2008a). The competition of phenolic compounds with the substrate for enzyme active site or enzyme substrate specificity phenomenon could also account for the observation.

The oxidation of glycated collagen or glycoxidation presents a risk of complications in diabetic condition. Hedge *et al.* (2002) demonstrated the effect of a methanolic extract of FM and kodo millet on glycation and collagen cross-linking. The results showed inhibited glycation for collagen which was incubated with glucose (50 mM) and 3 mg of the FM extract. The inhibitory effect of the FM extract was attributed to the antioxidative activities of phenolic compounds present in the extract and other phytochemicals extracted from the seed coat. The effect of a methanolic extract of FM on collagen cross-linking was evaluated (Hedge *et al.*, 2005) and the results showed that the extract, strongly inhibited the glycation reaction compared to the synthetic antioxidants (aminoguanidine, butylated hydroxyanisole). The inhibitory effect was attributed to the phenolic compounds, especially ferulic acid, which has been shown to offer renal-protective effects through improved glycaemic control and renal-structural changes involved in the inhibition of oxidative stress (Hedge *et al.*, 2005; Choi *et al.*, 2011). A study of Kumari and Sumathi (2002) showed that the consumption of FM-based diets significantly lowered the blood-plasma glucose levels in individuals with non-insulin dependent *diabetes mellitus*, due to higher fibre content. Besides the fibre content, the presence of some antinutritional factors, in whole FM flour that could reduce starch digestibility and absorption may be responsible for the lower glycaemic response (Shobana *et al.*, 2007; Gupta *et al.*, 2017). Previously, the study of Geetha and Parvathi (1990) had shown that the supplementation of diets with *ragi* resulted in a higher reduction in fasting and postprandial glucose levels than supplementation with other millets. The hyperglycaemic,

hypcholesterolaemic, nephroprotective, and anticataractogenic properties of FM were demonstrated by Shobana *et al.* (2010). In the study, reduced fasting hyperglycaemia and partial reversal of abnormalities in the serum albumin, urea, and creatinine status were observed in streptozotocin-induced diabetic rats. Hypercholesterolaemia, hypertriacylglycerolaemia, nephropathy, and neuropathy associated with diabetes were significantly reversed in a diabetic group, fed the diet containing the FM seed-coat matter (Shobana *et al.*, 2010; Devi *et al.*, 2014). In addition, FM-based diets were found to enhance the antioxidant status and better control the blood glucose levels in rats (Rajasekaran *et al.*, 2004). These effects result from the high dietary-fibre content of the grain and the presence of antinutritional factors known to reduce starch digestibility and glucose diffusion. In the same vein, the phenolic compounds present in the grain may also play a role in the observed effect, as they have been shown to be potential inhibitors of carbohydrate-degrading enzymes.

In the later stage of diabetes, dermal injuries are known to worsen, resulting in deep wounds and amputation risk, if not properly treated. In a study by Rajasekaran *et al.* (2004), the effect of FM feeding on dermal wound-healing of induced diabetic rats was evaluated. In the experiment, feeding of diabetic rats with FM for a period of 4 weeks resulted in an improved wound-healing process. The effect was suggested to be a result of the synergistic activities of different phenolic antioxidants and minerals present in the grain. Consistent with the findings, Hegde *et al.* (2005) observed a similar effect on the dermal wound-healing process in rats upon topical application of an aqueous paste of FM flour. The observation was attributed to the high calcium and amino acid (cysteine and methionine) content of the grain, which were suggested to participate in the wound-healing process, although the effect could also result from the antioxidative and anti-inflammatory properties of the grain phenolic compounds. In addition, the antimicrobial activities of a FM polyphenol extract, as well as the individual phenolic compounds were determined by Banerjee *et al.* (2012). The millet extract was found to exhibit

proliferative inhibitory activities against *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Streptococcus pyogenes*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Serratia marsescens*, *Klebsiella pneumonia*, and *Yersinia enterocolitica*. It was suggested that the high phenolic content of millet may have caused the inhibition of microbial enzymes and oxidation of the microbial membranes thus resulting in the proliferation of bacterial cells. In the study, the phenolic compound, quercetin was found to exert the highest anti-proliferative effect among the phenolic compounds evaluated. This compound is widely known for its pharmaceutical properties including antioxidant, anti-apoptotic and anti-proliferative effects.

2.8. Conclusion

It is evident from the available literature that FM contains a significant amount of essential health-promoting compounds that are both bioaccessible and bioactive. As recommended by several authors, these findings require further validation on human subjects as well as their underlying mechanism of action to well establish FM's health associated claims (Udeh *et al.*, 2017). In the future, research on possible diverse application of FM should be encouraged to foster extensive utilisation of the grain, especially in resource poor areas where the grain is native. Research is needed to further identify and quantify the bioactive compounds, particularly flavonoids, as well as their chemical structure and structure-activity properties, which would aid in describing the bioactivities of the grain (Chandrasekara and Shahidi, 2012). An optimisation of the processing methods, with respect to the availability and bioaccessibility of FM bioactive compounds will be desirable, as it will assist in predicting the optimal conditions suitable for ensuring a significant amount of bioaccessible bioactive compounds in the grain for food use (Hithamani and Srinivisan, 2014; Gull *et al.*, 2016).

CHAPTER 3

EFFECT OF MALTING ON PHYSICOCHEMICAL PROPERTIES, MINERALS AND PHYTIC ACID CONTENT OF FINGER MILLET FLOUR

3.1. Introduction

A number of inorganic minerals are essential for numerous biological activities, which are important for human performance and survival and therefore should be provided in the diet. Minerals constitute a large proportion of the components of the body intracellular fluid where they act as cofactors of several enzyme systems, neuronal transmission, muscle contraction and ionic balance. Beside their bodily functions, minerals play an important role in the treatment of metabolic conditions such as diabetes, hypertension, cardiovascular disorder, anaemia, osteoporosis and immune abnormalities, among others (Sasaki *et al.*, 1999; Goldhaber, 2003; WHO/FAO, 2004; Fraga, 2005). Deficiency, in the body, of minerals like calcium, potassium, magnesium, iron, and zinc are common, causing widespread nutritional disorders in the world, particularly in developing economies (WHO, 2001; WHO/FAO, 2004; Platel *et al.*, 2010). Another major concern of mineral deficiency surrounds those on gluten-free diets. Gluten-free foods which are necessary in the diet of people who are allergic to gluten or wheat, and in other individuals who voluntarily, follow gluten-free foods, have been shown to contain less valuable minerals than gluten-containing food products (Gliszczyńska *et al.*, 2017). Poor accessibility of minerals from plant foods is a major contributing factor to these individuals' deficiency and associated health problems (Gibson *et al.*, 2006; Gupta *et al.*, 2017).

Finger millet is a rich source of essential minerals, which makes it an ideal cereal grain for use as a functional food ingredient. FM is processed either by soaking, germination, malting or fermentation with the resultant flour or extract used for specialised preparations. It has been recently used as composite flour for making biscuit due to its high calcium content (Krishnan

et al., 2012; Sharma *et al.*, 2017). Due to its high phytate (Sripriya *et al.*, 1997; Mamiro *et al.*, 2001; Afify *et al.*, 2011; Jha *et al.*, 2015) and polyphenol (Khrishnan *et al.*, 2012; Tako *et al.*, 2015), minerals in FM are low, thus presenting ground for concern. Platel *et al.* (2010) reported that malting had no significant influence on the mineral content of FM. Mamiro *et al.* (2001) did not find any significant change in calcium, zinc and iron content of soaked and germinated FM. Sripriya *et al.* (1997) however, showed that germination was effective in increasing trace elements like copper, zinc and manganese. Information on the mineral composition of FM and how the minerals are affected by malting period and phytic acid content is relatively scarce and inconclusive. In this study, we report on the application of inductively-coupled plasma spectrometer as a tool to study essential minerals present in malted FM. Changes in the physicochemical properties and phytic acid content of FM were also monitored.

3.2. Materials and methods

3.2.1. Cereal grain samples

Local FM (brown and dark brown coloured) and red-coloured sorghum grain varieties were purchased from retail outlet in Thohoyandou, Limpopo Province, South Africa. Megazyme K-PHYT assay kit was purchased from Megazyme International, Ireland, Bray Business Park, Bray Co. Wicklow, A98 YV29, Ireland. All other chemicals used were of analytical grade.

3.2.2. Malting of the cereal grain samples

The cereal grains were malted according to the method of Chethan *et al.* (2008a). Two (2) varieties of FM, namely, brown FM (BFM) and dark brown FM (DBFM) were used in this study. Sorghum was used as an external reference, in an attempt to prepare a comparable FM malt flour that could be used in malted and fermented food and beverage. One hundred gram

(100 g) portion of the FM and sorghum samples were initially soaked in water for 24 h at 25 °C in a growth chamber (Labcon, model L.T.G.C. South Africa); the grains were spread on a clean cheese cloth and maintained moist by sprinkling water periodically at intervals of 24 h. The sprouted grains were kilned for 8 h at 50 °C, after which a characteristic malt aroma was developed. Appendix ii shows the unmalted and malted FM and sorghum grain varieties. The kilned grains were milled into fine flour in a stainless steel grinder and stored in airtight polyethylene bags at - 20 °C until analysis.

3.2.3. Physicochemical properties of the cereal grain samples

The pH of the milled flour was determined according to AOAC (2000) method number 943.02, using a pH meter. Colour determination was done using Lovibond spectrophotometer (Model LC 100) according to manufacturer's instruction. Upon calibration of the spectrophotometer, the unmalted and malted FM as well as the sorghum grains were placed in cuvettes and inserted in the device for colour measurement. Distilled water was used as blank for each determination.

3.2.4. Determination of mineral content of the cereal flours

The mineral content of the cereal grain samples was determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES) and mass spectroscopy (ICP-MS), and atomic absorption spectrometer (AAS). To solubilise the acid-extractable elemental content of the samples for analysis using inductively coupled plasma atomic emission spectroscopy (ICP-AES) and mass spectroscopy (ICP-MS), digestion was performed on a Mars CEM microwave digester (CEM Corporation, North Carolina, USA) using ultra-pure HNO₃ or HNO₃ + HCl at elevated temperature and pressure. After a cooling period, extractants were made up to 50 ml volume with deionised water, then analysed by ICP-AES and/ or ICP-MS for the selected analytes. Major elements were analysed on a Thermo iCap 6200 ICP-AES

(Thermo Fisher Scientific, Waltham, USA). The instrument was calibrated with National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) traceable standards to quantify selected elements. A NIST-traceable quality control standard of a separate supplier were analysed to verify the accuracy of the calibration before the sample analysis. Where sample had undergone a digestion step, the results were corrected for the dilution factor resulting from the digestion procedure. Cobalt and selenium were analysed on an Agilent 7900 quadrupole ICP-MS. Samples were introduced via a 0.4 ml/min micro-mist nebulizer into a peltier-cooled spray chamber at a temperature of 2 °C, with a carrier gas flow of 1.05 l/ min. The elements were analysed under He-collision mode to remove polyatomic interferences.

The mineral content of FM and sorghum flours were also analysed with AAS according to AACC (1999) method number 40-70.01. Ten grams (10 g) of the flour samples were charred and allowed to become an ash in a muffle furnace at 500 °C overnight. The resultant ash cake was dissolved in 10 mL concentrated HCl, boiled and allowed to evaporate to dryness. The residue was dissolved in 2 N HCl and filtered through a filter paper, while rinsing the filter paper and residue, thoroughly, with distilled water. The filtrate was made up to 100 mL with distilled water and mixed well. Calibration for measurements was done using commercial mineral standards. The mineral contents were determined directly using an atomic absorption spectrometer (AA 240 FS, Agilent Technologies, Waldbronn, Germany). Standard flame condition (instrument mode: absorbance; flame type: air/acetylene; air flow: 13.50 L/min; acetylene flow: 2.00 L/min; lamp current: 5.0 mA) for each mineral was maintained during the analysis.

3.2.5. *Determination of the phytic acid and total phosphorus content of the cereal flours*

Phytic acid and total phosphorus content of the unmalted and malted finger millet and sorghum varieties were determined using Megazyme kit (K-PHYT, Megazyme, Bray, Ireland)

according to manufacturer's instruction. Accordingly, 1 g of the flour was extracted with 20 ml of 0.66 M HCl for 24 h at room temperature. An aliquot of the extract was centrifuged at 4470 g for 20 min, and the supernatant was neutralised with 0.5 ml 0.75 M NaOH. The neutralised extract was subjected to enzymatic dephosphorylation to determine free and total phosphorus. For free phosphorus, 0.05 ml of the neutralised extract was mixed with 0.62 ml of distilled water, 0.20 ml 0.02 % w/v NaN_3 , pH 5.5 sodium acetate buffer, then, vortexed and incubated at 40 °C for 10 min. Afterwards, 0.02 ml of distilled water, 0.20 ml MgCl_2 , ZnSO_4 , 0.02 % NaN_3 , pH 10.4 buffer was added, vortexed and incubated at 40 °C for another 15 min; the reaction was stopped with 0.30 ml of 50 % trichloroacetic acid (TCA) [$\text{C}_2\text{HCl}_3\text{O}_2$]. For total phosphorus, 0.05 ml of the neutralised extract was mixed with 0.60 ml of distilled water, 0.20 ml 0.02 % w/v NaN_3 , pH 5.5 sodium acetate buffer and 0.02 ml phytase enzyme. The mixture was vortexed and incubated at 40 °C for 10 min before the addition of 0.2 ml MgCl_2 , ZnSO_4 , 0.02 % NaN_3 , pH 10.4 buffer and alkaline phosphatase suspension, vortexed and incubated at 40 °C for 15 min. The reaction was stopped by the addition of 50 % 0.30 ml $\text{C}_2\text{HCl}_3\text{O}_2$ (TCA). The mixtures were centrifuged at 2860 g for 20 min. Colour reagent for colorimetric determination of dephosphorylated extract was prepared using 10 % w/v ascorbic acid, 1 M sulphuric acid and 5 % w/v ammonium molybdate. One millilitre (1 ml) of dephosphorylated extract (free and total phosphorus) was mixed with 0.5 ml of the colour reagent, vortexed and incubated at 40 °C for 1 h; absorbance was read at 655 nm. Absorbance for the standard was recorded using the standard solution supplied along with the kit. Total phosphorus and phytic acid contents were calculated as:

$$\text{Phosphorus (g/ 100 g)} = \text{absorbance for standard} \times 0.1112 \times \Delta A_{\text{phosphorus}}$$

where: $\Delta A_{\text{phosphorus}}$ = Difference between the total phosphorus and free phosphorus

$$\text{Phytic acid (g/ 100 g)} = \frac{\text{Phosphorus}}{0.282}$$

3.2.6. Statistical analysis

Measurement was conducted in triplicates and results presented as mean values and standard deviation. Statistical analysis was conducted using the one way analysis of variance (ANOVA) and the mean values for each experiment were compared using Duncan's multiple comparison test ($p < 0.05$ confidence levels). Pearson's correlation test was used to evaluate the significance of correlations. The mean values were considered to be statistically significant at $*p < 0.05$ and $**p < 0.01$. SPSS 24 for windows (SPSS Inc., Chicago, Illinois, USA) statistical software package was used to conduct the statistical analysis.

3.3. Results

3.3.1. Physicochemical properties of finger millet and sorghum malt flours

The effect of malting period on the pH and colour properties of the FM is presented in Table 5 and Figure 3, respectively. Malting did not result in a marked decrease in pH of the FM and sorghum grains for the entire malting periods. For the grain colour, the unmalted and malted sorghum grains had significantly higher ($p < 0.05$) L^* (lightness) values than the corresponding brown and dark brown FM grains. Lower a^* (redness) values were observed for unmalted and malted DBFM compared to the BFM and sorghum varieties. There was no significant ($p > 0.05$) change in yellow colour (b^*) as a result of malting for DBFM and sorghum grains except for BFM. Malting resulted in lower chroma (C^*) values for DBFM compared to BFM and sorghum grain. In general, the DBFM had the least L^* (0 h = 19.80 ± 0.75 , 24 h = 18.73 ± 0.95 , 48 h = 18.50 ± 0.62 , 72 h = 19.20 ± 1.76 , 96 h = 18.57 ± 0.31) values compared to BFM and sorghum grains.

Table 5. Effect of malting on pH, phytic acid and total phosphorus of finger millet and sorghum grain varieties.

Cereal grains	Malting period (h)				
	0	24	48	72	96
pH					
Dark brown finger millet	$6.61 \pm 0.01^{a y}$	$6.53 \pm 0.02^{b x}$	$6.50 \pm 0.01^{b z}$	$6.43 \pm 0.02^{c z}$	$6.53 \pm 0.02^{b z}$
Brown finger millet	$6.46 \pm 0.01^{a x}$	$6.35 \pm 0.01^{b y}$	$6.25 \pm 0.02^{c f}$	$6.37 \pm 0.01^{b y}$	$6.35 \pm 0.01^{b y}$
Sorghum	$6.48 \pm 0.02^{a x}$	$6.50 \pm 0.00^{a x}$	$6.37 \pm 0.00^{b x}$	$6.50 \pm 0.00^{a x}$	$6.60 \pm 0.23^{c x}$
Phytic acid (g / 100 g)					
Dark brown finger millet	$0.34 \pm 0.14^{a z}$	$0.70 \pm 0.00^{b y}$	$0.84 \pm 0.00^{c z}$	$0.76 \pm 0.15^{d y}$	$0.92 \pm 0.14^{e y}$
Brown finger millet	$0.65 \pm 0.00^{a y}$	$0.53 \pm 0.01^{b y}$	$0.70 \pm 0.03^{c y}$	$1.07 \pm 0.01^{d y}$	$0.86 \pm 0.03^{e y}$
Sorghum	$0.29 \pm 0.01^{a x}$	$0.38 \pm 0.26^{b x}$	$0.45 \pm 0.00^{c x}$	$0.36 \pm 0.00^{b x}$	$0.67 \pm 0.00^{d x}$
Total phosphorus (g/ 100 g)					
Dark brown finger millet	$0.09 \pm 0.00^{a x}$	$0.19 \pm 0.00^{b z}$	$0.24 \pm 0.00^{c z}$	$0.21 \pm 0.00^{d z}$	$0.26 \pm 0.00^{e y}$
Brown finger millet	$0.18 \pm 0.00^{a y}$	$0.15 \pm 0.00^{b y}$	$0.19 \pm 0.00^{c y}$	$0.30 \pm 0.00^{d y}$	$0.24 \pm 0.01^{e y}$
Sorghum	$0.08 \pm 0.00^{a x}$	$0.11 \pm 0.00^{b x}$	$0.13 \pm 0.00^{c x}$	$0.10 \pm 0.00^{b x}$	$0.19 \pm 0.78^{d x}$

^{abc} Mean within each row for each group, not followed by the same superscript, are significantly different ($p < 0.05$).

^{xyz} Mean within each column for each group, not followed by the same superscript, are significantly different ($p < 0.05$).

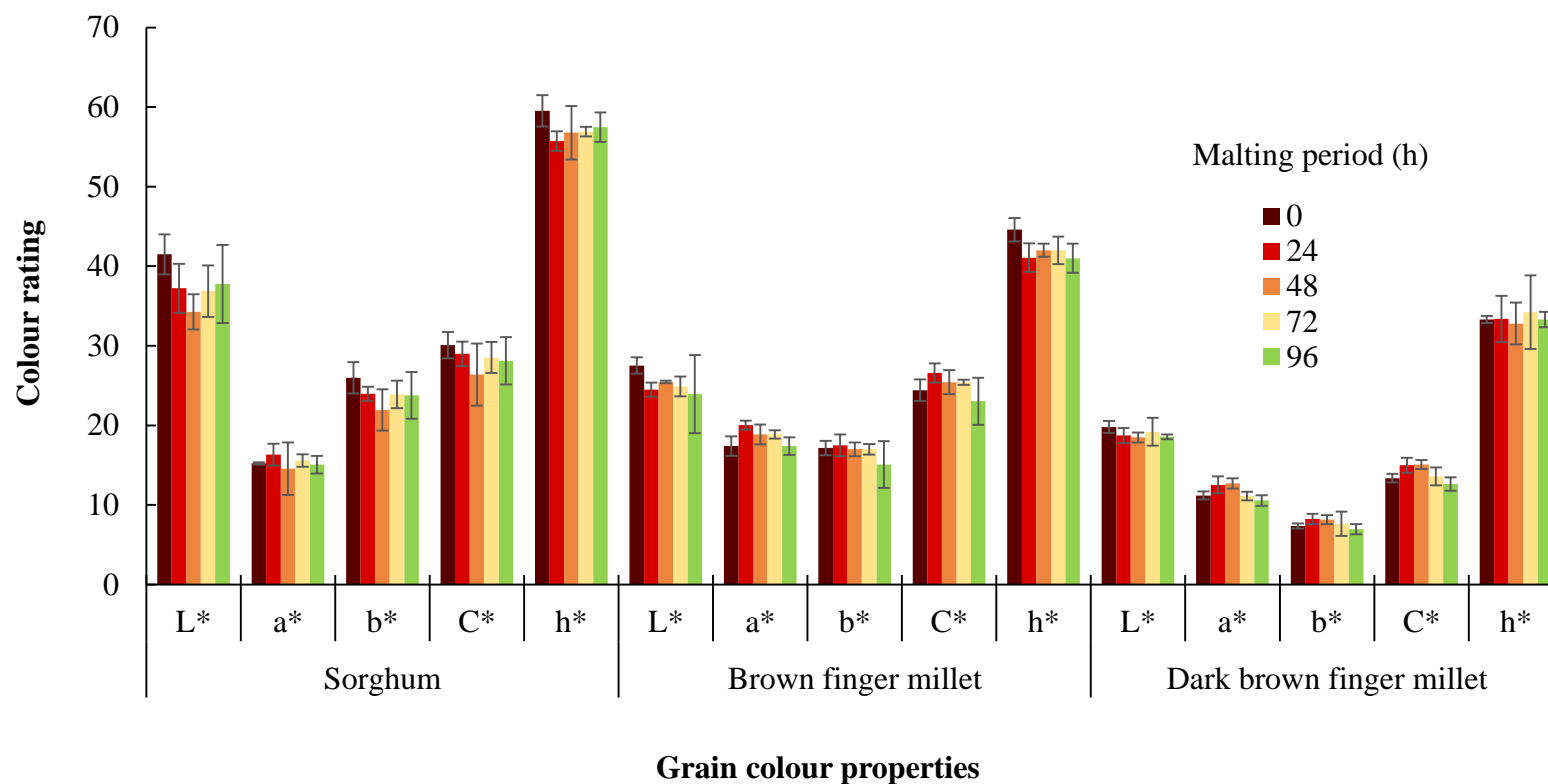


Figure 3. Effect of malting period on grain colour of finger millet and sorghum grain varieties. Error bars are standard error of mean ($n = 3$). L^* = Positive for whiteness and negative for black; a^* = positive for red and negative for green; b^* = positive for yellow and negative for blue; C^* = Chroma; h^* = Hue.

3.3.2. Mineral content of the unmalted and malted finger millet and sorghum flours determined using ICP-AES/ ICP-MS and AAS

Calcium (Ca)

The FM varieties were found to contain a higher amount of Ca than the sorghum grain, ranging from 4408.30 – 4524.93 mg/ kg in DBFM, from 3888.80 – 4483.67 mg/ kg in BFM and from 199.43 – 227.53 mg/ kg in the sorghum grain (Figure 4a). Significant decrease in the Ca content of the grains was observed at 24 h of malting which later increased. At 96 h of malting, a significantly higher ($p < 0.05$) amount of calcium was observed for BFM, whereas for the DBFM, 48 h of malting resulted in higher amounts of Ca compared with 96 h. Similar observations made for DBFM was recorded for the sorghum malt.

Magnesium (Mg)

The Mg content of the unmalted and malted grains are shown in Figure 4b. The amount ranged from 2250 – 2374.37 mg/ kg in DBFM, from 2106.37 – 2272.93 mg/ kg in BFM and from 1476.83 – 1531.93 mg/ kg in the sorghum grain. A significant ($p < 0.05$) decrease in Mg content of the food grains was observed at 24 and 72 h of malting. At 96 h of malting, a significantly ($p < 0.05$) higher amount of Mg was observed for BFM and the sorghum grain, whereas the same was higher at 48 h DBFM malt.

Phosphorus (P)

The unmalted and malted FM varieties and sorghum grain were found to contain high amounts of P which ranged from 3871.40 – 3871.40 mg/ kg in DBFM, from 3657.83 – 3930.10 mg/ kg in BFM and from 2303.27 – 2395.40 mg/ kg in the sorghum grain (Figure 4c).

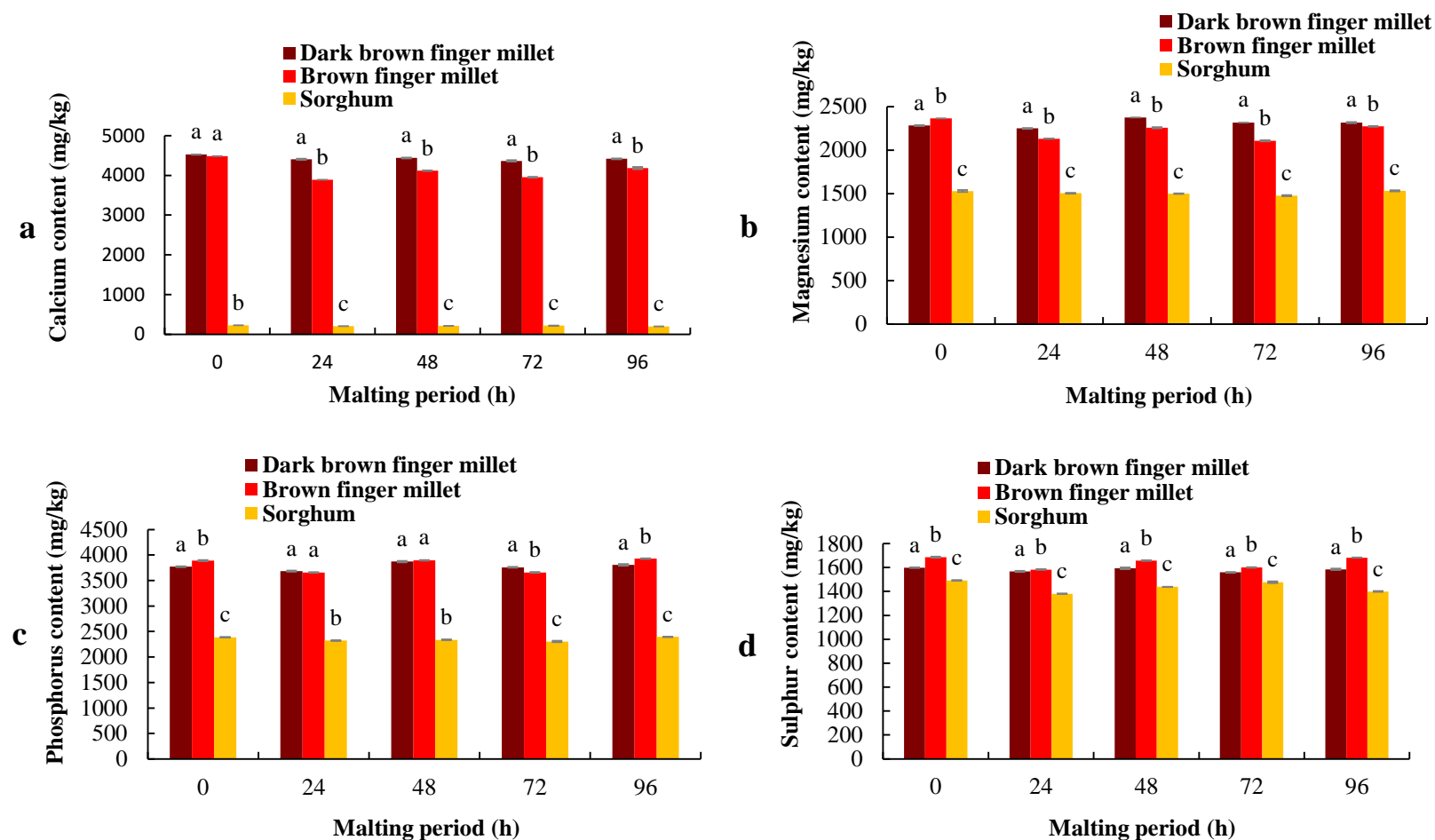


Figure 4. Calcium, magnesium, phosphorus and sulphur contents of finger millet and sorghum grain varieties analysed with inductively coupled atomic emission spectroscopy and inductively coupled plasma mass spectroscopy. (a) calcium; (b) magnesium; (c) phosphorus; (d) sulphur. Mean values \pm standard error for different data labels on each period are significant at $p < 0.05$.

A significant ($p < 0.05$) decrease in P content of the grains was observed at 24 h of malting, which later increased with malting time. Malting for 96 h resulted in a significantly ($p < 0.05$) higher amount of P for the BFM and sorghum varieties, while for DBFM the highest amount of P was observed at 48 h.

Sulphur (S)

The S content of the unmalted and malted grains ranged from 1559.13 – 1597.37 mg/kg in DBFM, from 1582.27 – 1686.60 mg/kg in BFM and from 1380.60 – 1492.07 mg/kg in the sorghum grain (Figure 4d). A significant ($p < 0.05$) decrease in the S content of the cereal grains was observed at 24 h of malting which later increased. At 96 h of malting, a higher amount of S was observed for BFM, whereas for DBFM the same was higher at 48 h which was not significantly different from the amount observed at 96 h. For the sorghum grain, malting for 72 h resulted in higher amounts of S compared to 48 and 96 h periods.

Copper (Cu)

Higher amount of Cu was observed in the FM varieties compared to the sorghum grain and were in the range of 7.07 – 7.60 mg/kg in DBFM, from 6.97 – 7.77 mg/kg in BFM and from 5.03 – 5.30 mg/kg in the sorghum grain (Figure 5a). Reduced ($p < 0.05$) Cu content was observed during 24 and 72 h of malting which later increased. Increase in the Cu content of BFM were observed at 48 and 96 h which did not differ significantly from the unmalted grain. Although 96 h of malting increased Cu content of DBFM, the amount was significantly lower than the amount observed at 48 h malt.

The Cu content (mg/ 100 g) of the FM and sorghum grains obtained using AAS are presented in Figure 9a and ranged from 2.10 to 3.48 in DBFM, from 2.12 to 2.95 in BFM and from 1.45 to 2.21 in sorghum.

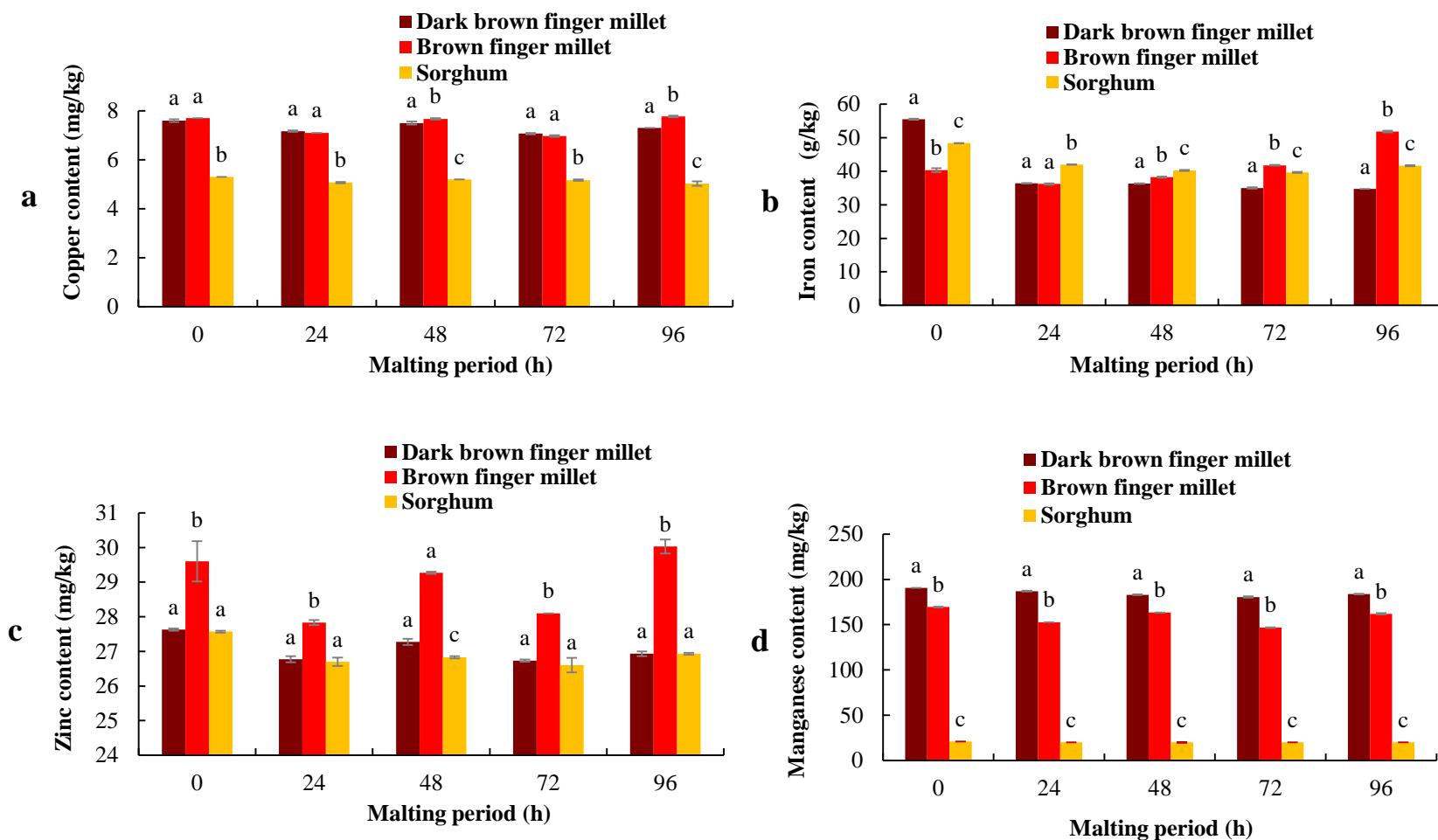


Figure 5. Copper, iron, zinc, and manganese contents of finger millet and sorghum grain varieties analysed with inductively coupled atomic emission spectroscopy and inductively coupled plasma mass spectroscopy. (a) copper; (b) iron; (c) zinc; (d) manganese. Mean values \pm standard error for different data labels on each period are significant at $p < 0.05$.

