BIO-ACCESSIBILITY OF PHYTOCHEMICALS FROM SORGHUM [*SORGHUM BICOLOR* (L.) MOENCH] SPROUTS TREATED WITH NOVEL TECHNOLOGIES

By
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DEDICATED

To

My Beloved Parents

DECLARATION

The work reported in this thesis was carried out by me under the supervision of **Dr. Muhammad Kamran Khan**, Assistant Professor, Department of Food Science, Nutrition and Home Economics, GC University, Faisalabad, Pakistan.

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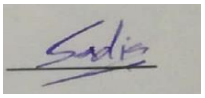

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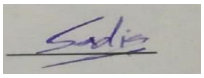
Student/Author Signature: 

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Sadia Hassan

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LIST OF ABBREVIATIONS

Abbreviation	Complete name
AGE	Advanced Glycation End
CVD	Cardiovascular disease
DPPH	2,2-diphenyl-1-picrylhydrazyl.
EMF	Electromagnetic Fields
EM	Electromagnetic
EHF	Extra High Frequency
FAO	Food and Agriculture Organization
FRAP	Ferric reducing antioxidant potential
GP	Germination Percentage
HDL	High-Density Lipoprotein
IR	Infrared Radiation
ICRISAT	International Crop Research Institute for the Semi-Arid Tropics
IVPD	*In vitro* Protein Digestibility
LDL	Low-Density Lipoprotein
LB	Liebermann-Burchard Reagent
MFs	Magnetic Fields
MW	Microwave
ORAC	Oxygen Radical Absorbance Capacity
ROS	Reactive Oxygen Species
RL	Root length
SL	Shoot length
SL/RL	Ratio of shoot length and root length
SVI	Seedling Vigor Index
TFAAs	Total Free Amino Acids
TW	Total weight
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
UM	Ultrasound and microwave combined treated
USDA	United States Department of Agriculture
US	Ultrasound
AGE	Advanced Glycation End

ABSTRACT

Germination generally increases the nutritive value of seeds by modulating phytochemical composition depending on processing conditions. Therefore, the current study was conducted to compare the effect of emerging processing techniques such as ultrasound (US) and microwave (MW) on phytochemicals composition of sorghum sprouts. Overnight steeped sorghum grains were divided into four groups. The first group was designated as control without applying any treatment. Second group was subjected to ultrasonic treatment using 40% & 60% amplitude for 5 & 10 min. Third group was exposed to microwave treatment at 450 W & 700 W power levels for 15 & 30 sec. Similarly, fourth group was treated with combined MW & US application for different intervals. Microwave and sonication processing significantly improved the germination percentage and growth parameters, hence the phytochemical profile improvement. After germination, sprouts subjected to different quantitative analysis showed that with increased germination significant reduction was observed in anti-nutritional components such as alkaloid, saponin, tannin and phytate contents while contents of total free amino acids, sterols, and digestible proteins and carbohydrates increased. The oil yield and fatty acid composition increased with increasing amplitude of processing techniques. Antioxidant activities and HPLC analysis for individual phenolic compounds also improved significantly with high germination. In-vitro protein digestibility was significantly higher while bio-accessibility of polyphenol and flavonoid was insignificantly affected. From the present investigation, ultrasound processed treatment US_1 (40% amplitude for 5 min) and microwave processed treatment MW_2 (700 watt for 5 sec) are found paramount to improve the composition and bio-accessibility of phytochemicals through enhanced germination of seeds.

Keywords: Sorghum, germination, ultrasound, microwave, antioxidant activity, phytochemicals, bio-accessibility.

CHAPTER 1

INTRODUCTION

Whole grains cereals are excellent source of vital phytochemicals. Their abundance generally depends upon type of cereal, site of presence within the cereal grain and most importantly its processing (Belobrajdic & Bird, 2013). Cereal grains are used as staple food in various regions such as Asian and African. They are also rich source of phenolic compounds therefore, widely used in many functional foods (Van Hung, 2016). Almost 85% of the food energy is fulfilled through the cereal grains. Sorghum (*Sorghum bicolor* (L.) Moench) is world's major cereal crop. It is known for its grain, stems and leaves. Sorghum grain is used as a staple food in 30 different countries, approximately 500 million people lived in these semi-arid tropical countries (Kumari et al., 2016). Sorghum is 5th most important cereal grain crop consumed as staple food in the world after wheat, rice, corn and barley (Proietti et al., 2015). Production of sorghum is easier and economical as compared to other cereal crops because it can withstand the harsh conditions of the environment. Approximately 35% sorghum is grown for human consumption and it also grown for other purposes e.g. it is used for fodder, alcohol production and other industrial productions (Kangama & Rumei, 2005).

Chemically it contains different phytochemicals which includes polyphenol, sterols, and policosanols. Among these policosanols and plant sterols are found in plant oils and wax, on the other hand phenolic compounds increase the plant immunity against various pests and maladies. Two major categories of phenolic compounds present in sorghum are flavonoids and phenolic (Polumahanthi & Nallamilli, 2014). Flavonoids including tannins and anthocyanins were separated from seeds of the sorghum (in red color variety) as the vital constituents (Guarjardo-Flores & Rooney, 2011). Phytochemicals are secondary metabolites of sorghum. These compounds are biologically active but did not take part in vital activities of cell metabolism. These compounds provide numerous health benefits other than linked by macronutrients and micronutrients (Saxena et al., 2013). The diet containing phytochemical from plant origin proved helpful for the human beings against numerous degenerative maladies. We are also aware that most of the ancient medicines were obtained from the plants (Iqbal et al., 2015). Many characteristics of sorghum plant are associated with the presence of phytochemicals such as aroma, color, flavor etc. They also protect plant from harm of maladies

and other environmental stress such as drought, pollution, and pathogenic attack (Shalini & Velavan, 2017). Surprisingly, more than thousand types of phytochemical are known and still many are not known. Approximately four thousand phytochemical are cataloged and almost 150 phytochemicals have been studied in detail (Sharma et al., 2010). Important classes of phytochemicals are phenols, tannins, alkaloids, glycosides, volatile oils, resins, and mucilages besides the main four classes (proteins, lipids, carbohydrates and nucleic acids) (Saxena et al., 2013). Among these compounds phenolic compounds are more important and popular because they have the properties of antioxidation and anti-inflammation.

Bioavailability, metabolism, cellular and molecular mechanisms of polyphenols have been studied in detail (Reinisalo et al., 2015). Fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices are rich source of polyphenols. Phenolic compounds occupy various places in the plants such as seeds, flowers, fruits, leaves, stems and roots (Upadhyay & Dixit, 2015). Polyphenols are classified by chemical characteristics, physical characteristics and protective functions (Shalini & Velavan, 2017). Lipids also perform vital role in human health and lipids are group of phytochemicals (Alabdulkarim et al., 2012). Lipids act as source of energy, essential fatty acids, sterols and structural components to membranes; transport medium of metabolic fuel; and protect and carry to lipophilic vitamins (Nagao & Yanagita, 2008).

Experts are extensively working on healthy nutrition from last decades and they are giving more attention to determine the biological value of the nutritional sprouts (Laila & Murtaza, 2014). In Asian region germinated have long been used to improve the nutrition of diet of consumers (Fazaeli et al., 2012). During germination, a new plant grows from the seed at rest if the moisture content is optimum, oxygen is sufficient for the respiration and the temperature is suitable (Li et al., 2015). Sprout developed from the seed after germination contain bioavailable proteins, lipids, vitamins, minerals and phenolic components. Biologically, during germination large/complex compounds are catalyzed into small and simple compounds which are readily available for metabolism (Helal, 2016). Sorghum is use as staple food and principle source of energy, protein, vitamins and minerals by millions of people living in Asia, Africa and the semi-arid tropics (Maunder, 2002; Raihanatu et al., 2011). But the existence of various anti-nutritional factors creating complexes with many other food components reduces the nutritional value of sorghum grain and also decrease its organoleptic

characteristics (Ogbonna et al., 2012). Various processing techniques such as soaking, germination and cooking can enhance nutritional and functional characteristic of plant seeds (Kajihausa et al., 2014). These techniques individually or in combination have the ability to enhance the nutritional quality of sorghum grains by eliminating the anti-nutritional components (Raihanatu et al., 2011). During soaking, germination and cooking amount of anti-nutrient e.g. phytate, trypsin inhibitor, tannin etc. decreased. Germination alone have the ability to develop functional foods which help in sustaining the health and exert positive effects on the micro-biota of human (Marton et al., 2010). Many essential compounds such as polyphenol are not biologically available to impart their valuable characteristics. Biological availability of such compounds greatly depends upon their release from the complex food matrix. Germination process separate the anti-nutritional constituents such as phytate or mineral therefore, make it bio-accessible (Oghbaei & Prakash, 2016).

Application of new and emerging novel technologies in various fields is one of the main challenges for the improvement of the food quality. Seed pre-treatments including physical and chemical treatments are widely used to enhance plant performance. Various physical treatments for example microwaves and electric irradiations are popular techniques to improve seed enactment (de Sousa Araújo et al., 2016). These physical technologies affect the biochemical and physiological process in growing seeds without harmfully affecting the environment therefore, it can enhance the plant performance. Chemical technologies are also widely used as they are cost effective but create burden of their disposal in environmental cycle (Sharma et al., 2015). Recently, ultrasound gain the attention of experts. The process of cavitation contains series of phenomena including the creation, development and the collapse of micro-bubbles produced in a liquid when ultrasound waves travel through the medium (López-Ribera & Vicient, 2017). Ultrasonic waves change the structure and function of the bio-molecules by acting as an alternative stress on cells and tissues. After Ultrasound treatment effectively improve the biological activity, high efficiency, save energy, reduce process time and also enhance mass transfer. Seed treatment with ultrasound offers the high productivity in food industry and biotechnology processes (Machikowa et al., 2013). Promotion of cell growth can be achieved by the use of ultrasound waves in proper intensity and duration which stimulate seed physiological activities or increase its enzymatic activities (Chowdhury et al., 2014).

Microwave treatment also effective as high frequency microwaves have positive effects on plant growth, germination and enzymatic activity (Soran et al., 2016). Electromagnetic irradiation has special consideration as the pre-sowing treatment of seeds among different physical methods used (Yanenko et al., 2004) and microwaves is a form of electromagnetic energy which emit non-ionizing radiations (EM) spectrum (de Sousa Araújo et al., 2016). Thermal effect is produced after the application of microwave irradiation which result due to the interaction with charged particles and polar molecules known as heat. Different biological material absorb different amount of energy on placing in such radiation field (Aladjadjiyan, 2010). Different effects have been recognized after microwave application such as improved crop characteristics of seeds of different crops, improved germination etc. Such effects were produced due to the active biochemical process enhancing the more absorption of nutrients (Morozov et al., 2013).

Nowadays, bio-accessibility of nutrient is the major issue. Like many other food processing methods, germination proved to be an effective method which improve energy and nutritional values of sorghum-based foods. In last decades, polyphenols from the plant source has become the major area of health-related research projects. Beside improving nutritional value of food, novel processing technologies have also reduced the losses during the processing. Evidence related to the effect of food processing on the bio-accessibility of polyphenol and their contents is not enough. Keeping in view the whole context, the present study was designed to explore the effect of novel food processing on phytochemical profile and the phenolic contents during germination of sorghum seeds. The main objectives of this study set to be attained are here;

1. Optimization of parameters for ultrasonic and microwave treatments in response to germination potential of sorghum grains and phytochemical composition of their sprouts.
2. Assessment of bio-accessibility of phytochemicals from treated sorghum sprouts through protein digestibility and phenolic compounds availability in different *in vitro* analysis.

CHAPTER 2

REVIEW OF LITERATURE

Around the globe, the recent trend towards the utilization of organic resources enhance the nutritional value of food for human health. Among the different feasible solutions one of the most renowned way out is the bioactive moieties of sprouts. Whole grain bioactive components such as antioxidants and phytochemicals have not received as much attention as well as during the last decades when whole-grain cereals have received. Among cereals, sorghum is a rich source of phytochemicals having high antioxidant activity and potential to significantly affect the human health. Food scientists and technologists notice new emergent technologies like ultrasound (US) and microwave (MW) for their effectiveness. Hence, the objective of this present research project was to investigate the effect of novel technologies on phytochemicals composition of sorghum grains through sprouting. The literature related to different aspects of the present study has been reviewed under the following headings.

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2.1. Sorghum: an overview

Recently trends toward diet-based therapies are increasing to overcome the health complication. In this context, cereal consumption is rising because they exhibit health promoting components apart from diet (Rakická et al., 2013). *Sorghum bicolor* (L.) is an ancient cereal grain member of grass family *Poaceae* tribe *Andropogonae* has long been a vital staple food in the semi-arid tropics of Africa. Around 2000 BC archeological facts cultivation of sorghum began and later on extended to arid areas of Asia, China and India (El Rabey et al., 2012). Sorghum is a close relative of sugarcane and name was derived from Italian word *Sorgo* which was further derived from Latin word *Syricum* which means grain of Syria. Its name was suggested by Person who named it as *Sorghum bicolor* (L.) Moench (Berenji et al., 2011). It is an annual summer and multipurpose cereal satisfying various human needs. It is believed that about 5000 years ago, to be originated from wild sorghum in Ethiopia, from where it extended to other different parts of world (Dossou-Aminon et al., 2015). The seed head, panicle, stalk, roots and leaves of sorghum deliberately vary in size and shape (Anami et al., 2015). The main anatomical composition of grain is the pericarp (outer layer), testa or seed coat, the endosperm tissue and the germ (de Morais Cardoso et al., 2017). The caryopses or kernels of the panicle may vary in shape and size but usually range from 4-8 mm in diameter and spherical to tear drop shaped (Pruett, 2012). The average caryopsis consists of three main components: 84% endosperm, 10% germ and 6% pericarp. The germ contains the scutellum and embryonic axis (Yan et al., 2014).