The FM varieties had higher Cu content than sorghum both in the un-malted and malted forms. Malting for 24 h was found to reduce Cu of FM varieties, thereafter a progressive increase occurred up to 96 h malting for FM and sorghum. At 96 h of malting, the highest Cu content was recorded for DBFM (3.48 mg/ 100 g), followed by BFM (2.95 mg/ 100 g) and sorghum grain (2.21 mg/ 100 g).

Iron (Fe)

The Fe content of the grains ranged from 34.70 to 55.50 mg/ kg in DBFM, from 36.20 to 51 mg/ kg in BFM and from 39.67 to 48.37 mg/ kg in the sorghum (Figure 5b). Significant decrease in the Fe content of the grains was observed at 24 h of malting which later increased significantly ($p < 0.05$), with malting time for BFM up to 96 h. At 96 h, highest Fe content of 51.83 mg/ kg was observed for BFM compared with other malting periods and grain varieties. For DBFM, a slight increase in Fe content was observed at 48 h which later declined significantly at 96 h of malting.

Zinc (Zn)

The amount of extractable zinc in the unmalted and malted grains ranged from 26.77 – 27.63 mg/ kg in DBFM, from 28.10 – 30.03 mg/ kg in BFM and from 26.60 – 27.57 mg/ kg in the sorghum grain (Figure 5c). Reduced ($p < 0.05$) Zn content was observed at 24 h of malting for the cereal grains. The Zn content later increased at 48 h of malting and remained constant up to 96 h for sorghum grain. Malting for 96 h resulted in higher amounts of Zn in BFM. For DBFM, higher amount of Zn was recorded at 48 h of malting and this later decreased and remained constant up to 96 h period.

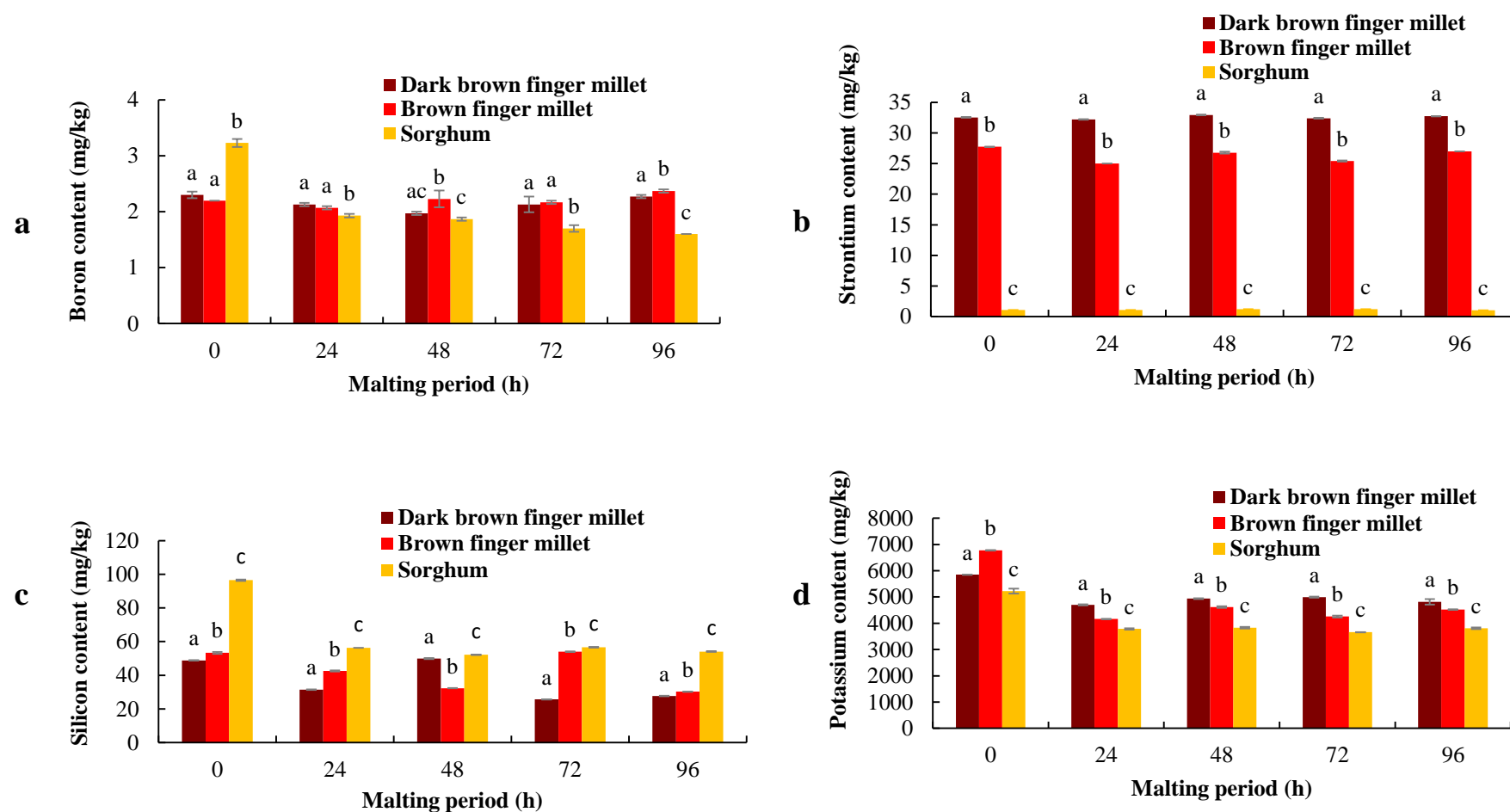


Figure 6. Boron, strontium, silicon and potassium contents of finger millet and sorghum grain varieties analysed with inductively coupled atomic emission spectroscopy and inductively coupled plasma mass spectroscopy. (a) boron; (b) strontium; (c) silicon; (d) potassium. Mean values \pm standard error for different data labels on each period are significant at $p < 0.05$.

The Zn content (mg/ 100 g) of the sorghum, unmalted and malted DBFM/ BFM analysed with AAS were in the range of 1.98 - 2.14, 1.83 - 2.34 and 2.01 - 2.24, respectively (Figure 9b). Both FM varieties had reduced Zn content after 24 h of malting. Increase in Zn content of DBFM was observed after 24 h to a final value of 2.34 mg/ 100 g at 96 h of malting. Similar to DBFM, the Zn content of BFM increased after 24 h to similar levels as in the unmalted BFM grain at 72 h and 96 h periods. No significant change in Zn content was recorded for the entire malting period in sorghum grain.

Manganese (Mn)

The amount of Mn present in the unmalted and malted cereal grains ranged from 180.50 – 190.63 mg/ kg in DBFM, from 146.73 – 169.43 mg/ kg in BFM and from 19.93 – 20.97 mg/ kg in the sorghum grain (Figure 5d). Higher Mn content was observed for the FM varieties compared to the sorghum grain. A significant ($p < 0.05$) decrease in the Mn content of the grains was observed at 24 h of malting which remained constant up to 96 h for sorghum. For the DBFM, higher amount of Mn was observed at 96 h, which was not significantly different from the 48 h malt. For BFM, a higher amount of Mn was observed at 48 h of malting compared with other malting periods.

AAS data show that FM varieties had higher levels of Mn (mg/ 100 g) compared to sorghum and they were in the range of 6.73 - 10.26 in DBFM, from 3.65 - 9.4 in BFM and from 0.85 - 2.22 in sorghum (Figure 9c). There was a significant ($p > 0.05$) decrease in Mn content of DBFM after 24 h malting, subsequently, the Mn content of DBFM increased to levels either higher than or close to those in unmalted DBFM. For the BFM, a significant reduction in Mn content was recorded at 24 h of malting, thereafter, there was a steady increase from 48 h to levels similar to unmalted BFM at 72 and 96 h of malting. For the sorghum grain, increase in the Mn content was only observed at 48 h.

Boron (B)

The amount of boron (B) from the unmalted and malted food grains are presented in Figure 6a and they were in the range of 1.97 – 2.30 mg/ kg in DBFM, from 2.07 – 2.37 mg/ kg in BFM and from 1.60 – 3.23 mg/ kg in the sorghum grain. The B content of the sorghum grain was significantly ($p < 0.05$) higher than those of the FM varieties. Unlike the sorghum grain, the FM varieties showed insignificant ($p > 0.05$) decrease in B content during 24 h of malting. At 48 h of malting, however, significant ($p < 0.05$) decrease in B content of DBFM was observed, which later increased significantly at 72 h and 96 h to levels closer to those in the unmalted counterpart. For the BFM, no significant ($p < 0.05$) difference in the amount of B was observed within 48 and 72 h of malting. At 96 h of malting, the B content increased significantly ($p < 0.05$) close to the amount present in the unmalted grain with a marginal decrease in B content of the sorghum grain compared to the FM malt varieties.

Strontium (Sr)

The FM varieties were found to contain higher amount of Sr than the sorghum grain, which ranged from 32.20 to 32.93 mg/ kg in DBFM, from 25 to 27.73 mg/ kg in BFM and from 1.10 to 1.03 mg/ kg in the sorghum grain (Figure 6b). A significant ($p < 0.05$) decrease in Sr content of the BFM were observed at 24 and 72 h of malting which later increased. Although, a significant ($p < 0.05$) increase in the Sr content of BFM was observed at 48 h, malting at 96 h resulted in higher Sr content which was close to the amount in the unmalted grain. For the DBFM, a slight ($p < 0.05$) decrease in Sr content was observed during 24 h of malting which later increased. Malting for 48 and 96 h resulted in increase in the Sr content of DBFM which were slightly higher than the amount present in the unmalted grain. The amount of Sr content in 48 h DBFM malt, however, was higher than in the 96 h malt. For the sorghum grain, significant increase in Sr content was observed at 48 h which later declined at 96 h of malting.

Silicon (Si)

The Si contents of the unmalted and malted grains are presented in Figure 6c and it ranged from 25.63 – 49.97 mg/ kg in DBFM, from 30.23 – 53.30 in BFM and from 52.13 – 96.47 mg/ kg in the sorghum grain. A significant ($p < 0.05$) decrease in content was observed at 24 h of malting for the grains. A further decrease in content of BFM and the sorghum grains was observed at 48 h unlike in DBFM where the malting period resulted in higher amounts of the mineral. Conversely, malting at 72 h resulted in higher amounts in the BFM and sorghum grains unlike in the DBFM where a significant decrease in Si was observed. Generally, 96 h malting resulted in decrease of Si content of the cereal grains.

Potassium (K)

Compared with other minerals, the grains were found to contain higher amount of K which ranged from 4698.80 – 5850.93 mg/ kg in DBFM, from 4161.73 – 6775.03 in BFM and from 3659.63 – 5223.50 mg/ kg in the sorghum grain (Figure 6d). A significant ($p < 0.05$) decrease in K content of the grains was observed at 24 h of malting thereafter an increase which remained constant up to 96 h malting of the sorghum grain. A significantly ($p < 0.05$) high amount of K was observed at 48 h malt of FM varieties which did not differ significantly from the amounts observed at 72 and 96 h DBFM malt. Although a significant increase in K content was observed at 96 h malting of BFM, this was lower than the amount recorded at 48 h.

Sodium (Na)

The Na content of the grains ranged from 23.60 – 33.97 mg/ kg in DBFM, from 18.97 – 22.70 mg/ kg in BFM and from 5.33 – 13.40 mg/ kg in the sorghum grain (Figure 7a). Malting for 48 h resulted in higher ($p < 0.05$) amount of Na content of the grains, which later decreased significantly and remained constant up to 96 h for the FM varieties.

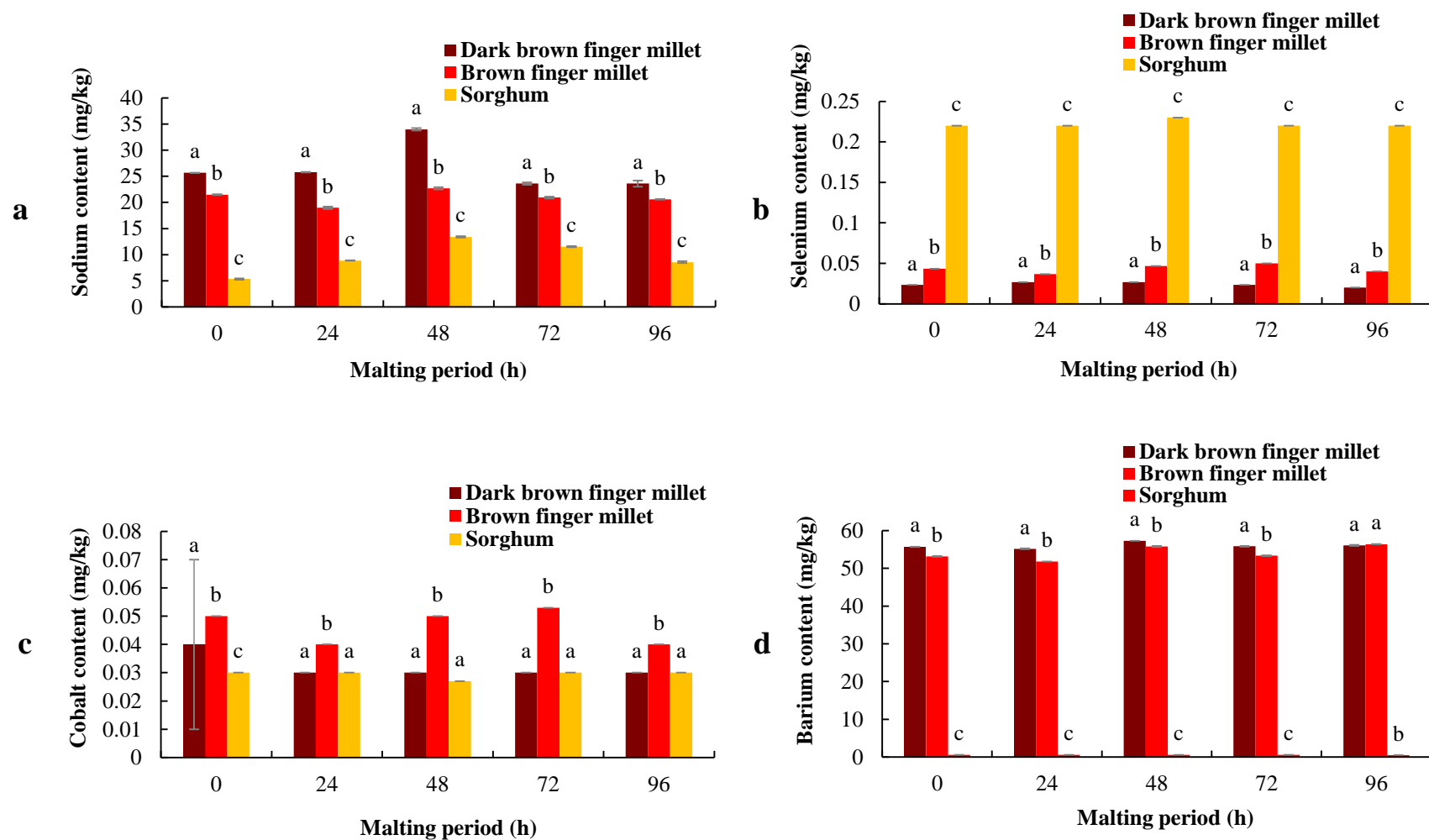


Figure 7. Sodium, selenium, cobalt and barium contents of finger millet and sorghum grain varieties analysed with inductively coupled atomic emission spectroscopy and inductively coupled plasma mass spectroscopy. (a) sodium; (b) selenium; (c) cobalt; (d) barium. Mean values \pm standard error for different data labels on each period are significant at $p < 0.05$.

For the sorghum grain, a further decrease was observed after 72 h malting. Unlike other minerals, 24 h of malting resulted in an increase in the Na content of the BFM and sorghum varieties, with a statistically insignificant increase in the same for DBFM.

The Na content (mg/ 100 g) of unmalted and malted FM and sorghum grains determined from using AAS are presented in Figure 9d. The FM varieties had higher Na content than the sorghum with values in the range of 1.73 - 4.4 in BFM, from 1.56 - 4.01 in DBFM and from 1.1 - 2.27 in sorghum grain. Malting reduced the Na content of the FM and sorghum grains. A significant decrease in Na content of the FM varieties was observed at 24 and 48 h of malting which remained constant up to 96 h for the BFM. A further decrease in the Na content was observed at 96 h for DBFM while a decrease in the Na content of sorghum grain was only observed at 48 h, beyond which no significant decrease / increase was observed.

Selenium (Se)

Lower amount of Se was found in the grains and it ranged from 0.02 – 0.037 in DBFM, from 0.037 – 0.05 mg/ kg in BFM and from 0.22 – 0.23 mg/ kg in the sorghum grain (Figure 7b). The sorghum grain contained higher amount of Se compared to the FM varieties. The malting process did not result in any significant change in Se content of DBFM and the sorghum grain. For BFM, significant ($p < 0.05$) decrease was observed at 24 h of malting. A statistically significant ($p < 0.05$) increases were observed at 48 and 72 h periods, which were higher than the amount in the unmalted grain. Malting for 96 h significantly reduced the amount of Se compared to 48 and 72 h BFM malt.

Cobalt (Co)

The grains were found to contain relatively low amounts of Co (Figure 7c). Malting did not affect the amount of Co in the sorghum grain, however, it resulted in changes in the FM

varieties and was in the range of 0.03 – 0.04 mg/ kg in DBFM and from 0.04 – 0.05 mg/ kg in the BFM. An initial decrease ($p < 0.05$) in Co content was observed at 24 h for the DBFM which remained constant for the entire malting period. A similar initial decrease was observed for BFM after which there was an increase, therefore, higher amounts of Co was recorded at 48 and 72 h periods.

Barium (Ba)

The FM varieties were found to contain higher amount of Ba compared to the sorghum grain, and were in the range of 55.17 – 56.04 mg/ kg in DBFM, from 51.80 – 56.37 mg/ kg BFM and from 0.5 – 0.6 mg/ kg in the sorghum grain (Figure 7d). The unmalted DBFM was found to contain a significantly ($p < 0.05$) high amount (55.67 mg/ kg) of Ba compared to the BFM (53.13 mg/ kg). A significant decrease in Ba content was observed at 24 h of malting for the FM varieties after which there was an increase. A significantly ($p < 0.05$) higher amount of Ba was recorded at 96 h of malting for the FM varieties compared to other malting periods. No significant change in the Ba content was observed for the sorghum grain for the entire malting period.

Aluminium (Al)

The Al content of the unmalted and malted cereal grain varieties ranged from 3.63 – 12.33 in DBFM, from 8.03 – 13.20 in BFM and from 10.23 – 19.57 in the sorghum grain (Figure 8). The unmalted sorghum grain contained high amount of Al followed by the BFM and the least for DBFM. A significant ($p < 0.05$) decrease in the Al content of the cereal grains was observed at 24 h of malting, after which there was a further decrease up to 72 h for the DBFM and sorghum grains. Increase in the Al content was recorded for the DBFM and sorghum grains at 96 h of malting. A significantly ($p < 0.05$) higher amount of Al was recorded at 72 h of malting for the BFM compared with other malting periods.

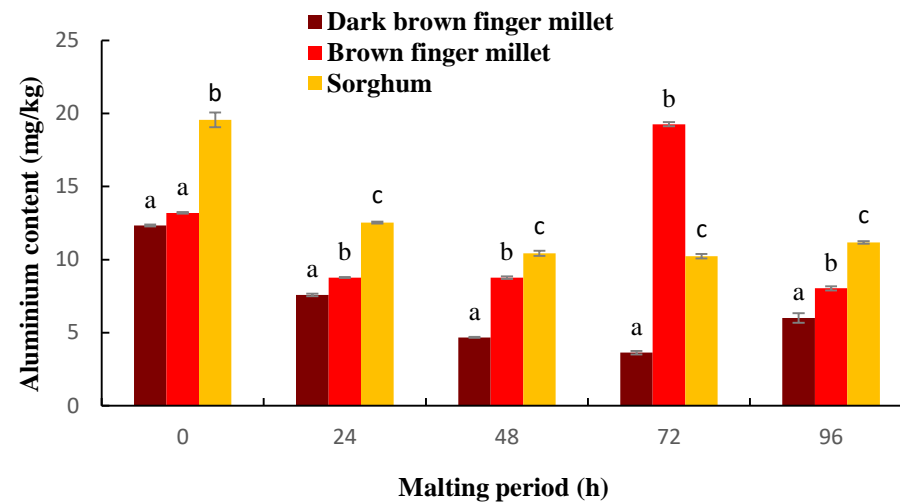


Figure 8. Aluminium content of finger millet and sorghum grain varieties analysed with inductively coupled atomic emission spectroscopy and inductively coupled plasma mass spectroscopy. Mean values \pm standard error for different data labels on each period are significant at $p < 0.05$.

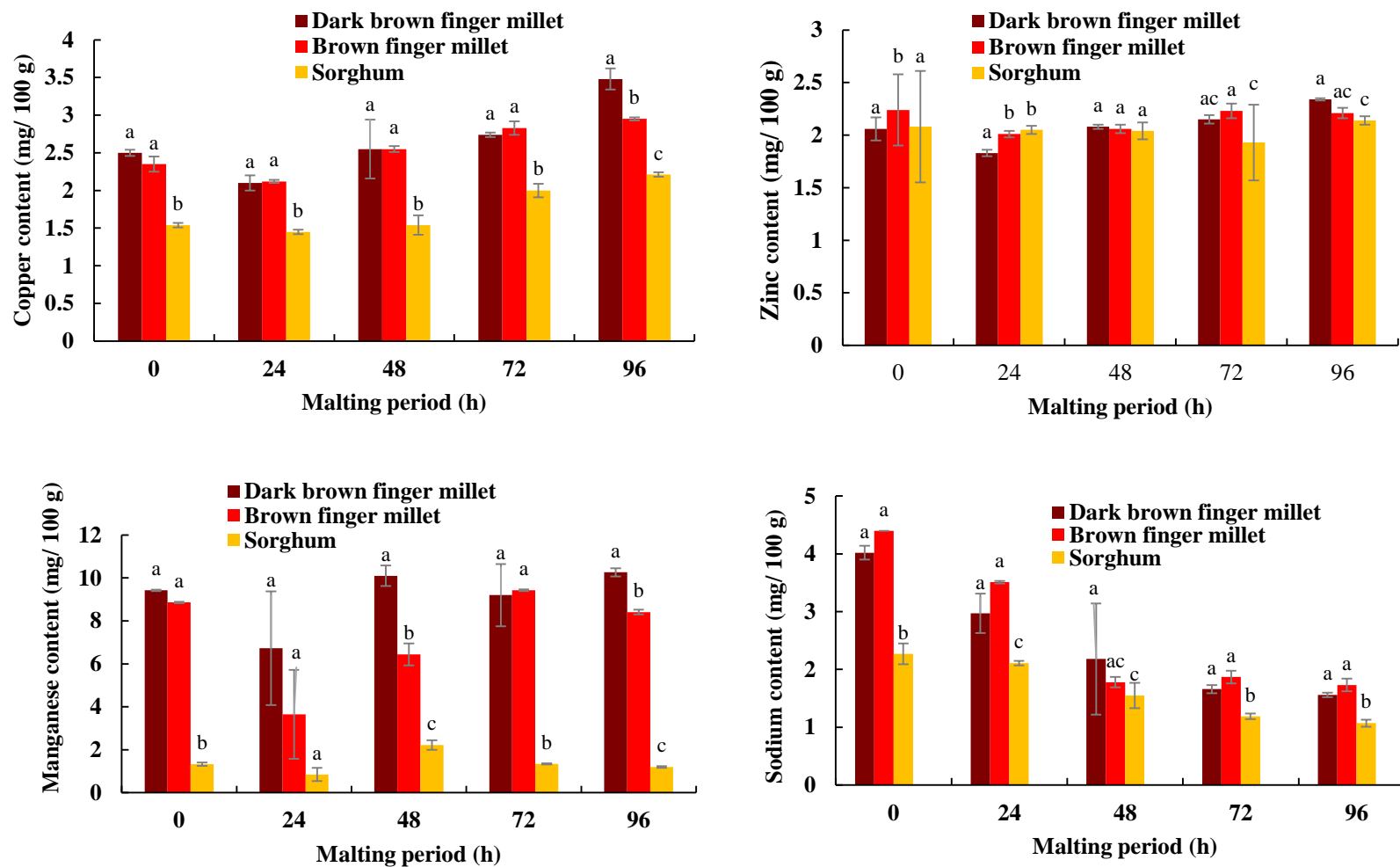


Figure 9. Copper, sodium, zinc and manganese contents of finger millet and sorghum grain varieties analysed with atomic absorption spectrometer.

(a) copper; (b) zinc; (c) manganese; (d) sodium. Mean values \pm standard error for different data labels on each period are significant at $p < 0.05$.

3.3.3. Phytic acid and total phosphorus content of finger millet and sorghum malt flours

The phytic acid content of the unmalted and malted grains ranged from 0.3363 – 0.9186 g/ 100 g in DBFM, from 0.5271 – 1.0680 g/ 100 g in BFM and from 0.2889 – 0.6725 g/ 100 g in the sorghum grain (Table 5). Malting for 24 h resulted in a significant ($p < 0.05$) decrease in the phytic acid content of BFM unlike for DBFM and sorghum grain malt. A significant ($p < 0.05$) increase in the phytic acid content of the cereal grain malt was observed at 48 h of malting. A further increase was observed at 72 h of malting for the BFM, whereas the same was reduced significantly ($p < 0.05$) for the DBFM and sorghum grain malt. Conversely at 96 h of malting, reduced phytic acid content was observed for BFM, but phytic acid increased in DBFM and sorghum grain malts.

The total phosphorus content of DBFM, BFM and sorghum grain were in the range of 0.0948 – 0.2590, from 0.1486 – 0.3012 and from 0.0815 – 0.1896 g/ 100 g respectively (Table 5). Unlike the BFM, malting for 24 h significantly ($p < 0.05$) increased the total phosphorus content of DBFM and sorghum grain. A further increase in the total phosphorus content was observed at 48 h for the grain malts which also increased for BFM. After 72 h of malting, a total phosphorus content decrease was observed for DBFM and the sorghum grain malt, but increased significantly for BFM. A similar trend observed at 96 h of malting for phytic acid content of the grains was also recorded for total phosphorus. In general, malting had a similar effect on the phytic acid and total phosphorus content of each grain malt.

3.4. Discussion

3.4.1. Physicochemical properties of finger millet and sorghum malt flours

The physicochemical properties of the FM and sorghum varieties were not strongly affected by malting, particularly the pH, which became slightly acidic. The changes noted in the pH of FM and sorghum malt are comparable with the report of Sripriya *et al.* (1997), where germination did not result in a significant decrease in pH (5.8 to 5.7) of 24 h germinated FM.

The primary objective of kilning or heat treatment during malting is to develop a characteristic dark or dark brown colour, which is achieved through Maillard reaction of sugars and amino acids in malted grains (Bellaio *et al.*, 2013; Baidab *et al.*, 2016). The changes in the colour properties of the FM and sorghum grain varieties are attributed to the effect of Maillard reaction and the distinct colour types of the cereal grains, which were slightly modified during malting.

3.4.2. Mineral content of the unmalted and malted finger millet and sorghum malt flours

The FM and sorghum grain varieties contain a number of nutritionally essential macro- and micro-elements, which were affected by malting. The amount of calcium, phosphorus, potassium, magnesium, iron, zinc, manganese, copper and sodium obtained are comparable with the results of Sripriya *et al.* (1997), Shashi *et al.* (2007), Platel *et al.* (2010) and Khirishnan *et al.* (2012). Higher amounts of calcium (388.9 – 452.5 mg/ 100 g), phosphorus (365.8 – 393.10 mg/ 100 g), magnesium (211.0 – 237.4 mg/ 100 g), zinc (2.68 – 3.0 mg/ 100 g), manganese (14.7 – 19.1 mg/ 100 g), copper (0.7 – 0.78 mg/ 100 g) and sodium (1.9 – 3.4 mg/ 100 g) were observed in this study compared to the value ranges (Ca, 295.7 – 340.15 mg/ 100 g; P, 240 - 292 mg/ 100 g; Mg, 66 – 110 mg/ 100 g; Zn, 1.38 – 2.4 mg/ 100 g; Mn, 3.4 – 3.7 mg/ 100 g; Cu, 0.16 – 0.54 mg/ 100 g; Na, 0.60 – 0.95 mg/ 100 g) reported by other researchers - Sripriya *et al.* (1997), Shashi *et al.* (2007), Platel *et al.* (2010) and Krishnan *et al.* (2012). The observation could be as a result of the technique and instrument used for the determination. The combination of two strong acids - nitric and hydrochloric acids - was found to better solubilise and extract the minerals unlike in other studies where hydrochloric acid was the main extraction solvent. Also, a high amount of sulphur (158.4 – 168.7 mg/ 100 g) was observed in the FM varieties followed by strontium (2.5 – 3.3 mg/ 100 g), silicon (2.6 – 5.4 mg/ 100 g) and boron (0.2 – 0.24 mg/ 100 g), while cobalt (0.003 – 0.005 mg/ 100 g) and selenium (0.002 – 0.005 mg/ 100 g) were found in lower amounts. Aluminium and barium were also present in FM varieties in amounts comparable to copper and iron, respectively.

Zinc and manganese contents of unmalted FM obtained using AAS, are similar with the amounts 2.4 and 5.98 mg/ 100 g reported by Platel *et al.* (2010) and Krishnan *et al.* (2012), except for copper, where a higher amount was observed (Figure 8a). The sodium content in the range of 0.60 - 0.95 mg/100 g was reported on varieties of unmalted FM (Shashi *et al.*, 2007). A high amount of 11 mg/ 100 g Na was reported in the review of Chandra *et al.* (2016) for raw FM. The values agree with the sodium contents obtained in this study. The discrepancy in the amount of copper content could be attributed to genetic differences, soil type, agronomical practices and or analytical technique employed (Yamunarani *et al.*, 2016).

The initial 24 h malting resulted in a decrease of the minerals except for sodium. A similar observation has been reported by other researchers (Krishnan *et al.*, 2012; Malleshi and Desikachar, 1986; Afify *et al.*, 2011). A loss in sodium content was observed with malting time. Since biosynthesis or degradation of minerals is not expected during malting, the loss of the minerals may be due to leaching during soaking and the germination stages of the grains. The reduction might also have resulted from a loss of the minerals through the utilisation of other nutrients by the growing embryo. The increase observed for sodium within 24 h – 48 h of malting (Figure 7a) could be attributed to its highly water soluble nature and single oxidation state that enables early release from the grain components compared with other minerals.

Significant increases were noted in the mineral composition of the grains at 48 h up to 96 h of malting. At 48 h of malting, significant increases in iron, magnesium, phosphorus, sulphur and zinc were observed for DBFM, whereas for BFM and the sorghum grain, the same were found to increase significantly at 96 h period. At 48 h, the strontium content of the grains was higher compared to other malting periods. Also, 48 h of malting resulted in an increased silicon content of DBFM, which decreased by the same amount in BFM and sorghum grain, however, at 72 and 96 h, respectively, silicon was found in higher amounts in BFM and sorghum grains. Manganese content was higher at 48 h of malting for BFM but was highest for DBFM at 96 h period. Calcium, copper and boron were found in a significantly higher amount

at 96 h of malting in FM varieties unlike in sorghum where higher amounts of the minerals were observed at 48 h period. Selenium and cobalt contents were relatively low and malting did not result in any significant change in their amounts. The increase in the mineral content of the malted grains observed after 24 h period, could be due to an accumulative effect resulting from the dissociation and or reconfiguration of phytate complexes and other mineral-binding components of the grain during malting, as well as the presence of organic compounds that enhance the release of minerals, like ascorbic acids (Sripriya *et al.*, 1997; Mamiro *et al.*, 2001; Hemalatha *et al.*, 2007; Krishnan *et al.*, 2012; Yu *et al.*, 2012). A varietal difference which is a dependent factor in the degradation of phytate complexes was also found to play a major role in the amount of extractable minerals from the grains. The differences in extractable minerals, particularly at 48 and 96 h of malting were seen as a function of varietal differences. Differences in mineral [calcium, phosphorus, iron, magnesium, zinc, sodium and potassium] content of different FM genotypes have been studied (Shashi *et al.*, 2007). The results on the mineral content of the malted FM are in contrast with the reports of Mamiro *et al.* (2001) and Platel *et al.* (2010) who found no significant effect of malting in the mineral content of FM. Nkama *et al.* (2015), however, reported an increase in the calcium content of sorghum grain germinated for 24 h period. Badau *et al.* (2005), also reported an increase in zinc content with malting time for pearl millet varieties.