The combo of anthocyanin, anthocyanidin pigments and other flavonoid compounds are responsible for the color of the sorghum pericarp (Dykes & Rooney, 2006). Condensed tannin lies in pigmented testa (Wu et al., 2012). Likewise, other cereals the endosperm tissue of sorghum, is composed of the aleurone layer, corneous (vitreous), floury (opaque) and peripheral areas. The peripheral endosperm is extremely hard, dense and resistant to digestion

and water penetration. Corneous endosperm has continuous protein matrix with the starch physically surrounded by the protein. In the center of the granule floury endosperm is present having few protein bodies and discontinuous protein matrix (Ring et al., 1988). In the semi-arid tropical regions drought tolerant sorghum is a very important dietary supplement (Proietti et al., 2015). Hence there is a need for the further characterization and classification of more than 7000 varieties of sorghum in terms of their nutritional value (Pontieri et al., 2012). Based on tannin contents, sorghum is often classified as tannin sorghum, white sorghum and mixed sorghum (Hikeezi, 2010). Sorghum is classified as white, red, black and brown on the basis of appearance and extraction efficiency of phytochemicals. White sorghum has no detectable level of anthocyanins or tannins with low level of total extractable phenol while red sorghum having a red pericarp containing significant level of extractable phenols but has no tannins. In this manner, brown sorghum due to having pigmented testa contains significant level of tannins and black sorghum having black pericarp has high level of anthocyanins (Austin, 2008). Moreover on the basis of the extractable tannin sorghum is further categorized into Type I (red pericarp has no significant amount of tannin on extraction with 1% acidified methanol), Type II (Early Hegari) and Type III (Early Sumac variety has extractable level of tannin in both acidified methanol and methanol alone) (Adetunji et al., 2013).

2.2. Production

Sorghum is primarily belongs to tropical regions but at the time has vast region over the world includes Africa, China, Central India, South America and the United States (Macauley & Ramadjita, 2015). In Africa, it accounts over 43% of cultivated area. In 100 B.C it was introduced in Indo-Pak subcontinent. Moreover, it has been cultivated successfully on a large scale. The largest growers of sorghum all over the world are Indo-Pak regions, USA and Nigeria. Among all the countries, Australia and USA were the leading exporters while Mexico was the largest importer during 2012-13 (Ishida & Malaga, 2015). There is a variation in the crop characteristics varies depending upon the location and agronomic practices. Among 105 countries of Asia, Africa, Oceania and the Americas, sorghum has been grown on 40 million hectares in which USA, India, Mexico, Nigeria, Sudan and Ethiopia are worth mentioning, while Australia, Argentina, Brazil, Burkina Faso, China, Chad, Cameroon, Egypt, Mali, Niger and Tanzania cultivates as well (Muui et al., 2013). In Pakistan the annual production of sorghum is 0.21 M ton with an average yield of 620 kg/ H (Habib et al., 2013). The cultivated

area has been increased by more than 60% in last two decades. In Pakistan the cultivated area has also been increased while limited increase in yield due to limited resources (Habib et al., 2013; Taylor et al., 2006).

Sorghum is a healthy cereal crop used by human being's cause of emerging global warming scenario. Sorghum is cultivated in a wide range of temperature and soil without affecting yield from Africa to Asia to Americas (Iqbal & Iqbal, 2015). Sorghum can be grown in low fertility, moderate acidity and high alkaline soil, but gives best yield is in fertile, well drained soils having pH ranges 6.0–6.5 while frost, shade and sustained flooding effects its yield (Clark, 2008; Food and Agriculture Organization [FAO], 2012). A vast range of sorghum cultivars has been introduced round the globe depends upon yield, dry matter, morphological traits and quality parameters (Borrell et al., 2014). In Pakistan Rari-S-4, SPV-462, CSV-15, RS-29, PARC-SV-10, YSS-9, PARC-SS-2, Johar (Hussain et al., 2011), JS-2002, JS-263, MR Sorghum-2011, Hegari, Pak-China-1, Sandal Bar, F-7017 and F-114 (Rana et al., 2014) are cultivated on a vast area. According to a study conducted by Hussain et al. (2011) among all cultivars, SPV-462, CSP-15 and Johar have higher grain yield. Sorghum varieties released in different regions of southern Africa are Phofu, Mahube, Mmabaitse, BSH1 (Botswana); Pirira 1, Pirira 2 (Malawi); Tegemeo, Pato (Tanzania); SV-1, SV-2 (Zimbabwe) and Macia, Mamonhe (Mozambique) (Olembo et al., 2010). According to ICRISAT (International Crop Research Institute for the Semi-Arid Tropics) during 1975 to 2011 there are 250 improved cultivars available in Asia (83), Africa (132) and America (35). The top three individual country beneficiaries from ICRISAT research and materials are India (41 cultivars) followed by Mali (33) and China (24) (Charyulu et al., 2013).

2.3. Utilization as/in food products

Among the countries of Asia and Africa its grains are used as food in the form of flat breads and porridges by 55% and as feed (33%) in America (Muui et al., 2013). Above 35% of sorghum is cultivated primarily for human consumption worldwide (Okpala et al., 2013). Sorghum is most amenable cereal and can be used after processing levels including primary (boiling, fermentation, milling, popping and roasting), secondary (beverages preparation, brewing, baking and steaming) and tertiary (fortification of sorghum with artificial and natural additives as well as making composite flour with other cereals) (Olosunde et al., 2015). Processed sorghum seeds and flour are main sources of proteins for a large number of the

population (Raihanatu et al., 2011). The main foods produced by sorghum includes thin porridge (Africa and Asia), stiff porridge (West Africa), injera and bread (Ethiopia), traditional beers (Africa), baked products (USA, Japan, and Africa) etc. (Dicko et al., 2006).

Sorghum cultivars are being used in a variety of food products. White sorghum is used in different products such as cookies, snacks and ethnic foods (Pontieri et al., 2010). This flour is very useful in food products as it does not contain strong flavor and color so, for these reasons this is more desirable over maize flour (Pontieri et al., 2013). White sorghum is used as substituent of wheat in the America by the peoples having wheat gluten allergy (Ratnavathi & Patil, 2013). In Africa sorghum varieties having moderate content of tannin are used as staple food and in alcoholic beverages (Schnitzenbaumer, 2013). In Asian and African countries, sorghum has been used traditionally in a vast range of products such as couscous, snacks, malted beverages and fermented or unfermented breads (Saleh et al., 2013). In developed countries, there have been attempts to introduce sorghum into the food market (Dahlberg et al., 2011). Black sorghum containing tannin might be more advantageous in products for the health market (Casas Moreno et al., 2015). Brown sorghum is used in cakes, cookies, chocolate and muffins (Abdelghafor, 2015). Sorghum has been recognized to have a considerable potential to be used in numerous beverages and a variety of food products for humans (Iqbal & Iqbal, 2015). In many countries like India and Sudan sorghum is consumed as whole grain like rice (Awika & Rooney, 2004; Iqbal & Iqbal, 2015). In developing countries sorghum bread is used as an alternative to wheat. It is suggested that by the addition of xanthan gum to sorghum flour the characteristics of wheat flour has been developed (Padalino et al., 2016).

According to a research by the consumption of sorghum grains there is a positive effect on health. Based on the study of sorghum food products are not toxic for celiac patients (Pontieri et al., 2013). The effects of different sorghum hybrids on food characteristics were studied by developing several gluten free sorghum products (de Oliveira Pineli et al., 2015). Sorghums bran contains high levels of antioxidants (Dykes & Rooney, 2007). Antioxidants are defined as any substance that delays, prevents or removes the oxidative damage from a target molecule either by stabilizing or deactivating free radicals before they interact with the cells (Halliwell, 2007). Food items having functional properties are very important for the human health, especially for the optimum growth of the growing children (Omueti et al., 2009). In

developing countries low energy and nutrient density in weaning foods is a major cause of improper growth and undernutrition among newly born and young children (Mosha & Vicent, 2005). High quality foods have high nutrient density, low viscosity, low bulk density and appropriate texture along with high energy, protein and micronutrient contents. It should have a consistency that allows easy consumption (Balasubramanian et al., 2014). Sorghum is being used in weaning programs after fortification with legumes. Sorghum and soybean blend has shown improvement of protein, ash and carbohydrate contents of weaning food quality (Menure, 2017). Weaning food prepared from germinated sorghum appeared to be a promising food with improved energy and nutrient density (Tizazu et al., 2010). Sorghum based weaning food supplemented with legume and oil seeds produced blends with high protein and energy along with improved functional and sensory characteristics (Asma et al., 2006). Weaning foods prepared from germinated and fermented maize, sorghum and soybean showed improved nutritional quality and balanced amino acid profile (Kunimboa et al., 2015).

2.4. Health benefits of sorghum

Amongst cereals based diets, sorghum has momentous decrease in plasma cholesterol and non-HDL-cholesterol fractions of plasma due to low soluble fiber content i.e. β-glucans (Burdette, 2007). Moreover, increase in consumption, sorghum lipids up to 5 % significantly reduced the plasma triglycerides (Lee et al., 2014). Epidemiological data regarding the mortality rate due to cardiovascular complications quantified the lower whole grain consumption (Aune et al., 2016). The beneficial effects of cereal brans are subsidizing by sterols while the other components of whole grains such as fiber and polyphenols also have major role in averting cardiovascular complications. In this consequence when there is a limitation of *in vivo* studies, low-tannin sorghum grains fed to guinea pigs at 58% of diet caused significant cholesterol lowering effect as compared with rolled oats, wheat and pearl millet (Kaur et al., 2014).

The pharmacological aspects of sorghum wax concentrated on the pericarp surface of the grain also has peculiar health related properties because of fatty aldehydes (46%), hydrocarbons (0.7%), triacylglycerol (1%), wax and sterol esters (1.4%), fatty acids (7.5%), fatty alcohols (41%) (Hwang et al., 2002; Taylor et al., 2006). Therefore, sorghum wax fatty alcohol is characterized by the primary long-chained alcohols classified as policosanols whereas wax esters presence identified as ‘‘long-chained lipids’’ (Hwang et al., 2002).

Sorghum kernels contain 0.2–0.3% of long-chained lipids ,4–5% acids, 37–44% policosanols and 44–55% aldehydes (Adhikari et al., 2006). In addition, they also contain approximately 800 ppm policosanol contents. It has been sanctioned that mixed alcohols encompassing both octacosanol and triacontanol which are present in sorghum kernels and dried distillers' improve the LDL/HDL ratio by raising the high-density lipoprotein (HDL) and lowering the low-density lipoprotein (LDL) (Hargrove et al., 2004). Findings of several recent studies are supporting the claims that long-chain fatty alcohols, aldehydes and acids are interconverted in cellular metabolism, so they might lower the cholesterol. Policosanols are useful source for therapy and CVD hindrance (Rizzo, 2014).

AGE products can lead to activation of different reactions linked to the pathogenesis of diabetes such as production of nuclear factor-kB, pro inflammatory cytokines and reactive oxygen species Advanced Glycation End product (AGE) formation is introverted by *in vitro* analysis of ethanolic sorghum extract (Stefoska-Needham et al., 2015). Furthermore, nexus between sorghum bran and rice as well as wheat and oat bran extracts was more valuable in reducing the AGE products formation (Burdette, 2007). Sorghum tannins are complex compounds formed by binding of carbohydrates and proteins which cannot be further fragmented through digestive enzymes therefore without drastically affecting the nutrients absorption (Awadelkareem, 2015). Digestion enzymes such as trypsin, amylase, chymotrypsin directly binds with tannins progressively slow down digestion (Barrett et al., 2013). They also bind with intestinal brush-border bound amino acid transporters thus rendering their activity (Awika, 2011). Indeed, sorghum cereal due to their slower digestibility or 'staying power' in the stomach different ethnic groups in Africa give preference to utilization of high-tannin sorghum varieties (Proietti et al., 2015).

The integral treatment for coeliac disease is completely abstain the consumption of wheat, barley and rye (Troncone et al., 2008). Sorghum is not closely associated with *Triticeae* cereals and considered safe food for celiac patients (Balakireva & Zamyatnin, 2016). Meanwhile the phenolic contents in substantial levels may be present in sorghum grains hence it is scrutinized a good raw material for gluten-free baked products like cakes, snacks , bread and pasta (Kaur et al., 2014). Dykes and Rooney (2006) summarized health endorsement properties of sorghum in functional foods as nutraceuticals and its antioxidant activity. It was found that as compared to other cereals like rice bran (78%) and soybean (13%), the distillery

residues of dietary tannin-sorghum inhibited 63–97% oxidation of linoleic acid (Zaroug, 2015). Consequently, during winter sorghum residues maintenance of normal blood fluidity averted red blood cells hemolysis by enhancing the integrity of erythrocyte membrane and blood thinning of fish blood cells. The pharmacological health appraisal of sorghum residues are due to its antioxidant and anti-inflammatory properties of the tannins and different polyphenols (Mathanghi, 2012). The vigilant potential of sorghum again cancer prevalence designated through several epidemiological studies (Stefoska-Needham et al., 2015). The anti-carcinogenic properties of sorghum bioactive moieties have been resulted through *in vitro* studies. Sorghum tannins demonstrated positive melanogenic activity and anti-carcinogenic activity against human melanoma cells by decreasing the development of human melanoma colony cells (Barh et al., 2013). Worldwide, consumption status of sorghum persistently reduced incidences of esophageal cancer (Jideani et al., 2014).

2.5. Nutritional value of sorghum

Nutritional composition of sorghum grains differs and depend upon cultivar, genotype and the environmental circumstances. Sorghum is a good source of several micro and macronutrients along with also contain satisfactory level of antioxidants. It has similarity with other grains like rice, wheat and corn regarding its nutritional and chemical composition. 100 grams of sorghum grains are source of energy value ranged from 296.1 to 356.0 kcal (Martino et al., 2012; United States Department of Agriculture [USDA], 2012). Worldwide in different countries people do not have enough food supply thus suffer from issues of food-insecurity therefore they make sorghum as a part of their diet and they also have idea about how to use it in different ways. Due to having complex carbohydrates it's a good way of getting food energy (Kahlon & Chiu, 2014). Similar to other grains (rice, corn, and wheat) sorghum contain polysaccharides as the major constituent along with starch and non-starch, protein and lipid (Martino et al., 2012; USDA 2012). Regarding nutritional aspects sorghum grain has similarity with maize grain but in size sorghum grain is smaller than of maize (Holding, 2014). Yellow endosperm and occurrence of xanthophyll in sorghum grain are other exciting feature regarding increased nutrition of sorghum (Iqbal & Iqbal, 2015). Both aleurone layer and germ of sorghum also composed of other nutrients like proteins, lipids, vitamins, minerals and enzymes. Thus, presence of B-complex vitamins, carbohydrate with high energy value declare

that due to existence of innate protein and several other dietary proteins sorghum can be applied in protein's needed purposes.

2.5.1. Polysaccharides and carbohydrates

Sorghum is similar to maize regarding its nutritional composition, in addition to the antioxidants and resistant starch present in it. Major component present in sorghum is starch then followed by protein, non-starch polysaccharides and fat (Sarwar et al., 2013). Mesocarp layer of the pericarp contained little contents of starch, major contents exist in the endosperm. Sorghum grains composed of two types of starch polymers linear amylose (20-30%) and highly branched amylopectin (70-80%) (Kaufman, 2014). Some waxy sorghum types mainly composed of amylopectin only (Ahmed et al., 2016). Non-starch polysaccharides are mainly present in the pericarp but also found in small amount in the cell walls of endosperm. ß-glucans and arabinoxylans are the main components of these non-starch polysaccharides (Stefoska-Needham et al., 2015). Sorghum is composed of about 6.5% resistant starch (Niba & Hoffman, 2003; Stefoska-Needham et al., 2015).

The amount and composition of starch such as polysaccharides is greatly depending upon genetic characteristics of the sorghum grains along with the environmental conditions (Hill et al., 2012). Few varieties of sorghum grain contain 32.1 and 72.5 g /100 g of polysaccharides which are composed of amylopectin (81.0-96.5%) and amylose (3.5-19.0%) (Shegro et al., 2012; Udachan et al., 2012). Variation in composition of amylose and amylopectin effects the physic-chemical properties like gelatinization, gelling and retro-gradation, additionally digestibility of sorghum starch also improved (Singh et al., 2010). As there is a strong association between starch granules, proteins and tannins, therefore starch has lowest digestibility as compared to other cereals (Barros et al., 2012; Mkandawire et al., 2013). Starch of the sorghum is overall divided into three categories depending upon its digestibility such as slowly digestible starch comprising 30.0-66.2%, secondly is quickly digestible comprising 15.3-26.6% and third is indigestible comprising 16.7-43.2% to overall the starch (Mkandawire et al., 2013). Besides these starchy proportion, non-starchy compounds are also present in the sorghum which includes water insoluble dietary fibers (75.0-90.0%), arabinoxylans present in abundance, and water soluble dietary fibers (10.0-25.0%) (Martino et al., 2012; USDA, 2012).

2.5.2. Proteins and total free amino acids (TFAAs)

Fewer amount of protein also present in sorghum which is classified into two groups' i.e. prolamins and non-prolamins. Among these proteins prolamins present in major amount i.e. 79% while among non-prolamines mostly included albumins, globulins, and glutelins, and their concentration is 7 to 15g/100g (Martino et al., 2012). The kafirins are the major example prolamins which are present in sorghum and these proteins are further subdivided into three major groups' i.e. α-kafirins (66-84%), β-kafirins (8-13%) and γ-kafirins (9-21%) (Mokrane et al., 2010). Kafirins proteins are spherical in shape and are stored in endoplasmic reticulum. δ-kafirins are present in the inner region in the encapsulated form while β and γ-kafirins are peripheral protein (Wu et al., 2013). Digestibility of these proteins depends upon their conformation. The digestibility of the protein of other cereals like wheat and maize is higher than protein of sorghum especially cooked protein (Moraes et al., 2012). The main reason for low digestibility of sorghum protein is the formation of disulfide bonds which make it resistant to peptidase (Belton et al., 2006). Another reason to reduce digestibility of sorghum protein is presence of phenolic contents which react with kafirins and reduce its digestibility 50% (Taylor et al., 2007). Other factors may also influence the digestibility such as interaction of protein with non-proteins for example reactions of starch, non-starchy compounds, lipids and phytate, additionally endogenous factors may also reduce their digestibility such as nature and organization of proteins within the sorghum grain (da Silva et al., 2011; Ezeogu et al., 2008). Besides these factors few processes such as fermentation and germination may enhance the digestibility of the proteins up to two times (ELKhier & Abd-Al Raheem, 2011).

During the processing of sorghum grain amount of digestible protein such as glutenins and albumins increased while amount of indigestible proteins like kafirins, especially β and γ forms are reduced intentionally (Kumar et al., 2012; Wu et al., 2013). Various genetically modified varieties of sorghum had been developed which have up to 23 to 102% higher digestibility than other varieties (da Silva et al., 2011; Kumar et al., 2012). The α-kafirins reduce the nutritive value of sorghum grain as it didn't digest easily in human digestive system (Wu et al., 2013). Cysteine is a sulphur containing amino acid is richly found in the β and γ-kafirins and it has ability to form di-sulphide bond therefore reduces the accessibility of α-kafirins to enzymatic degradation (Wong et al., 2009). Digestibility of these proteins could be improved by changing the protein morphology along with reduction in disulfide bond in these

indigestible protein (Mehlo et al., 2013). Chemistry of the sorghum protein reveals that it is rich in glutamic acid and nonpolar amino acids such as proline, leucine, and alanine, while lysine is its major limiting amino acid (Mesa-Stonestreet et al., 2010; Moraes et al., 2012).

However, sorghum protein may be deficit in five essential amino acids namely methionine, cysteine, isoleucine, valine, and threonine, which play important rule in human nutrition (Moraes et al., 2012). Recently such varieties had been developed by the researchers which contain 52 to 115% more lysine than other varieties (Kumar et al., 2012; Taylor & Taylor, 2011). These varieties contain high amount of lysine which characteristically reduces the levels of kafirin protein, on the other side increase the lysine rich non kafirin protein in the sorghum grain (Shewry, 2007). Besides these, kafirins have an important function that it didn't produces the allergic reactions in consumers while protein present in other cereals grains like wheat containing gliadin, rye containing secalin, and barley which is rich in hordein may trigger the allergic responses in the consumers (Mesa-Stonestreet et al., 2010). Sorghum grain contain proteins which didn't produce any toxic or allergic effect such as gliadin, which have been proved through various studies (Pontieri et al., 2013). Therefore, sorghum-based diet is safe for the people suffering with celiac disease.

Nutritive value of food, specially linked with protein not only relied on amino acid profile but also depend upon concentration of essential amino acids. Pattern and quantity of essential amino acids is also used determination of quality in nutritional protein (Afify et al., 2012c). Food components contained amino acids in both free form or bound form either peptide linked or through non-peptide bonded polymers. Total amino acids present in food can be analyzed through hydrolyzation of proteins by following different methods that depend upon stability of amino acids (Peace & Gilani, 2005). Raw sorghum contains free amino acids within range of 0.66 to 1.03 mg/g. Germination process increased their contents ranged from 8.78 to 9.94 mg/g and this increase may be occurred due to the action of proteolytic enzymes (Afify et al., 2012c). Chavan et al. (1981) found 1.20 mg/g of free amino acids content in sorghum after 72 h of germination. Free amino acids of sorghum increased by 2 folds at 3rd day of germination. Germination caused degradation of the stored protein to free amino acids by hydrolytic enzymes to meet the seed requirements and the embryo growth. Protein degradations indicated the interference with metabolic systems work on reserve protein by the enzymes proteases (Yang et al., 2016). In another study concentration of amino acids were discovered in the seeds

of sorghum. And it was observed that steeped samples were proved to be best in total amino acids (57.71 g/100 g crude protein) compared to germinated (53.37 g/100 g c.p.) and raw (37.91 g/100 g c.p.) (Adeyeye, 2008).

2.5.3. Lipids

Sorghum grain contains little amount of lipids in range of 1.24 to 3.07 g/100g. These lipids are rich in unsaturated fatty acids which are in the range of 83 to 88% (Martino et al., 2012). Polyunsaturated fatty acids are in higher amount as compared to mono-unsaturated fatty acids (Hadbaoui et al., 2010). Sorghum is also a rich source of some fatty acids including linoleic acid (45.6-51.1%), oleic acid (32.2-42.0%), palmitic acid (12.4-16.0%), and linolenic acid (1.4-2.8%) (Afify et al., 2012b; Hadbaoui et al., 2010). It has been discovered through several research studies that sorghum grains composed of higher contents of fat as compared with wheat grains. Sorghum germ is composed of approximately 3% content of crude fat primarily consisted of polyunsaturated fatty acids (Lindsay, 2010).

2.6. Bioactive compounds: a sorghum plus

Phytochemicals essential cellular, non-nutritional bioactive compounds are present in all type of grains, fruits, vegetables. They are linked with reducing the danger of various long-lasting diseases (Kiran et al., 2012). So far more than 5000 phytochemicals have been recognized but still large number of these compounds is not recorded (Chandra et al., 2012). Sorghum composed of different phytochemicals is a good source of these superior constituents including anthocyanins, phenolic acids, policosanols and sterols according to genotype of sorghum (Althwab et al., 2015). Due to different possible health benefits provided phytochemicals like antioxidant activity and cholesterol lowering ability phytochemicals have attained more consideration than before (Nasri et al., 2014).

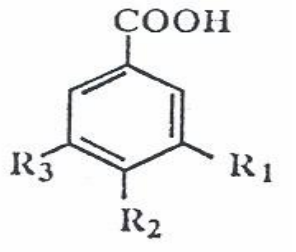
2.6.1. Phenolic compounds

Phenolic compounds are the mostly studied and important group of phytochemicals. Mainly sorghum bran is composed of high contents of phenolic compounds in bran portion (Awika & Rooney, 2004; Burdette, 2007). All varieties of sorghum are composed of phenolic compounds but have variations in contents according to genotypes and environment (Dykes et al., 2005; Wu et al., 2016). Phenolic compounds are basically classified into phenolic acids and flavonoids (Afify et al., 2012a).

2.6.1.1. Phenolic acids

Phenolic acids can be classified into two groups namely hydroxybenzoic acid and their derivatives and hydroxycinnamic acid along with their derivatives (figure 1). High antioxidant activity of these compounds were investigated in various studies and therefore proved beneficial for human health (Kamath et al., 2004). Phenolic contents in the sorghum are in the range of 135.5 to 479.40 µg/g (Chiremba et al., 2012), among phenolic compounds the protocatechuic is present in larger amount i.e. in the range of 150.3 to 178.2 µg/g, while ferulic acid present in the range of 120.5 to 173.5 µg/g along with little amount of the p-coumaric (41.9 to 71.9 µg/g), syringic (15.7 to 17.5 µg/g), vanillic (15.4 to 23.4 µg/g), gallic (14.8 to 21.5 µg/g), caffeic (13.6 to 20.8 µg/g), cinnamic (9.8 to 15.0 µg/g), and p hydroxybenzoic (6.1 to 16.4 µg/g) acids (Svensson et al., 2010). Phenolic acids present in vegetables and fruits have good bioavailability as their major amounts present in these entities are either as free or as combined form with such a compound which could be easily hydrolyzed in human gastrointestinal track (Hole et al., 2012). While phenolic acids present in cereals including sorghum are in bound form with lignin or arabinoxylans chains which caused their unavailability (Abdel-Aal et al., 2012). Therefore, such bounded forms could not be hydrolyzed by the human digestive system and remain intact until reached in colon where these compounds are fermented by the microbes (Saura-Calixto, 2010).

A. Benzoic acid derivatives

Benzoic acid derivatives	**Functional group position**				**Structure of benzoic acid**
Name of acids	**R_1**	**R_2**	**R_3**	**R_4**	
Gallic	H	OH	OH	OH	
Protocatechuic	H	OH	OH	H	
p- hydroxybenzoic	H	H	OH	H	
Gentisic	OH	H	H	OH	
Salicylic	OH	H	H	H	

B. Cinnamic acid derivatives

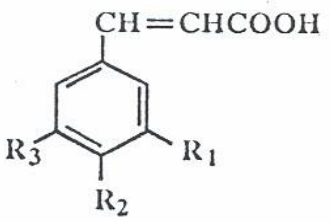
Cinnamic acid derivatives	Functional group position			Structure of cinnamic acid
Name of acids	**R_1**	**R_2**	**R_3**	
Caffeic	OH	OH	H	
Ferulic	OCH_3	OH	H	
p-coumaric	H	OH	H	
Sinapic	OCH_3	H	OCH_3	

Figure 1. A & B phenolic acids reported in sorghum (Adapted from Sikwese, 2005).

2.6.1.2. Flavonoids

Outer layer of the sorghum grain is a good source of flavonoids. Concentration and profile of the flavonoids is varies depending upon the color and thickness pericarp and/or the presence of the (Dykes et al., 2009). Genetic and various environmental factors attribute the physical characteristics of the sorghum grain (Taleon et al., 2012). Anthocyanins, flavones, and flavanones are the three major classes of the flavonoids which are majorly present in the sorghum grain (figure 2). Up to 79 % of the total flavonoids content is anthocyanins which primarily belongs to the class of 3-deoxyanthocyanidins (Shih et al., 2007; Taleon et al., 2012). On the other hand, little amounts of methoxylated 3 deoxyanthocyanidins for example 5-methoxy-luteolinidin, 7-methoxy-luteolinidin, and 7 methoxy-apigeninidin are also present in the sorghum grain along h methoxylated 3 deoxyanthocyanins i.e. 5 methoxy-luteolinidin 5-glucoside, 5-methoxy-luteolinidin 7-glucoside, 7 methoxy-luteolinidin 5-glucoside, and 7-methoxy-apigeninidin 5-glucoside additionally few other non-methoxylated 3-deoxyanthocyanins such as apigeninidin 5-glucoside and luteolinidin 5-glucoside were also observed (de Morais Cardoso et al., 2014).