The majority of minerals found in the FM cultivars are indispensable for human nutrition and health, and the impact of malting showed that FM could be exploited in addressing various health conditions. Calcium, iron, zinc, manganese, potassium, magnesium, copper among others, are involved in various biological functions for maintaining good health. Calcium in the form of calcium phosphate provides rigidity to the skeleton and calcium ions play a role in most metabolic processes. Ionic balance of calcium in the body is important in mediating hormonal functions on target organs through several intracellular signalling pathways, such as the phosphoinositide and cyclic adenosine monophosphate systems

(WHO/FAO, 2004). Calcium deficiency gives rise to osteoporosis, a severe health condition in which bone loss occurs, which often lead to bone fracture (Nordin, 1960; WHO/FAO, 2004).

Iron has several vital functions in the human body. It serves as a carrier of oxygen to the tissues from the lungs by red blood cell haemoglobin and as a transport medium for electrons (WHO/FAO, 2004). It is important for growth, cognitive performance especially in infants, resistance to infections and alleviation of iron deficiency anaemia. Zinc plays a central role in the immune system, affecting a number of aspects of cellular and humoral immunity (Shankar and Prasad, 1998). Zinc acts as a co-factor to a large number (> 300) of enzymes that are involved in the synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids as well as in the metabolism of other micronutrients. Clinical symptoms of severe zinc deficiency in humans include growth retardation, delayed sexual and bone maturation, skin lesions, impaired appetite, increased susceptibility to infections and behavioural changes (Hambridge *et al.*, 1987; WHO/FAO, 2004). Except for barium and aluminium, other minerals that were found like strontium and silicon have been implicated with bone mineralisation, prevention of osteoporosis and diabetes, among other health conditions (Marie *et al.*, 1985; Martin, 2013). The results show that malting of FM for 96 h or 48 h depending on the cultivar, allows its utilisation as a functional food ingredient for the alleviation of diseases associated with mineral deficiencies.

3.4.3. *Phytic acid and total phosphorus content of finger millet and sorghum malt flours*

The effects of malting on phytate-mineral complexes have been reported by researchers like, Sripriya *et al.* (1996); Sehgal and Kawatra (1998); Badau *et al.* (2005); Krishnan *et al.* (2012); Jha *et al.* (2015). Finger millet contains a high amount of phytate (417 mg/100 g) compared with sorghum (295 mg/ 100 g), barley (278 mg/100 g), rice (160 mg/ 100 g) and maize (414 mg/ 100 g) (Sripriya *et al.*, 1996; Hemalatha *et al.*, 2007). Several processing techniques, especially malting, have been shown to reduce the amount of phytate in cereal

grains, wherein phytase, a phytate-specific enzyme is activated, resulting in the dephosphorylation of inositol phosphates or phytate forms (Luo *et al.*, 2014). This process often results in phytate complex dissociation and or reconfiguration with principal products being inorganic phosphates, inositol phosphate monomers, divalent and trivalent mineral ions, proteins as well as amino acids. Enzymatic hydrolysis of phytate in FM during germination was observed by Mbithi-Mwikya *et al.* (2000), wherein phytate values decreased from 0.35 g/ 100 g in the raw sample to 0.02 g/ 100 g after 96 h of germination. Mamiro *et al.* (2001) has shown that 48 h germination reduced 49.2 % of the total phytic acid content of FM. In another report, germination for 72 h was found to significantly reduce the phytate content in pearl millet compared with 48 h of germination (Sehgal and Kawatra, 1998). The phytic acid content of FM and sorghum grain varieties obtained in this study are comparable with the report of Hemalatha *et al.* (2007), who found 417 mg/ 100 g (0.417 g/ 100 g) and 295 mg/ 100 g (0.295 g/ 100 g) for FM and sorghum, respectively. The result is also comparable with the report of Mbithi-Mwikya *et al.* (2000) and Sripriya *et al.* (1997), who found 0.35 g/ 100 g in FM and 0.6 g/ 100 g in FM.

The present study showed that malting had a variable effect on the phytic acid content of the cereal grain varieties, as previously described. A malting time-dependent decrease in phytic acid of the FM grains was not observed as previously reported by Mbithi-Mwikya *et al.* (2000). Decrease in the phytic acid content of grains were observed, particularly at 24 h, 72 h and 96 h of malting which varied for the FM varieties and sorghum grain malts. On the other hand, the increase in phytic acid content was observed at 48 h and 96 h of malting which also varied for the malt flour. This observation is contrary to the report of Mbithi-Mwikya *et al.* (2000), who showed a steady decrease in phytate content of 0.35 g/ 100 g in the raw sample to 0.02 g/100 g after 96 h of germination. The discrepancy is as a result of the difficulty in estimating accurately the phytic acid content of processed grains which can contain high amounts of other *myo*-inositol forms (that is, IP₃, IP₄, and IP₅) that would co-elute with phytic

acid (IP₆) and contribute to overestimation of phytic acid content (Kasim and Edwards, 1998; Lehrfeld and Morris, 1992; Qvirist *et al.*, 2015). This is not the case for unprocessed grain where phytic acid comprise at least 97 % of the total inositol phosphates, hence the coherence in phytic acid content of the unmalted FM varieties, in line with the report of others.

Phytic acid is present in cereal grains in the form of phytate complexes. During malting, phytases are activated then hydrolyse phytates (Mamiro *et al.*, 2001; Luo *et al.*, 2014). Phytase hydrolyses, the hexa form of phytic acid (IP₆, *myo*-inositol 1, 2, 3, 4, 5, 6-hexakisphosphate) into lower forms such as IP₅, IP₄, IP₃, IP₂, IP₁ and *myo*-inositol (Agte *et al.*, 1997). In the process, lower *myo*-inositol phosphate forms IP₅, IP₄ and IP₃ are simultaneously being formed as products from the dissociation and or reconfiguration of other higher inositol phosphates already present, like IP₅ and IP₄ (Qvirist *et al.*, 2015). This sequence of enzymatic reaction in turn increases the amount of inorganic phosphate and lower phytic acids, which co-elute as total phytic acid content. It is suggested that the differential effect of malting on the phytic acid content of cereal grains could be a result of the combined formation and dissociation of intermediate inositol phosphates during malting. Unlike the phytic acid content, the malting periods virtually resulted in malting time-dependent increase in phosphorus content of the malt flours. The observation is a result of the inorganic phosphorus released during malting, through hydrolysis of lower inositol phosphates together with the principal form of phytic acid (IP₆).

Pearson correlation coefficients between mineral content, phytic acid content, total phosphorus content and pH of the FM and sorghum grain malt are presented in Table 6. A significant positive correlation ($p < 0.05$) was observed between phytic acid, calcium, magnesium, potassium, manganese, and copper for the malting periods 24 and 72 h, suggesting that changes in phytate content of the grain malt contributed to the release of the minerals. No significant correlation ($p > 0.05$) was observed between phytic acid, selenium and boron.

Table 6. Pearson correlation coefficients between phytic acid, total phosphorus, pH and minerals of finger millet and sorghum grain varieties.

Response variables	TP	pH	Ca	Mg	K	Zn	Cu	Mn	P	S	Na	Si	Co	Se	Sr	B	Fe
PHYT	-.999*	-.998*	1.000*	.998*	.999*	-1.000*	1.000*	1.000*	-.938*	1.000*	1.000*	.999*	-.944	.982	1.000**	.971	-.835
TP		-.998*	1.000*	.998*	.999*	-.998*	-.603	1.000**	1.000*	1.000*	1.000**	.999*	-.943	.982	-.982	.944	-.835
pH			.929	.998*	.999*	1.000**	.866	-1.000**	.968	.964	.968	.910	1.000**	-.929	-1.000**	-.982	.990
Ca				1.000*	.997*	1.000*	.980	1.000**	1.000*	.999*	.999*	.998*	-.965	.956	.995	1.000**	.999*
Mg					1.000**	.999*	1.000**	.998*	1.000**	1.000**	1.000**	.998*	-.963	.971	.999*	.963	1.000**
K						.964	.960	.999*	1.000**	.999*	1.000*	.999*	.957	.909	.999*	.960	1.000*
Zn							-.500	1.000*	.999*	1.000*	.961	1.000*	1.000**	-.500	.945	1.000*	1.000*
Cu								.945	.976	.969	1.000**	1.000*	-1.000**	1.000**	.866	1.000**	1.000**
Mn									1.000**	.999*	1.000**	1.000*	-1.000**	-.715	.992	1.000**	.999*
P										1.000**	.999*	.999*	-.961	-.806	.998*	1.000**	.999*
S											.999*	.999*	-.956	-.945	1.000*	1.000*	1.000*
Na												1.000*	-.945	-.866	1.000**	.866	1.000*
Si													-.945	-1.000**	.982	.993	.997*
Co														.945	-.866	1.000**	-.277
Se															-.866	-1.000**	-.866
Sr																.993	1.000**
B																	.999*

Pearson correlation coefficients *, ** indicate significance at $p < 0.05$ and 0.01 . PHYT: phytic acid; TP: total phosphorus, pH; power of hydrogen, Ca; calcium, Mg; magnesium, K; potassium, Zn; zinc, Cu; copper, Mn; manganese, P; phosphorus, S; sulphur, Na; sodium, Si; silicon, Co; cobalt, Se; selenium, Sr; strontium, B; boron, Fe; iron.

A significant negative correlations between phytic acid, total phosphorus, and zinc content were observed. Among the minerals, significant positive and negative correlation coefficients were recorded, especially, for cobalt, copper and manganese where negative correlation was observed. Apparently, there are limited studies that have described the interaction between minerals, and phytic acid content of FM. Sripriya *et al.* (1997) observed a decreased phytate/ Zn molar ratio from 19.2 to 7.8 for 48 h germinated FM which suggests a negative interaction of phytic acid and zinc availability as observed in the present study.

The pH of plant food plays an important role in availability and/ or bioavailability of minerals both in food material and during digestion in the human gut. Under acidic conditions, transition/ dissociation of mineral into ionic state occurs which result in precipitation under increasing pH, thereby limiting their bioavailability (Skibsted, 2016). In addition, pH affects the hydrolysis of phytate and organic acid complexes which are important for mineral accessibility in cereal grains. The present study showed a significant negative correlation ($p < 0.05$) between pH and phytate content of the malted grains, thus increase in acidity contribute to changes in phytate which then affect mineral availability in finger millet.

3.5. Conclusion

Application of ICP-AES and ICP-MS revealed that FM is a rich source of both macro and trace elements in amounts that were not previously reported, and that malting for 72 – 96 h increased certain minerals. Malting for 48 and 96 h had a better impact on the mineral composition which varied for the grain varieties. The majority of the minerals, including magnesium, calcium, phosphorus, iron, zinc and copper were enhanced at 96 h of malting for BFM, whereas for DBFM the reverse was the case, except for manganese and strontium. The selenium and cobalt contents of FM and sorghum were relatively low and were not affected by malting. The changes in phytic acid content of the grain malt suggest that phytate, the principal form of phytic acid present in cereal grains, undergoes dissociation and/ or reconfiguration

during malting rather than a degradation of phytic acid. A varietal difference was found to play an important role on how a processing method affects the mineral and phytic acid contents of FM and sorghum grains. This study provides a rationale for increased utilisation of FM grain as a functional food ingredient and also shows that malting for 3 – 4 days can improve the mineral content of the flour to alleviate its deficiencies in children and adult foods.

CHAPTER 4

EFFECT OF MALTING ON PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF FLOUR OF FINGER MILLET VARIETIES

4.1. Introduction

Research studies on FM phenolics have increased owing to their role in disease risk reduction, health improvement and wellness. Regular consumption of FM-based diets has been associated with a reduced risk of diabetes, cataract and gastrointestinal tract disorder (Kumari and Sumathi, 2002; Devi *et al.*, 2014). Hyperglycaemic, hypocholesterolaemic, nephroprotective and anticataractogenic, and other health improvement properties like antioxidant and antimicrobial activity of FM have been reported (Shobana *et al.*, 2010; Shahidi and Chandrasekara, 2013).

Finger millet contains a variety of phenolic compounds, which are predominantly phenolic acids and flavonoids. These compounds are a large family of secondary metabolites with antioxidative properties (Rajasekaran *et al.*, 2004; Viswanath *et al.*, 2009; Devi *et al.*, 2014). Several attempts at characterisation of FM phenolic composition have been made to elucidate the health-associated claims. Chethan *et al.* (2008a) identified nine (9) phenolic acids, namely, gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, syringic, ferulic, cinnamic and coumaric acids, and a flavonoid (quercetin) in FM malt. Protocatechuic acid was identified as the major phenolic acid in FM by Subba Rao and Muralikrishna (2002). Ferulic, caffeic and coumaric acids, along with trace amounts of syringic were reported by Subba Rao and Muralikrishna (2001). Other flavonoids like catechin, epicatechin, taxifolin, luteolin, tricetin, myricetin, apigenin, vitexin, procyanidin B1 and B2, narigenin, diadzein and kaempferol were reported in the reviews of Dykes and Rooney (2006) and Shahidi and Chandrasekara (2013). The majority of the phenolic compounds of FM, however, have not been well elucidated or quantified and information on the effect of malting on the phenolic composition and antioxidant

activity of FM is scarce. This study investigated the phenolic composition and antioxidant activity of FM varieties, and how they are affected by malting period.

4.2. Materials and methods

4.2.1. Analytical reagents

Folin-Ciocalteu's reagent, gallic acid and catechin standards, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2'-azinobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) and Trolox standard were purchased from Rochelle Chemicals, Johannesburg, South Africa. All the solvents and phenolic standards used were of analytical, ultra-performance liquid chromatography-mass spectrometer (UPLC-MS) grade.

4.2.2. Malting and preparation of phenolic extracts of FM and sorghum flours

The FM and sorghum grains were malted as described in section 3.2.2. One gram (1 g) of the grain malt flour was refluxed with 15 ml of methanol containing 15 % formic acid for 1 h at 40 °C. The extract was centrifuged at 14, 851 g for 6 min and pipetted into vials and stored at 4 °C, before analysis. Extraction was also done using 20 ml of methanol containing 1 % hydrochloric acid for 2 h at 60 ± 5 °C. The mixtures were centrifuged at 4470 g for 20 min and the supernatants were separated and analysed for individual phenolic compounds, total polyphenol content and antioxidant activity.

4.2.3. Identification and quantification of phenolic compounds of FM and sorghum flours

Separation and identification of the phenolic compounds in the extracts were conducted on a Waters Synapt G2 quadrupole time-of-flight mass spectrometer system (MS) [Milford, MA, USA]. It was fitted with a Waters Ultra-performance liquid chromatograph (LC) and photo diode array (PDA) detector. Separation was achieved on a Waters BEH C18, 2.1x100 mm column with 1.7 μ m particles. A gradient was applied using 0.1 % formic acid (solvent A)

and acetonitrile containing 0.1 % formic acid (solvent B). The gradient started at 100 % solvent A for 1 min and changed to 28 % B over 22 min in a linear pattern. It changed to 40 % B over 50 sec and a wash step of 1.5 min at 100 % B, followed by re-equilibration to initial conditions for 4 min. The flow rate was 0.3 ml/ min and the column was kept at 55 °C and the injection volume was 2 µL. Data was acquired in MS^E mode which consisted of a low collision energy scan (6 V) from m/z 150 to 1500 and a high collision energy scan from m/z 40 to 1500. The high collision energy scan was done using a collision energy ramp of 30-60 V. The photo diode array detector was set to scan from 220 - 600 nm. The mass spectrometer was optimised for best sensitivity, a cone voltage of 15 V, desolvation gas was nitrogen at 650 L/ h and desolvation temperature 275 °C. The instrument was operated with an electrospray ionisation probe in the negative mode. Sodium formate was used for calibration and leucine encephalin was infused in the background as lock mass for accurate mass determinations. Individual peaks and phenolic compounds were identified by comparing the retention time and spectra of each peak with known standards under identical conditions.

4.2.4. Total phenolic content of FM and sorghum flours

Total phenolic contents of unmalted and malted FM and sorghum flour extracts were determined according to the method of Singleton *et al.* (1999). Briefly, 0.1 ml of the acidified methanolic sample extract was mixed with 5 ml distilled water in a 50 ml volumetric flask. Folin-Ciocalteu's reagent [1:2 dilution with water] (2.5 ml) and 7.5 ml 15 % sodium carbonate solution were added, mixed thoroughly, made up to 50 ml and allowed to react for 30 min. The absorbance of the reaction mixture was read at 760 nm with a 96 well microplate spectrophotometer. A calibration curve was prepared using a standard solution of gallic acid at concentrations of 0.1, 0.5, 1, 2.5 (determination coefficient $R^2 = 0.9993$), and result was expressed as mg of gallic acid equivalent (GAE) per g of the sample.

4.2.5. Total flavonoid content of FM and sorghum flours

Total flavonoid content was determined spectrophotometrically according to Zhishen *et al.* (1999). Extract (0.1 ml) was mixed with 4.9 ml distilled water and NaNO₂ (0.3 ml 5 % w/v) was added. After 5 min, AlCl₃ (0.3 ml 10 % w/v) and at 6 min, 2 ml 1 M NaOH were added, and immediately the volume was made up to 10 ml with distilled water. The mixture was vortexed and the absorbance was read at 510 nm. A calibration curve was prepared using a standard of quercetin (determination coefficient $R^2 = 0.9994$). The result was expressed as mg quercetin (QE) per g of the sample.

4.2.6. DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging activity of FM and sorghum flours

The DPPH radical scavenging activity was determined according to the method described by De Ancos *et al.* (2002). An aliquot (10 µl) of the acidified methanolic extract of FM and sorghum flours was mixed with distilled water (90 µl) and 3.9 ml of methanolic 0.1 mM DPPH solution. The mixture was thoroughly vortexed and kept in the dark for 30 min before absorbance was read at 515 nm. The result was expressed as percentage inhibition of the DPPH radical calculated from the following equation:

$$\% \text{ Inhibition of DPPH} = [\text{Abs control} - \text{Abs sample} / \text{Abs control}] \times 100;$$

where: Abs control is the absorbance of the DPPH solution without the sample extract.

4.2.7. ABTS (2, 2'-azinobis-3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging activity of FM and sorghum flours

The ABTS radical scavenging activity of the unmalted and malted cereal flours were determined according to the methods of Arnao *et al.* (2001) and Thaipong *et al.* (2006) with slight modification. Equal volumes (10 ml) of 7.4 mM 2, 2'-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and 2.6 mM potassium persulfate, both prepared in distilled water, were

mixed and allowed to react for 12 h, at room temperature in the dark to obtain ABTS radical cation. The solution was then diluted by mixing 1 ml ABTS^{•+} solution with 60 ml of pH 7.4 phosphate buffer containing 0.1 M NaOH to obtain an initial absorbance of 0.098 at 734 nm. Fresh extracts (150 µL) of the cereal flours were allowed to react with 2850 µL of the ABTS^{•+} solution for 5 min and the absorbance was read at 734 nm. The standard curve was obtained by dissolving Trolox in phosphate buffer (pH 7.4) and reacting with 2850 µL of the ABTS^{•+} solution for 5 min. The results were obtained by extrapolation of absorbance of the sample extract with ABTS, using the equation generated from the standard curve. Results were expressed as µM Trolox equivalent (TE)/ g.

4.2.8. Ferric reducing antioxidant power of FM and sorghum flours

The reducing power assay was determined according to the method of Oyaizu (1986). Briefly, 100 µl of the extract was placed in a test tube and the volume adjusted to 1 ml with methanol. Accurately, 2.5 ml 0.2 M phosphate buffer (pH 6.6) and 2.5 ml 1 % potassium ferricyanide were added to the tube and vortexed. The mixture was left for 20 min at 50 °C in water bath. After incubation, 2.5 ml 10 % (w/v) trichloroacetic acid was added and the mixture centrifuged at 4470 g for 20 min. Exactly 2.5 ml of the supernatant was taken and mixed with 2.5 ml distilled water and 0.5 ml 0.1 % (w/v) ferric chloride in a test tube. The absorbance was measured at 700 nm. Higher absorbance of reaction mixture with the sample extract indicates higher reducing power.

4.2.9. Statistical analysis

Results of the experiment were analysed as described in section 3.2.6.

4.3. Results

4.3.1. Phenolic composition of finger millet and sorghum malt flours

The phenolic compounds of FM and sorghum malt flours were identified by comparing their retention times and mass spectral data with that of standards (where available). Table 7 shows the retention time and mass spectral characteristics of identified phenolic compounds of the cereal grains, using formic acid-methanol and HCl-methanol. Figure 10 – 11 shows mass spectra of identified phenolic compounds in the unmalted FM and sorghum grains. Similar mass spectra were confirmed in the malted grains. The phenolic compounds identified in the extracts from FM and sorghum grains were benzoic acid derivative, flavan-3-ols, flavononol, proanthocyanidins, flavonols and flavanones.

4.3.2. Changes in phenolic content of the finger millet and sorghum malt flours

Table 8 shows changes in identified phenolic compounds of FM and sorghum grain varieties during malting. Protocatechuic acid was the only phenolic acid detected in the formic acid in methanol extracts of the cereal grains. From the same extraction solvent, catechin was found to be the major flavonoid in the FM varieties followed by epicatechin with taxifolin found in small amounts. Table 9 shows a comparison of quantified phenolic compounds of FM (BFM & DBFM) grains using two extraction solvents/ acidification methods compared with other studies. It is noted from the table that the acidification was the principal variable affecting the extractability of the phenolic compounds particularly flavonoids.

A decrease in the total phenolic content for DBFM was observed up to 96 h of malting, particularly for protocatechuic acid (7.27 to 4.38 $\mu\text{g/g}$) and epicatechin (16.18 to 11.34 $\mu\text{g/g}$). For BFM and sorghum grains, decreases were observed in total phenolic content after 24 and 48 h of malting which later showed increases at 72 and 96 h for protocatechuic acid, catechin and epicatechin. Taxifolin was not detected in DBFM, however, it was found in BFM.

Table 7. Retention time and mass spectra characteristics of phenolic compounds of the cereal grain extracts with UV absorption maxima of 280 nm.

Extraction solvents	t_R (min)	$[M-H]^-$ (m/z)	MS/MS fragments (intensity %)	Tentatively identified compounds
15% Formic acid – methanol	<i>Benzoic acid derivatives</i> 7.87	153	153 (100)	Protocatechuic acid
	<i>Flavan-3-ols</i> 11.17	289	289 (100), 245 (30), 203 (21), 123 (19), 109 (14)	Catechin
	13.32	289	289 (100), 245 (40), 203 (31), 137 (40)	Epicatechin
	<i>Flavononol</i> 17.05	303	303 (100), 285 (11), 236 (9), 191 (4), 175 (5)	Taxifolin
1% HCl-methanol	<i>Flavan-3-ols</i> 12.22	289	289 (100), 245 (84), 209 (72), 177(61), 137(47), 109 (37)	Catechin
	<i>Proanthocyanidins</i> 10.20	575	575 (100)	Proanthocyanidin A1/ A2
	<i>Flavonols</i> 24.18	303	303 (100)	Quercetin
	<i>Flavanones</i> 21.88	301	301 (100), 269 (89)	Hesperitin

t_R = retention time; $[M-H]^-$ = negative ion mode; MS = mass spectra.

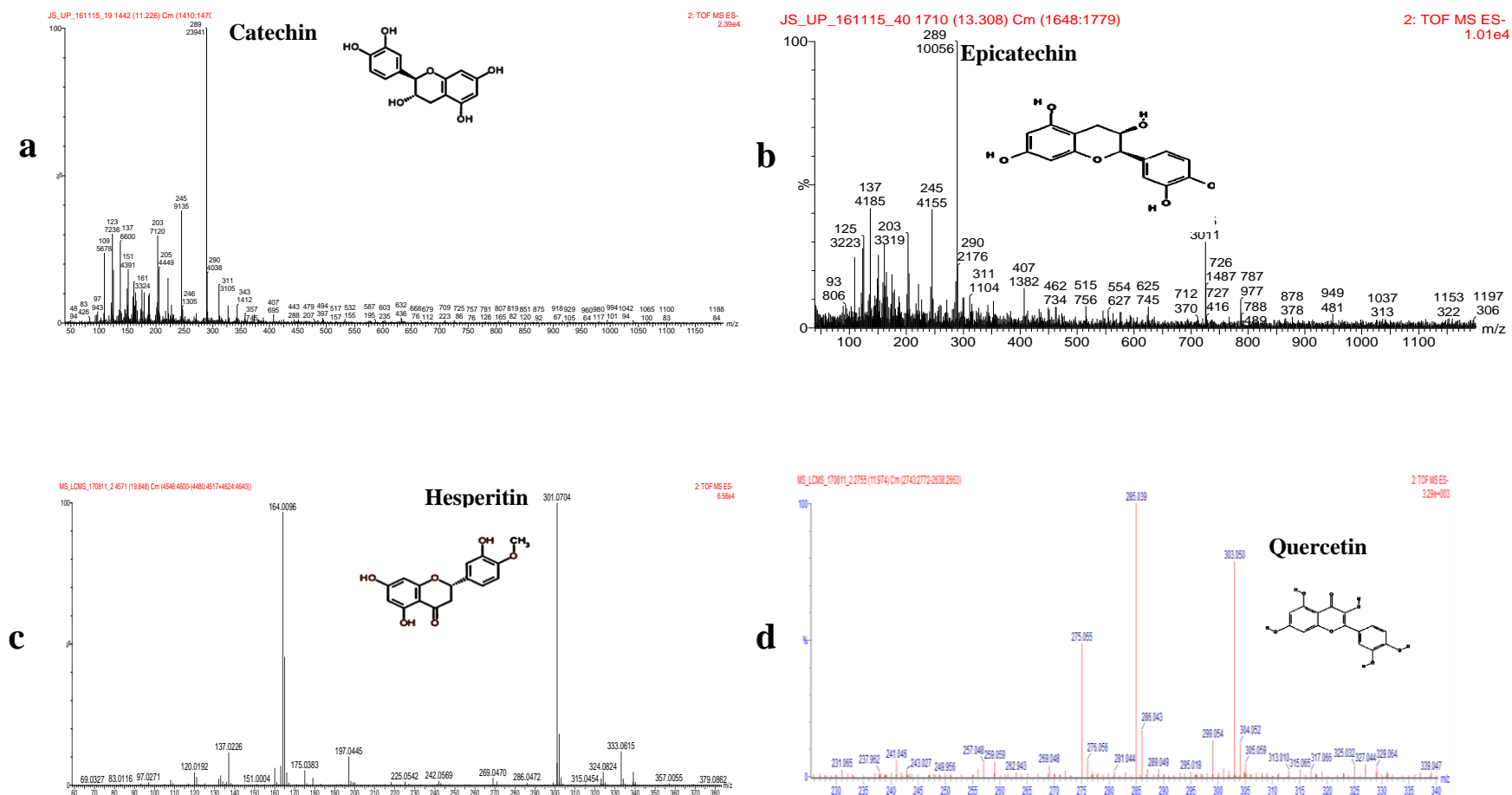


Figure 10. Mass spectra of a) catechin, b) epicatechin, c) hesperitin and d) quercetin of the cereal grains.

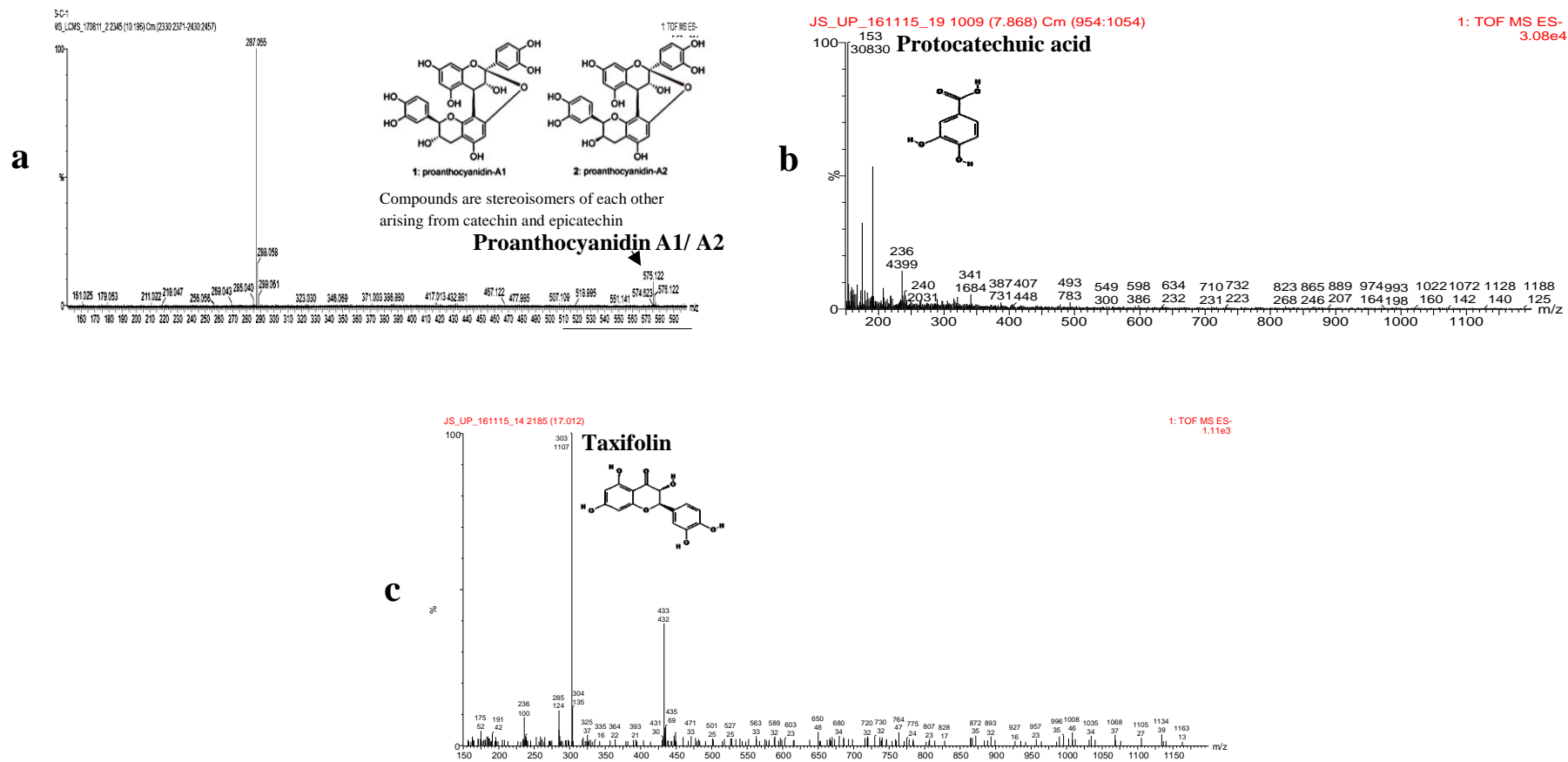


Figure 11. Mass spectra of a) proanthocyanidin A1/ A2, b) protocatechuic acid and c) taxifolin of the cereal grains.

Table 8. Changes in content ($\mu\text{g/g}$) of phenolic compounds in formic acid- and HCl-methanol extracts from the cereal grains.

Grains/ compounds	Malting period (h)									
	15% formic acid-methanol					1% HCl-methanol				
	0	24	48	72	96	0	24	48	72	96
<i>Dark brown finger millet (DBFM)</i>										
PA	7.3 \pm 0.1 ^a	6.7 \pm 0.2 ^b	5.9 \pm 0.0 ^c	4.9 \pm 0.2 ^d	4.4 \pm 0.1 ^e	Nd	Nd	Nd	Nd	Nd
CA	25.9 \pm 0.4 ^a	24.8 \pm 0.6 ^a	25.0 \pm 0.5 ^a	23.2 \pm 0.6 ^b	22.1 \pm 0.0 ^b	10.4 \pm 0.6 ^a	Nd	Nd	Nd	Nd
ECA	16.2 \pm 0.1 ^a	14.7 \pm 0.3 ^b	13.8 \pm 0.3 ^b	11.5 \pm 0.9 ^c	11.3 \pm 0.2 ^c	Nd	Nd	Nd	Nd	Nd
QE	Nd	Nd	Nd	Nd	Nd	46.7 \pm 2.9 ^a	41.7 \pm 1.3 ^b	23.3 \pm 1.6 ^c	25.1 \pm 2.8 ^c	27.8 \pm 1.9 ^c
Hp	Nd	Nd	Nd	Nd	Nd	96.3 \pm 13.8 ^a	9.2 \pm 3.7 ^b	Nd	Nd	Nd
PAC A1	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
PAC A2	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Total	49.4 \pm 0.6	46.2 \pm 1.1	44.7 \pm 0.8	39.6 \pm 1.7	37.8 \pm 0.3	153.4 \pm 17.3	50.9 \pm 5.0	23.3 \pm 1.6	25.1 \pm 2.8	27.8 \pm 1.9
<i>Brown finger millet (BFM)</i>										
PA	14.8 \pm 0.0 ^a	4.3 \pm 0.1 ^b	2.8 \pm 0.0 ^c	4.7 \pm 0.1 ^d	4.6 \pm 0.2 ^d	Nd	Nd	Nd	Nd	Nd
CA	27.9 \pm 0.3 ^a	20.7 \pm 0.4 ^b	17.9 \pm 0.4 ^c	20.9 \pm 0.1 ^b	21.9 \pm 0.0 ^d	1.4 \pm 0.0 ^a	Nd	Nd	Nd	Nd
ECA	17.4 \pm 0.8 ^a	9.8 \pm 0.4 ^b	7.7 \pm 0.1 ^c	10.4 \pm 0.2 ^b	10.4 \pm 0.1 ^b	Nd	Nd	Nd	Nd	Nd
QE	Nd	Nd	Nd	Nd	Nd	66.2 \pm 4.4 ^a	54.2 \pm 1.8 ^a	43.6 \pm 4.9 ^a	42.1 \pm 2.4 ^a	38.9 \pm 5.7 ^a
TX	0.4 \pm 0.3 ^a	0.11 \pm 0.03 ^a	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Hp	Nd	Nd	Nd	Nd	Nd	96.7 \pm 35.7 ^a	Nd	Nd	Nd	Nd
PAC A1	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
PAC A2	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Total	60.5 \pm 1.4	34.9 \pm 0.9	28.4 \pm 0.5	36.00 \pm 0.40	36.9 \pm 0.3	164.3 \pm 40.1	54.2 \pm 1.8	43.6 \pm 4.9	42.1 \pm 2.4^a	38.9 \pm 5.7^a
<i>Sorghum</i>										
PA	5.0 \pm 0.5 ^a	2.1 \pm 0.0 ^b	1.9 \pm 0.0 ^b	3.15 \pm 0.03 ^c	3.2 \pm 0.1 ^c	Nd	Nd	Nd	Nd	Nd
CA	0.6 \pm 0.0 ^a	0.3 \pm 0.0 ^b	0.3 \pm 0.0 ^b	0.33 \pm 0.01 ^{bc}	0.4 \pm 0.0 ^c	24.6 \pm 5.8 ^a	9.2 \pm 2.3 ^b	Nd	Nd	Nd
ECA	2.9 \pm 0.2 ^a	3.9 \pm 0.0 ^{bd}	3.8 \pm 0.0 ^b	4.38 \pm 0.08 ^c	4.3 \pm 0.0 ^{cd}	Nd	Nd	Nd	Nd	Nd
QE	Nd	Nd	Nd	Nd	Nd	84.9 \pm 5.6 ^a	63.4 \pm 1.7 ^b	41.9 \pm 3.5 ^c	37.0 \pm 1.9 ^c	23.1 \pm 1.9 ^d
TX	3.4 \pm 0.9 ^a	4.5 \pm 1.2 ^{bc}	4.1 \pm 1.0 ^b	4.7 \pm 0.7 ^{bc}	4.7 \pm 0.7 ^{bc}	Nd	Nd	Nd	Nd	Nd
Hp	Nd	Nd	Nd	Nd	Nd	1636.9 \pm 241.4 ^a	1210.0 \pm 256.0 ^a	244.5 \pm 87.8 ^b	52.50 \pm 15.2 ^{b,c}	32.9 \pm 8.4 ^c
PAC A1	Nd	Nd	Nd	Nd	Nd	22.1 \pm 3.3 ^a	13.5 \pm 8.3 ^a	Nd	Nd	Nd
PAC A2	Nd	Nd	Nd	Nd	Nd	26.9 \pm 4.5 ^a	20.5 \pm 6.8 ^a	4.2 \pm 0.0 ^b	Nd	Nd
Total	8.5 \pm 0.7	6.3 \pm 0.0	6.0 \pm 0.0	7.86 \pm 0.12	7.9 \pm 0.1	1795.4 \pm 260.6	1316.6 \pm 275.1	290.6 \pm 91.3	89.5 \pm 17.1	56.0 \pm 10.3

Nd, not detected; PA, protocatechuic acid; CA, (+)-catechin; ECA, (-)-epicatechin; QE, quercetin; TX, taxifolin; Hp, hesperitin; PAC A1, proanthocyanidin A1; PAC A2, proanthocyanidin A2; Values are mean of triplicate experiments; Mean values in a row with different superscripts are significantly different at $p < 0.05$.

Table 9. Comparison of phenolic composition of unmalted finger millet of present study with previous studies.

Phenolic compounds	Quantity (µg/ g)	Extraction solvent(s)		References
<u><i>Phenolic acids</i></u>				
Gentisic acid	^{a)} 4.96	1% HCl-methanol	N/A	Hithamani & Srinivasan (2014)
Vanillic acid	^{b)} 1.00	N/A	70% ethanol	Subba Rao & Muralikrishna (2002)
Gallic acid	^{a)} 3.91, ^{b)} 4.5	1% HCl-methanol,	N/A	Hithamani & Srinivasan (2014), Subba Rao & Muralikrishna (2002)
Syringic acid	^{a)} 60.0	1% HCl-methanol	N/A	Hithamani & Srinivasan (2014)
Salicylic acid	^{a)} 5.12	1% HCl-methanol	N/A	
Caffeic acid	^{a)} 4.24, ^{b)} 5.87	1% HCl-methanol	70% ethanol	Subba Rao & Muralikrishna (2002), Hithamani & Srinivasan (2014)
Ferulic acid	^{b)} 18.8	N/A	70% ethanol	Subba Rao & Muralikrishna (2002)
Sinapic acid	^{a)} 11.0	1% HCl-methanol		Hithamani & Srinivasan (2014)
Protocatechuic acid	^{b)} 45	N/A	70% ethanol	Subba Rao & Muralikrishna (2002), Present study
	7.27 – 14.82	15% formic acid-methanol		
<i>p</i> -hydroxybenzoic acid	^{a)} 4.41	1% HCl-methanol	N/A	Hithamani & Srinivasan (2014)
<i>p</i> -coumaric acid	^{a)} 1.81, ^{b)} 1.30	1% HCl-methanol	70% ethanol	Subba Rao & Muralikrishna (2002), Hithamani & Srinivasan (2014)
<i>t</i> -cinnamic acid	^{a)} 1.55	1% HCl-methanol	N/A	Hithamani & Srinivasan (2014)
<u><i>Flavonoids</i></u>				
Catechin	25.93 – 27.85	15% formic acid-methanol	N/A	Present study
	0.45 – 10.41	1% HCl-methanol	N/A	Present study
Epicatechin	16.18 – 17.43	15% formic acid-methanol	N/A	Present study
Taxifolin	0.41	15% formic acid-methanol	N/A	Present study
Quercetin	^{a)} 3,	1% HCl-methanol	N/A	Chethan <i>et al.</i> (2008)
	46.68 - 66.20	1% HCl-methanol	N/A	Present study
Hesperitin	183.11 – 427.07	1% HCl-methanol	N/A	Present study

^{a)} Phenolic compound quantified with methanol acidified with HCl/ formic acid, ^{b)} Phenolic compound quantified with ethanol.

Malting for 24 h decreased taxifolin content of BFM beyond which it was not detected. Taxifolin was detected in commensurable amount in sorghum grain. Unlike the BFM, an increase in taxifolin content was observed at 24 up to 72 h of malting after which there was no significant change.

Hesperitin was found in higher amounts followed by quercetin for HCl-methanol extracts of the FM and sorghum flours. A high amount of hesperitin (1636.9 $\mu\text{g/g}$) was recorded for the unmalted sorghum grain compared to 96.7 $\mu\text{g/g}$ and 96.3 $\mu\text{g/g}$ for unmalted BFM and DBFM, respectively (Table 8). A significant decrease in total phenolics was observed after 96 h of malting for BFM and sorghum flours. For DBFM, decrease in total phenolics was observed until 72 h of malting beyond which there was no significant change. A loss of catechin for both FM varieties was observed at 24 h of malting and at 48 h for sorghum grain; a similar loss was observed for hesperitin for BFM at 24 h of malting. For DBFM, a decrease and loss of hesperitin were observed at 24 and 48 h of malting, respectively. A decrease in quercetin content was observed until 96 h of malting for sorghum grain. A decrease in quercetin content up to 48 h of malting was observed for DBFM beyond which there was no significant change. There was no significant change in quercetin content for BFM during the malting period.

4.3.3. Total phenolic and total flavonoid content of finger millet and sorghum malt flours

The total phenolic contents (TPC) of the unmalted and malted FM and sorghum flours are presented in Table 10. The TPC of DBFM, BFM and sorghum grain ranged from 3.90 – 4.68, 4.41 – 7.09 and 17.23 – 19.26 mg GAE/ g, respectively. The sorghum grain had higher TPC compared to the FM varieties for the unmalted samples and at all malting periods. A statistically insignificant ($p > 0.05$) increase in TPC of the sorghum grain was observed at 24 h of malting which was not significantly different for other malting periods. A similar observation was recorded for the DBFM. For the BFM, a significant decrease in TPC was observed at 24 and 48 h of malting and thereafter, an increase which remained constant up to 96 h of malting.

Table 10. Effect of malting on total phenolic content of finger millet and sorghum grains.

	Malting period (h)				
	0	24	48	72	96
Cereal grains	Total phenolic content (GAE mg/g)				
Dark brown finger millet	4.42 ± 0.30 ^{a z}	4.68 ± 0.00 ^{a y}	4.64 ± 0.14 ^{a y}	3.90 ± 0.41 ^{a z}	4.33 ± 0.22 ^{a y}
Brown finger millet	7.09 ± 0.49 ^{a y}	5.39 ± 0.05 ^{b y}	4.41 ± 0.16 ^{c y}	5.22 ± 0.36 ^{b y}	5.02 ± 0.14 ^{b y}
Sorghum	17.23 ± 1.09 ^{a x}	18.92 ± 2.27 ^{a x}	18.56 ± 0.47 ^{a x}	17.54 ± 0.38 ^{a x}	19.26 ± 0.13 ^{a x}
	Total flavonoid content (QE mg/g)				
Dark brown finger millet	0.92 ± 0.15 ^{a y}	0.95 ± 0.07 ^{a y}	0.66 ± 0.07 ^{ab y}	0.72 ± 0.07 ^{ab y}	0.35 ± 0.19 ^{b y}
Brown finger millet	5.45 ± 0.27 ^{a y}	2.98 ± 0.33 ^{b y}	2.01 ± 0.15 ^{c y}	1.25 ± 0.17 ^{d y}	2.03 ± 0.18 ^{c y}
Sorghum	22.21 ± 3.49 ^{a x}	22.15 ± 3.37 ^{a x}	27.77 ± 1.56 ^{a x}	28.37 ± 2.99 ^{a x}	25.29 ± 2.28 ^{a x}

GAE: gallic acid equivalent, QE: quercetin equivalent. ^{abc} Mean values within each row not followed by the same superscript are significantly different ($p < 0.05$). ^{xyz} Mean values within each column not followed by the same superscript are significantly different ($p < 0.05$).

The total flavonoid contents (TFC) of the FM and sorghum flours are presented in Table 10. Values ranged from 0.35 – 0.95 QE mg / g in DBFM, from 1.25 – 5.45 QE mg / g in BFM and from 22.15 – 28.37 QE mg / g in the sorghum grain. Malting did not result in a statistically significant ($p > 0.05$) change in the TFC of the sorghum grain. For the BFM, a four-fold decrease in TFC was observed from 24 up to 72 h, which later increased. An insignificant increase in TFC was observed for DBFM at 24 h of malting which later decreased and remained constant up to 72 h. A further decrease ($p < 0.05$) in TFC of DBFM malt was observed at 96 h. High amounts of TPC and TFC were found at 24 h of malting for the FM varieties.

4.3.4. DPPH (2, 2 - diphenyl-1-picrylhydrazyl) free radical scavenging activity of the finger millet and sorghum malt flours

The unmalted and malted cereal grains showed DPPH radical scavenging activity, which was in the range of 46.79 – 70.16 in DBFM, from 48.05 – 60.82 in BFM and from 54.73 – 65.89% in the sorghum grain (Table 11). Malting did not result in a significant ($p > 0.05$) change in the DPPH radical scavenging activity of the BFM and sorghum grain varieties. For DBFM, an increased ($p < 0.05$) DPPH radical scavenging activity was observed for the 24 h malt.

4.3.5. ABTS (2, 2'-azinobis-3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging activity of finger millet and sorghum malt flours

The antioxidant capacity of the malted FM and sorghum grains determined by ABTS assay were in the range of 9.78 – 10.32 $\mu\text{M TE/ g}$ in DBFM, 9.76 – 10.63 $\mu\text{M TE/ g}$ in BFM and 9.79 – 10.26 $\mu\text{M TE/ g}$ in sorghum grain (Table 11).

Table 11. Effect of malting on antioxidant activities of finger millet and sorghum grains.

	Malting period (h)				
	0	24	48	72	96
Cereal grains	DPPH radical scavenging activity (%)				
Dark brown finger millet	51.00 ± 3.34 ^{a x}	70.16 ± 2.03 ^{b z}	61.76 ± 3.48 ^{ac x}	46.79 ± 9.71 ^{c x}	69.16 ± 5.98 ^{ac xz}
Brown finger millet	56.60 ± 1.69 ^{a x}	51.29 ± 1.97 ^{a y}	48.05 ± 7.64 ^{a x}	60.82 ± 7.64 ^{a x}	48.49 ± 1.46 ^{a xy}
Sorghum	64.85 ± 8.31 ^{a x}	61.15 ± 2.39 ^{a x}	54.73 ± 1.53 ^{a x}	65.89 ± 5.13 ^{a x}	62.67 ± 6.51 ^{a x}
	Iron reducing activity				
Dark brown finger millet	0.8904 ± 0.01 ^{a y}	0.8531 ± 0.01 ^{b y}	0.7975 ± 0.01 ^{c z}	0.9647 ± 0.01 ^{d y}	0.9798 ± 0.01 ^{d x}
Brown finger millet	0.9956 ± 0.01 ^{a x}	0.9199 ± 0.01 ^{b y}	0.9358 ± 0.03 ^{ab y}	0.9517 ± 0.02 ^{ab y}	0.9961 ± 0.03 ^{a x}
Sorghum	1.0375 ± 0.02 ^{a x}	1.0224 ± 0.03 ^{a x}	1.0134 ± 0.02 ^{a x}	1.0153 ± 0.01 ^{a x}	1.0101 ± 0.01 ^{a x}
	ABTS (μM TE/g)				
Dark brown finger millet	10.32 ± 0.07 ^{a y}	9.92 ± 0.05 ^{b x}	9.93 ± 0.04 ^{b y}	9.78 ± 0.21 ^{b y}	9.84 ± 0.08 ^{b y}
Brown finger millet	9.76 ± 0.10 ^{a x}	9.76 ± 0.15 ^{a x}	10.57 ± 0.02 ^{b x}	10.63 ± 0.11 ^{b x}	10.30 ± 0.05 ^{b x}
Sorghum	9.93 ± 0.11 ^{ab x}	9.79 ± 0.15 ^{b x}	10.23 ± 0.03 ^{a x}	10.02 ± 0.19 ^{ab x}	10.26 ± 0.03 ^{a x}

DPPH: 2, 2 - diphenyl-1-picrylhydrazyl, ABTS: 2, 2'-azinobis-3-ethylbenzthiazoline-6-sulfonic acid estimated as Trolox equivalent (TE),

^{abc} Mean values within each row not followed by the same superscript are significantly different ($p < 0.05$). ^{xyz} Mean values within each column not followed by the same superscript are significantly different ($p < 0.05$).

Malting for 24 h resulted in a significant ($p < 0.05$) decrease in ABTS radical quenching activity of the DBFM and sorghum grain malt, which remained unchanged up to 96 h for DBFM. For the sorghum malt, an increase in the ABTS radical quenching activity was observed at 48 h of malting, which remained constant up to 96 h of malting. For the BFM, no significant change in the ABTS radical quenching activity was observed at 24 h of malting. A significant ($p < 0.05$) increase was recorded at 48 h of malting, which remained constant up to 96 h period for BFM. Although a slight increase was observed at 72 h of malting, it was not significantly different from the value observed at 48 h and 96 h.

4.3.6. Ferric reducing antioxidant power of finger millet and sorghum malt flours

Similar to the DPPH radical scavenging activity, the cereal grains showed increased iron reducing activity (Table 11). The iron reducing activity of the malted FM and sorghum grains were in the range of 0.7975 – 0.9798 in DBFM, from 0.9199 – 0.9961 in BFM and from 1.0101 – 1.0375 in sorghum grain. High iron reducing activity was observed for the sorghum grain compared to the FM varieties both for the unmalted and malted grains. Malting did not result in any significant ($p > 0.05$) change in the iron reducing activity of the sorghum grain. For BFM, an increase in iron reducing activity with malting time was observed up to 96 h. For DBFM, a reduced iron reducing activity was observed within 24 and 48 h of malting, thereafter an increase, which was significantly ($p < 0.05$) high at 96 h of malting.

4.4. Discussion

4.4.1. Changes in phenolic composition of the finger millet and sorghum malt flours

In most FM studies, a number of phenolic acids have been isolated for aqueous solvents acidified with HCl (Subba Rao and Muralikrishna, 2002; Chethan and Malleshi, 2007; Chethan *et al.*, 2008a; Hithamani and Srinivasan, 2014). Although the phenolic compounds isolated

were few, this discrepancy with previous reports on the phenolic composition of FM is attributed to instrument of extraction/ acidification method on the stability/ extractability of the phenolic compounds. The study showed the presence of more of flavonoids than phenolic acids for the FM varieties; and also the presence of hesperitin, which has not been reported in the grain. It was found that the extraction solvents or acidification methods favoured the release of flavonoids than the phenolic acids (protocatechuic acid) of FM (Table 8). Conversely, the HCl-methanol extract showed high amount of total phenolics compared with formic acid-methanol extract. The high extractability of HCl could be attributed to the strength of the acid in disrupting cell wall matrix/ structure and/ or bonds associated with the phenolic compounds. Ivanova *et al.* (2009) used a lower concentration of HCl in aqueous solvents (acetone and methanol) for extraction of phenolics as 0.1% HCl does not cause as much hydrolysis of flavonoids as 1% HCl. This study highlighted the effect of the strength of acid/ concentration and aqueous solvent on extraction of phenolic compounds. Beside the identified phenolic compounds, the UPLC-MS data (appendix iii) also indicated the presence of several other peaks that could not be identified or matched with known phenolic compounds, thus revealing the complex chemical composition of the grain. Further study is required to assess the effect of acidification method/ solvent and instrument of extraction to further describe the phenolic composition of FM grain.

The phenolic composition of the cereal grains studied were affected by the malting periods. The observed decrease in phenolic compounds of the cereal grains particularly at 24 h of malting could be attributed to initial loss of hydrolysed phenolic compounds during steeping. The decrease and complete loss of catechin, hesperitin, and taxifolin at and after 24 h of malting could be due to oxidation or bioconversion of the phenolic compound to other active or less active forms. Leaching of the phenolic compounds during germination could also have contributed to the loss observed. Conversely, increases were observed at 72 and 96 h of malting

which varied for the FM and sorghum varieties. Similar observation was reported by Subba Rao and Muralikrishna (2002), Chethan *et al.* (2008a) and Hithamani and Srinivasan (2014). Structural breakdown of grain cell wall is a key biochemical modification that occurs during malting. This process often result in the activation of various enzymes like esterases, ligninolytic and carbohydrate metabolising enzymes which hydrolyses conjugated or bound phenolic compounds into their free forms (Subba Rao and Muralikrishna, 2002; Hur *et al.*, 2014). The increase in individual phenolic compounds of the cereal grains could be due to the hydrolytic action of the grain's endogenous and microbial enzymes on various bound or conjugated and condensed phenolic compounds and / or their synthesis, followed by storage in seeds instead of their migrating to shoots during malting. Furthermore, varietal differences among the grains could also account for the observed changes in total phenolic compounds during malting.

4.4.2. Total phenolic and total flavonoid content of finger millet and sorghum malt flours

The total phenolic and flavonoid content of the FM varieties (Table 10) were in the range obtained by Hithamani and Srinivasan (2014) who found 10.2 and 5.54 mg/ g for total phenolic and total flavonoid contents in FM. The values are also comparable with the results of Krishnan *et al.* (2012) who reported 18 mg/ g of total phenolic content (TPC) of FM. Despite the initial decrease in TPC during 24 h of malting, the total phenolic and flavonoid contents of the FM varieties were found to concentrate in the 24 h malt compared to other malting period. Variation in TPC of cereal grains during processing, are known to result from enzymatic hydrolysis, bioconversion and oxidative degradation among other factors. The decrease in TPC for BFM and TFC for both FM varieties after 24 h could be due to loss of hydrolysed phenolic compounds during steeping and germination. The high TPC observed at 24 h compared to other malting period for the FM varieties could be due to the total phenolic compounds retained at

the malting period which could be linked with the grain membrane structure. Also, varietal differences may have influenced the observed decrease in total phenolic and flavonoid contents in the FM varieties. These findings agree with the result of other researchers who have reported similar decrease in TPC and TFC of malted FM, although for a period of 48 h (Krishnan *et al.*, 2012; Hithamani and Srinivasan, 2014).

4.4.3. Changes in antioxidant activity of finger millet and sorghum malt flours

The antioxidant properties of FM have been studied by several researchers, however limited information on the antioxidant activity of the millet at different malting periods were reported. Chandrasekara *et al.* (2012) have reported the antioxidant activity of whole grains, cooked, dehulled and hulls of FM. Viswanath *et al.* (2009) also evaluated the antioxidant activity of milled fractions of the millet and in both studies, FM exhibited a measure of antioxidant activities. A similar observation was recorded in the present study. The unmalted and malted cereal grains exhibited DPPH radical scavenging activities. Malting did not result in any significant ($p < 0.05$) increase in DPPH radical scavenging activity for BFM and sorghum flours except for DBFM, which was significantly ($p < 0.05$) higher at 24 h. A concentration-dependent DPPH radical scavenging activity was not observed as expected for the sorghum flour, although it contained higher total phenolic compared to the millet varieties.

The unmalted cereal flours were able to quench ABTS radicals, although to a lesser extent compared to the values reported by Siwela *et al.* (2007) who found high antioxidant activity for FM varieties (37.9 – 195.4 mM trolox equivalent/ kg). The ABTS radical quenching activity obtained for sorghum grain in the present study, however, is similar to the report of Awika *et al.* (2005) wherein antioxidant activity of 9.8 mM trolox equivalent/ kg was observed. The malting process did not bring about a significant change/ increase in the ABTS radical scavenging activity of the cereal flours beyond 48 h of malting. Generation and reactivity time

of ABTS radicals with sample extract are considered as important factors for estimating antioxidant activity (Arnao *et al.*, 2001; Thaipong *et al.*, 2006). The low antioxidant activity of the FM varieties obtained in this study could be a result of the technique applied in conducting the assay wherein the reaction time of the ABTS radicals and the sample extracts was shortened by 5 – 6 min compared to the reactivity time of 30 min used by Siwela *et al.* (2007). The time difference could allow for increased reactivity of ABTS radicals with cereal flour extracts, thus resulting in higher values for the assay. There are no reports on the effect of malting on the antioxidant capacity of finger millet using ABTS assay. The changes observed in the ABTS radical quenching activity of the cereal grains in the present study are attributed to the presence of phenolic compounds with antioxidant properties such as protocatechuic acid, quercetin, catechin, epicatechin, and hesperitin; minerals (e.g. Zn, Fe, Cu, Mn) and other organic compounds such as, citric acid (Fardet *et al.*, 2008; Healthgrain, 2011; Wyrzykowski *et al.*, 2011; Abdel-Salam *et al.*, 2014).

The phenolic extracts of the unmalted and malted cereal flours were able to reduce Fe^{3+} to Fe^{2+} . Increased iron reducing activity was observed for the FM varieties, which were significantly ($p < 0.05$) higher at 96 h of malting. In a whole grain cereal there are different substances that contribute directly or indirectly to the antioxidant activities. Polyphenol content of plant foods is well known to have a parallel effect on the antioxidant activity, whereas vitamin E and minerals, such as iron, zinc, manganese, selenium, and copper act as co-factor in antioxidant enzymes - sulphur-containing amino acids, methionine and cysteine - that are precursors of glutathione, an endogenous antioxidant (Fardet *et al.*, 2008; Healthgrain, 2011). The observed changes in the iron reducing activity could not be entirely attributed to the total phenolic content, but also to other components of the malt, such as citric acid and minerals with antioxidant effects.

Pearson correlation coefficient test showed no significant correlation between the DPPH, ABTS and iron reducing assays for FM and sorghum malts (Table 12). The iron reducing activity assay was used to draw conclusion on the effect of malting time on the grain malts. This was based on the fact that iron reducing assay not only reflect the antioxidant effect of phenolics present, but also accounts for other components of the malt that may have increased the antioxidant activity like an increase in minerals.

Table 12. Pearson correlation coefficients between DPPH, ABTS and FRAP assays of finger millet and sorghum beverages.

Response variables	DPPH	ABTS	FRAP
<i>Dark brown finger millet</i>			
DPPH	1.000	-0.349	-0.128
ABTS	-0.349	1.000	-0.205
FRAP	-0.128	-0.205	1.000
<i>Brown finger millet</i>			
DPPH	1.000	-0.051	-0.075
ABTS	-0.051	1.000	-0.047
FRAP	-0.075	-0.047	1.000
<i>Sorghum</i>			
DPPH	1.000	-0.398	-0.091
ABTS	-0.398	1.000	-0.105
FRAP	-0.091	-0.105	1.000

Pearson correlation coefficients *, ** indicate significance at $p < 0.05$ and 0.01 .

DPPH: 2, 2-diphenyl-1-picrylhydrazyl;

ABTS: 2, 2'-azinobis-3-ethylbenzthiazoline-6-sulfonic acid;

FRAP: Ferric reducing antioxidant power.

4.5. Conclusion

Malting period affected the phenolic composition, total phenols and antioxidant activity of the two FM grain varieties. Results obtained show the presence of catechin, epicatechin, taxifolin and hesperitin; flavonoids that were not previously identified or quantified in FM studies. The loss of some phenolic compounds were observed with malting time, whereas other phenolics showed increases beyond malting period, commonly used in processing of FM.

Increase in quercetin, catechin, epicatechin and protocatechuic acid contents of the FM and sorghum grain varieties were observed at 72 and 96 h of malting. Total phenolic content was concentrated in 24 h malt of the FM varieties beyond which no significant increases were observed. This could be important in the production of FM malt with high total phenols that can be used in food product development, like geriatric and adult foods, in order to reduce oxidative stress associated with aging and various lifestyle disorders. The two FM grain varieties exhibited DPPH, ABTS radical scavenging and iron reducing activities. The iron reducing activity of the FM varieties increased with malting time. A similar observation was recorded for the ABTS radical scavenging activity of the FM varieties. This finding support the fact that, a high phenol content of plant foods does not necessarily translate into high antioxidant activity, but is a result of a synergy between total phenols and profile/ types, released organic components and minerals with antioxidant properties.

Varietal difference was found to play a noticeable role on how malting affected the phenolic composition of the two FM grain varieties. This was evident in the high total phenolics of unmalted BFM compared to DBFM, and increases that were noted during 72 – 96 h of malting for catechin, epicatechin and protocatechuic acid contents, unlike DBFM where a decrease in the phenolic compounds were observed with malting time. The study provides scientific rationale for FM grains' increased utilisation as a source of health-beneficial compounds, and also on the malting conditions that could be useful in food preparations for health promotion.

CHAPTER 5

EFFECT OF FERMENTATION AND MICROBIAL SOURCE ON THE PHYSICOCHEMICAL PROPERTIES, CITRIC ACID, TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF NON-ALCOHOLIC FINGER MILLET MALT BEVERAGE

5.1. Introduction

Finger millet has been used in several traditional foods, like fermented and non-fermented porridges, pancake-like flatbreads, and alcoholic and non-alcoholic beverages (ICRISAT/FAO, 1996; Siwela *et al.*, 2007). Beside its nutritional properties, FM has also been used for certain medical purposes, particularly in the management of diabetic conditions (Reddy *et al.*, 2008; Kumar *et al.*, 2016). Relatively few studies have described the nutritional, biochemical and health beneficial properties of these traditional foods. With regard to sorghum, there are popular fermented food products that are documented for their health properties (Towo *et al.*, 2006; Kayode *et al.*, 2007) but information on FM products is comparatively scarce.

Fermentation is predominantly used in food processing for improving the nutritional and health-promoting components of plant foods through biochemical modifications (Minamiyama *et al.*, 2003; Kataoka, 2005). Zvauya *et al.* (1997) describes the biochemical changes occurring during production of a traditional non-alcoholic beverage from malted FM, wherein increase in sugar content and decrease in pH was observed for the fermented beverages. Sripriya *et al.* (1997) reports an increase in total phenolics of FM malt slurry fermented using the grain's microflora, although Rani and Anthony (2014) showed a fermentation-dependent decrease in total phenolics of fermented FM grain. In both studies, the fermentation period using the grain's natural microflora resulted in reduced total phenols,

however the impact on the total phenolics and antioxidant activity was inconclusive. Information on the phenolic compounds and the effect of fermentation period on the antioxidant activity of FM malt beverage is limited. The aim of the study, therefore, was to monitor the effect of fermentation period and microbial source on the physicochemical properties, phenolic compounds and antioxidant activity of malt beverage from two FM varieties.

5.2. Materials and methods

5.2.1. Materials

Lactobacillus fermentum ATCC 9338 was purchased from Anatech Instruments, Sloane Park, South Africa. de Man, Rogosa and Sharpe (MRS) agar and MRS nutrient broth were purchased from Lab M Limited, United Kingdom. Folin-Ciocalteu's reagent, gallic acid and quercetin standards, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2'-azinobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) and Trolox standard were purchased from Rochelle Chemicals and Laboratory Equipment, Johannesburg, South Africa. All other chemicals used were of an analytical grade.

5.2.2. Malting, mashing and fermentation of the cereal flours

The cereal grains were malted as described in section 3.2.2. Fermentation of FM and sorghum malt flours for the beverage was done according to the method of Chelule *et al.* (2010) with improvements outlined in Figure 12. The flour from grain malt was prepared by mashing 1:9 w/v flour to water in a 250 ml volumetric flask using a water bath. The mash temperature was gradually raised from 25 °C and held at 65 °C for 10 min. The temperature of the mash was further raised to 85 °C for 15 min.

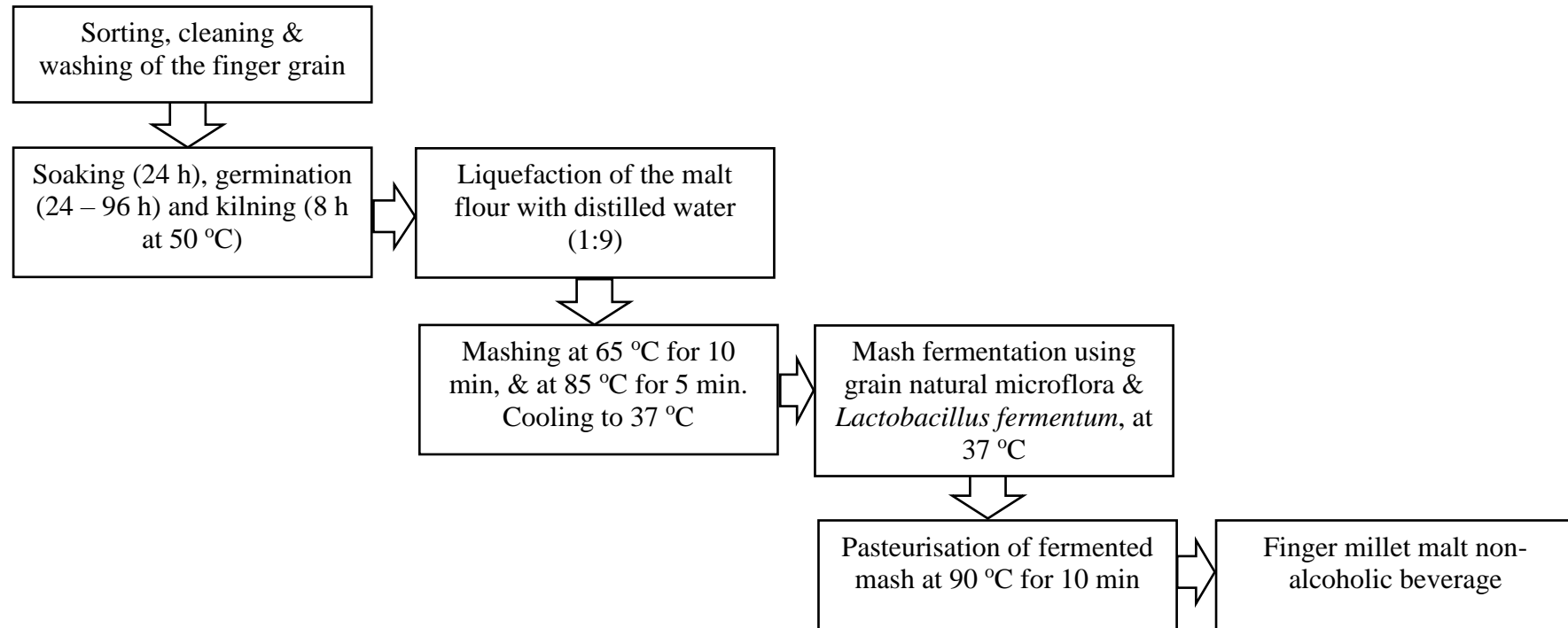


Figure 12. Flow diagram for the production of finger millet malt non-alcoholic beverage. Adapted from Chelule *et al.* (2010).