The color and anti-oxidant activity of the sorghum is attributed to sorghum 3-deoxyanthocyanidins (Awika & Rooney, 2004). Varieties of the sorghum which contain pericarp and black colored testa contain 3 to 4 times higher amounts of the total 3-deoxyanthocyanidins (5.4 to 6.1 mg/g) when compared with other red and brown colored varieties of sorghum grain (1.6 to 2.8 mg/g) (Awika et al., 2004). It was stated that bran of black sorghum contains more concentration of anthocyanins as comparative to other fruits (blueberries, grapes and strawberries) thus showed higher antioxidant functions. Thus, direct relationship of anthocyanin contents and antioxidant properties established black sorghum a

good source regarding antioxidant activities (Kumari et al., 2013). Black sorghum bran also composed of high level of flavan-4-ols compared to other sorghum types which mostly contain dominately apiforol and luteoforol (Schoeman, 2014). Aglycone type of luteolin and apigenin overcome the sorghum grain while total flavones ranged from 0-386 µg/g (Dykes et al., 2011). Among flavanones present in sorghum, the aglycone forms of eriodictyol and naringenin are major one (Dykes et al., 2009; 2011). White colored varieties of the sorghum contain smallest number of flavones while largest amount of these compounds was found in lemon-yellow pericarp i.e. 474-1780 µg/g (Dykes et al., 2011).

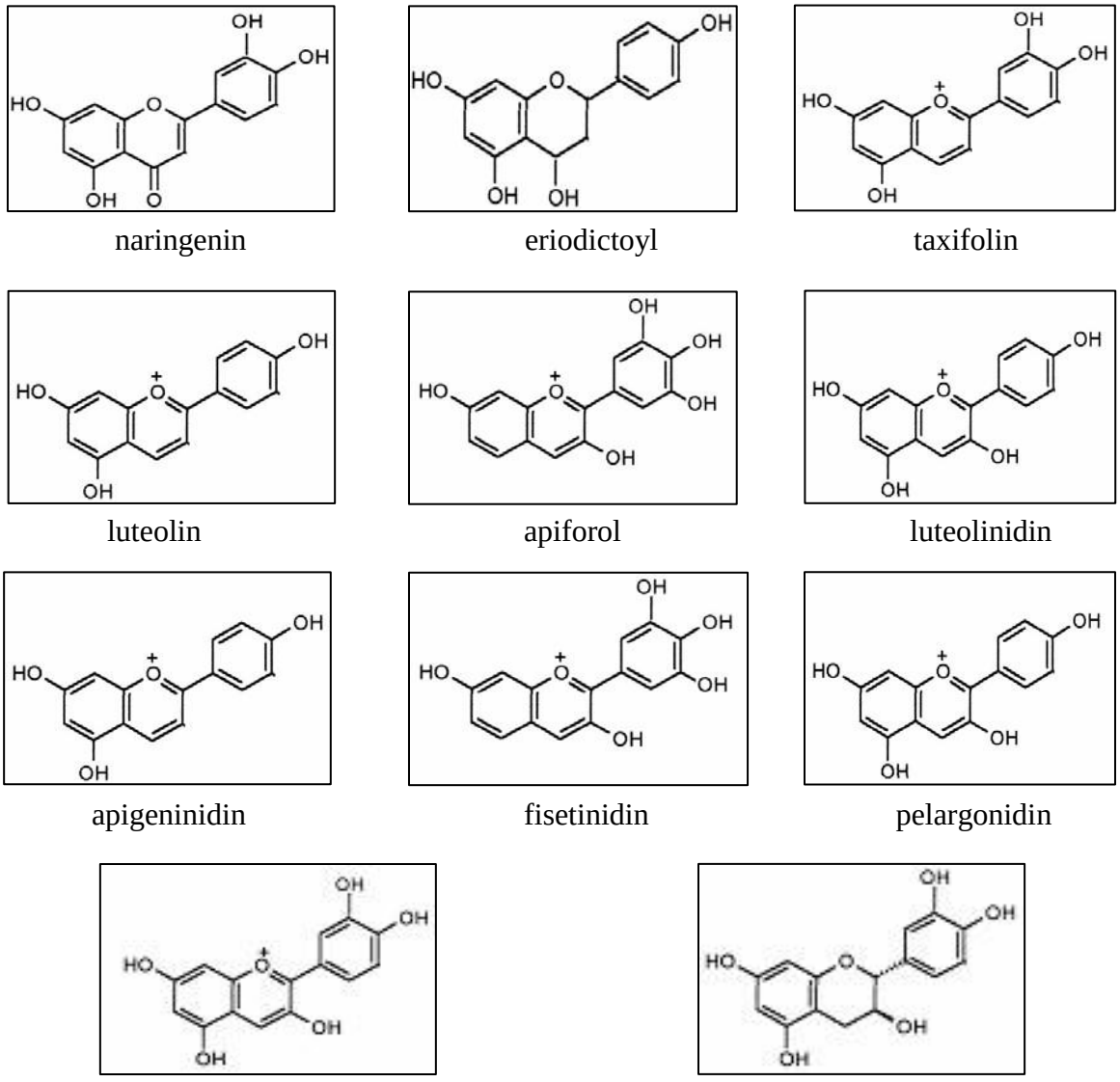


Figure 2. Some major flavonoids reported in sorghum [Adapted from Awika and Rooney (2004)].

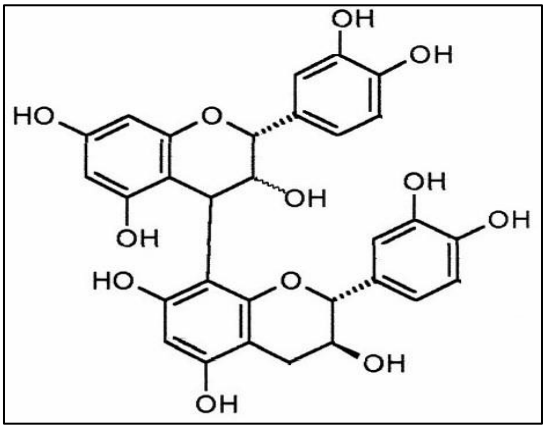


Figure 3. Structure of condensed tannin [(Adapted from Awika and Rooney (2004)].

2.6.2. Polycosanols and sterols

Polycosanols are the examples of long chained lipids mainly extracted from the kernel of sorghum grain and are present in the range of 33.4 to 44% (Hwang et al., 2005). Polycosanols have many physiological functions and sorghum is the main source of these compounds. Various amounts of total polycosanols are found in polished or unpolished sorghum grain i.e. 74.5 mg/100g in the unpolished dry kernel while 9.8 mg/100g in polished in the dry kernel (Hwang et al., 2005). The concentration of sorghum sterols is present in the range of 4.13 to 24.45 µg/g, on dry weight basis, and mainly depends upon environmental conditions during its growth (Chung et al., 2013). As compared to fruits, vegetables, and other cereal grains sorghum is the rich source of sterols in the food supply. Three main types of sterols are found in the sorghum namely sitosterol (44.8 to 48.2%), campesterol (26.1 to 38.0%) and stigmasterol (17.3% to 25.6%) while 200 types of sterols are present in vegetables (Delgado-Zamarreño et al., 2009; Ye et al., 2010). Sterols perform as competitor regarding absorption of cholesterol in GI tract thus has efficiently decrease the cholesterol level (Brauner et al., 2012). Cereal grains mainly contained these compounds in their wax and bran portions (Burdette, 2007). Corn and sorghum contain the sterols content as 0.9 mg/g and nearly 0.5 mg/g respectively (Liangli et al., 2012). Sterols may occur in grains in several forms including as free forms, conjugated form with sugars esters of fatty acid esters. Different types of free sterol are recognized in sorghum like campesterol, sitosterol and stigmasterol (Lee et al., 2011). Other kinds of sterols esterified with fatty acid and ferulates were also analyzed in sorghum grain (Lee et al., 2011; Singh et al., 2003). Cereal grains also contain stanol other kind of sterols. Stanol are not frequently studied compounds but they also provide same health

benefits as sterols. Ostlund Jr (2002) stated that 10% of sterols in diet contained stanols (Racette et al., 2009).

2.7. Anti-nutritional factors

Idris et al. (2007) reported that antinutritional factors (i.e. alkaloids, saponins, tannins and phytate) formed complexes by binding the food constituents which influenced on the human health. Moreover, the alkaloids, saponins, tannins, and phytate decreased the digestibility of proteins and carbohydrates by reducing the activity of enzymes (Mohammed et al., 2010) and also influenced on the absorption of divalent minerals into a living system. Germination process altered the grains physiochemical properties, taste and shapes which resulted in soft kernel, enhanced the nutrients value and decreased the anti-nutrients. It has been postulated that germinated cereals/pseudo-cereals or sprouts were more nutritious as compared to the cereal and pseudocereals grains (Rozan et al., 2000)

2.7.1. Alkaloids

Alkaloids are being used in defensive mechanisms against herbivores. Presence of alkaloids linked with bitter taste and toxicity when consumed by the humans and animals. Presence of alkaloids also impaired the protein metabolism process (Clements et al., 1996). However, many beneficial process are also associated with presence of alkaloids in plants such as these compounds are accumulated in underground parts of a plant, take part in metabolic processes, initiate root growth and leach down in to the soil, which cause a barrier to microorganisms (Peneva, 2006). It had long been reported that alkaloid contains plants had been used as dyes, spices, drugs or poisons and also well known for their CNS activities (Lewis & Elvin-Lewis, 2003). Higher concentration of alkaloid contents was reported in *S. bicolor* (1.63 mg.100 g-1) followed by *T. aestivum* (1.26 mg.100 g-1) and *P. typhoides* (1.13 mg.100 g-1) (Okwu & Orji, 2007b). The reduction in alkaloids, during the initial phases of hydrolysis of germination and the gradually increase during the early synthesis phase, follows the pattern of the primary food reserve in the seed and showed that alkaloids are easily take part in the metabolism and are perhaps not metabolic end products (Kamal & Ahmad, 2014). Different processing techniques (cooking, fermenting, soaking and germination) were investigated in the lupin bean samples. It was determined that the range of the percentage alkaloids present in the unprocessed and processed sweet bean was from 1.76 – 0.31% while in case of bitter lupin bean it was from 6.03% to 3.78% therefore, from these results it can be concluded that

processing technologies reduced the significant level of alkaloids after treatments. Similar results were achieved after cooking, fermenting and soaking. On the other hand, after germination significant higher amounts of alkaloids were observed in lupin seeds (Abeshu & Kefale, 2017).

2.7.2. Phytates

Phytate reduced the availability of the minerals to the human and sorghum contained phytate like other grains. The levels of phytate in sorghum are same as reported in wheat, barley and maize, but showed lower levels as compared to soybeans and other oilseeds. Waniska and Rooney (2000) suggested that the supplementation required with other mineral sources in those community where sorghum is a major part of their diet because the mineral content in sorghum was low due to the presence of phytate. Cereal grains such as rice, maize, millet, sorghum and wheat reportedly contain phytate in the range of 5.58 to 6.23 mg g-1 (Azeke et al., 2011). Rice, maize and millet contains similar phytate while significant difference was observed in the phytate of sorghum and wheat. Mostly phytate contents were developed during the maturation plant seeds therefore it greatly affected by the different factors such as degree of maturation during harvest, genetics, environmental variations, locality, irrigation conditions, soil type, year, and fertilizer application (Wu et al., 2009). It was observed that products of legumes contain higher amounts of phytate as compared to cereal foods. Significant amount of dietary phytate was reported in cereal grain. It has been reported that single molecules of phytate-bound with six divalent cations and metal bound at least two molecules of phytate, associated with redox reaction (Graf & Eaton, 1990). Germination process reduced the phytate by using an enzyme (phytase). During germination process of wheat, barley, rye, and oats, phytase enzymes hydrolyzed the phytate to phosphate and myoinositol phosphates (Larsson & Sandberg, 1992).

2.7.3. Saponins

Saponins are naturally present in plants. Saponins are categorized as steroidal saponins, triterpenoid saponins and steroidal glycoalkaloids (Oyekunle et al., 2006). Saponins are classified as either triterpenoids, steroids, steroidal glycoalkaloids because these compounds are glycosylated. Triterpenoid saponins (biologically active) were reportedly act as defenses secondary metabolites in plants (Buchanan et al., 2000). Gemede and Ratta (2014) reported that saponins used as an antibiotic, antifungal, antiviral, hepatoprotective anti-inflammatory

and anti-ulcer in many scientific studies on medicine and drugs. Study conducted by Okwu and Orji (2007a) to evaluate the secondary metabolite constituents in different grains reported 0.18-0.20 mg/100g of saponin contents in sorghum grains. In chickpea saponin content was found to be 0.44 % which was reduced to 0.34% after germination (Mittal et al., 2012). The amount and allocation of these naturally occurring substances were depended on plant varieties, structure, growth and ripening. Differences in the saponin quantity, structure and distribution have been reported to helpful in plant protection (Moses et al., 2014). *J. curcus* seeds showed 58.17% reduction in saponins after germination (Arab et al., 2010). Heat processing of seeds resulted in formation of extractable complex between saponins and sugar or amino acids. Enzymic degradation is a possible explanation of the saponin lost during germination (Mittal et al., 2012).

2.7.4. Tannins

The variety of sorghum which contains pigmented testa is a good source of tannins while other cereal grains such as rice, wheat, and maize, didn't contain these compounds. Tannins present in sorghum varied based on type, quantity and their distribution. Based on tannin contents, sorghum can be classified into three classes such as type I i.e. no significant level in sorghum, while in type II those tannins are included which can only be extracted with acidified methanol and type III contain those tannins which can be extracted by using methanol and acidified methanol. Oligomers or polymers of catechins (flavan 3-ols and/or flavan-3,4-diols) condensed together to give rise to tannins in the sorghum (Awika & Rooney, 2004). Tannins found in sorghum usually contain high degree of polymerization than 10 and high molecular weight (figure 3) (Awika et al., 2003). Sorghum variety with black testa contain higher amount of tannins, usually in sorghum these compounds are present in the range of 0.2 to 48.0 mg/g (Dykes et al., 2013). Amount and type of tannins is greatly affected by the seasonal changes. Therefore, care should be given to the environmental factors before selection and breeding of sorghum variety to achieve the beneficial health effects (Mkandawire et al., 2013).