The role of these different initial and later temperatures was to activate and subsequently inactivate enzymes particularly β - and α -amylases, and also to reduce the microbial load of the mash. Reducing the microbial load was necessary to maintain an active fermentation, since high concentration of fermenting microorganisms negatively affect fermentation process due to increased/ mixed fermentative activities. The total plate count of the mash indicated low microbial load after mashing; before mashing, it was 40×10^6 CFU/ ml, and after mashing 6.67×10^6 CFU/ ml. The culture condition and inoculum preparation of *L. fermentum* was done following the method of Gao *et al.* (2009) with slight modification. The *L. fermentum* cells were allowed to grow for 48 h at 37 °C, harvested at 4470 g for 15 min and washed twice using sterile water before use. The FM grain microflora was prepared by soaking 3 g of the unmalted grain in distilled water and it was allowed to incubate for 20 min at 37 °C. Fermentation of the mash was performed using the grain natural microbial flora and *L. fermentum* separately for 96 h at 37 °C in an incubator (IncoTherm, Labotech, model 297, South Africa). The fermented mash batches were withdrawn at interval of 24 h and pasteurized at 90 °C for 10 min to obtain the FM non-alcoholic beverages. The production was repeated twice before an analysis of the physicochemical properties, phenolic compounds and antioxidant activity of the beverage.

5.2.3. Viscosity, pH and sugar content of the finger millet and sorghum grain malt beverages

The viscosity of the FM non-alcoholic beverages was determined using Brookfield viscometer (Sansas calibration laboratory, USA, Model, RVDVE230) [spindle 7, speed 100 rpm, temp. 25 °C]. The pH of the beverages was determined directly, using digital pH meter (Crison instruments, EU) calibrated with standard buffer at 25 °C. The sugar content (°brix) of the beverages was determined using automatic refractometer (Labex, Japan) according to the manufacturer's instruction.

5.2.4. Preparation of phenolic extracts of the finger millet and sorghum grain malt beverages

Phenolic extracts were prepared by refluxing 5 g of the FM and sorghum malt beverage with 20 ml of methanol containing 1% HCl for 2 h at 60 ± 5 °C (Hithamani and Srinivasan, 2014). The mixtures were centrifuged at 4470 g for 20 min and the supernatants were separated and used to analyse for phenolic compounds, total phenolic and antioxidant activity.

5.2.5. Determination of the phenolic compounds of the finger millet and sorghum grain malt beverages

The phenolic compounds were determined according to the procedure described in section 4.2.3.

5.2.6. Determination of citric acid content of the finger millet and sorghum grain malt beverages

The citric acid content of the FM and sorghum malt beverages was quantified following the procedure described in section 4.2.3.

5.2.7. Total phenolic content of the finger millet and sorghum grain malt beverages

The total phenolic content of sample extracts were determined according to the procedure described in section 4.2.4.

5.2.8. Total flavonoid content of the finger millet and sorghum grain malt beverages

Total flavonoid content was determined based on the method in section 4.2.5.

5.2.9. *DPPH (2, 2 - diphenyl-1-picrylhydrazyl) free radical scavenging activity of the finger millet and sorghum grain malt beverages*

The DPPH radical scavenging activity was determined following the procedure stated in section 4.2.6.

5.2.10. *ABTS (2, 2'-azinobis-3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging activity of the finger millet and sorghum grain malt beverages*

The ABTS radical scavenging activity of the beverage samples were determined as described in section 4.2.7.

5.2.11. *Ferric reducing antioxidant power of the finger millet and sorghum grain malt beverages*

The iron reducing power of the beverage extract was determined according to the method described in section 4.2.8.

5.2.12. *Statistical analysis*

Data obtained were analysed as described in section 3.2.6.

5.3. Results

5.3.1. *Viscosity, pH and sugar content of the finger millet and sorghum grain malt beverages*

The viscosities (cP) of the non-alcoholic beverages ranged from 26.9 – 66.0 in DBFM, 25.2 – 60.9 in BFM and 28.4 – 40.4 in the sorghum grain malt for fermentation using the grain natural microflora, whereas viscosities for the *L. fermentum* fermented non-alcoholic beverages ranged from 25.6 – 66.0 cP in DBFM, 19.1 – 60.9 cP in BFM and 23.6 – 30.4 cP in the sorghum grain malt (Figure 13).

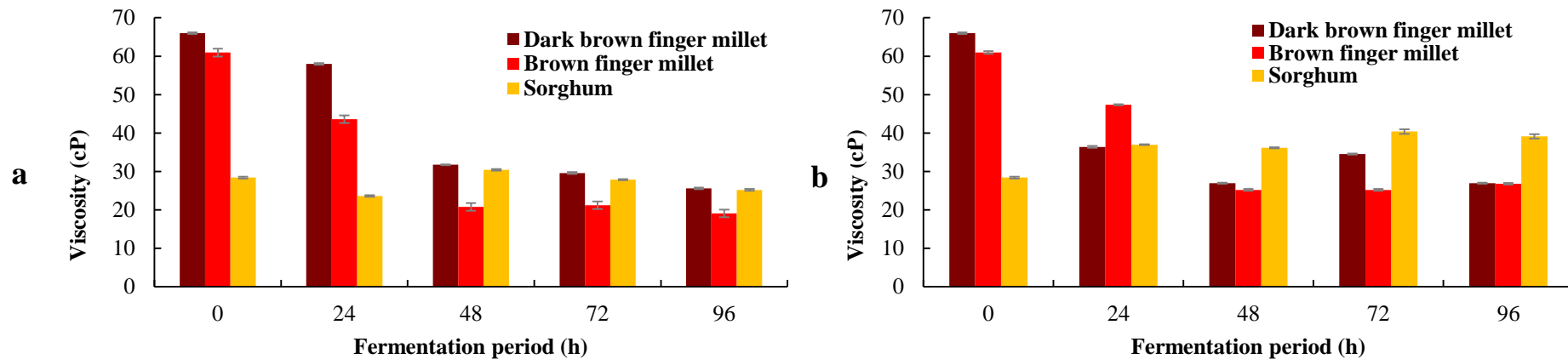


Figure 13. Effect of fermentation and microbial source on viscosity of finger millet and sorghum malt beverages. (a) fermentation with *Lactobacillus fermentum*; (b) fermentation with grain microbial flora. Mean values and standard error are significant at $p < 0.05$.

A steady decrease in the viscosities of the DBFM and BFM malt non-alcoholic beverages was observed during 24 – 48 h of fermentation, which remained constant up to 96 h for BFM beverage using the grain natural microflora. Increased viscosity was observed at 72 h of fermentation for DBFM, after which there was a decrease after 96 h of fermentation, however, an increase in the viscosity of the sorghum malt non-alcoholic beverage with the grain natural microflora was noted within 24 – 48 h of fermentation. A further increase in the viscosity of the sorghum malt non-alcoholic beverage was recorded at 72 h of fermentation, which later decreased. For the non-alcoholic beverages fermented with *L. fermentum*, - a decrease in viscosity with fermentation time was observed for the FM malt non-alcoholic beverage fermented with *L. fermentum*. For the sorghum malt non-alcoholic beverage, a decrease in the viscosity was observed at 24 h of fermentation, which later increased significantly at 48 h, however, a steady decrease in the viscosity of the sorghum non-alcoholic beverage was observed after 48 h up to 96 h of fermentation for using *L. fermentum*.

A significant ($p < 0.05$) decrease in the pH of the non-alcoholic beverages was observed during fermentation of the malt for both microbial sources (Figure 14). Increased acidity of the BFM (pH, 6.3 – 4.1) and sorghum (pH, 6.7 – 4.2) beverage was observed at 24 h up to 96 h of fermentation using *L. fermentum*. A similar observation was recorded for the DBFM (pH, 6.4 – 4.4) beverage up to 72 h of fermentation, thereafter a slight increase in the pH. Fermentation with the grain natural microflora also resulted in a decrease of the pH of the non-alcoholic beverages. Increase in the acidity of the DBFM (pH, 6.4 – 3.9) beverage was observed up to 96 h of fermentation using the grain natural microflora. For the BFM and sorghum beverages, a decrease in the pH was observed for other fermentation periods, except at 72 h where a slight increase was recorded.

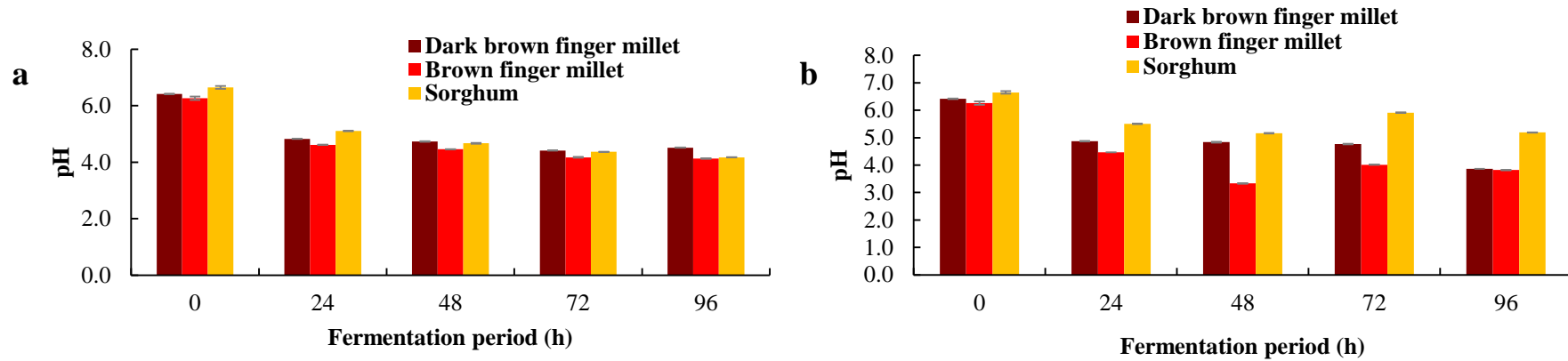


Figure 14. Effect of fermentation and microbial source on pH of finger millet and sorghum malt beverages. (a) fermentation with *Lactobacillus fermentum*; (b) fermentation with grain microbial flora. Mean values \pm standard error are significant at $p < 0.05$.

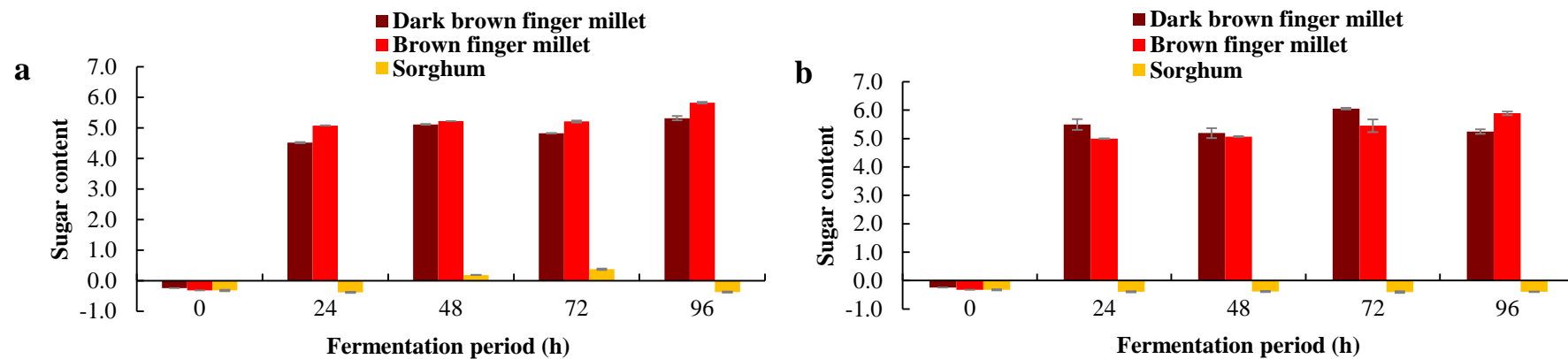


Figure 15. Effect of fermentation and microbial source on sugar content of finger millet and sorghum malt beverages. (a) fermentation with *Lactobacillus fermentum*; (b) fermentation with grain microbial flora. Mean values \pm standard error are significant at $p < 0.05$.

An increase in the sugar content ($^{\circ}\text{Brix}$) with fermentation time was observed at 24 h up to 96 h for the BFM ($^{\circ}\text{Bx}$, 5.1 – 5.8) non-alcoholic beverage using *L. fermentum* (Figure 15). Similar increase in the sugar content of DBFM ($^{\circ}\text{Bx}$, 4.5 – 5.3) beverage was observed using *L. fermentum* except at 72 h of fermentation, wherein a decrease was recorded. For fermentation using the grain natural microflora, a steady increase ($p < 0.05$) in the sugar content was also observed for the BFM ($^{\circ}\text{Bx}$, 5.0 – 5.9) non-alcoholic beverage at 24 h up to 96 h of fermentation, whereas the same increased for the DBFM ($^{\circ}\text{Bx}$, 5.5 – 6.1) beverage at 24 h up to 72 h of fermentation, which later decreased. Brix value of the unfermented FM beverages were negative. Similar negative values were recorded for the sorghum beverage samples, except at 48 h and 72 h fermentation with *L. fermentum*, where increases were noted.

5.3.2. Phenolic composition of the finger millet and sorghum grain malt beverages

The phenolic compounds of the FM and sorghum malt non-alcoholic beverages were identified by comparing their retention times, UV-visible and mass spectral data with that of standards. Table 13 shows the retention time and mass spectra characteristics of identified phenolic compounds of the FM and sorghum beverages. Figure 16 - 18 shows mass spectra of identified phenolic compounds in the FM and sorghum grain malt beverages. The classes of phenolic compounds tentatively identified in the beverage extracts were benzoic acid derivative, flavan-3-ols, flavononol and flavonols.

5.3.3. Changes in the phenolic content of the finger millet and sorghum grain malt beverages

The phenolic compounds of the FM and sorghum beverages are presented in Table 14. Flavonoids found in the FM beverages were catechin and epicatechin, whereas taxifolin and kaempferol together with the catechin and epicatechin were found in the beverage made from sorghum malt. Protocatechuic acid was the only phenolic acid found in the beverages.

Table 13. Retention time and mass spectra characteristics of phenolic compounds of malt beverage extracts at UV absorption maxima of 280 nm.

t_R (min)	$[M-H]^-$ (m/z)	MS/MS fragments (Intensity, %)	Tentatively identified compounds
<i>Benzoic acid derivatives</i>			
8.70	153	153 (100)	Protocatechuic acid
<i>Flavanols</i>			
12.03	289	289 (100), 245 (5)	Catechin
14.08	289	289 (100)	Epicatechin
<i>Flavononol</i>			
15.40	303	303 (100), 285 (49)	Taxifolin
<i>Flavonols</i>			
10.23	285	285 (100)	Kaempferol

t_R = retention time; $[M-H]^-$ = negative ion mode; MS = mass spectra.

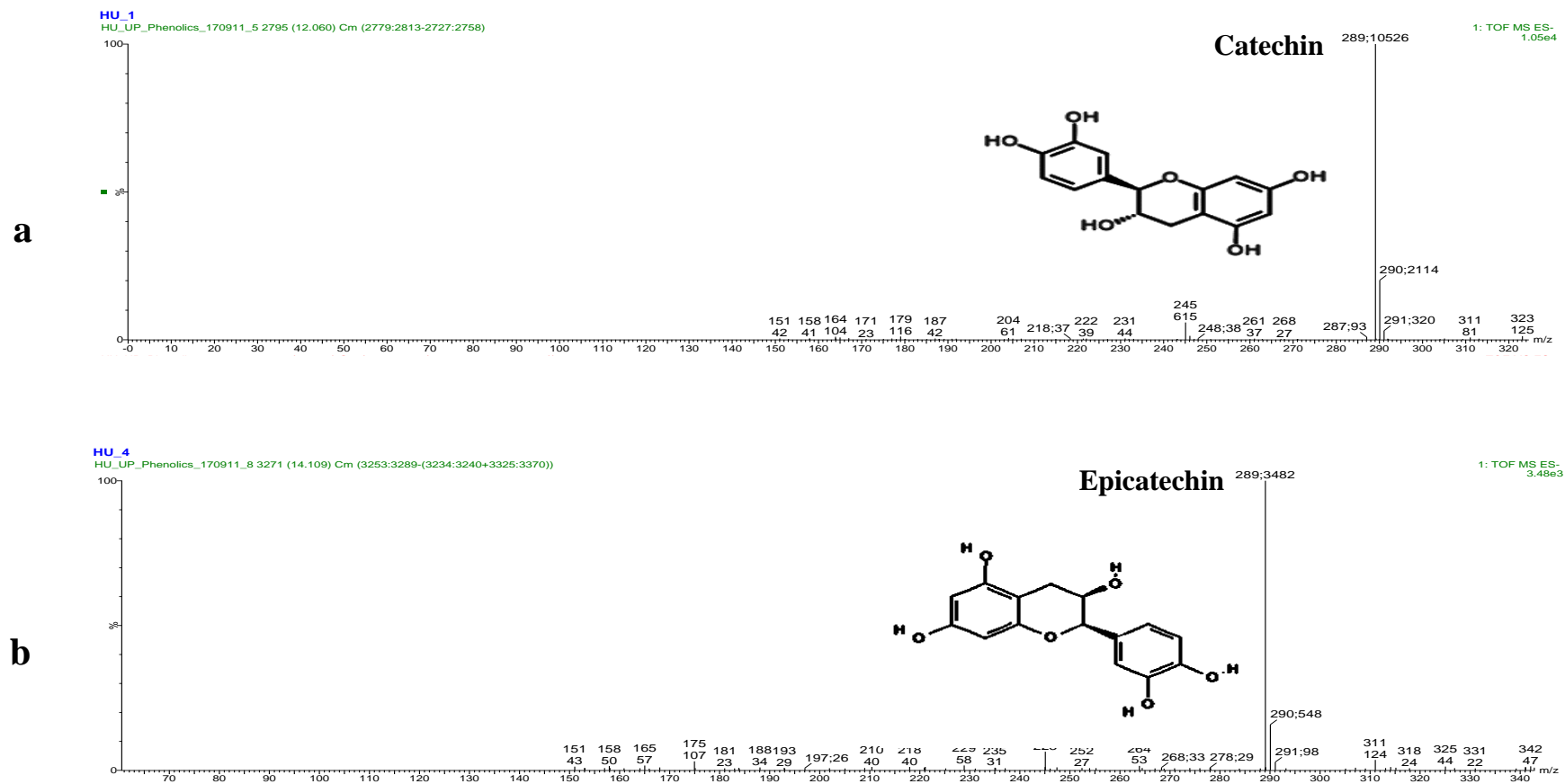


Figure 16. Mass spectra of catechin and epicatechin found in finger millet and sorghum malt non-alcoholic beverages: a = catechin b = epicatechin.

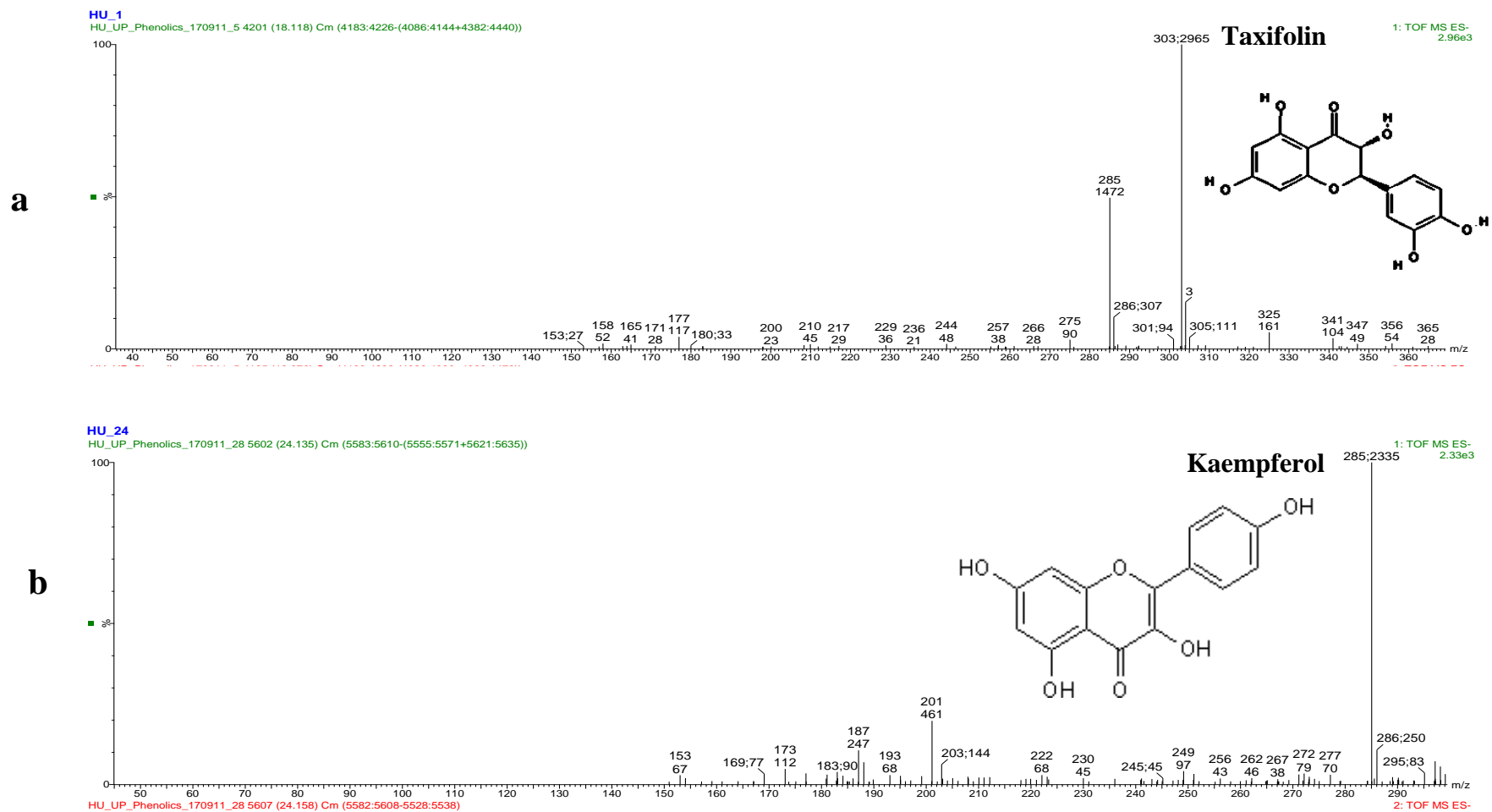


Figure 17. Mass spectra of taxifolin and kaempferol found in sorghum malt non-alcoholic beverage: a = taxifolin b = kaempferol.

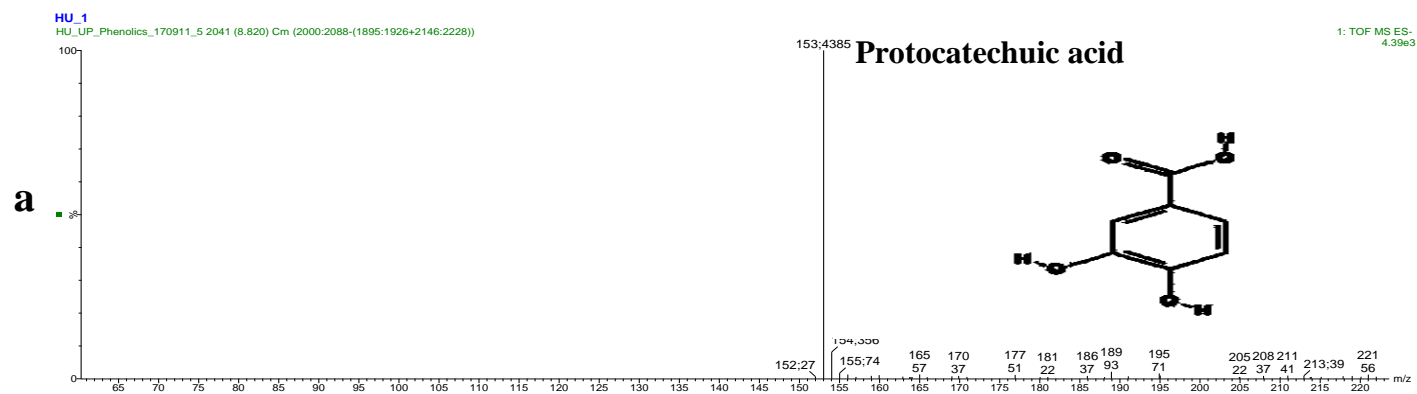


Figure 18. Mass spectra of protocatechuic acid found in finger millet and sorghum malt non-alcoholic beverages: a = protocatechuic acid.

Table 14. Changes in the content ($\mu\text{g/g}$) of phenolic compounds of finger millet and sorghum malt beverages.

Beverage samples/ compounds	Fermentation period (h)									
	0	24	48	72	96	0	24	48	72	96
	Grain natural microflora					<i>Lactobacillus fermentum</i>				
<i>Dark brown finger millet</i>										
PA	0.90 ± 0.14 ^a	0.75 ± 0.07 ^a	0.70 ± 0.14 ^a	0.30 ± 0.00 ^b	0.40 ± 0.14 ^b	0.90 ± 0.14 ^a	0.70 ± 0.14 ^{abc}	0.75 ± 0.21 ^{bc}	0.50 ± 0.00 ^{ab}	0.55 ± 0.28 ^c
CA	6.25 ± 0.28 ^a	5.10 ± 0.14 ^b	6.05 ± 0.21 ^a	2.50 ± 0.64 ^c	1.85 ± 0.85 ^c	6.25 ± 0.28 ^a	4.10 ± 0.00 ^b	2.85 ± 0.64 ^c	1.45 ± 0.35 ^d	1.40 ± 0.14 ^d
ECA	1.25 ± 1.13 ^a	0.90 ± 0.00 ^b	1.00 ± 0.00 ^{ab}	0.45 ± 1.06 ^c	0.30 ± 0.00 ^c	1.25 ± 1.13 ^a	0.65 ± 0.21 ^b	0.40 ± 0.14 ^{cb}	0.25 ± 0.07 ^{cb}	0.20 ± 0.00 ^c
Total	8.40 ± 1.55	6.75 ± 0.21	7.96 ± 0.35	2.95 ± 1.70	2.55 ± 0.99	8.40 ± 1.55	5.45 ± 0.35	4.00 ± 0.99	2.20 ± 0.42	2.15 ± 0.42
<i>Brown finger millet</i>										
PA	1.70 ± 0.28 ^a	0.70 ± 0.00 ^b	0.50 ± 0.14 ^c	0.95 ± 0.07 ^{bd}	1.00 ± 0.00 ^{bd}	1.70 ± 0.28 ^a	1.25 ± 0.21 ^a	0.95 ± 0.21 ^{ab}	1.40 ± 0.14 ^b	0.80 ± 0.07 ^b
CA	7.10 ± 0.35 ^a	3.50 ± 0.78 ^b	2.85 ± 0.28 ^b	6.00 ± 0.42 ^a	5.75 ± 0.21 ^a	7.10 ± 0.35 ^a	4.40 ± 0.14 ^b	3.30 ± 0.42 ^c	3.65 ± 0.07 ^{cb}	2.60 ± 0.14 ^{cd}
ECA	1.45 ± 0.99 ^a	0.55 ± 0.78 ^b	0.55 ± 0.99 ^b	1.00 ± 0.00 ^{ab}	0.90 ± 0.84 ^b	1.45 ± 0.99 ^a	0.90 ± 0.00 ^b	0.55 ± 0.07 ^c	0.70 ± 0.00 ^{bc}	0.50 ± 0.00 ^c
Total	10.25 ± 1.62	4.75 ± 1.56	3.90 ± 1.41	7.95 ± 0.49	7.65 ± 1.05	10.25 ± 1.62	6.55 ± 0.35	4.80 ± 0.70	5.75 ± 0.21	3.90 ± 0.21
<i>Sorghum grain</i>										
PA	2.35 ± 0.49 ^a	1.75 ± 0.21 ^{ab}	1.30 ± 0.14 ^{bc}	1.25 ± 0.07 ^{bc}	1.40 ± 0.21 ^{bc}	2.35 ± 0.49 ^a	1.55 ± 0.14 ^b	1.50 ± 0.28 ^b	1.25 ± 0.35 ^b	1.40 ± 0.63 ^b
CA	5.35 ± 1.34 ^a	5.35 ± 1.13 ^a	5.10 ± 1.48 ^a	4.70 ± 2.05 ^a	5.65 ± 1.91 ^a	5.35 ± 1.34 ^a	2.80 ± 0.28 ^b	4.15 ± 0.07 ^{ba}	4.35 ± 1.20 ^{ba}	3.60 ± 0.71 ^{bc}
ECA	0.15 ± 1.06 ^a	0.15 ± 0.99 ^a	0.20 ± 0.00 ^a	0.25 ± 1.13 ^a	0.25 ± 1.20 ^a	0.15 ± 1.06 ^a	0.20 ± 0.00 ^a	0.10 ± 0.00 ^a	0.15 ± 0.07 ^a	0.10 ± 0.00 ^a
TX	1.85 ± 0.85 ^a	2.00 ± 0.00 ^a	1.65 ± 0.78 ^b	1.75 ± 0.85 ^{ba}	1.75 ± 1.06 ^{ba}	1.85 ± 0.85 ^a	1.00 ± 0.42 ^b	0.80 ± 0.00 ^b	1.15 ± 0.07 ^b	1.00 ± 0.14 ^b
KF	1.15 ± 0.78 ^a	1.50 ± 0.92 ^{ab}	1.65 ± 0.71 ^b	1.35 ± 0.92 ^{ab}	1.75 ± 1.13 ^b	1.15 ± 0.78 ^a	Nd	Nd	1.20 ± 0.14 ^a	1.05 ± 0.21 ^a
Total	10.85 ± 4.52	10.75 ± 3.25	9.90 ± 3.11	9.30 ± 5.02	10.80 ± 5.51	10.85 ± 4.52	5.55 ± 0.84	6.55 ± 0.35	8.10 ± 1.83	7.15 ± 1.69

Values given are mean values of duplicate experiments; mean values in a row with different superscripts are significantly different at $p < 0.05$. PA, protocatechuic acid; CA, (+)-catechin; ECA, (-)-epicatechin; TX, taxifolin; KmF, kaempferol; Nd, not detected.

The FM beverages as well as the sorghum beverage were found to contain a high amount of catechin compared to other phenolic compounds detected. A significant decrease in the total phenolic compounds was observed during fermentation of the DBFM (8.40 – 3.55 µg/ g; 8.40 – 2.15 µg/ g), BFM (10.25 – 3.90 µg/ g; 10.25 – 3.90 µg/ g) and sorghum (10.85 – 9.30 µg/ g; 10.85 – 5.55 µg/ g) beverages using the grain microbial flora and *L. fermentum* respectively.

A decrease in catechin and epicatechin was observed during 24 h of fermentation for the DBFM beverage using both microbial sources. No significant ($p > 0.05$) change in protocatechuic acid of the DFM beverage was observed during the 24 h fermentation period. Similar decrease in the phenolic compounds was observed in the BFM beverage. Unlike the grain microbial flora, no significant ($p > 0.05$) decrease in protocatechuic acid was observed beyond 48 h of fermentation for the BFM malt fermented with *L. fermentum*. For the sorghum beverage, no significant change ($p > 0.05$) was observed for catechin and epicatechin at 24 h up to 96 h of fermentation using the grain microbial flora. For *L. fermentum*, no significant change in catechin and epicatechin was observed at 24 h up to 72 h, which later remained constant up to 96 h for epicatechin in the sorghum beverage.