Plant tannins proved to be beneficial but reduced the bioavailability of other components including minerals, proteins, and starch present in the sorghum (Barros et al., 2012). The reduction in availability of these components is not only due to tannins but also with degree of polymerization (Kaufman et al., 2013; Mkandawire et al., 2013). The tannins

which shows this effect mainly have higher molecular weight or sometime highly complex structure usually have more than 10 DP (Barros et al., 2014; Mkandawire et al., 2013). Tannins with polymeric form made complex bound with starch due to strong attraction between them and therefore, made it resistant starch which is unable to digest by human digestive system (Barros et al., 2014). Tannins are proved to be 15 to 30 times more effective regarding anti-oxidant activity when compared to phenolic compounds despite their anti-nutritional effect.

Therefore, tannins have been extensively investigated for their beneficial effect on human beings. Oligomers of tannins were extensively studied for their functional effects in human beings as they are strong anti-oxidant (Beecher, 2004). Up to 19% antioxidant activity of oligomers were studied. And these oligomers proved to be health promoting as they show immunomodulatory, anticancer, antioxidant, antiradical, anti-inflammatory, vasodilatory, cardioprotective, anti-thrombotic, and anti-UV actions effect in *in vitro* and *in vivo* studies (Floegel et al., 2010). Oligomeric belongs to tannins and polymeric form of proanthocyanidins are not assimilated by animals as well as humans (Crozier et al., 2010). But few studies show that traces of dimers of B1 and B2 procyanidins were found in the plasma of human beings (Donovan et al., 2006). On the other hand, this low availability of tannins is not observed present in sorghum. Diversified compounds are produced from these indigestible tannins in the colon of human beings by the action of intestinal micro-organisms (Selma et al., 2009). Although there is lack of detail study but functional effects of the plant tannins are characterized due to their readily absorbed colonic catabolized products and phenolic acids (Crozier et al., 2010).

2.8. Effect of germination on bioactive compounds

Germination is a natural processing technique used for biological activation of grains to improve their nutritional and functional properties (Hefni & Witthöft, 2011). Germination technique can induce several changes in composition by several steps like breaking the nutrient complex, their return into circulation system and finally their accumulation in stored form. Complex nutrients like carbohydrate, lipid and protein is break down through germination into simpler smaller one like carbon and nitrogen. The resultant smaller molecules are further used for photosynthetic reactions and in plant growth mechanism (Theodoulou & Eastmond, 2012). Hung et al. (2012) reported that nutritional profile of germinated waxy wheat was more improved compared to non-germinated due to having high contents of dietary fiber, free amino

acid and phenolic compounds. According to other work, germination of wheat grains for 102 hours of germination at 20-25˚C, showed two times higher contents of α-tocopherol minerals (Ozturk et al., 2012). Similarly germinated wheat showed 3.6-times higher content of folate (Koehler et al., 2007). Afify et al. (2012a) subjected sorghum varieties (Dorado, Shandaweel-6 and Giza-15) to germinate for 72 hours; and results showed germinated samples have high level of free amino acids 8.78 to 9.94 mg/g compared to non-germinated 0.66 to 1.03 mg/g. Other research conducted on sorghum investigated 0.82±0.01 g/100 g of TFAA in raw sorghum which increased up to 1.66±0.07 g/100 g after 72 h of germination (Yang et al., 2016).

Protein contents of soaked and germinated soybean, sorghum and barley grains significantly increased from 29.09 % to 34.99 %, 7.25 % to 9.85 % and 11.25% to 13.85 % respectively (Sonone, 2014). It was reported by Tatsadjieu et al. (2004) that prolonged germination increased the protein contents of sorghum. Carbohydrates of sorghum starches after 5 to 7 days of sprouting were in the range of 79.39%, and 78.64% (Otutu et al., 2014). In another research continuous increment in carbohydrate contents were observed in sorghum grains as sprouted days increased (Mbaeyi & Onweluzo, 2010). Several anti-nutrient constituents like trypsin inhibitors, phytates, tannins were decreased up to significant level while health beneficial bioactive substances are improved. Thus, germination process is helpful in exerting good influence on human health through reducing the several diseases risks (Sangronis & Machado, 2007). Raihanatu et al. (2011) also observed a significant reduction ranged 2.08% to 14.58% of tannin content in five local varieties of sorghum after application of combined processing technologies (sprouting and fermentation). Nour et al. (2015) reported that raw sorghum flour contains 0.15 mg/100g of tannin, which was insignificantly ($p \geq 0.05$) reduced after sprouting up to 0.13 mg/100g. Similarly, in another investigation Chilomer et al. (2010) reported remarkable reduction in alkaloids contents of lupin sprouts during germination. Zheng et al. (2005) who studied germinated beans, lentils and peas along with cotyledons of germinated mung bean (*Phaseolus aureus*) seeds for their decreased alkaloid contents. Megat Rusydi and Azrina (2012), investigated that phytate contents of germinated soybean and peanut were significantly reduced 21.74% and 38.11% respectively as compared to non-germinated

Olawoye and Gbadamosi (2017) reported a significant difference of saponins in processed amaranth flour from 4.962 to 2.94 mg/100 g. Regarding phenolic substances effect

of germination has been studied in various edible grains. Through several studies in cereal grains it was shown that germination process could enhance the amount of solvent-extractable phenolic compounds. Various researchers reported that increased level of water soluble phenolic constituents through germination can be credited to *de novo* synthesis and transformation process (Kim et al., 2013; Tang et al., 2014). In different *in vitro* studies of cereal grains enhanced polyphenol levels and antioxidant action has been described like wheat (Hung et al., 2012), rye and sorghum (Donkor et al., 2012). Germinated buckwheat (Alvarez-Jubete et al., 2010) and germinated rice (Imam et al., 2012) were also reported to have increased total polyphenol content following germination. Wheat sprouts gave higher values of total phenolic compounds and showed increased TPC from 41,458 mg GAE /100g to 197,083 mg GAE /100g after germination (Elzamzamy, 2014). TPC of rice at various germination durations were remarkably enhanced after germination (Moongngarm & Khomphiphatkul, 2011). Zhang et al. (2015) showed that amount of total flavonoid content in buckwheat increased by increasing germination duration (4.17±0.11 to 11.69± 0.87 mg RE/g). It was also observed that concentration of these active compounds significantly altered as germinated for 48 to 72 h.

Phattayakorn et al. (2016) observed remarkably higher TFC in germinated Hang rice (92.66 mg CE/100g) when compared with non-germinated rice (3.66 mg CE/100g). Sorghum seed showed higher FRAP values after germination i.e. 4.26 - 4.88 mmol Fe^{2+} equiv/100 g DM. Therefore, it can be concluded that antioxidant capacity is depends upon both germination and time (Kayodé et al., 2013). Wheat and barley were also investigated for their antioxidant activity and higher (49.08% & 79.75% respectively). DPPH scavenging activity observed as compared to non-germinated which gave only 21.78% & 35.58% respectively (Elzamzamy, 2014). Afify et al. (2012c), investigated IVPD in raw sorghum ranged 50.94 to 52.09%, additionally, germination increased the protein digestibility up to 70 to 78%. In another study carried by Nour et al. (2015) sprouting effected IVPD% in raw sorghum flour (51.2%) which improved to 65.03% after sprouting. Native bio-accessible polyphenols of sorghum grain were 0.60 mg/g and after the sprouting little improvement was observed total bio-accessible polyphenols i.e. 0.64 mg/g. Native bio-accessible flavonoids of sorghum grain were 0.09 mg/g and after the sprouting 0.10 mg/ (Hithamani & Srinivasan, 2014a).

2.9. Pre-sowing seed processing techniques

For facilitating sowing process, protecting seeds and improving their growth and development in severe environmental situations, now different techniques had been established. Scientist recommended different techniques for improving germination according to performance of the seeds for temperature and water availability. These processing techniques can be grouped as physiological, physical and biological (Belal et al., 2013; Hanci et al., 2014). Different research work regarding these techniques is available. Halmer (2006) described practical aspects of seed treatment and classified it as conditioning, protection and physiological enhancements. Black and Bewley (2000) discovered mechanism regarding physiological enhancement mainly seed priming. Moreover, Pietruszewski and Martínez (2015) described effect of magnetic field regarding crop development and yield. To stimulate plant growth, as substitute of chemical methods physical methods appeals more consideration of agricultural producers. These physical methods more efficiently improve food quality without damaging its safety (Aladjadjiyan, 2011). The attention regarding use of physical techniques to stimulate plant growth and development has extended. Among these techniques most important are use of microwave and ultrasound process (de Sousa Araújo et al., 2016). Exchange of energy between the environment and cell is responsible for all living procedures. Introduction of energy in to cell is the main event of physical processing which generates situations for molecular transformations to make availability of essential materials to plant cell (Govindaraj et al., 2017). Application of these techniques in agriculture field will promote more stimulating and high production of enzymes, protein along with safety of environment (Al Mashhdani & Muhammed, 2016).

2.9.1. Ultrasound processing

At present for improving crop production and increasing their yield ultrasonic waves are also applied (Nazari & Eteghadipour, 2017). According to scientists, treatment of ultrasound can improve the seed inactivity through ultrasonic waves that is considered as an easy, safe and time-saving approach. Ultrasonic waves are form of mechanical waves with more than 20,000 Hz frequency while humans cannot read this range of frequency (Liu et al., 2016; Ramteke et al., 2015). These waves do not injure plant cells and tissue due to having less strong intensity. Germination of seeds and formation of seedlings are survival stages of plants in case of adverse environmental conditions (de Melo et al., 2015). Ultrasonic processing is an

exclusive and efficient techniques among already used methods of pre-sowing treatments. It's a unique method due to its simplicity, less cost, multipurpose and environmentally safe features (Goussous et al., 2010; Liu et al., 2016). It can stimulate the reaction rate thus has ability to change the nature of substances (Aladjadjiyan, 2007). It is one the novel physical methods inducing an alternative stress for cells and tissues through means of interaction in which acoustically persuaded cavitation mechanism is applied resulting in diverse chemical and thermal changes (Yaldagard et al., 2008c). Ultrasonic waves stimulate the degree of substances uptake by due to its mechanical reaction (Liu et al., 2016). All these reactions primarily generate through cavitational events resulting from production, growth and violent breakdown of microbubbles produced sonication liquid due to changes in pressure (Leong et al., 2011).

Effect of ultrasound treatment has been studied in various kind of seeds to accelerate their germination process such as barley, carrot, maize, radish, rice and sunflower (Wang et al., 2012; Yaldagard et al., 2008b). Results of the studies demonstrated that destructive or stimulating effects of sonication waves are influenced by various factors including type and variety of crop, frequency of sonication and exposure time duration (da Silva & Dobránszki, 2014; Machikowa et al., 2013). Sonication is applied in different crops to induce high and fast germination and growth like barley (Yaldagard et al., 2008a), Norway spruce (Rîşca et al., 2007), and other crops (Goussous et al., 2010). Optimum parameters of sonication can results into quick and uniform germination rate (Machikowa et al., 2013). Application of these waves cause alternations in cellular membrane resulting in enhanced nutrients absorption and transport within grains (Liu et al., 2016; Rîşca et al., 2007). Current research works have been discovered application of suitable intensity of sonication waves for proper time would boost the enzymatic reactions and improve growth by stimulating rate of several physiological activities occurring in cells (Awad et al., 2012; Liu et al., 2003).

Goussous et al. (2010) applied ultrasonic waves on vegetable seeds at 40 kHz for 5-60 minutes and observed high growth rate index of sonicated seeds. Rîşca et al. (2007) applied ultrasonic waves on Norway spruce seeds for 50 and 60 seconds and observed 40% increase in seed germination (40%), root length (32%) and shoot length (5-8%). Several studies stated that slight ultrasonic treatment improved the enzyme based reactions but according to some reports high intensity of sonication can reduce the activity of alpha-amylase (Yaldagard et al., 2008a; 2008b; 2008c). Stimulatory effect of ultrasound waves is linked with inducing high

fluidity rate of cell wall and membrane (Liu et al., 2003; Toth, 2012). Different studies showed that sonication treatment enhanced the enzymes based events activity, for example alpha-amylases contributing metabolic reaction of maltose and maltodextrins (Goussous et al., 2010; Yaldagard et al., 2008c). While unfavorable results were also observed resulting from adverse reaction of enzymes occurred due to disturbance of structural units of cells including membranes and the cytoskeleton (Shekari et al., 2015). Although ultrasound technique has been applied for different objectives like for enhanced germination and the increased germination has ability of improving production in field of biotechnology and the food industry (Yaldagard et al., 2008b).

2.9.2. Microwave processing

According to available work in literature microwave radiations had shown both good and adverse results regarding germination in different plants. Therefore, it is proposed that stimulatory effects of microwave treatment depend upon different factors like exposure time duration, radiation frequency and also environmental circumstances (Aladjadjiyan, 2007; 2012). In some studies application of radiation with high power level did not promote seed germination. Use of medium power level of radiations exhibited enhanced germination rate, development and also produced high level of enzymes (Al Mashhdani & Muhammed, 2016). Rajagopal (2009) exposed grains of wheat, barley and rye to microwave power of 650 W, 2.45 GHz for 30 seconds and observed their higher germination rate. Similarly, Aladjadjiyan (2010) treated lentil seeds for 5, 10, 15, 20 and 25 seconds with progressively increasing intensity and temperature and in result higher activity of α-amylase was observed. Khalafallah and Sallam (2009) also examined the influence of microwave irradiation of (2.45 GHz) on wheat and obtained enhanced germination efficiency. Furthermore, in other study stimulatory effect on germination rate and growth parameters in various vegetable plant varieties was observed at high power level of microwave treatment (Radzevičius et al., 2013).

Regarding application in agriculture field microwave treatment has been employed mostly for disinfection the grains as pre-sowing treatment (Aladjadjiyan, 2010). Microwave radiations generate thermal properties when they interact with charged and polar molecules, and this thermal reaction induce the agitation action defined as heat (Puligundla et al., 2013). As the oscillating electric field absorb the energy which in result rise the temperature thus induce the thermal influences. After absorption microwave radiations cause alteration of

electron orbit in plant tissues, induce the movement of ions and revolution of dipoles leading to heating process (Crețescu et al., 2013). As compared to traditional methods of heating, microwave techniques is more effective as its fast and has discriminatory heating capacity (Sun et al., 2016). There is restricted number of research work explaining about microwave radiations influencing plants. All the biological constituents absorbs energy according to their dielectric properties (Buffer, 1993; Sun et al., 2016). Electromagnetic microwave irradiations were effectively applied for devastation of the microorganisms before storage in various seeds such as rice, soybeans and wheat (Aladjadjiyan, 2002).