A significant ($p < 0.05$) increase in the phenolic compounds of the BFM beverage was observed after 48 h fermentation using the grain microbial flora. No significant difference in the amount of the individual phenolic compounds was recorded after 48 h up to 96 h of fermentation using *L. fermentum*. For the DBFM beverage, significant decreases were observed after 48 h of fermentation for both microbial sources. Similar observation was also recorded for the phenolic compounds of the sorghum beverage for both microbial sources except for taxifolin and kaempferol where significant increase were observed particularly at 96 h of fermentation. Despite the initial decrease in phenolic compounds of the beverages, fermentation for 24 h retained high amount of the total phenolic compound, compared to other fermentation periods particularly for the FM beverages.

5.3.4. Changes in the citric acid content of the finger millet and sorghum grain malt beverages

The FM malt beverages were found to contain higher amount of citric acid compared to the sorghum malt beverage (Figure 19). The citric acid content of the beverages fermented with the grain microbial flora and *L. fermentum* were in the range of: 0.90 – 3.70; 1.90 – 3.70 (DBFM), 2.25 – 5.70; 1.70 – 5.70 (BFM), and 1.10 – 1.45; 0.85 – 1.45 (sorghum malt). Fermentation with both microbial sources was found to reduce the citric acid content, particularly at 24 h of fermentation using *L. fermentum*. A decrease in citric acid content of BFM beverage was observed up to 48 h of fermentation using *L. fermentum*, which later remained constant up to 96 h. A similar observation in the citric acid content of the BFM beverage was also recorded during fermentation with the grain microbial flora. Unlike the BFM beverage, a further decrease in the citric acid of the DBFM beverage was up to 72 h of fermentation, which later remained constant up to 96 h of fermentation with *L. fermentum*. A decrease in the citric acid of the DBFM malt fermented with the grain microbial flora was observed within 24 – 48 h of fermentation, and later was undetected at 96 h. A decrease in the citric acid content of the sorghum malt beverage was observed up to 48 h of fermentation, which remained constant up to 96 h of fermentation with *L. fermentum*. A similar decrease in the citric acid of the sorghum malt beverage was observed up to 48 h of fermentation with the grain microbial flora, after which it was not detected for the rest of the fermentation period.

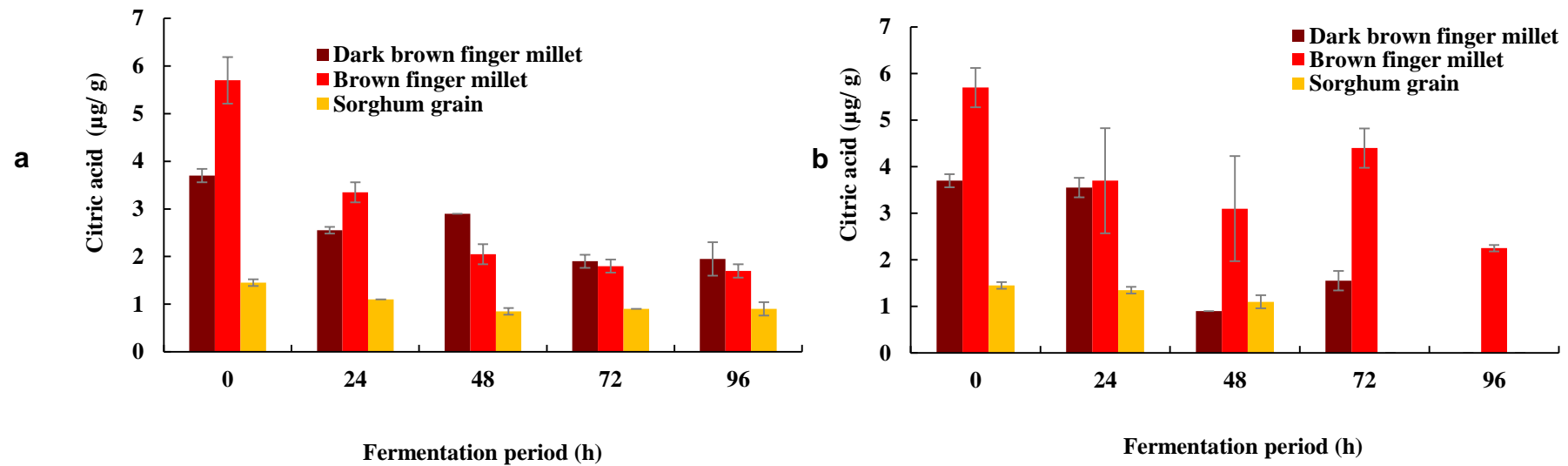


Figure 19. Effect of fermentation on the citric acid content of the grain malt non-alcoholic beverages: (a) fermentation with *Lactobacillus fermentum*, (b) fermentation with grain microbial flora. Mean values \pm standard error are significant at $p < 0.05$.

5.3.5. Total phenolic and total flavonoid contents of finger millet and sorghum grain malt beverages

The total phenolic content (TPC) of the non-alcoholic beverages are presented in Figure 20 and they were in the range of 0.7489 – 1.957 mg GAE/ g in DBFM, from 0.6129 – 2.8541 mg GAE/ g in BFM and from 0.9800 – 5.2335 mg GAE/ g in sorghum grain malt. Fermentation for 24 h resulted in a significant ($p < 0.05$) decrease in the TPC of the grains fermented with both microbial sources. A further decrease in TPC was observed at 48 h for BFM and sorghum malt with *L. fermentum* after which there was a steady increase which was higher and above other fermentation periods at 96 h of fermentation. For DBFM non-alcoholic beverage fermented with *L. fermentum*, an increase in TPC was observed at 48 and 72 h of fermentation which later declined at 96 h. For the DBFM non-alcoholic beverage fermented with the grain microflora, a decrease in TPC was observed at 24 and 48 h of fermentation which then remained constant up to 96 h. A similar decrease was noted at 24 and 48 h of fermentation for BFM non-alcoholic beverage, which later increased and remained constant up to 96 h using the grain microflora.

The total flavonoid content (TFC) of the non-alcoholic beverages ranged from 0.2607 – 0.7097 mg QE/ g in DBFM, from 0.2760 – 0.8736 mg QE/ g in BFM and from 0.4606 – 1.6461 mg QE/ g in sorghum grain malt (Figure 21). A decrease in TFC was observed up to 48 h of fermentation using *L. fermentum* for the two varieties of FM malt. A significant ($p < 0.05$) increase in TFC of the FM varieties was observed at 72 h of fermentation, which later declined at 96 h. For sorghum non-alcoholic beverage, an initial decrease in TFC was observed at 24 h of fermentation which later increased significantly ($p < 0.05$) at 48 and 72 h compared to other fermentation periods. An initial decrease at 24 h of fermentation in TFC was noted for the non-alcoholic beverages using the grains natural microflora. No significant ($p > 0.05$) change in TFC was observed for the DBFM except for the initial decrease at 24 h of fermentation.

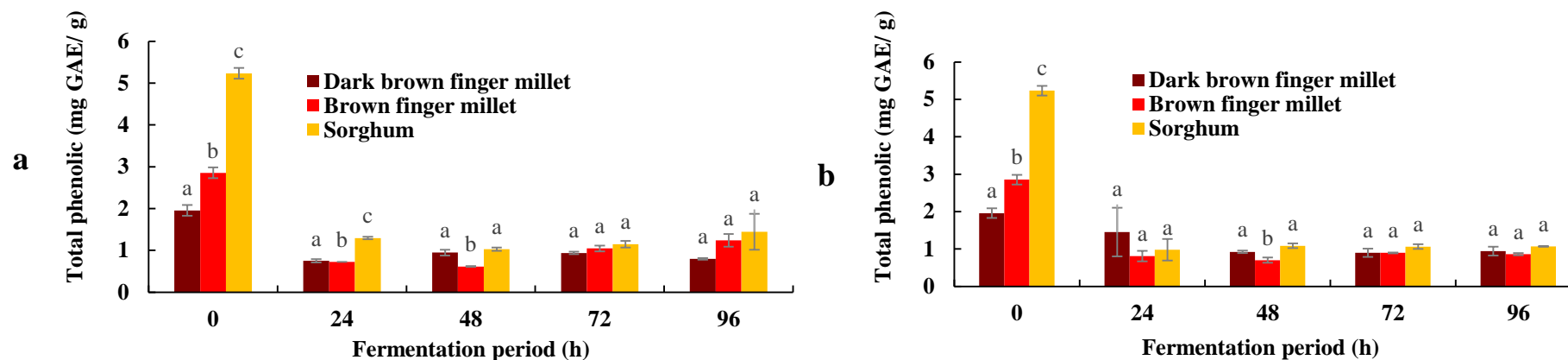


Figure 20. Effect of fermentation time and microbial sources on total phenolic content of finger millet and sorghum malt non-alcoholic beverages. (a) Fermentation with *L. fermentum*, (b) fermentation with grain microbial flora. Mean values \pm standard error for different data labels on each period are significant at $p < 0.05$.

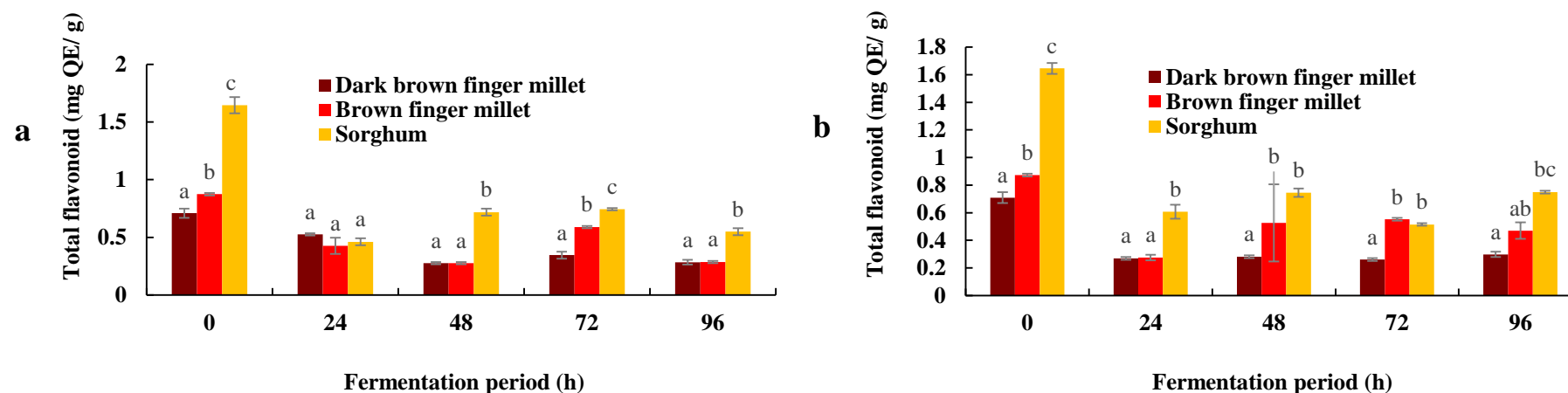


Figure 21. Effect of fermentation time and microbial sources on total flavonoid content of finger millet and sorghum malt non-alcoholic beverages. (a) Fermentation with *Lactobacillus fermentum*, (b) fermentation with grain microbial flora. Mean values \pm standard error for different data labels on each period are significant at $p < 0.05$.

Increase in TFC was observed after 24 h of fermentation for BFM and sorghum non-alcoholic beverages. Fermentation for 72 h resulted in significantly ($p < 0.05$) higher TFC for BFM compared to other fermentation periods.

5.3.6. DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging activity of finger millet and sorghum malt beverages

The DPPH radical scavenging activity of the non-alcoholic beverages are shown in Figure 22, and they were in the range from 64.90 – 89.82% in DBFM, from 69.20 – 89.47% in BFM and from 80.73 – 89.82% in sorghum malt. In general, fermentation with both microbial sources resulted in an initial decrease in DPPH radical scavenging activity of the non-alcoholic beverages. No significant ($p > 0.05$) change in DPPH scavenging activity was observed after 24 up to 72 h of fermentation particularly for *L. fermentum*. Fermentation for 96 h significantly ($p < 0.05$) reduced the DPPH radical scavenging activity of the FM non-alcoholic beverages for both microbial sources. Unlike the FM non-alcoholic beverages, no significant change in DPPH radical scavenging activity for the sorghum beverage was observed for the entire fermentation period for both microbial sources.

5.3.7. ABTS (2, 2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging activity of finger millet and sorghum malt beverages

The antioxidant capacity of the non-alcoholic beverages determined by ABTS radical scavenging activity assay was in the range of 9.92 – 10.87 $\mu\text{M TE/g}$ in DBFM, from 9.83 – 10.92 $\mu\text{M TE/g}$ in BFM and from 9.56 – 10.47 $\mu\text{M TE/g}$ in sorghum malt (Figure 23). Fermentation for 24 h did not result in any statistically significant ($p > 0.05$) change in the ABTS radical scavenging activity of the non-alcoholic beverages except for the DBFM non-alcoholic beverage where a slight increase was observed.

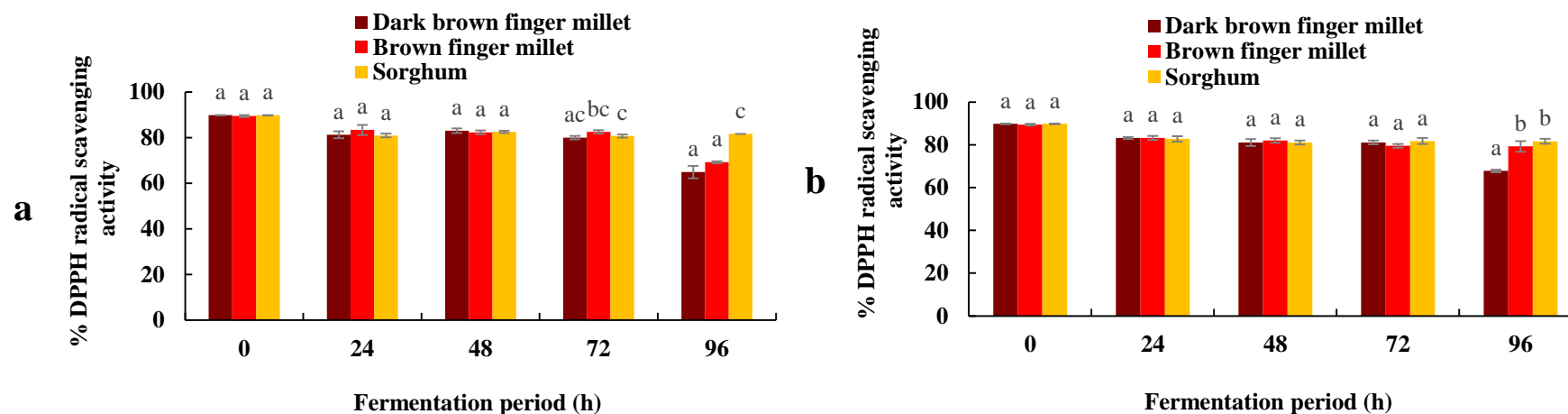


Figure 22. Effect of fermentation time and microbial sources on DPPH (2, 2'-diphenyl-1-picrylhydrazyl) radical scavenging activity of finger millet and sorghum non-alcoholic beverages. (a) Fermentation with *Lactobacillus fermentum*, (b) fermentation with grain microbial flora. Mean values \pm standard error for different data labels on each period are significant at $p < 0.05$.

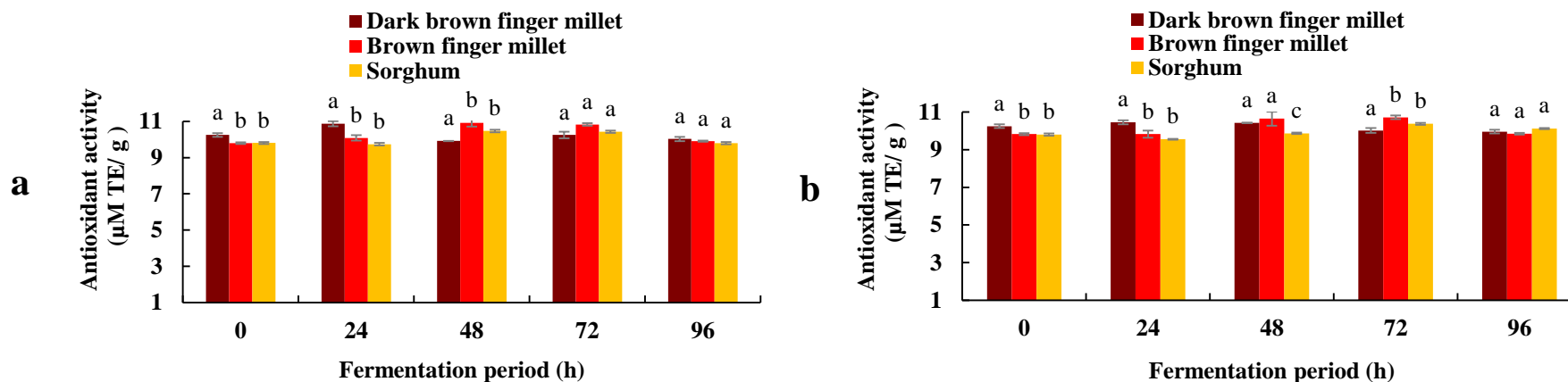


Figure 23. Effect of fermentation time and microbial sources on antioxidant activity of finger millet and sorghum non-alcoholic beverages estimated using ABTS (2, 2'-azinobis-3-ethylbenzthiazoline-6-sulfonic acid) cations. (a) Fermentation with *Lactobacillus fermentum*, (b) fermentation with grain microbial flora. Mean values \pm standard error for different data labels on each period are significant at $p < 0.05$.

A significant ($p < 0.05$) increase in the ABTS radical scavenging activity of the BFM and sorghum non-alcoholic beverages was observed at 48 and 72 h of fermentation using *L. fermentum*. An increase in the ABTS radical scavenging activity of the BFM non-alcoholic beverage was observed at 48 h of fermentation, which remained constant up to 72 h for both microbial sources. A similar effect was observed for sorghum malt non-alcoholic beverage using *L. fermentum*. Unlike the DBFM non-alcoholic beverage, fermentation for 96 h resulted in reduced ABTS radical scavenging activity for the BFM and sorghum non-alcoholic beverages for both microbial sources.

5.3.8. Ferric reducing antioxidant power of finger millet and sorghum malt beverages

The iron reducing activity of the FM and sorghum malt non-alcoholic beverages are presented in Figure 24, and they were in the range of 0.4416 – 0.6042 in DBFMG, from 0.4913 – 0.6493 in BFMG and from 0.503 – 0.6974 in sorghum. An initial decrease in the iron reducing activity of the non-alcoholic beverages was observed at 24 h of fermentation with both microbial sources. A significant ($p < 0.05$) increase in iron reducing activity was observed at 72 h of fermentation for the FM non-alcoholic beverages, which later declined significantly at 96 h especially for the natural microflora. A similar effect was observed for the sorghum non-alcoholic beverage at 96 h of fermentation.

5.4. Discussion

Indigenous fermented cereal beverages play an important role in the diet of many rural homes in Africa and Asia, serving as a refreshing ready-to-drink beverage, for weaning and for geriatric and adult complementary foods (Zvauya *et al.*, 1997). In southern African, the use of FM as food and food product is not common, and few research has been conducted on the grain, despite its agronomic, nutritional and health advantages.

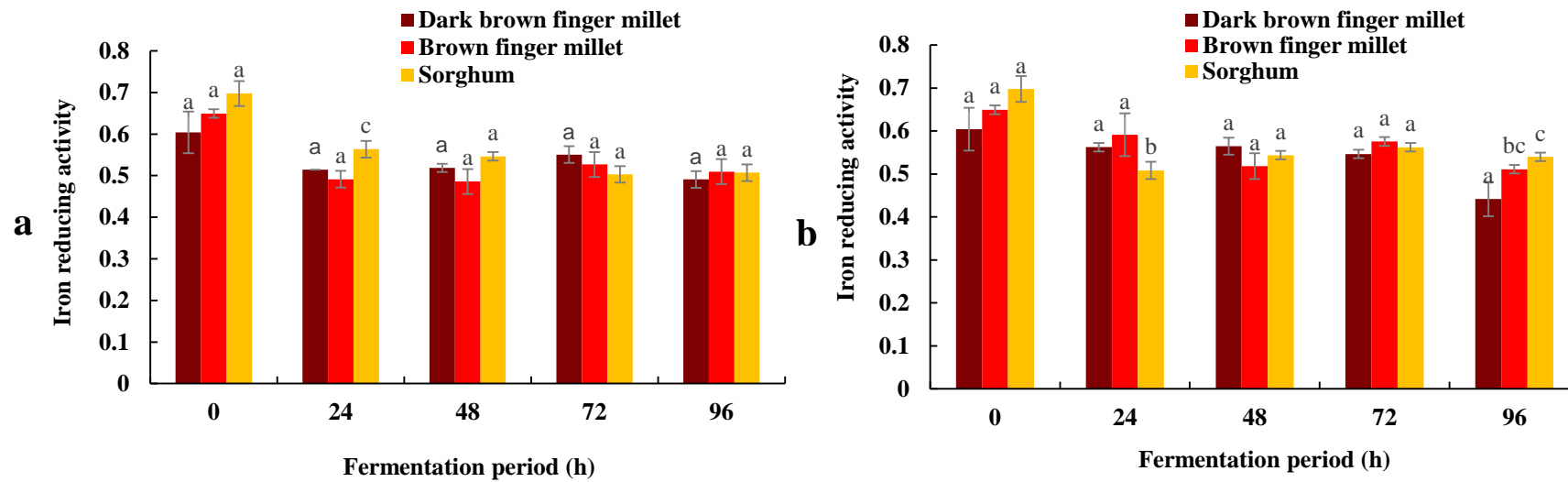


Figure 24. Effect of fermentation time and microbial sources on iron reducing activity of finger millet and sorghum malt non-alcoholic beverages. (a) Fermentation with *Lactobacillus fermentum*, (b) fermentation with grain microbial flora. Mean values \pm standard error for different data labels on each period are significant at $p < 0.05$.

Several studies have described the nutritional and nutraceutical potential of FM grain (Sripriya *et al.*, 1996; Sripriya *et al.*, 1997; Kumari & Sumathi, 2002; Platel *et al.*, 2010; Shobana *et al.*, 2010; Hithamani and Srinivasan, 2014; Devi *et al.*, 2014), however, information on the nutraceutical properties of FM products is scanty, particularly in Southern Africa. There are relatively few or no studies that have described the physicochemical and phenolic compounds of FM beverage products, therefore, this study produced and evaluated the health-beneficial properties of FM malt non-alcoholic beverage from two varieties of FM simulating the typical production steps used by Chelule *et al.* (2010) for commercialised sorghum beverage product - *amahewu*.

5.4.1. Viscosity, pH and sugar content of finger millet and sorghum malt beverages

Starch retrogradation, which is largely dependent on time and temperature of processing or cooking, is often accompanied by a series of physical changes, such as increased viscosity and turbidity of pastes, exudation of water and increased degree of crystallinity (Hoover *et al.*, 2010; Wang *et al.*, 2015). The results on the physicochemical properties of two varieties of FM non-alcoholic beverage show an increase in the viscosity of the products upon mashing, with corresponding decrease in the same with fermentation time. This observation could be as a result of the change in time and temperature on starch retrogradation, as well as the water used as medium for inoculation and activities of the microorganism on digestible starch present during production. The FM beverage viscosities are within free-flowing liquid or drinkable consistency of < 1 Pa.s of gruels for infant (Gopaldas *et al.*, 1988; Tréche and Mbome, 1999; Mouquet and Tréche, 2001).

A typical change in pH and sugar contents of fermenting cereal grains was observed during fermentation, with an increase in sugar content and corresponding decrease in pH of the non-alcoholic beverages. Increased acidity is associated with lactic acid fermentation by lactic

acid bacteria as well as other acid producing microorganisms that participate in the fermentation of sugars and organic acids to produce lactic acid and other secondary fermentation metabolites. The increased sugar content of the beverages is attributed to enzymatic activities of fermenting microorganisms that breaks starch oligosaccharides through cleavage of amylose and amylopectin into maltose and glucose. The sugar content of the unfermented FM as well as the sorghum non-alcoholic beverage were below 0 °Bx. A similar observation was recorded in the work of Zvaunya *et al.* (1997), although, there is no clear reason for this observation. Since starch retrogradation affects starch digestibility, which in turn affects available sugars (Wang *et al.*, 2015), the observation may, however, have resulted from time and temperature lag on the digestibility of retrograded starch, and enzyme denaturation during mashing and pasteurisation.

5.4.2. Changes in phenolic compounds and total phenolic content of finger millet and sorghum malt beverages

Fermentation period and microbial sources were found to affect the phenolic compounds, total phenolic and antioxidant activity of malt beverages from the two varieties of FM. The use of pure culture of *L. fermentum* showed a fermentation-time dependent decrease in the phenolic compounds of the FM malt beverages compared to the use of the grain's microbial flora. This could be as a result of the predominant fermentative activity of *L. fermentum* unlike the grain's microbial flora where a mixed culture of microorganisms participated in the fermentation.

The initial decrease in the phenolic compounds and the total phenolics of the malt beverages are comparable with the observation of Towo *et al.* (2006), who reported a decrease in the total phenolics of fermented sorghum gruel. Taxifolin and kaempferol were not detected in the FM beverages but were found in the sorghum beverage at considerable amounts, and did

not show a marked decrease during the fermentation periods. Fermentation with *L. fermentum* showed a modest decrease in the phenolic compounds with the fermentation time for the FM and sorghum malt beverages, with the 24 h fermented beverage retaining high total phenolics compared to other fermentation period. Increase in catechin, epicatechin, protocatechuic acid, kaempferol and taxifolin, however, were observed, particularly for the BFM and sorghum malt beverages at 72 and 96 h of fermentation using the grain's microbial flora. Several other peaks that could not be matched to any known phenolic compound were found in the chromatograms of the fermented beverage samples (Figure 25 - 27), which is an indication of the presence of other compounds other than phenolics in the malt beverage.

The combination of malting and fermentation resulted in a drastic reduction in total phenolics of FM and sorghum beverages. This additive effect is primarily due to increased oxidation of phenolic compounds as a result of high mashing and pasteurisation temperatures used during production of the beverage, and as well as the kilning temperature and germination of the grains (Hur *et al.*, 2014). A reduction in phenolic compounds during lactic acid fermentation of cereals has been reported by other authors Beta *et al.* (2000); Towo *et al.* (2006); Rani and Anthony (2014). Degradation and synthesis of phenolic compounds and other macromolecules are typical of fermentation processes (Bhat *et al.*, 1998; Kim *et al.*, 2011; Hur *et al.*, 2014). The reduction in total phenolics during fermentation could be a result of the loss of volatile compounds or mixed metabolic utilisation of the phenolic compounds by the different microbial flora present in the grain malt.

Varietal differences in the grain could also be an important factor that affected the phenolic composition of the beverages such that the different microbial flora that are pertinent to the FM malt varieties, may have different mechanism for utilisation of the phenolic compounds or their esters for their metabolic activities during fermentation.

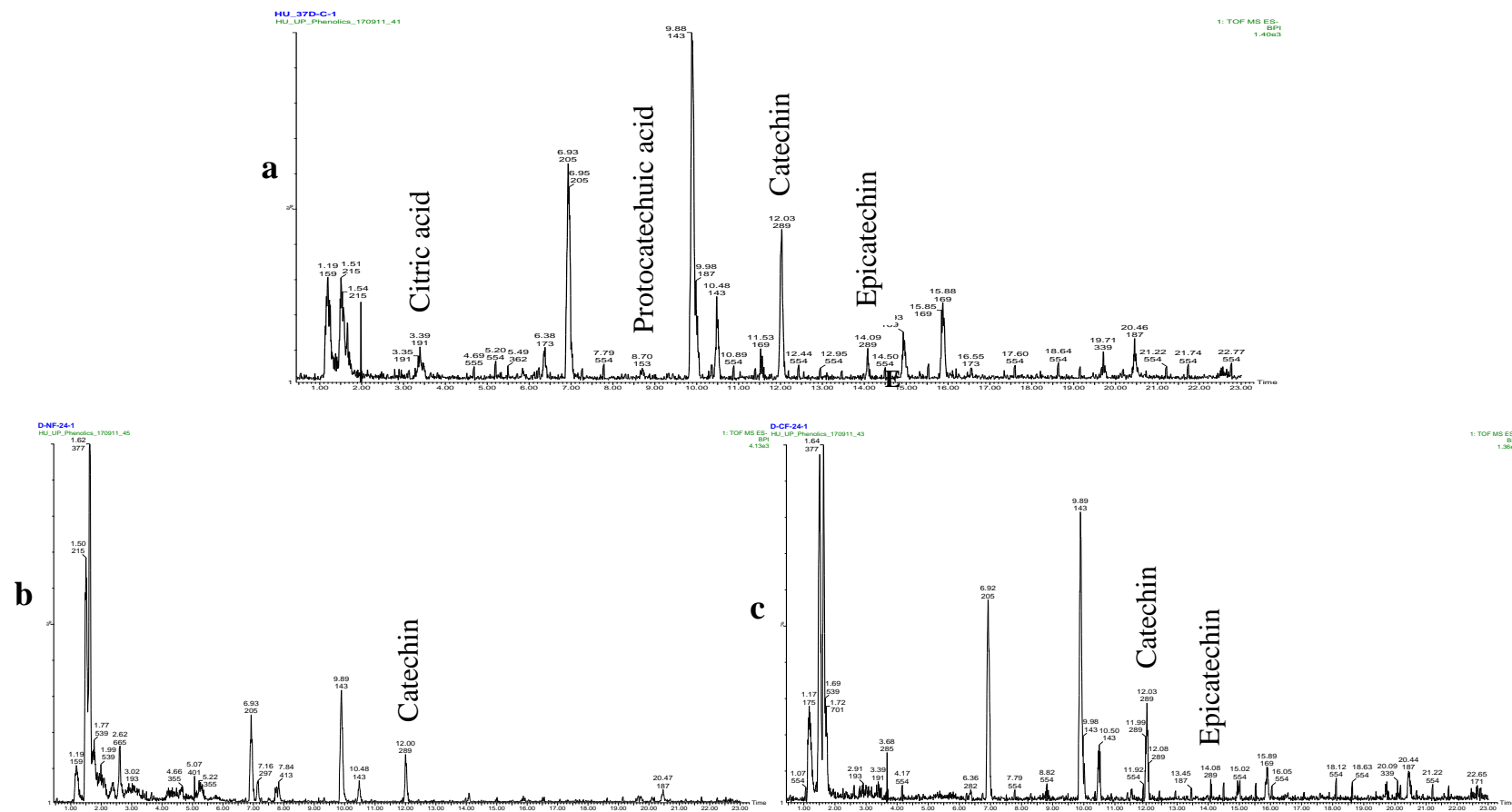


Figure 25. Chromatograms of some phenolic compounds of dark brown finger millet malt non-alcoholic beverage. a, b, c) unfermented and fermentation using grain microbial flora and *Lactobacillus fermentum* for dark brown finger millet beverage.

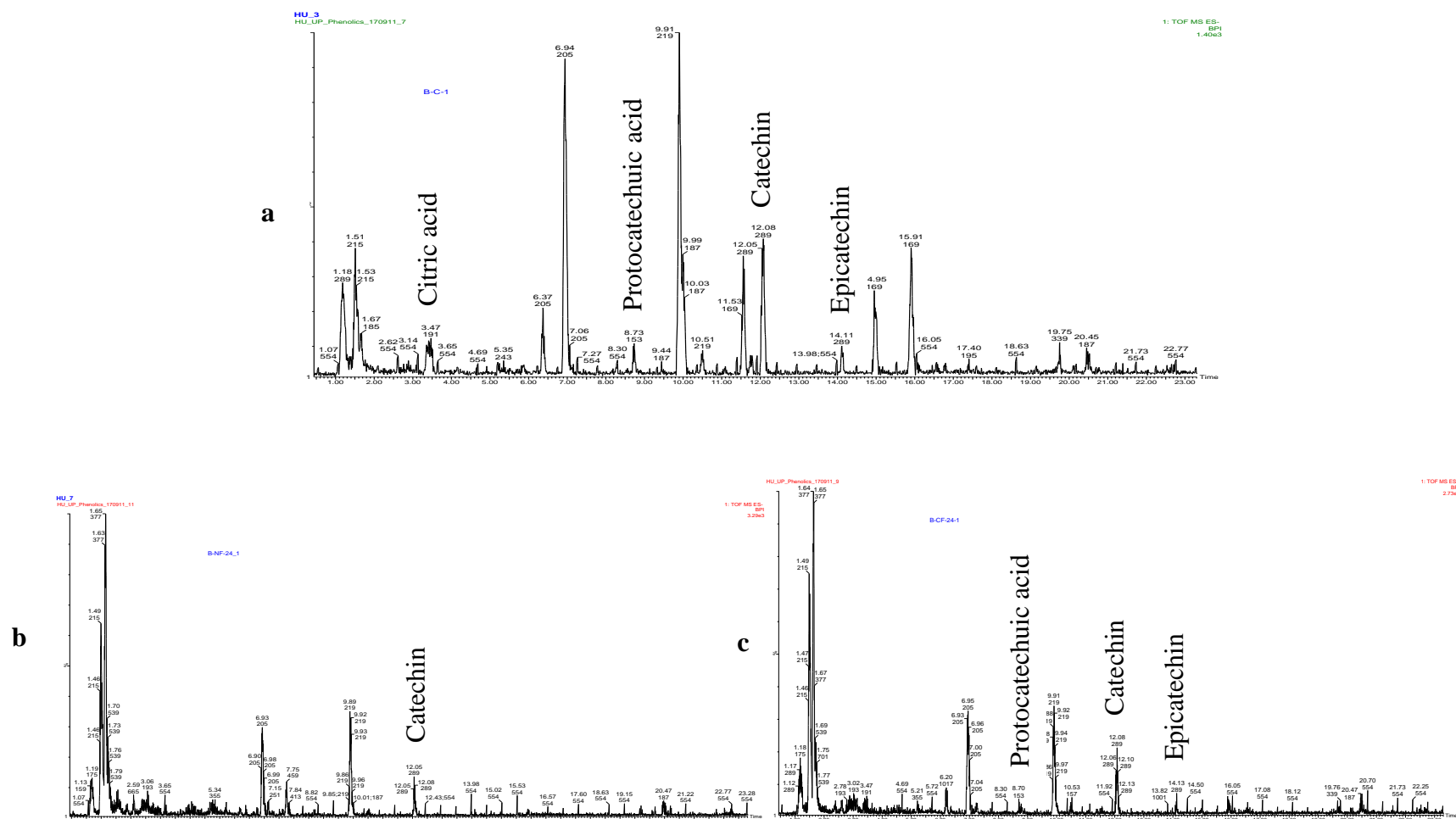


Figure 26. Chromatograms of some phenolic compounds of brown finger millet malt non-alcoholic beverage. a, b, c) unfermented and fermentation using grain microbial flora and *Lactobacillus fermentum* for brown finger millet beverage.