Rajagopal (2009) also studied similar results in barley, rye and wheat grains regarding decontamination by using a pilot scale microwave at industrial level at 2.45 GHz. Aladjadjiyan (2010) treated soybean seeds with microwave radiations for 6 to 12 minutes and analyzed improved distribution of triglycerides in their seed coat. Chen (2006) treated *Isatis indigotica* seeds with microwave as pre-sowing treatment and stated their improved resistance in seedlings against UV-B stress, enhanced performance of various enzymes like catalase, peroxidase, and superoxide dismutase. Stimulatory action of microwave radiations with improved outcomes has been analyzed on bean (Aladjadjiyan, 2010) and some ornamental plant species (Aladjadjiyan, 2002). Treatment of wheat grains with microwave radiations also promoted enhanced germination ratee (Oprica, 2008). According to many researchers electromagnetic microwave irradiation has positively affected germination rate of seeds, growth and development of seedlings and meristem cells, chlorophyll level, respiration rate, enzyme bases and photochemical reactions (Hozayn et al., 2014; Qados & Hozayn, 2010).

2.9.3. Other processing techniques

The physical methods are way to enhance food quality without having any safety issue (Aladjadjiyan, 2011). With time, awareness regarding use of physical techniques to affect plant growth has increased (Soltani et al., 2006). Therefore, for improving and facilitating the germination process different physical processing techniques have been developed. All the physical methods import energy into the cells which is absorbed in molecules by the electrons. This absorbed energy is transformed in other form of energy which in result accelerates the different metabolic reactions and thus stimulation of plant development (Govindaraj et al., 2017). Possible approaches other than ultrasound and microwave include the treatment with magnetic field, plasma, gamma irradiation, laser irradiation and sound waves.

Exposure of plants to magnetic fields also results into the speedy germination, uniform establishment and crop yield (Pietruszewski & Kania, 2010). With enhancing the germination rate, growth and yield magnetic fields also decrease the outbreak of pathogenic diseases (Balouchi & Sanavy, 2009; De Souza et al., 2006). Use of MFs for seed invigoration includes finding the MF dose affecting to influence germination, seedling growth and consequently the yield (da Silva & Dobránszki, 2016). The MFs dose for exposure is the combination of its flux density and exposure duration. With stationary or changed magnetic field the flux density of MFs changes. Magnetic field treatment affects preliminary growth stages after seed germinated and further increases their germination % (Aladjadjiyan, 2002). Different studies discovered that use of magnetic-treated water for seed priming improved the seed germination rate and plant growth (Morejon et al., 2007). In addition to plant growth MFs had a positively affected the photochemical activities, respiration rate and enzyme reactions (Martinez et al., 2000). Ahmad et al. (2007) proposed that MFs-perception/signaling mechanism is mediated through the blue light photoreceptors called cryptochromes. Physiological mechanism behind the MFs treatment influencing the germination and growth is still not fully understood. However, the paramagnetic characteristics of chloroplast cause the stimulation of different metabolic activities in seed after MF treatment (Aladjadjiyan & Ylieva, 2003). In other study conducted by Racuciu et al. (2008) stated that some enzymatic activities were enhanced after application to magnetic field.

A new development in agriculture field related to germination and plant growth is application of plasma treatments (Hayashi et al., 2011; Klämpfl et al., 2012). Through several research work, scientists stated about improvement in germination and growth mechanism by plasmas with different gases (Jiayun et al., 2014). Different types of plasma treatments are adopted to improve seed germination and plant growth such as microwave, magnetic and atmospheric plasma (Zhou et al., 2011). Non-thermal plasma radiations as priming technique assisted in enhancing the plant development is used as substitute to scarification, stratification (Dhayal et al., 2006). After plasma application, thin layers of hydrophobic and hydrophilic nature are produced and these thin layers can be beneficial for various cultivation conditions and seed types (Volin et al., 2000). The seeds having high moisture can be vulnerable to imbibition chilling injury but hydrophobic coatings delay water absorption in seeds sown in cold and wet soil thus improve seeds viability by decreasing water uptake (Kavak & Eser,

2009). In other studies, water uptake and germination stimulation improved by increasing hydrophilicity and etching (Bormashenko et al., 2012).

Among infrared radiation (IR), gamma (γ) radiation is a high-energy form of IR produces from Cobalt-60 having ability of penetrating and interacting with biological material (Moussa, 2006). According to new developments in agriculture, depending upon the dose of IRs, gamma rays can positively affect product quality, yield of grains, precocity and salinity tolerance (Kiong et al., 2008). As compared to conventional seed invigoration techniques, IRs activate the different biochemical reactions in seeds without causing any DNA damage and disturbing their structural integrity (Bhosale & More, 2013). Among various IRs treatments, application of the γ-rays on seeds is now getting more consideration mainly focused on use of their reduced dose rate and/or total dose. At low dose γ-rays act as an actual 'priming' treatment thus boost up germination % and seedling growth. However, it has been verified that their biological effects mainly depend upon the intensity, dose level and exposure duration. Gamma-rays directly affect the cell components at different levels such as membranes, proteins, and nucleic acids (Kovacs & Keresztes, 2002). Several scientists stated that reactive oxygen species (ROS) resulted from water radiolysis are main regulators produced in seed from γ-ray's application. These ROS regulators perform as signaling molecules thus in results activate and amplify stress and antioxidant responses (Borzouei et al., 2010).

Sound also known as acoustic energy can be diffused through gases, liquids, and solids as an oscillatory concussive pressure. Audible sound having frequency between 20 Hz and 20 kHz can be hear by human beings. Among environmental factors affecting plant growth as compared to moisture, light, wind and temperature, little data is available regarding audible sound affecting plants (Hassanien et al., 2014). Application of sound waves as seed invigoration not only improved plant resistance against disease but also minimize requirements of chemical fertilizers (Carlson, 2013; Junfang, 2012). The mechanism underlying the sound waves influencing growth has not been properly discovered. Up till now it has also been stated that sound wave stimulation enhanced the transcription level and activate the stress-induced genes (Xiujuan et al., 2003). Sound waves also stimulate the opening of leaf stomata which in results increase the uptake of spray fertilizer and dew. Furthermore, the sound energy could interact and may be converted or reserved as chemical energy, which in result helps to stimulate the photosynthetic reactions (Meng et al., 2012). According to earlier research musical sound

could significantly affect the seeds sprouted showing that sound vibrations can directly influence the biologic systems (Creath & Schwartz, 2004).

Laser radiations has remarkable characteristic including monochromatic, polarization, coherence and high density, therefore all these characteristics make laser irradiations applicable in all field of biology and plant growth (St Dinoev et al., 2004). Different factors including type of laser radiation, wavelength, intensity and the exposure duration can induce changes in physiological state of seeds. These changes in result can trigger or hinder the plant development and disease resistance (Vasilevski, 2003). Different experiments on vegetables, cereals, peas, wheat, radishes and corn, revealed the positive effect of laser irradiations on germination and disease resistance (Nenadić et al., 2008). While other different experiments discussed the effect of laser irradiation as pre-sowing treatment, both in cereals (basic seeds are rice, maize) and in vegetables (Gładyszewska, 2006). In laboratory and field different experimental works have been carried out showing impact of laser irradiations on different seeds, seedlings and plants (Aladjadjiyan & Kakanakova, 2008; Vasilevski, 2003). According to scientific literature in different experimental study authors stated that laser light treatment of seeds leads to series different effects such as increased energy potential, accelerated maturity, enhanced disease resistance, improved alpha-amylase action and free radical's concentration (Hernandez et al., 2010). All these effects in result lead to seed dormancy inactivation; uniform and enhanced rate, percentage, energy of germination higher seed vigor, positive impact on respiration, photosynthesis, and chlorophyll, carotenoid content in irradiated seeds (Aladjadjiyan & Kakanakova, 2008; Wu et al., 2007).

CHAPTER 3

MATERIALS AND METHODS

This study was conducted in different labs of IHFS-GCUF and Department of Food Science and Technology, University of Nebraska-Lincoln, USA (food processing lab, applied research & engineering lab, value added processing lab and food grade lab).

3.1. Reagents and standards

All the reagents (HPLC and analytical grade) and standards used in this study were purchased from Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan), Duksan (Korea), and Merck (Merck KGaA, Darmstadt, Germany).

3.2. Instrumentation

Ultrasonic processor (Sonics & materials. Inc; model: VCX750); microwave oven **(**Homage; model: HDSO234S); incubator (Alto-shaam; model: 1000-TH-I); freeze dryer **(**Labconco; catalog number: 7750020); sieve screen (sieve number 35); centrifuge machine (Jouan GR 20, 222; model number: 11174651); microplate reader (Thermo fisher scientific; catalog number: VL0L00D0); UV/Vis spectrophotometer (Specord 200 PLUS, Germany); analytical balance (Mettler toledo; model: ML3002E / 03); vortex mixer **(**Labnet; model: VX 200); drying oven (Fisher scientific; model: 282A); desiccator (Thermo fisher scientific); water bath (thermo fisher scientific; model number: Precision SWB 27); mechanical shaker (thermo fisher scientific; model number: MaxQ 2000); rotary evaporator (Eyela, Japan); nitrogen analyzer (Leco; model: 630-300-300); soxhlet apparatus (Electromantle; model: EME30250/CEBX1); gas chromatograph apparatus (Hewlett packard – agilent; model: 6890); HPLC system (Agilent 1200 Series; Agilent Technologies Inc., Santa Clara, CA, USA); stirring hot plate (Corning PC-420D).

3.3. Procurement of raw material

Sorghum grains were procured from a local grain market of Faisalabad (Pakistan) & Lincoln, Nebraska (USA). Cleaning of sorghum grains was conducted through winnowing and hand sorting. Later grains were stored in high density polyethylene bags to keep away from moisture uptake and contamination until further use.

3.4. Grains sterilization and steeping

Sorghum grains were placed into four groups along with control for applying different techniques and each group was further divided into four treatments (100 g in each treatment). Initially, grains were soaked for 5 minutes in 5% sodium hypochlorite (NaOCl) solution for surface sterilization to avoid fungal invasion and mold growth during germination. Afterwards, grains were washed with distilled water several times till they attained neutral pH (Hamidi et al., 2013). After washing grains were steeped in water for further 22 hrs at room temperature. Afterward, the water was drained off and grains were washed properly with distilled water.

3.5. Study plan

The wet sorghum grains were divided into following four groups (C, US, MW & UM). The detailed treatment plan of all groups is given in following table.

Table: Treatment plan for processing of sorghum grains

Ultrasonic group (US)			**Microwave group (MW)**			**Combined group (UM)**		
Treat-ment	Time (min)	Ampli-tude (%)	Treat-ment	Time (sec)	Power (watt)	Treat-ment	Time US (min): MW (sec)	amplitude (%): power (watt)
Control								
US_1	5	40	MW_1	15	450	UM_1	5:15	40:450
US_2	5	60	MW_2	15	700	UM_2	5:15	60:700
US_3	10	40	MW_3	30	450	UM_3	10:30	40:450
US_4	10	60	MW_4	30	700	UM_4	10:30	60:700

Note: The selected treatment conditions for ultrasound (US) and microwave (MW) processing were based on some preliminary trials.

3.5.1. Control (C) group

The first group weighing 100 g (3 replicates = 100 g per each replicate) of sorghum grains was subjected to germination without application of any technique and it was designated as control group.

3.5.2. Ultrasound (US) processed group

The second group of sorghum grains was treated with ultrasound including optimization of treatment time and ultrasound amplitude with some modifications as described by Machikowa et al. (2013). This group was divided into four treatment levels (US_1, US_2, US_3, US_4) (3 replicates = 100 g for each treatment per each replicate) and subjected to sonication

using different time and amplitude values. Two amplitude levels at 40% and 60% for 5 and 10 minutes were used at constant temperature of 35 ℃. Pulsed on and pulsed off time was 5 seconds and 10 seconds, respectively. All ultrasonic treatments were applied by using ultrasonic processor having power of 750 W, frequency 20 kHz and volts 230 VAC~ 50/60Hz NOM.

3.5.3. Microwave (MW) processed group

Third group was given microwave treatment by using two variables of time duration and power level and was also divided into four treatment levels (MW_1, MW_2, MW_3, MW_4) (3 replicates = 100 g for each treatment per each replicate). All treatments were subjected to microwave technique for two power levels 450 watts; 700 watts at two different time durations 15 seconds; 30 seconds (Aladjadjiyan, 2010). Overnight steeping of grains enhanced the absorption of microwave energy. All treatments were treated through microwave oven having rated voltage of 230 V~, rated frequency 50 Hz, rated input 1250 W, rated output 800 W and microwave frequency of 2450 MHz.

3.5.4. Combined ultrasonic and microwave processed group (UM)

Both treatments were applied in combination to fourth group, similarly this group was also divided into four treatments (UM_1, UM_2, UM_3, UM_4) (3 replicates = 100 g for each treatment per each replicate). Each treatment was exposed to combination of microwave (450 W, 700 W) and sonication (40%, 60%) techniques for specific time intervals.

3.6. Germination process

All the untreated (control) and treated grains were subjected to germination through method reported by Nour et al. (2015) with little modification. About 100 seeds from each treatment (per each replicate) were germinated separately for analyzing rent growth parameters. Grains placed on moist paper towel in germination trays were covered with other sheet of paper towel. The trays were shifted in to incubator at 25±2 °C for 48 hrs to conduct germination. Trays were watered two or three times daily to stimulate the germination activity. The grains were considered to be germinated when both the root and shoot had emerged up to ≥ 0.5 cm (Geressu & Gezaghegne, 2008).

3.7. Growth parameters

After germination, all sprouts from control and treated groups were collected for analysis of growth parameters. Sprouts were measured for the shoot length (SL) and root length (RL) in cm, total weight in gram (g). The germination % (GP) of grains, seedling vigor index (SVI) and RL/SL ratio were also measured (Aladjadjiyan, 2010). GP was calculated according to the following formula:

$$GP = (N_g / N_t) \times 100$$

where N_g refers to total number of germinated grains, N_t is a total number of grains used.

SVI was calculated by following formula (Abdul-Baki & Anderson, 1973):

SVI= seedling length (cm) x germination percentage (%)

Where; Seedling length= Root length + Shoot length

3.8. Freeze drying

After germination, all sprouts of control and treated groups washed properly by distilled water were subjected to drying by using freeze dryer at relative system vacuum level of 7.0 Pascal and -48 °C collector temperature for 48 hours. After drying the samples were ground and crushed into a fine powder by stainless-steel grinder and sieved by 500 µm sieve screen. The resultant flour of all treatments was packed into air tight plastic containers and stored in refrigerator at 4 °C until further use for different analysis.

3.9. Quantitative analysis of phytochemical constituents

The quantitative phytochemical analysis of samples (powder/extract) were performed to quantify or ascertain the presence of some active constituents by employing conventional or standard protocols. These active compounds include alkaloids, tannins, phytates, saponins, sterols and total free amino acids. Most of analysis were based on the principle of colorimetric titration involving quantitative estimation of colors. This means the quantity of a substance in a mixture can be measured, by allowing the substance to bind with color forming chromogens. The difference in color results in the difference in the absorption of light, which was measured through spectrophotometer.

3.9.1. Determination of alkaloids

Total alkaloid contents were analyzed gravimetrically by protocol of Harborne (1973) as cited by Eze et al. (2014). Five grams of the dried sprout powder sample was added into 50

mL of 10% acetic acid solution in ethanol to form a ratio of 1:10 (10%). The mixture was properly mixed by using vortex mixer, properly covered with aluminum foil, allowed to stand for about 4 hours at 28 °C before filtration. Later filtered through Whatman No. 42 grade filter paper. The filtrate was concentrated up to one quarter by evaporation using hot plate. Alkaloids were completely precipitated by drop wise addition of concentrated (57%) ammonium hydroxide up to a final pH of 10. Solution allowed to settle, and precipitates were filtered. Later precipitates washed with 1% ammonium hydroxide solution subjected to drying in an oven at 60 °C for 30 min. After that cooled into desiccator and reweighed until a constant weight. The weight of the alkaloid was determined by following weight difference formula.

$$\text{Alkaloid (\%)} = W_2 - W_1 / \text{weight of sample} \times 100$$

Were W_1 = weight of empty filter paper

W_2 = weight of filter paper + alkaloid precipitate

Alkaloid content was expressed as mg/100 g of dried matter (DM).

3.9.2. Saponins determination

Quantification of saponin contents was carried out by method of Obadoni and Ochuko (2002) with minor alterations as described by Okwu and Orji (2007b). A 20g powder sample mixed with 200 mL of 20 % aqueous ethanol was stirred for half hour and heated with continuous stirring for 4 h at 45 °C. The mixture was filtered and residues re-extracted with fresh 200 mL of 20 % aqueous ethanol. The combined extract was concentrated to 40 mL by rotary evaporator at 40 °C. Later concentrate was moved into separating funnel and extracted with 20 mL diethyl ether. The aqueous layer was kept, extracted again with 30 mL n-butanol while the ether layer was removed. Then n-butanol extract was washed with 10 mL of 5 % sodium chloride. The remaining solution was evaporated, and samples were dried in oven at 40 °C. Saponin contents were calculated by given formula:

$$\text{Saponin (\%)} = \text{Final weight of sample} / \text{initial weight of sample} \times 100$$

Where final weight of sample is the weight of residue after drying. Saponin contents were expressed as mg/100 g of dried matter (DM).

3.9.3. Determination of tannins

Tannin contents were estimated according to vanillin- HCl assay (Price et al., 1978) described by Dykes et al. (2005). Briefly, 1 mL sorghum extract was put into two culture tubes named as sample and sample control. Then incubated in a water bath at 30 °C for 5 min.

Vanillin reagent was prepared by mixing equal amounts of vanillin (1%) and HCl (8%) solutions. 5.0 mL of vanillin reagent was added at 1 min intervals to the sorghum extract while 5.0 mL of 4% HCl was added to the sample control. Tubes were again incubated in a water bath at 30 °C for 20 min, finally absorbance was measured at 500 nm using UV/Vis spectrophotometer. The standard used was catechin (50-250 µg/mL). Tannin contents were expressed as mg catechin equivalent per 100 g of dry matter (mg CE/100 g DM).

3.9.4. Phytate contents determination

The phytate contents were determined as per method of Latta and Eskin (1980) described by Tizazu et al. (2011). A powder sample of 0.15 g was extracted with 10 mL 2.4% HCl in a mechanical shaker for one hour at room temperature and later centrifuged at 3000 rpm for 30 minutes. One milliliter of Wade reagent was added to 3 mL of the sample solution and the mixture was vortexed for 5 sec. Analytical grade sodium phytate (20-100 µg/mL) was used as standard. The absorbance was measured at 500 nm using deionized water as blank through UV/Vis spectrophotometer. Phytate contents were expressed as mg sodium phytate equivalent per 100 g of dry matter (mg SPE/100 g DM).

3.9.5. Total free amino acids content (TFAAs)

The TFAA contents of the sorghum sprout were determined based on method of Rosen (1957) described by Afify et al. (2012c). The powder was accurately weighed (2 g) and placed in a 250 mL round bottom flask. Boiling water was added three times. The volume of the first addition of water was 100 mL. The solution underwent reflux extraction for 1 h and was filtered while hot. The volume of the second addition of water was 50 mL. The solution was boiled for 0.5 h and filtered while hot. The volume of the third addition of water was 50 mL. The solution was boiled for 0.5 h and filtered while hot. The filtrate that was merged three times was concentrated to 100 mL as the test solution. 0.5 mL of water extract was added to 1 mL of sodium carbonate buffer salts and 2 mL of 2% ninhydrin solution in a tube. Tubes were heated in a water bath for 15 min and allowed to cool at room temperature. A constant volume of water was added to the solution and agitated. Each sample solution's absorbance was measured at 566 nm. Arginine (0-12 µg/mL) was used as a standard to prepare a calibration curve. Total free amino acids contents were expressed as mg arginine equivalent per g of dry matter (mg AE/g DM).

3.9.6. Determination of sterols

Liebermann-Burchard (LB) reagent was employed for the quantitative estimation of the sterol contents in the sample extracts (Daksha et al., 2010) as described by Nancy and Ashlesha (2015). LB reagent was prepared by adding 0.5 mL of concentrated sulphuric acid in 10 mL of acetic anhydride. To 1 mL of each sample extract, chloroform was added to make up the volume to 5 mL in a test tube. Then 2 mL of LB reagent was added and mixed well. These tubes were then covered with black paper and kept in the dark for 15 min to avoid any exposure to light. The reaction mixture turned green, which was spectrophotometrically measured at 640 nm. Cholesterol (1.158-5.79 mg/mL) was used as the standard to prepare a calibration curve. Sterol contents were expressed as mg cholesterol equivalent per gram of dry matter (mg CHE/g DM).

3.10. Proximate composition

3.10.1. Determination of proteins

Protein contents were calculated through combustion method by nitrogen analyzer according to AOAC method 992.15 (2002) as described by Ragaee et al. (2006). The powder sample (0.3 g) was subjected to combustion at 1150 °C in furnace. Before analyzing actual samples, measurements were optimized by running blank and standard samples. The protein contents were calculated by multiplying nitrogen by a factor (6.25 for sorghum grains). Calibration of nitrogen analyzer was done with an EDTA standard and crude protein (%) was expressed as nitrogen * 6.25.

3.10.2. Determination of carbohydrates

The contents of total carbohydrate were determined by the phenol sulfuric acid method (Krishnaveni et al., 1984) as described by Ebeid et al. (2015). 0.1 g sample was hydrolyzed with 5 mL of 2.5 N HCl in water bath for 3 hours. Sample was cooled at room temperature and sodium carbonate was added until bubbling stops. Later samples centrifuged, and supernatant was made to 100 mL with distilled water. 0.2 mL of this sample was pipetted out and made up 1 mL volume with distilled water. After that 1.0 mL phenol reagent was added followed by 5.0 mL of 96% sulphuric acid. Tubes were stayed at 25-30 °C for 20 min and absorbance was read at 490 nm. Glucose was used as standard (60-100 µg/mL) to prepare a calibration curve. Carbohydrate contents were calculated as g glucose/100 g of dried weight.

3.10.3. Lipids content determination

Lipid contents were estimated using a soxhlet apparatus with hexane for 8 h. Powder sample was hydrolyzed with aqueous solution of 6N HCL in reflux for 8 h before hexane extraction (Hadbaoui et al., 2010). Acidic hydrolysis performed to facilitate the fatty acids extraction. Percentage of oil yield was calculated as follows.

Oil yield (%) = (Oil extract from sprout (g) / Initial weight of sprout powder (g)) × 100

3.11. Fatty acid profile

Fatty acid profile of extracted oil was analyzed by method Ce 1f–96 mentioned in AOCS (1998). The 50 µL oil sample was methylated with 4 mL KOH-MeOH solution (1 M) at room temperature for 1 h to convert fatty acids into their methyl esters. The resultant fatty acid methyl esters were extracted with GC grade n–hexane and subjected to gas chromatograph equipped with an auto sampler, flame-ionization detector and supelco wax column. About 1 µL sample was injected with Helium (1 mL/min) as a carrier gas onto the column, at given operating conditions like column oven temperature 160 °C at 0 minutes with subsequent increase of 3 °C/min until 180 °C. The column oven temperature was increased from 180 °C to 220 °C at 1 °C/min and was held for 7.5 min at 220 °C. Split ratio was 50 % with injector 240 °C and detector 250 °C temperatures. Peak areas and total fatty acids composition were calculated for each sample by retention time using Varian Chem Station Software. The standards of fatty acids methyl esters were also run under the same conditions for comparison with experimental samples.

3.12. Sample extraction

All ground samples were extracted through method described by Mohankumar and Vaishnavi (2012). 1 g of powder sample was centrifuged with 20 mL of 70% methanol (1:20 w/v). The mixture was subjected for extraction under agitation for 2 hours at room temperature (vortexed per 20 min to shake vigorously). After that supernatant was poured out and the precipitates were again extracted with 20 mL of 70% methanol. Both supernatants were combined and centrifuged at 5000 rcf for 10 min. The supernatant was collected in air tight tube and covered with aluminum foil. All extracts were stored at 4 °C in the dark till further quantitative analysis.

3.13. Phenolic profile

3.13.1. Total phenolic content (TPC)

TPC was estimated by Folin–Ciocalteau method of Singleton and Rossi (1965) as described by Hithamani and Srinivasan (2014a). To 1 mL of the methanolic extract, 4 mL of Folin–Ciocalteau reagent diluted with water (1:5 H_2O) was added. After 3 min, 5 mL of Na_2CO_3 (7.5%, w/v) was added. Samples were agitated and incubated for 1 h in 40 °C water bath. After that absorbance at 760 nm was determined. The calibration curve was performed with gallic acid (100-800 μg/mL) and results were expressed as mg gallic acid equivalent per gram of dry matter (mg GAE/g DM).

3.13.2. Total flavonoid content (TFC)

TFC of the methanolic extract of samples was analyzed according to aluminium chloride colorimetric method (Zou et al., 2004) as described by Lallianrawna et al. (2013). 1.5 mL of extract was mixed with 75 μL of 5% $NaNO_2$ solution. After 6 min, 150 μL of 10% $AlCl_3.6H_2O$ was added in it and kept at room temperature for 6 more minutes. Later 0.5 mL of 1M NaOH added and volume was made up to 2.5 mL with deionized water. Solution was vortexed, and absorbance was measured at 510 nm. Standard quercetin (50-250 μg/mL) was used to prepare a calibration curve. The total flavonoid contents were expressed as the mg quercetin equivalent per gram of dry matter (mg QE/g DM).

3.14. Radical scavenging activity

As there is no standardized method to evaluate the radical scavenging potential of foods and biological systems, it is recommended to evaluate it by various methods (Frankel & Meyer, 2000).

3.14.1. DPPH assay

The DPPH assay was based on the slightly modified method of Brand-Williams et al. (1995) also described by Zou et al. (2011). Briefly, 100 μL of methanolic extract was added to 3.9 mL methanolic solution of DPPH (0.0025 g/100 mL CH_3OH). After 60 min of retention time in the dark, the absorbance at 515 nm was recorded to determine the concentration of the remaining DPPH. The percentage inhibition of DPPH of the test sample and known solutions of trolox were calculated by the following formula:

$$\% \text{ Inhibition} = 100 * (A_0 - A)/A_0$$

Where A_0 was the beginning absorbance at 515 nm, obtained by measuring the same volume of solvent, and A was the final absorbance of the test sample at 515 nm. The calibration curve between absorbance and known solutions of trolox was then established. Trolox standard solutions were prepared at a concentration ranging from 100 to 1000 µM.

3.14.2. Ferric reducing antioxidant potential assay (FRAP Assay)

The FRAP assay was performed according to methods described by Sutharut and Sudarat (2012) and also used by Alka et al. (2013). The 200 µL of methanolic extract of each sample was mixed with 1.3 mL of the FRAP reagent. FRAP reagent consisted of 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3$ in a ratio of 10:1:1 (v/v/v). After 30 min of incubation at 37 °C, absorption was measured at 595 nm using a spectrophotometer. The absorbance changes in the test mixture were compared to those obtained from standard mixture of ferrous sulphate ($FeSO_4.7H_2O$) (0.15 mmol/L–1.40 mmol/L). FRAP values expressed as mmol of Fe (II) equivalent per gram dry matter (mmol FE/g DM).