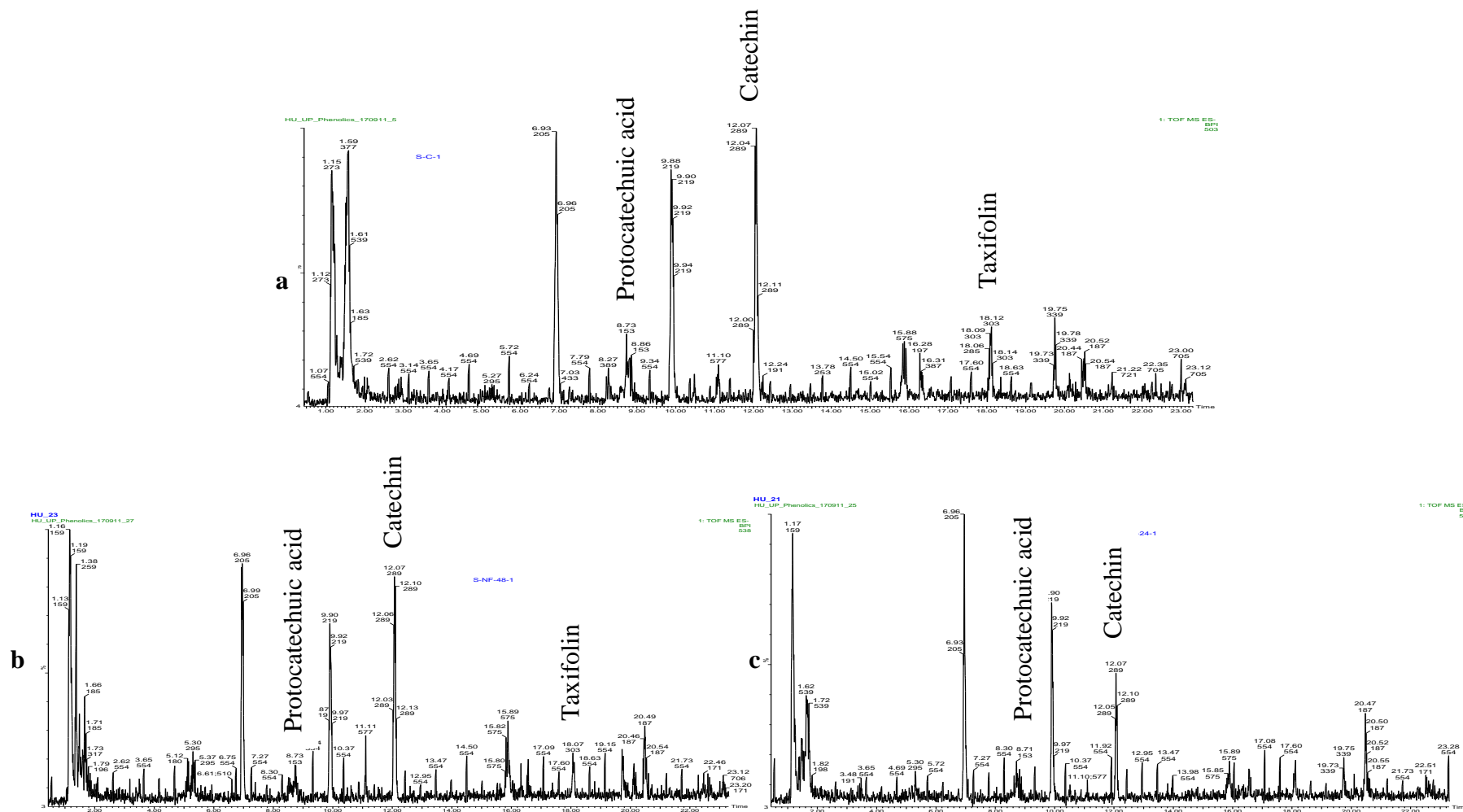


Figure 27. Chromatograms of some phenolic compounds of sorghum malt non-alcoholic beverage. a, b, c) unfermented and fermentation using grain microbial flora and *Lactobacillus fermentum* for sorghum beverage.

In the report of Towo *et al.* (2006), it was suggested that the reduction in the amount of total phenolics during fermentation could be due to the effect of acidic environment and rearrangement of the phenolic structures. The addition of water, amount of beverage sample, mashing and pasteurisation temperatures may have also resulted in low extractability of the phenolic compounds.

Microbial enzymes, such as glucosidase, amylase, phytase, tannase, esterase, invertase or lipase are produced during fermentation, which are capable of hydrolysing glucosides, break down cell walls as well as starch. The enzymatic hydrolysis consequently disintegrate plant cell wall matrix and subsequently facilitate the release of phenolic compounds and other components of the plant food (Hur *et al.*, 2014). Conversely, the increase observed in the individual phenolic compounds, total phenolic and flavonoid content during fermentation with both microbial sources, could suggest the release and bioconversion of bound and / or condensed phenolic compounds into their free forms as a result of microbial hydrolysis reaction (acidic and enzymatic), as well as a shift in dry matter composition during fermentation. Kayode *et al.* (2007) also noted these phenomena during the fermentation of opaque sorghum malt alcoholic beverage.

5.4.3. Changes in the citric acid content of finger millet and sorghum malt beverages

The FM malt beverages were found to contain higher amounts of citric acid compared to the sorghum malt beverage. These varieties of cereal grain showed a decrease in citric acid content with fermentation time in the resulting malt beverage. At 96 h of fermentation with the grain's microbial flora, citric acid was no longer detected in DBFM and sorghum malt beverages, and was further reduced in the BFM malt beverage. Lactic acid bacteria are known to ferment a wide range of sugars (mainly glucose and fructose) and convert certain organic acids such as citric acid and malic acid into lactic acid and, depending on the type of the lactic acid bacteria strain, acetic acid, ethanol and carbon dioxide are also produced (De Vuyst and Weckx, 2016). Thus, the decrease in the citric acid content of the beverages during fermentation could be due

to its consumption or conversion into lactic acid and acetic acid by fermenting microbes or *Lactobacillus spp.*, particularly *L. fermentum*. Citric acid is heat labile and could be destroyed by heat treatment (Wyrzykowski *et al.*, 2011), which is typical of the production steps used in the study. In other words, the decrease in the citric acid content of the beverages could be partly explained by the effect of temperature on the citric acid either during mashing or pasteurisation step.

5.4.4. Antioxidant activity of finger millet and sorghum malt beverages

Information on the antioxidant activity of fermented FM malt beverage is scarce. In this study, the non-alcoholic beverage from FM malt exhibited significant DPPH and ABTS radical scavenging, and iron reducing activities. Research has shown that fermentation processes are very good for antioxidant preservation owing to moderate temperature and limited oxygen access which provides suitable condition for reduced lipid oxidation. It has also been shown that antioxidant activity of fermented food increases due to hydrolytic action of enzymes either from the plant food or microorganisms on conjugated phenolic compounds, sugar residues and esters of organic acids, lipid and amines (Grajek and Olejnik, 2010; Hur *et al.*, 2014). The antioxidant activity of the non-alcoholic beverage is attributed to the presence of the isolated flavonoids and phenolic acid and / or release of esterified polymeric phenolic compounds and organic acids as well as the lactic acid bacteria in themselves that exhibits antioxidant effect.

Various factors such as microorganism species, pH, temperature, solvent, water content, fermentation time, kind of food/ substrate and aerobic conditions can affect antioxidant activity of fermented plant-based foods (Hur *et al.*, 2014). The results of the present study show that fermentation time and substrate type affected the antioxidant activity of the beverages. Although no statistical change was observed up to 72 h of fermentation, the two varieties of FM malt showed a fermentation-time dependent decrease in DPPH radical scavenging and iron reducing activities which was significantly reduced at 96 h of fermentation especially with *L. fermentum*.

Loss of phenolic compounds and decrease in antioxidant activity during fermentation have been reported. A decrease in antioxidant activity at the late stage of solid-state-fermentation of pineapple waste mixed with soy flour using *Rhizopus oligosporus* was reported by Correia *et al.* (2004). In the study, it was suggested that the decrease in antioxidant activity at the late stage of fermentation occurred as a result of further hydrolysis of phenolic compounds, perhaps to a lesser antioxidative forms during the fermentation stage. Kim *et al.* (2011) also reported a decrease in flavonol glycosides during tea fermentation and that the reduction could be due to oxidative degradation. In the study, the tea catechins were transformed to theaflavins and thearubigin, which resulted in the loss of total soluble phenolics and antioxidant activity (Kim *et al.*, 2011). This phenomenon could explain the observed decrease in antioxidant activity of the non-alcoholic beverages at 96 h of fermentation, wherein a further hydrolysis of free and/ or depolymerisation of phenolic compounds as a result of microbial hydrolysis or their oxidation and / or decomposition during pasteurisation.

Pearson correlation coefficients of the antioxidant activity assays of FM and sorghum beverages show a strong positive correlation between DPPH and iron reducing activities of the beverages fermented with the grain microbial flora and *L. fermentum* (Table 15). This result further support the time-dependent decrease in antioxidant activities of the beverages observed during fermentation. No significant correlation was found for ABTS assay against DPPH and iron reducing activity. The three antioxidant assays were used to estimate the antioxidant activities of the malt beverages owing to their advantages in terms of cost and time of running the assays as well as the use of simple machine, a spectrophotometer, which is commonly available.

Table 15. Pearson correlation coefficients between DPPH, ABTS and FRAP assays of finger millet and sorghum beverages.

Response variables	Grain microbial flora			<i>Lactobacillus fermentum</i>		
	DPPH	ABTS	FRAP	DPPH	ABTS	FRAP
<i>Dark brown finger millet</i>						
DPPH	1.000	0.463	0.890**	1.000	0.234	0.718**
ABTS	0.463	1.000	0.452	0.234	1.000	0.195
FRAP	0.890**	0.452	1.000	0.718**	0.195	1.000
<i>Brown finger millet</i>						
DPPH	1.000	0.234	0.718**	1.000	0.234	0.718**
ABTS	0.234	1.000	0.195	0.234	1.000	0.195
FRAP	0.718**	0.195	1.000	0.718**	0.195	1.000
<i>Sorghum</i>						
DPPH	1.000	-0.242	0.744**	1.000	-0.275	0.914**
ABTS	-0.242	1.000	0.004	-0.275	1.000	-0.385
FRAP	0.744**	0.004	1.000	0.914**	-0.385	1.000

Pearson correlation coefficients *, ** indicate significance at $p < 0.05$ and 0.01 .

DPPH: 2, 2-diphenyl-1-picrylhydrazyl;

ABTS: 2, 2'-azinobis-3-ethylbenzthiazoline-6-sulfonic acid;

FRAP: Ferric reducing antioxidant power.

5.5. Conclusion

The results show that malt from the two varieties of FM could be utilised in the production of non-alcoholic beverage with enhanced antioxidant activity using either the grain natural microflora or pure culture of *L. fermentum*. Both microbial sources had relatively similar effect on the antioxidant activity of the malt grain fermented particularly at 96 h. Fermentation of the FM varieties for 24 h caused the retention of a high amount of total phenolic compounds and had high antioxidant activity, relative to other fermentation periods. Fermentation for 96 h was found to negatively affect the antioxidant activity of the beverages. Beverages produced using malt from the two varieties of FM contained a good amount of citric acid which showed a decrease with fermentation time, especially at 96 h of fermentation. The acidity level and sugar content of the FM beverages were found to be typical for preservation of a beverage product. The FM beverages show a drinkable/ free-flowing liquid consistency, which however, requires a corresponding

sensorial description. This study has demonstrated that a non-alcoholic beverage can be derived from two varieties of finger millet grains, and also provides an ideal fermentation limit for enhanced health promoting compounds. Further study on the proximate, microbial, and sensory evaluation are required to validate the utilisation of malt from FM in the production of non-alcoholic beverage. Investigation should focus on the energy, carbohydrate, protein, fat, ash and crude fibre content of FM beverage as well as total bacteria, yeast and mould count, and overall acceptability in terms of colour, aroma, mouth-feel and flavour.

CHAPTER 6

GENERAL CONCLUSION AND RECOMMENDATION

6.1. General conclusion

The present study has shown that finger millet (FM) has a potential use as functional food and in food products. The results on minerals, phytic acid, pH and colour properties of the two varieties of FM show that FM contains various essential elements that are important for nutrition. The use of inductively coupled plasma spectroscopy for analysis of the mineral composition revealed that the FM contains a higher amount of calcium than was widely reported in the literature. This property was also observed for phosphorus, sulphur, potassium, zinc and manganese. The grain also contains trace amount of essential minerals like selenium and cobalt that are clinically useful for certain health conditions. Heavy metals aluminium and barium were also found in considerable amounts. The use of atomic absorption spectrometer for analysis of the minerals provided amounts that were lower compared to the results obtained using inductively coupled plasma spectroscopy particularly for zinc, copper, manganese and sodium. It can be seen that the use of advanced technique provided better understanding of FM with regard to its mineral composition. The sensitivity level of inductively coupled plasma spectroscopy over atomic absorption spectrometer in determining the minerals, was a major strength in the experiment.

The widely used germination periods of 6 – 48 h, restricts other beneficial changes that could be derived from FM grain. Although major changes in the mineral composition were observed at 48 and 96 h of malting, which varied between the two FM varieties, certain minerals from the FM varieties like calcium, magnesium, iron, zinc and manganese could be enhanced beyond 48 h germination period. Most of the essential minerals of the brown FM were more enhanced at 96 h of malting than at 48 h. For the brown FM variety, a malting time-dependent increase in the mineral content beyond 48 h was observed for iron, zinc, copper, cobalt, sulphur,

silicon and boron. Insight into the beneficial effect of malting for longer germination period was achieved.

The two varieties of FM grain were found to contain a high amount of phytic acid which did not show a malting time-dependent decrease. The variation in phytic acid content of the malted grains could be a result of a co-elution of phytic acid resulting from the dissociation and / or reconfiguration of inositol phosphate isomers during the malting periods. Varietal differences were seen as an important determining factor that influenced the mineral composition and phytic acid content of the malted FM grain varieties. There was no substantial effect on the pH of the FM during malting, however, correlations were established for the changes in the pH and phytic acid, and how they affected the mineral composition. Correlations between the minerals of FM as affected by malting was described. The colour properties of the cereal grains were enhanced, somewhat during malting, which probably may have been closely associated with the colour types and browning reaction. Also, the malting technique employed showed that germination of the cereal grains in heap under white light, was more effective than spreading the grains, even without light.

Malting affected the phenolic composition and antioxidant activity of FM grain. The phenolic compound hesperitin was newly identified and quantified in the grain. Other previously identified flavonoids such as catechin, epicatechin, and taxifolin were also quantified. The beneficial impact of malting on the antioxidant activity was noted beyond 48 h of malting for the FM varieties. The study shows that malting for 72 – 96 h of germination has a positive effect on the compounds catechin, epicatechin, quercetin and protocatechuic acid, and antioxidant activity, which varied for the FM varieties. The discrepancy in the trends observed for phenolic compounds analysed with UPLC-MS as compared with total phenolic and flavonoid contents, could be attributed to non-specificity and or sensitivity of the assay methods used for total phenolic and flavonoid contents. The use of water bath for polyphenol extraction instead of rotary evaporator

was a weakness in the identification and quantification of the phenolic compounds especially the phenolic acids, however, it resulted in isolation of a new compound, hesperitin.

Finger millet malt flour shows potential for use in preparation of a non-alcoholic beverages with ideal physicochemical and nutraceutical properties. The FM malt non-alcoholic beverage had a pH and sugar content which are typical of lactic fermented beverage with drinkable consistency. Citric acid which is an important antioxidant (Abdel-Salam *et al.*, 2014), was found in high amounts in the FM malt beverages. Successive decrease in the citric acid content of the FM beverages with fermentation time, was noted particularly for *L. fermentum* beverages. Fermentation had a tremendous impact on the phenolic composition, total phenolic content and antioxidant activity of the FM malt non-alcoholic beverage. Fermentation of the malt flour beyond 72 h negatively affected the antioxidant activity of the FM malt beverages. Fermentation for 24 h retained high amount of the citric acid and phenolic compounds, and had a better impact on the antioxidant activities of the non-alcoholic beverage using the grain's microflora, and pure culture of *L. fermentum*.

6.2. Recommendation

The study therefore recommends that the cultivation and diverse use of FM in South Africa should be considered an important issue. The ability of finger millet to grow on South Africa soil was observed during a viability trial in the agriculture farm, School of Agriculture, University of Venda. This observation requires a grounded agronomic investigation to confirm its viability and/or sustainability as a crop in South African soil. The finger millet should be further investigated for other minerals like iodine, chloride, nickel and chromium which are also important in human nutrition. It is strongly recommended that an investigation be conducted on the bioaccessibility of minerals and phenolic compounds of the FM malt non-alcoholic beverage at optimal fermentation condition established in the study. Further studies on the nutritional composition, microbial profile

and proliferation, and sensory properties of the beverage are required to draw a firm conclusion on the use of FM malt for non-alcoholic beverage production in South Africa.

REFERENCES

- AACC. (1999). International Method. Elements by atomic absorption spectrophotometry – Inorganic constituents. American Association of Cereal Chemists. <http://methods.aaccnet.org/summaries/40-70-01.aspx>. Accessed: 26-06-2016.
- Abdel-Salam, O. M. E., Youness, E. R., Mohammed, N. A., Morsy, S. M. Y., Omara, E. A., & Sleem, A. A. (2014). Citric acid effects on brain and liver oxidative stress in lipopolysaccharide-treated mice. *Journal of Medicinal Food*, 17, 588-598.
- Afify, A. E. M. R., El-Beltagi, H. S., El-Salam, S. M. A., & Omran, A. A. (2011). Bioavailability of iron, zinc, phytate and phytase activity during soaking and germination of white sorghum varieties. *PlosOne*, 6, e25512. DOI:10.1371/journal.pone.0025512.
- Agte, V. V., Gokhale, M. K., & Chiplonkar, S. A. (1997). Effect of natural fermentation on in vitro zinc bioavailability in cereal-legume mixture. *International Journal of Food Science and Technology*, 31, 29–32.
- Amadou, I., Gounga, M. E., & Le, G. W. (2013). Millets: Nutritional composition, some health benefits and processing – a review. *Emirate Journal of Food Agriculture*, 25, 501-508.
- Annor, G. A., Tyl, C., Marcone, M., Ragaei, S., & Marti, A. (2017). Why do millets have slower starch and protein digestibility than other cereals? *Trends in Food Science and Technology*, 66, 73-83.
- Anthony, U., Moses, L. G., & Chandra, T. S. (1998). Inhibition of *Salmonella typhimurium* and *Escherichia coli* by fermented flour of finger millet (*Eleusine coracana*). *World Journal of Microbiology and Biotechnology*, 14, 883–886.
- Anthony, U., Sripriya, G., & Chandra, T. S. (1996). Effect of fermentation on the primary nutrients in finger millet (*Eleusine coracana*). *Journal of Agricultural and Food Chemistry*, 44, 2616-2618.
- AOAC. (2000). Official methods of analysis. (17th ed.): Association of Official Analytical Chemists, Washington.
- Arnao, M. B., Cano, A., & Acosta, M. (2001). The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chemistry*, 73, 239-244.
- Awika, J. M., McDonough, C. M., & Rooney, L. W. (2005). Decorticating sorghum to concentrate healthy phytochemicals. *Journal of Agricultural and Food Chemistry*, 53, 6230-6234.
- Badau, M. H., Nkama, I., & Jideani, I. A. (2005). Phytic acid content and hydrochloric acid extractability of minerals in pearl millet as affected by germination time and cultivar. *Food Chemistry*, 92, 425-435.
- Baidab, S. F. A., Hamad, S. A., Ahmed, A. H. R., & Ahmed, I. A. M. (2016). Preparation of hulu-mur flavoured carbonated beverage based on feterita sorghum (*Sorghum bicolor*) malt. *International Journal of Food Studies*, 5, 120-130.

- Banerjee, S., Sanjay, K. R., Chethan, S., & Malleshi N. G. (2012). Finger millet (*Eleusine coracana*) polyphenols: Investigation of their antioxidant capacity and antimicrobial activity. *African Journal of Food Science*, 6, 362–374.
- Bellaio, S., Kappeler, S., Rosenfeld, E. Z., & Jacobs, M. (2013). Partially germinated ingredients for naturally healthy and tasty products. *Cereal Foods World*, 58, 55-59.
- Beta, T., Rooney, L. W., Marovatsanga, L. T., & Taylor, J. R. N. (2000). Effect of chemical treatments on polyphenols and malt quality in sorghum. *Journal of Cereal Science*, 31, 295-302.
- Bhat, T. K., Singh, B., & Sharma, O. P. (1998). Microbial degradation of tannins – a current perspective. *Biodegradation*, 9, 343-357.
- Briggs, D. E., Boulton, C. A., Brookes, P. A., & Stevens, R. (2004). Brewing Science and Practice. 1st ed, Woodhead Publishing Limited, Cambridge, UK. Chapter 1, pp. 1-2.
- Cardoso, C., Afonso, C., Lourenco, H., Costa, S., & Nunes, M. L. (2015). Bioaccessibility assessment methodologies and their consequences for the risk–benefit evaluation of food. *Trends in Food Science and Technology*, 41, 5–23.
- Chandra, D., Chandra, S., & Sharma, P. A. K. (2016). Review of finger millet (*Eleusine coracana* (L.) Gaertn): A power house of health benefiting nutrients. *Food Science and Human Wellness*, 5, 149-155.
- Chandrasekara, A., & Shahidi, F. (2011). Antiproliferative potential and DNA scission inhibitory activity of phenolics from whole millet grains. *Journal of Functional Foods*, 3, 159–170.
- Chandrasekara, A., & Shahidi, F. (2012). Bioaccessibility and antioxidant potential of millet grain phenolics as affected by simulated in vitro digestion and microbial fermentation. *Journal of Functional Foods*, 4, 226–237.
- Chandrasekara, A., Naczki, M., & Shahidi, F. (2012) Effect of processing on the antioxidant activity of millet grains. *Food Chemistry*, 133, 1–9.
- Chelule, P. K., Mokoena, M. P., & Gqaleni, N. (2010). Advantages of traditional lactic acid bacteria fermentation of food in Africa. In: Mendez-Vilas, A. (ed.), Current Research Technology and Education Topics in Applied Microbiology and Microbial Biotechnology Series 2. vol. 2. Formatex, Spain, pp. 1160–1167.
- Chethan, S., & Malleshi N. G. (2007). Finger millet polyphenols: Characterization and their nutraceutical potential. *American Journal of Food Technology*, 2, 582–592.
- Chethan, S., Sreerama, Y. N., & Malleshi, N. G. (2008a). Mode of inhibition of finger millet malt amylases by the millet phenolics. *Food Chemistry*, 111, 187-191.
- Chethan, S., Dharmesh, S. M., & Malleshi, N. G. (2008b). Inhibition of aldose reductase from cataracted eye lenses by finger millet (*Eleusine coracana*) polyphenols. *Bioorganic and Medicinal Chemistry*, 16, 10085–10090.

- Chitindingu, K., Benhura, M. A. N., & Muchuweti, M. (2015). *In vitro* bioaccessibility assessment of phenolic compounds from selected cereal grains: a prediction tool of nutritional efficiency. *LWT – Food Science and Technology*, 63, 575-581.
- Choi, R., Kim, B. H., Naowaboot, J., Lee, M. Y., Hyun, M. R., Cho, E. J., Lee, E. S., Lee, E. Y., Yang, Y. C., & Chung, C. H. (2011). Effects of ferulic acid on diabetic nephropathy in a rat model of type 2 diabetes. *Experimental and Molecular Medicine*, 43, 676–683.
- Connor, M. D., Walker, R., Modi, G., & Warlow, C. P. (2007). Burden of stroke in black populations in sub-Saharan Africa. *The Lancet Neurology*, 6, 269-278.
- Correia, R. T. P., McCue, P., Magalhaes, M. M. A., Macedo, G. R., & Shetty, K. (2004). Production of phenolic antioxidant by the solid-state bioconversion of pineapple waste mixed with soy flour using *Rhizopus oligosporus*. *Process Biochemistry*, 39, 2167–2172.
- De Ancos, B., Sgroppo, S., Plaza, L., & Cano, M. P. (2002). Possible nutritional and health-related value promotion in orange juice preserved by high-pressure treatment. *Journal of the Science of Food and Agriculture*, 82, 790-796.
- Devi, P. B., Vijayabharathi, R., Sathyabama, S., Malleshi, N. G., & Priyadarisini, V. B. (2014). Health benefits of finger millet (*Eleusine coracana* L.) polyphenols and dietary fibre: a review. *Journal of Food Science and Technology*, 51, 1021–1040.
- De Vuyst, L. & Weckx, S. (2016). The functional role of lactic acid bacteria in cocoa bean fermentation. In: Mozzi, F., Raya, R. R. & Vignolo, G. M. (2nd ed.): *Biotechnology of lactic acid bacteria: Novel application*. John Wiley & Sons, Ltd, UK. Chapter 16, 312-314.
- Dida, M. M., & Devos, K. M. (2006). Finger millet. In: *Cereals and Millets*. Kole, C. (ed). New York, NY, Springer, pp. 333-343. DOI: 10.1007/978-3-540-34389-9_10.
- Dykes, L., & Rooney L.W. (2006). Sorghum and millet phenols and antioxidants. *Journal of Cereal Science*, 44, 236–251.
- Dykes, L., & Rooney, L. W. (2007). Phenolic compounds in cereal grains and their health benefits. *Cereal Foods World*, 52, 105–111.
- Fardet, A., Rock, E., & Remesy, C. (2008). Is the *in vitro* antioxidant potential of whole-grain cereals and cereal products well reflected *in vivo*? *Journal of Cereal Science*, 48, 258-276.
- Fardet, A. (2010). New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? *Nutrition Research Review*, 23, 65-134.
- Fardet A (2014). Grain-based products, food structure and health potential: Holism and reductionism. *Journal of Nutritional Health and Food Engineering*, 1, 1-2.
- FAOSTAT. (2004). Food and Agriculture Organization Statistical Database. www.faostat.fao.org. Accessed: 8-27-2015.
- FAOSTAT. (2011). Food and Agriculture Organization Statistical Database. www.faostat.fao.org. Accessed: 8-27-2015.

- Fraga, C. G. (2005). Relevance, essentiality and toxicity of trace elements in human health. *Molecular Aspects of Medicine*, 26, 235-244.
- Gao, X., Qiao, S. Y., & Lu, W. Q. (2009). Determination of an economical medium for growth of *Lactobacillus fermentum* using response surface methodology. *Letters in Applied Microbiology*, 49, 556-561.
- Geetha, C., & Parvathi, E. P. (1990). Hypoglycaemic effect of millet incorporated breakfast on selected non-insulin dependent diabetic patients. *The Indian Journal of Nutrition and Dietetics*, 27, 316-320.
- Gibson, R. S., Perlas, L., & Hotz, C. (2006). Improving the bioavailability of nutrients in plant foods at the household level. *Proceedings of the Nutrition Society*, 65, 160-168.
- Gliszczyńska A., Klimczak I., & Rybicka I. (2017). Chemometric analysis of minerals in gluten-free products. *Journal of the Science of Food and Agriculture*, 98, 3041-3048.
- Goldhaber, S. B. (2003). Trace element risk assessment: essentiality vs. toxicity. *Regulatory Toxicology and Pharmacology*, 38, 232-242.
- Gopalan, C. (1981). Carbohydrates in diabetic diet. Bulletin of Nutrition Foundation, pp.3., India.
- Gopaldas, S., Deshpande, S., & John, C. (1988). Studies on a wheat-based amylase-rich food. *Food Nutrition Bulletin*, 10, 55-59.
- Grajek, W., & Olejnik, A. (2010). The influence of food processing and home cooking on the antioxidant stability in foods. In: Smith, Jim., & Charter, E. (ed.), Functional Food Product Development, Blackwell Publishing Ltd, United Kingdom, Chapter 9, pp. 194-197.
- Gull, A., Ahmad, G. N., Prasad, K., & Kumar, P. (2016). Technological, processing and nutritional approach of finger millet (*Eleusine coracana*) – a mini review. *Journal of Food Processing and Technology*, 7, 593. doi:10.4172/2157-7110.1000593.
- Gull, A., Jan, R., Nayik, G. A., Prasad, K., & Kumar, P. (2014). Significance of finger millet in nutrition, health and value added products: a review. *Journal of Environmental Science Computer Science and Engineering Technology*, 3, 1601-1608.
- Gupta, S. M., Arora, S., Mirza, N., Pande, A., Lata, C., Puranik, S., Kumar, J., & Kumar, A. (2017). Finger millet: A certain crop for an uncertain future and a solution to food insecurity and hidden hunger under stressful environments. *Frontiers in Plant Science*, 8, 643. DOI:10.3389/fpls.2017.00643.
- Hambridge K. M., Casey, C. E., & Krebs, N. F (1987) Zinc. In: Mertz W, ed. *Trace elements in human and animal nutrition*, 5th ed. Volume 2. Orlando, FL, Academic Press, 1987:1-137.
- Healthgrain forum (2011). Health-protective mechanisms of whole grain cereal – new hypothesis. www.healthgrain.org. Accessed: 14 August, 2015.
- Hedge, P. S., Chandrakasan, G., & Chandra, T. S. (2002). Inhibition of collagen glycation and cross linking *in vitro* by methanolic extracts of finger millet (*Eleusine coracana*) and kodo millet (*Paspalum scrobiculatum*). *Journal of Nutritional Biochemistry*, 13, 517-521.

- Hegde, P. S., Anitha, B., & Chandra, T. S. (2005). *In vivo* effect of whole grain flour of finger millet (*Eleusine coracana*) and kodo millet (*Paspalum scrobiculatum*) on rat dermal wound healing. *Indian Journal of Experimental Biology*, 43, 254–258.
- Hemalatha, S., Platel, K., & Srinivasan, K. (2007). Zinc and iron contents and their bioaccessibility in cereal grains and pulses consumed in India. *Food Chemistry*, 102, 1328-1334.
- Hithamani, G., & Srinivasan, K. (2014). Effect of domestic processing on the polyphenol content and bioaccessibility in finger millet (*Eleusine coracana*) and pearl millet (*Pennisetum glaucum*). *Food Chemistry*, 164, 55–62.
- Hithamani, G., & Srinivasan, K. (2017). Bioavailability of finger millet (*Eleusine coracana*) phenolic compounds in rat as influenced by co-administered piperine. *Food Bioscience*, 19, 101-109.
- Hoover, R., Hughes, T., Chung, H.J., & Liu, Q. (2010). Composition molecular structure, properties, and modification of pulse starches: a review. *Food Research International*, 43, 399–413.
- Hur, S. J., Lee, S. Y., Kim, Y., Choi, I., & Kim, G. (2014). Effect of fermentation on the antioxidant activity in plant-based foods. *Food Chemistry*, 160, 346-356.
- ICRISAT/FAO. (1996). The world sorghum and millet economies: facts, trends and outlook. International Crops Research Institute for the Semi-Arid Tropics/ Food and Agriculture Organization. Patancheru, India, pp. 1-2, 31, 40.
- Ivanova, V., Stefova, M., & Vojnoski, B. (2009). Assay of the phenolic profile of Merlot wines from Macedonia: Effect of maceration time, storage, SO₂ and temperature of storage. *Macedonian Journal of Chemistry and Chemical Engineering*, 28, 141-149.
- Jha, N., Krishnan, R., & Meera, M. S. (2015). Effect of different soaking conditions on inhibitory factors and bioaccessibility of iron and zinc in pearl millet. *Journal of Cereal Science*, 66, 46-52.
- Jideani, A. I., Takeda, Y., & Hizururi, S. (1996). Structure and physicochemical properties of starches from acha (*Digitaria exilis*), iburu (*D. iburua*) and tamba (*Eleusine coracana*). *Cereal Chemistry*, 73, 677-685.
- Jideani, A. I. O., Silungwe, H., Takalani, T., Anyasi, T. A., Udeh, H. O., & Omolola, A. (2014). Antioxidant-Rich Natural Grain Products and Human Health. In: Oguntibeju O. (ed), Antioxidant-antidiabetic agents and human health. InTech. Publisher, Rejeka, Croatia, pp. 167-186. DOI: 10.5772/57. ISBN 978-953-51-1215-0.
- Kamat, M. V., & Belavady, B. (1980). Unavailable carbohydrates of commonly consumed Indian foods. *Journal of the Science of Food and Agriculture*, 31, 194-202.
- Kataoka, S. (2005). Functional effects of Japanese fermented soy sauce (*shoyu*) and its components. *Journal of Bioscience and Bioengineering*, 100, 227–234.

- Kayode, A. P. P., Hounhouigan, J. D., & Nout, M. J. R. (2007). Impact of brewing process operations on phytate, phenolic compounds and in vitro solubility of iron and zinc in opaque sorghum beer. *LWT-Food Science and Technology*, 40, 834-841.
- Kikuzaki, H., Hisamoto, M., Hirose, K., Akiyama, K., & Taniguchi, H. (2002). Antioxidant properties of ferulic acid and its related compounds. *Journal of Agricultural and Food Chemistry*, 32, 2161-2168.
- Kim, Y., Goodner, K. L., Park, J. D., Choi, J., & Talcott, S. T. (2011). Changes in antioxidant phytochemicals and volatile composition of *Camellia sinensis* by oxidation during tea fermentation. *Food Chemistry*, 129, 1331-1342.
- Krishnan, R., Dharmaraj, U., & Malleshi, N. G. (2012). Influence of decortication, popping and malting on bioaccessibility of calcium, iron and zinc in finger millet. *LWT-Food Science and Technology*, 48, 169-174.
- Kumar, A., Babu, C., Reddt, V. C., & Swathi, B. (2016a). Anti-nutritional factors in finger millet. *Journal of Nutrition and Food Sciences*, 6, 491. DOI: 10.4172/2155-9600.1000491.
- Kumar, A., Metwal, M., Kaur, S., Gupta, A. K., Puranik, S., Singh, S., Singh, M., Gupta, S., Babu, B. K., Sood, S., & Yadav, R. (2016b). Nutraceutical value of finger millet [*Eleusine coracana* (L.) Gaertn.], and their improvement using omics approaches. *Frontiers in Plant Science*, 7, 934. DOI:10.3389/fpls.2016.00934.
- Kumari, L., & Sumathi, S. (2002). Effect of consumption of finger millet on hyperglycemia in non-insulin dependent diabetes mellitus (NIDDM) subjects. *Plant Foods for Human Nutrition*, 57, 205-213.
- Lafiandra, D., Riccardi, G., & Shewry, P. R. (2014). Review – Improving cereal grain carbohydrates for diet and health. *Journal of Cereal Science*, 59, 312-326.
- Latha, A. M., Rao, K. V., & Reddy, V. D. (2005). Production of transgenic plants resistant to leaf blast disease in finger millet (*Eleusine coracana* (L.) Gaertn.). *Plant Science*, 169, 657-667.
- Licker, R., Johnston, M., Foley, J. A., Barford, C., Kucharik, C. J., Monfreda, C., & Ramankutty, N. (2010). Mind the gap: How do climate and agricultural management explain the ‘yield gap’ of croplands around the world? *Global Ecology and Biogeography*, 19, 769-782.
- Liu, R. H. (2004). Potential synergy of phytochemicals in cancer prevention: mechanism of action. *Journal of Nutrition*, 134, 3479S-3485S.
- Liu, R. H. (2007). Whole grain phytochemicals and health. *Journal of Cereal Science*, 46, 207-219.
- Luo, Y., Xie, W., Jin, X., Wang, Q., & He, Y. (2014). Effects of germination on iron, zinc, calcium, manganese, and copper availability from cereals and legumes. *CyTA-Journal of Food*, 12, 22-26.

- Maggi-Capeyron, M. F., Ceballos, P., Cristol, J. P., Delbosc, S., Le Doucen, C., Pons, M., Leger, C. L., & Descomps, B. (2001). Wine phenolic antioxidants inhibit AP-1 transcriptional activity. *Journal of Agricultural and Food Chemistry*, 49, 5646-5652.
- Mahadevappa, V. G., & Raina, P. L. (1978). Lipid profile and fatty acid composition of finger millet (*Eleusine coracana*). *Journal of Food Science and Technology*, 15, 100-102.
- Malleshi, N. G., & Desikachar, H. S. R. (1986). Nutritive value of malted millet flours. *Plant Foods for Human Nutrition*, 36, 191-196.
- Malleshi, N. G., & Hadimani, N. A. (1993). Nutritional and technological characteristics of small millets and preparation of value added products from them. In: Advances in small millets. eds. Riley KW, Gupta SC, Seetharam A, Mushong JN. Oxford and IBH, New Delhi, pp 270-287.
- Mamiro, P. R. S., Van Camp, J., Mwikya, S. M., & Huyghebaert, A. (2001). In vitro extractability of calcium, iron, and zinc in finger millet and kidney beans during processing. *Food and Chemical Toxicology*, 66, 1271-1275.
- Manach, C., Scalbert, A., Morand, C., Remesy, C., & Jimenez, L. (2004). Polyphenols: Food sources and bioavailability. *American Journal of Clinical Nutrition*, 79, 727-747.
- Mathanghi, S. K., & Sudha, K. (2012). Functional and phytochemical properties of finger millet (*Eleusine coracana* L.) for health. *International Journal of Pharmaceutical, Chemical and Biological Sciences*, 2, 431-438.
- Marie, P. J., Skoryna, S. C., Pivon, R. J., Chabot, G., Glorieux, F. H., & Stara, J. F (1985). Histomorphometry of bone changes in stable strontium therapy. In: Trace substances in environmental health XIX, edited by D.D. Hemphill, University of Missouri, Columbia, Missouri, 193-208.
- Martin, K. R (2013). Silicon: The health benefits of a metalloid. In: Interrelations between essential metal ions and human diseases, metal ions in life sciences 13. Sigel, A., Sigel, H., Sigel, R. K. O. (eds). DOI 10.1007/978-94-007-7500-8_14, © Springer Science+Business Media Dordrecht, chapter 14: page 457.
- Mayosi, B. M., Flisher, A. J., Lalloo, U. G., Sitas, F., Tollman, S. M., & Bradshaw, D. (2009). The burden of non-communicable diseases in South Africa. *Lancet*, 374, 934-947.
- Mbithi-Mwikya, S., Van Camp, J., Yiru, Y., & Huyghebaert, A. (2000). Nutrient and antinutrients changes in finger millet (*Eleusine coracana*) during germination. *Lebensmittel-Wissenschaft and Technology*, 33, 9-14.
- McDonough, C. M., Rooney, L. W., & Earp, C. A. (1986). Structural characteristics of *Eleusine coracana* (finger millet) using scanning and fluorescence microscopy. *Food Microstructure*, 5, 247-256.
- Mgonja, M., Lenne, J. M., Manyasa, E., & Sreenivasaprasad, S. (2007). Finger millet blast management in East Africa. Creating opportunities for improving production and utilisation of finger millet. Proceedings of the First International Finger Millet Stakeholder Workshop, Projects UK Department for International Development –Crop Protection

- Programme, (Patancheru: International Crops Research Institute for the Semi-Arid Tropics), pp. 196.
- Minamiyama, Y., Takemura, S., Yoshikawa, T., & Okada, S. (2003). Fermented grain products, production, properties and benefits to health. *Pathophysiology*, 9, 221–227.
- Mouquet, C., & Tréche, S. (2001). Viscosity of gruels for infants: a comparison of measurement procedures. *International Journal of Food Sciences and Nutrition*, 52, 389–400.
- Naczki, M., & Shahidi, F. (2004). Extraction and analysis of phenolics in food. *Journal of Chromatography*, 1054, 95–111.
- NAAS. (2013). Role of millets in nutritional security of India. Policy paper No. 66, National Academy of Agricultural Sciences, New Delhi. p. 16.
- Nordin B. E. C (1960). Osteomalacia, osteoporosis and calcium deficiency. *Clinical Orthopaedics and Related Research*, 17:235–258.
- NRC. (1996). Lost crop of Africa. National Research Council. Volume I: Grains. Washington DC. The National Academic Press. pp 39–58.
- Nirmala, M., Subba Rao, M. V. S. S. T., & Muralikrishna, G. (2000). Carbohydrates and their degrading enzymes from native and malted finger millet (Ragi, *Eleusine coracana*, Indaf-15). *Food Chemistry*, 69, 175–180.
- Nkama, I., Gbenyi, D. I., & Hamaker, B. R. (2015). Effects of malting and roasting of millet and sorghum on protein digestibility, mineral availability, soluble sugar composition and consumer acceptability of *dakuwa*. *Indian Journal of Nutrition*, 2, 1–16.
- Obilana, A. B., & Manyasa, E. (2002). Millets. In ‘Pseudocereals and less common cereals; grain properties and utilization potential’. (Belton PS, Taylor JRN edition), Springer-Verlag, Berlin Heidelberg New York. pp. 177–217.
- Oyaizu, M. (1986). Studies on product browning reactions antioxidative activities of products of browning reaction prepared from glucosamine. *The Japanese Journal of Nutrition and Dietetics*, 44, 307–315.
- Pagano, A. E. (2006). Whole grains and the gluten-free diet. In: The Celiac Diet Series 2 ed. Parrish, C. R. (ed.), Charlottesville, VA, University of Virginia Health System, pp. 66–78.
- Platel, K., Eipeson, S. W., & Srinivasan, K. (2010). Bioaccessible mineral content of malted finger millet (*Eleusine coracana*), wheat (*Triticum aestivum*), and barley (*Hordeum vulgare*). *Journal of Agricultural and Food Chemistry*, 58, 8100–8103.
- Pradhan, A., Nag, S. K., & Patil, S. K. (2010). Dietary management of finger millet controls diabetes. *Scientific Correspondence*, 98, 763–765.
- Qvirist, L., Carlsson, N. G., & Andlid, T. (2015). Assessing phytase activity – methods, definitions and pitfalls. *Journal of Biological Methods*, 2, e16. DOI:10.144440/jbm.2015.58.

- Rajasekaran, N. S., Nithya, M., Rose, C., & Chandra, T. S. (2004). The effect of finger millet feeding on the early responses during the process of wound healing in diabetic rats. *Biochimica Biophysica Acta*, 1689, 190–201.
- Ramulu, P., & Rao, U. P. (1997). Effect of processing on dietary fibre content of cereals and pulses. *Plant Foods for Human Nutrition*, 50, 249-257.
- Rani, S., & Anthony, U. (2014). Effect of germination and fermentation on polyphenols in finger millet (*Eleusine coracana*). *International Journal of Food and Nutritional Sciences*, 3, 65-68.
- Rao R.S.P., & Muralikrishna, G. (2006). Water soluble feruloyl arabinoxylans from rice and ragi: changes upon malting and their consequence on antioxidant activity. *Phytochemistry*, 67, 91–99.
- Rao R.S.P., & Muralikrishna, G. (2007). Structural characteristics of water-soluble feruloyl arabinoxylans from rice (*Oryza sativa*) and ragi (finger millet, *Eleusine coracana*): variations upon malting. *Food Chemistry*, 104, 1160–1170.
- Reddy, V. D., Rao, K.V., Reddy, T. P., & Kishor, P. B. K. (2008). Finger millet. In: Compendium of transgenic crops plants; Transgenic Cereals and Forage Grasses, Kole C, and Hall TC (eds), Blackwell Publishing Ltd, Chapter 8, 191-198.
- Rohn, S., Rawel, H. M., & Kroll, J. (2002). Inhibitory effects of plant phenols on the activity of selected enzymes. *Journal of Agricultural and Food Chemistry*, 50, 3566–3571.
- Rosentrater, K. A., & Evers, A. D. (2018). Introduction to cereals and pseudocereals and their production. In: An introduction for student of food science and agriculture. Kent's Technology of Cereals (5th ed), Woodhead Publishing Series in Food Science, Technology and Nutrition. p. 58.
- Sasaki, S., Oshima, T., Teragawa, H., Matsuura, H., Kajiyama, G., & Kambe, M. (1999). Magnesium (Mg) status in patients with cardiovascular diseases. *The Japanese Journal of Clinical Pathology*, 47, 396-401.
- Sastri, B. N. (1989). The wealth of India: A dictionary of Indian raw materials and industrial products. CSIR, New Delhi, Vol. 3(D-E), pp. 160-166.
- Seetharam, A., Riley, K. W., & Harinarayana, G. (1986). Small millets in global agriculture. Proceedings of the First International Small Millets Workshop, Bangalore, India, October 29th – November 2nd. Oxford and IBH Publishing Co. PVT, Ltd. India.
- Sehgal, A. S., & Kawatra, A. (1998). Reduction of polyphenol and phytic acid content of pearl millet grains by malting and blanching. *Plant Foods for Human Nutrition*, 53, 93-98.
- Shahidi, F., & Chandrasekara, A. (2013). Millet grain phenolics and their role in disease risk reduction and health promotion: a review. *Journal of Functional Foods*, 5, 570–581.

- Shahidi, F., & Chandrasekara, A. (2015). Processing of millet grains and effects on non-nutrient antioxidant compounds. In: Preedy V. (ed.): Processing and Impact on Active Components in Food. San Diego, Elsevier Science Publishing Co. Inc.: 345–352.
- Shankar A. H., & Prasad, A. S (1998). Zinc and immune function: the biological basis of altered resistance to infection. *American Journal of Clinical Nutrition*, 68(Suppl.):S447–S463.
- Sharma, B., Gujral, H. S., & Solah, V. (2017). Effect of incorporating finger millet in wheat flour on mixolab behaviour, chapatti quality and starch digestibility. *Food Chemistry*, 231, 156–164.
- Shashi, B. K., Sharan, S., Hittalamani, S., Shankar, A. G., & Nagarathna, T. K. (2007). Micronutrient composition, antinutritional factors and bioaccessibility of iron in different finger millet (*Eleusine coracana*) genotypes. *Karnataka Journal of Agricultural Sciences*, 20, 583–585.
- Shibairo, S. I., Nyongesa, O., Onwonga, R., & Ambuko, J. (2014). Variation of nutritional and anti-nutritional contents in finger millet (*Eleusine coracana* (L.) Gaertn) genotypes. *Journal of Agriculture and Veterinary Science*, 7, 6–12.
- Shobana, S., Harsha, M. R., Platel, K., Srinivasan, K., & Malleshi, N. G. (2010). Amelioration of hyperglycaemia and its associated complications by finger millet (*Eleusine coracana* L.) seed coat matter in streptozotocin -induced diabetic rats. *British Journal of Nutrition*, 104, 1787–1795.
- Shobana, S., Sreerama, Y. N., & Malleshi, N. G. (2009). Composition and enzyme inhibitory properties of finger millet (*Eleusine coracana* L.) seed coat phenolics: Mode of inhibition of alpha-glucosidase and pancreatic amylase. *Food Chemistry*, 115, 1268–1273.
- Shobana, S., & Malleshi, N. G. (2007). Preparation and functional properties of decorticated finger millet (*Eleusine coracana*). *Journal of Food Engineering*, 79, 529–538.
- Shobana, S., Ushakumari, S. R., Malleshi, N. G., & Ali, S. Z. (2007). Glycemic response of rice, wheat and finger millet based diabetic food formulations in normoglycemic subjects. *International Journal of Food Science and Nutrition*, 58, 363–372.
- Singleton, V. L., Orthofer, R., & Lamule-Raentos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299, 152–178.
- Siwela, M., Taylor, J. R. N., de Milliano, W. A. J., & Duodu, K. G. (2007). Occurrence and location of tannins in finger millet grain and antioxidant activity of different grain types. *Cereal Chemistry*, 84, 169–174.
- Skibsted, L. H. (2016). Mineral nutrient interaction: Improving bioavailability of calcium and iron. *Food Science and Biotechnology*, 25, 1233–1241.
- Slavin, J. L. (2000). Mechanism for the impact of whole grain foods on cancer risk. *Journal of American College of Nutrition*, 19, 300–307.

- Sreeramulu, D., Reddy C. V. K., & Raghunath, M. (2009). Anti-oxidant activity of commonly consumed cereals, millets, pulses and legumes in India. *Indian Journal of Biochemistry and Biophysics*, 46, 112–115.
- Sripriya, G., Chandrasekharan, K., Murty, V. S., & Chandra, T. S. (1996). ESR spectroscopic studies on free radical quenching action of finger millet (*Eleusine coracana*). *Food Chemistry*, 57, 537–540.
- Sripriya, G., Anthony, U., & Chandra, T. S. (1997). Changes in carbohydrate, free amino acids, organic acids, phytate and HCL extractability of minerals during germination and fermentation of finger millet (*Eleusine coracana*). *Food Chemistry*, 58, 345–350.
- Subba Rao, M. V. S. S. T., & Muralikrishna, G. (2001). Non-starch polysaccharides and bound phenolic acids from native and malted finger millet (Ragi, *Eleusine coracana*, Indaf - 15). *Food Chemistry*, 72, 187–192.
- Subba Rao, M. V. S. S. T., & Muralikrishna, G. (2002). Evaluation of the antioxidant properties of free and bound phenolic acids from native and malted finger millet (Ragi, *Eleusine coracana* Indaf-15). *Journal of Agricultural and Food Chemistry*, 50, 889–892.
- Tako, E., Reed, S. M., Budiman, J., Hart, J. J., & Glahn, R. P. (2015). Higher iron pearl millet (*Pennisetum glaucum* L.) provides more absorbable iron that is limited by increased polyphenolic content. *Nutrition Journal*, 14, 11. DOI: 10.1186/1475-2891-14-11.
- Tan, X. L., Shi, M., Tang, H., Han, W., & Spivack, S. D. (2010). Candidate dietary phytochemicals modulate expression of phase II enzymes GSTP1 and NQO1 in human lung cells. *Journal of Nutrition*, 140, 1404–1410.
- Tatala, S., Ndossi, G., Ash, D., & Mamiro, P. (2007). Effect of germination of finger millet on nutritional value of foods and effect of food supplement on nutrition and anaemia status in Tanzanian children. *Tanzanian Health Research Bulletin*, 9, 77–86.
- Thaipong, K., Boonprakbo, U., Crosby, K., Cisneros-Zevallo, L., & Byrne, D. H. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, 19, 669–675.
- Tovey, F. I. (1994). Diet and duodenal ulcer. *Journal of Gastroenterology and Hepatology*, 9, 177–185.
- Towo, E., Matuschek, E., & Svanberg, U. (2006). Fermentation and enzyme treatment of tannin sorghum gruels: effects on phenolic compounds, phytate and in vitro accessible iron. *Food Chemistry*, 94, 369–376.
- Tréche, S., & Mbome, L. I. (1999). Viscosity, energy density and osmolality of gruels for infants prepared from locally produced commercial flours in some developing countries. *International Journal of Food Sciences and Nutrition*, 50, 117–125.
- Udeh, H. O., Duodu, K. G., & Jideani, A. I. O. (2017). Finger millet bioactive compounds, bioaccessibility, and potential health effects – a review. *Czech Journal of Food Sciences*, 35, 7–17.

- Viswanath, V., Urooj, A., & Malleshi, N. G. (2009). Evaluation of antioxidant and antimicrobial properties of finger millet (*Eleusine coracana*). *Food Chemistry*, 114, 340–346.
- Vitaglione, P., Napolitano, A., & Fogliano, V. (2008). Cereal dietary fibre: A natural functional ingredient to deliver phenolic compounds into the gut. *Trends in Food Science and Technology*, 19, 451-463.
- Wang, T., He, F., & Chen, G. (2014). Improving bioaccessibility and bioavailability of phenolic compounds in cereal grains through processing technologies: A concise review. *Journal of Functional Foods*, 7, 101-111.
- Wang, S., Li, C., Copeland, L., Niu, Q., & Wang, S. (2015). Starch retrogradation: A comprehensive review. *Comprehensive Reviews in Food Science and Food Safety*, 14, 568-585.
- Warwick, E., Cassidy, A., Hanley, B., Jouni, Z. E., & Bao, T. (2012). Effect of phytochemicals on phase II enzyme expression in infant human primary skin fibroblast cells. *British Journal of Nutrition*, 108, 2158-2165.
- Whent, M., Huang, H., Xie, Z., Lutterodt, H., Yu, L., Fuerst, E. P., Morris, C. F., & Luthria, D. (2012). Phytochemical composition, anti-inflammatory, and antiproliferative activity of whole wheat flour. *Journal of Agricultural and Food Chemistry*, 60, 2129-2135.
- WHO/FAO. (2004). Vitamin and mineral requirement in human nutrition. World Health Organisation and Food and Agriculture Organisation of the United Nations. Food and Agriculture Organisation of the United Nations, Rome, Italy.
- WHO. (2001). Iron deficiency anaemia assessment, prevention and control. World Health Organisation, Geneva.
- Wyrzykowski, D., Hebanowska, E., Nowak-Wicz, G., Makowski, M., & Chmurzynski, L. (2011). Thermal behaviour of citric acid and isomeric aconitic acids. *Journal of Thermal analysis and Colorimetry*, 104, 731-735.
- Yamunarani, R., Govind, G., Ramegowda, V., Thammegowda, H. V., & Guligowda, S. A. (2016). Genetic diversity for grain Zn concentration in finger millet types: potential for improving human Zn nutrition. *The Crop Journal*, 4, 229-234.
- Yu, L. L., Haley, S., Perret, J., & Harris, M. (2002). Antioxidant properties of hard winter wheat extracts. *Food Chemistry*, 78, 457-461.
- Yu, S., Cowieson, A., Gilbert, C., Plumstead, P., & Dalsgaard, S. (2012). Interactions of phytate and myo-inositol phosphate esters (IP₁₋₅) including IP₅ isomers with dietary protein and iron and inhibition of pepsin. *Journal of Animal Science*, 90, 1824-1832.
- Yun, K. J., Koh, D. J., Kim, S. H., Park, S. J., Ryu, J. H., Kim, D. G., Lee, J. Y., & Lee, K. T. (2008). Anti-inflammatory effects of sinapic acid through the suppression of inducible nitric oxide synthase, cyclooxygenase-2, and pro-inflammatory cytokines expressions via nuclear factor-kappa B inactivation. *Journal of Agricultural and Food Chemistry*, 56, 10265-10272.

- Zarnkow, M. (2014). Fermented foods: Beverages from sorghum and millet. *Encyclopedia of Food Microbiology*, 1, 839-845.
- Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64, 555-559.
- Zvauya, R., Mygochi, T., & Parawira, W. (1997). Microbial and biochemical changes occurring during production of *masvusvu* and *mangisi*, traditional Zimbabwean beverages. *Plant Foods for Human Nutrition*, 51, 43-51.

APPENDIX I. PUBLICATIONS/ CONFERENCES ATTENDED

Published journal papers:

Udeh, H. O., Duodu, K. G., & Jideani, A. I. O. (2018). Malting period effect on phenolic composition and antioxidant activity of finger millet (*Eleusine coracana* L. Gaertn) flour. *Molecules*, 23(9), 2091. DOI: <https://doi.org/10.3390/molecules23092091>.

Udeh, H. O., Duodu, K. G., & Jideani, A. I. O. (2018). Effect of malting period on physicochemical properties, minerals, and phytic acid of finger millet (*Eleusine coracana*) flour varieties. *Food Science and Nutrition*, 1-12. DOI: 10.1002/fsn3.696.

Udeh, H. O., Duodu, K. G., & Jideani, A. I. O. (2017). Finger millet bioactive compounds, bioaccessibility, and potential health effects – a review. *Czech Journal of Food Sciences*, 35(1), 7-17.

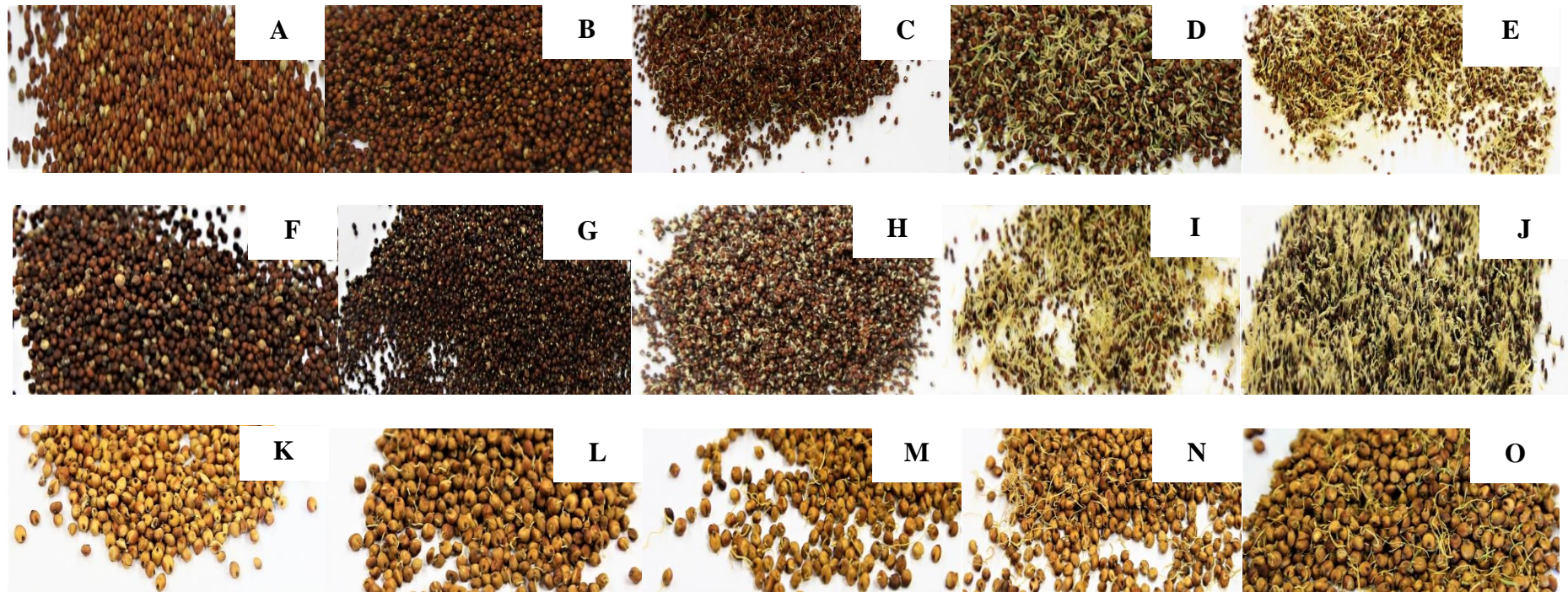
Conference papers:

H.O. Udeh, S.E. Ramashia, K.G. Duodu, & A.I.O. Jideani (2018). Fermentation and microbial source affect citric acid, phenolic compounds and antioxidant activity of non-alcoholic finger millet malt beverage. 5th International ISEKI_Food Conference, 3rd – 5th July, 2018, University of Hohenheim, Stuttgart, Germany.

H.O. Udeh, K.G. Duodu, & A.I.O. Jideani (2018). Effect of malting on the phenolic compounds and antioxidant activity of finger millet (*Eleusine coracana* L. Gaertn) varieties. Sorghum in the 21st Century, Century City, Cape Town, South Africa, 9th – 12th April, 2018.

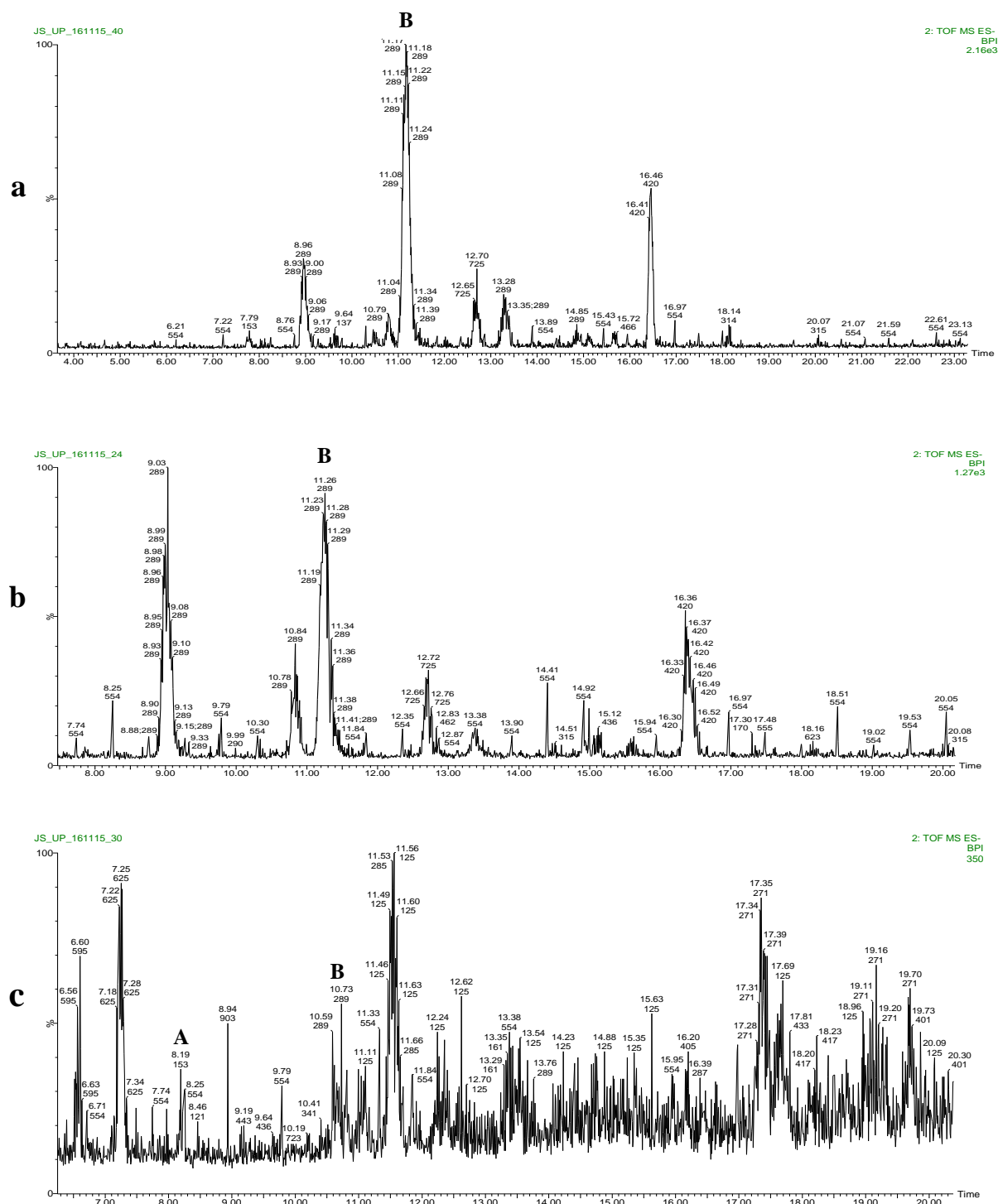
H.O. Udeh, K.G. Duodu, & A.I.O. Jideani (2017). Evaluating the effect of malting period on some physicochemical properties and mineral content of finger millet (*Eleusine coracana* L. Gaertn) varieties. IFT Annual Meeting & Food Expo, Las Vegas, Nevada, USA, 25th – 28th June, 2017.

APPENDIX II. DIAGRAMS OF UNMALTED AND MALTED FINGER MILLET AND SORGHUM GRAINS



Appendix II. Diagram of unmalted and malted finger millet and sorghum grains: A = unmalted brown finger millet; B = 24 h malted brown finger millet; C = 48 h malted brown finger millet; D = 72 h malted brown finger millet and E = 96 h malted brown finger millet. F = unmalted dark brown finger millet; G = 24 h malted dark brown finger millet; H = 48 h malted dark brown finger millet; I = 72 h malted dark brown finger millet and E = 96 h malted dark brown finger millet. K = unmalted sorghum; L = 24 h malted sorghum; M = 48 h malted sorghum; N = 72 h malted sorghum and E = 96 h malted sorghum.

APPENDIX III. CHROMATOGRAMS OF SOME PHENOLIC COMPOUNDS OF FINGER MILLET AND SORGHUM GRAINS



Appendix III. Chromatograms of some phenolic compounds of finger millet and sorghum grains extracted with formic acid in methanol: (a) brown finger millet, (b) dark brown finger millet, and (c) sorghum. (A) protocatechuic acid; (B) catechin.