3.14.3. Oxygen radical absorbance capacity (ORAC)

The ORAC assay was conducted with some modifications according to Huang et al. (2002) as described by Rhodes et al. (2017). A solution of 153 mM AAPH in 75 mM sodium phosphate buffer (pH 7.4) was prepared for immediate use. A 4×10^{-3} mM fluorescein stock solution in 75 mM sodium phosphate buffer (pH 7.4) was wrapped in foil and stored at 5 °C. A working solution of diluted fluorescein (1: 1,000) in 75 mM sodium phosphate buffer (pH 7.4) was prepared immediately before use. The exterior wells of the 96-well plate were filled with 300 µL of deionized water. Fluorescein working solution (150 µL) was added to all inner wells. Solutions (25 µL) of diluted extract, phosphate buffer, and diluted trolox were added to samples, blank, and standard wells, respectively, for a total volume of 175 µL. The plate was incubated at 37 °C for 30 min. All experimental wells were injected with 35 µL of AAPH solution using the plate reader injector and shaken for 10 seconds at maximum intensity. Fluorescence was monitored at 485 nm (excitation) and 528 nm (emission), with measurements taken from the top 1 min intervals for 60 min. Trolox was used to generate a standard curve (3.125-50 µmol TE/L). The antioxidant capacities of extracts were expressed as micromoles of trolox equivalent (TE) per gram of dry matter (µmol TE/g DM).

3.15. HPLC quantification of phenolic compounds

To obtain a profile of individual phenolic compounds extracts of processed germinated samples were analyzed by chromatographic analysis through HPLC system (Hithamani & Srinivasan, 2014a). HPLC system (Agilent 1200 Series; Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a diode array detector was used. 20 µL of filtered sample was analyzed using a C18 analytical column (250 × 4.6 mm; 5 µm; Agilent Technologies Inc., USA). Mobile phase was composed of 0.1% trifluoroacetic acid (solvent A) and 100% methanol (solvent B). Total run time was 60 min with a flow rate of 1.0 mL/ min. Gradient program was as follows: initial B concentration of 20% to 40% in 40 min which was maintained for 10 min and again to 20% B in the next 5 min and 5 min of post run for reconditioning. Peaks were recorded simultaneously at 270-370 nm. Respective standards of phenolic compounds were also run to identify and quantify the major phenolic compounds from sample extracts.

3.16. Bio-accessibility assessment

3.16.1. *In vitro* protein digestibility (IVPD)

In vitro protein digestibility was determined according to the method of Akeson and Stahmann (1964) as described by Afify et al. (2012c). About one-gram sample added to HCl (15 mL, 0.1 M), containing 1.5 mg pepsin and incubated at 37 °C for 3 h. The obtained suspension was neutralized with NaOH (7.5 ml, 0.2 M), then treated with 4 mg of pancreatin in 7.5 mL 0.2 M phosphate buffer (pH 8.0). One milliliter of toluene was added to prevent microbial growth and the mixture was gently shaken and incubated for additional 24 h at 37 °C. After incubation, the sample was treated with 10 mL of 10% trichloroacetic acid (TCA) to remove undigested protein and larger peptides and centrifuged at 50000 g for 20 min at room temperature. Protein in the supernatant was estimated using the Kjeldahl method (Horwitz, 2000). The percentage of protein digestibility was calculated by the ratio of protein in supernatant to protein in sample as equation:

Protein digestibility% = Nitrogen (in supernatant) – Nitrogen (in blank) \ Nitrogen (in sample) × 100

3.16.2. Bio-accessibility of polyphenols

Polyphenol bio-accessibility was analyzed through *in vitro* method described by Luten et al. (1996) having simulated gastrointestinal digestion with some alterations as mentioned by Hithamani and Srinivasan (2014a). For gastric digestion 10 g powder sample was incubated with pepsin at pH 3.0 for 2 hrs at 37 °C. At the end of gastric digestion, titratable acidity was determined in an aliquot of gastric digest as the amount of 0.2 M sodium hydroxide required to attain a pH of 7.5 in the presence of a mixture of pancreatin and bile extract dissolved in 0.1 M sodium bicarbonate (4 g pancreatin and 25 g bile extract per liter). Subsequently, intestinal digestion was simulated by suspending segments of dialysis tubing (molecular mass cut-off: 10 kDa) containing 25 mL aliquots of sodium bicarbonate solution, being equivalent in moles to the titratable acidity (sodium hydroxide needed to neutralize the gastric digest) in erlenmeyer flasks containing the gastric digest and incubated at 37 °C with shaking until the pH of the digest reached 5.0. Pancreatin–bile extract mixture (5 mL) was then added and incubation was continued for 2 h or longer until the pH of the digest reached 7.0. At the end of this simulated gastrointestinal digestion, the dialysate was analyzed for polyphenols by spectrophotometry as described in previous sections. The bio-accessible polyphenols present in the sample are the dialyzable portion of the total polyphenol which was expressed as percent bio-accessibility.

3.17. Statistical analysis

The data of all experiments obtained by applying each treatment was subjected to statistical analysis to determine their significance for the response. All experimental results were observed as mean value of three independent replicates. The data in the tables and figures represent mean values ± standard deviation. The results were evaluated for statistical significance that may exist between the mean values at a significance level of 5%, using analysis of variance (ANOVA) with Minitab 17 and LSD post hoc test with SPSS 21 Statistical Software. LSD post hoc test was used to evaluate the statistical significance in pair wise comparison of each treatment with other treatments.

CHAPTER 4

RESULTS AND DISSCUSSION

The increased awareness about phytochemicals has directed to consumption of sorghum in form of functional food recipes for improving human health. Worldwide, sorghum is an important cereal as the part of staple diet representing a main source of energy and protein. It is also a rich source of different phytochemicals having potential to significantly affect human health. Still no authenticated research information is available regarding effect of novel processing technologies on the sorghum germination characteristics and chemical composition. Therefore, sorghum sprouts were analyzed for compositional assay after application of different novel pre-germination seed invigoration techniques. The obtained data was further statistically analyzed to determine significance level; results of examined parameters are debated herein:

4.1. Germination parameters as indicators of morphological changes

Sorghum grains were exposed to different pre-germination processing treatments i.e. sonication, microwave individually and in combination to observe their effect on the seed germination frequency (%) and growth parameters (root length, shoot length, total weight, seedling vigor index and RL/SL ration).

4.1.1. Effect of ultrasound processing

Mean squares for germination parameters of ultrasound treated seeds has been presented in table 1. It is understandable from the results that the ultrasonic treatment significantly ($p \leq 0.01$) affected the germination frequency (G) along with root length (RL), shoot length (SL), total weight and seedling vigor index (SVI) while root length/shoot length ratio (RL/SL) was not significantly ($p > 0.05$) changed. Means for germination parameters in ultrasonic treated seeds are presented in table 2. The results showed that seed germination percentage increased at 40% (US_1: 94.00±3.00 %) & 60% (US_2: 85.00±1.00 %) of total ultrasonic amplitude for 5 minutes as compared to untreated control sprouts (78.00±2.00 %). However, increasing exposure time up to 10 minutes at same amplitude decreased seed germination. The stimulating efficiency of ultrasonic treatment on sorghum grains was observed at the amplitude level of 40% of the output power with the exposure times 5 minutes

(US_1). The variation in duration for ultrasonic amplitude has also resulted variation in the root and shoot lengths and total weight of sprouts. From the data, mean root length of the treated seedlings was ranged 1.40±0.60 to 2.80±0.10 cm, compared to 2.40±0.30 cm of the control treatment. A similar result was detected in shoot lengths of the processed seedlings which ranged 1.10±0.20 to 2.20±0.30 cm compared to 1.90±0.10 cm of the control. The total weight range for treated seeds was 1.51±0.06 to 2.56±0.04 g while that of control was 2.42±0.20 g. Treatment US_1 at the amplitude of 40% for duration of 5 minutes, produced maximum RL (2.80±0.10 cm), SL (2.20±0.30 cm) and TW (2.56±0.04 g). The size of the seedlings and weight was decreased at 40% (US_3) and 60% (US_4) amplitude for 10 min. Similarly, treatment US_1 & US_2 gave high values of RL/SL ratio 1.29±0.22 & 1.92±0.24, respectively. Seeds having higher SVI are more vigorous. It is clear from results that use of ultrasonic amplitude increased the SVI % than control (335.53±21.02 %). The duration of ultrasound exposure to seeds resulted in SVI % variations. The maximum SVI (US_1: 470.40±33.80 %) of seedling was observed at 5 minutes of ultrasonic exposure with 40% amplitude. While amplitude of 40% (US_3) and 60% (US_4) for 10 min decreased the SVI. Prolonged treatments of ultrasonic at higher amplitude for longer exposure time possibly impose injury on the embryo so decreased the germination parameters as shown in table 2. This study showed that the ultrasonic processing of sorghum grains with different amplitude and time levels used in these investigations caused statistically significant changes in the germination trend along with RL, SL, TW & SVI ($p \leq 0.05$) except RL/SL ratio ($p > 0.05$). According to LSD test regarding pair wise multiple comparison given in table 3 for germination % and SVI the pair wise comparisons of every treatment with other treatments showed significantly different effect on response ($p \leq 0.05$). Regarding TW treatment US_1, US_2 and control showed significantly different effect ($p \leq 0.05$) in pair wise comparisons with every other treatment. While for or RL/SL ratio all the comparisons were non-significant ($p > 0.05$). Long time duration of sonication (10 min) declined germination performance. It's possible sonication treatment of 10 min might be too high to be endured by small and fragile sorghum grains resulting into to cell lysis process.

Literature available on pre-sowing treatment using sonication support our findings. But there is no significant work regarding effect of ultrasound on sorghum grains germination. Results regarding orchid seeds treated with ultrasonic treatment at high amplitude (80-100%)

reduced the germination percentages up to 44-48% compared to control (Shin et al., 2011). Optimum ultrasonic conditions used for sunflower seeds at 40 kHz frequency were 40 to 60% amplitude for 5-10 minutes of exposure durations (Machikowa et al., 2013). These optimized conditions resulted maximum germination of 98% compared to control seeds. Goussous et al. (2010) also reported similar results in wheat seeds by using mild US duration and observed enhanced germination percentage. Another study by Shekari et al. (2015) showed increase in seedling length of sesame seeds when sonicated for 10 min and seedling length decreased on prolong exposure for 30 min. According to work described by Aladjadjiyan (2012) seedling length of lentil had direct relationship with US exposure time. Similarly 70% increase in SVI of common yarrow was investigated when seeds were treated with ultrasound for 5 minutes (Mirshekari et al., 2013). These results suggested that decreased germination at high ultrasonic amplitude for long exposure time was due to some alternations of metabolic reactions in treated seeds including endogenous hormonal balance (Chen et al., 2013).

It is confirmed that ultrasound applies its major effects by causing mechanical changes (acoustic cavitation) and disturbance of cell walls. Yaldagard et al. (2008c) and Chen et al. (2013) stated that ultrasonic application could fragment the seed shell causing larger porosity on the surface of barley grains by captivation of ultrasound (shock waves). Thus, increasing water uptake and water retention capacity in dry grains and result in better hydration. The extra absorbed water reacts freely and readily with the cell embryo, so, metabolic processes in the form of gibberellic acid release and activation of enzymes expedited, and increase the rate of enzyme-catalyzed hydrolysis reactions within the seeds (Yaldagard et al., 2008c). These phenomena may consequently lead to quicker germination and faster embryo growth. However, only ultrasound with proper amplitude and duration would increase the enzyme activities and stimulate physiological activities of cells, while at high amplitude there will be more damage to the cells or enzyme structure (Liu et al., 2003). In this study, the optimal ultrasonic amplitude was 40% for 5 minutes (US_1) to significantly ($p \leq 0.05$) enhance germination trend and to improve growth parameters (except RL/SL) than the control, while the ultrasonic amplitude of 60% for 10 minutes (US_4) resulted in the reduction of germination and growth parameters significantly ($p \leq 0.05$).

Table 1.

Mean squares of germination parameters for ultrasound processed sorghum sprouts

SOV	df	G	RL	SL	RL/SL	TW	SVI
Treatment	4	1023.90**	1.06500**	0.69900**	0.28092NS	0.695910**	57128.9**
Replicate	2	3.05NS	0.07200NS	0.12800NS	0.09902NS	0.000500NS	2206.1**
Error	8	4.30	0.14700	0.06800	0.42665	0.001400	174.4
Total	14						

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

G: germination %; RL: root length; SL: shoot length; RL/SL: ratio of root length & shoot length; TW: total weight; SVI: seedling vigor index

Table 2.

Germination parameters for ultrasound processed sorghum sprouts

Treatments	G (%)	RL (cm)	SL (cm)	RL/SL	TW (g)	SVI (%)
C	78.00 ± 2.00^{abcd}	2.40 ± 0.30^{ab}	1.90 ± 0.10^{abc}	1.27 ± 0.22^{ns}	2.42 ± 0.20^{abcd}	335.53 ± 21.02^{abcd}
US_1	94.00 ± 3.00^{abcd}	2.80 ± 0.10^{abc}	2.20 ± 0.30^{abc}	1.29 ± 0.22^{ns}	2.56 ± 0.04^{abcd}	470.40 ± 33.80^{abcd}
US_2	85.00 ± 1.00^{abcd}	1.90 ± 0.40^{a}	1.20 ± 0.50^{ab}	1.92 ± 0.24^{ns}	2.09 ± 0.02^{abcd}	263.43 ± 5.40^{abcd}
US_3	64.00 ± 2.00^{abcd}	1.50 ± 0.20^{ab}	1.30 ± 0.10^{ab}	1.17 ± 0.24^{ns}	1.56 ± 0.01^{abc}	179.33 ± 12.00^{abcd}
US_4	47.00 ± 1.50^{abcd}	1.40 ± 0.60^{ab}	1.10 ± 0.20^{ab}	1.23 ± 0.32^{ns}	1.51 ± 0.06^{abc}	116.70 ± 33.85^{abcd}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; US_1, US_2, US_3, US_4: different ultrasound processed germinated treatments

US_1: (5 min for 40%); US_2: (5 min for 60%); US_3: (10 min for 40%); US_4: (10 min for 60%)

G: germination %; RL: root length; SL: shoot length; RL/SL: ratio of root length & shoot length; TW: total weight; SVI: seedling vigor index

Table 3.

Multiple comparisons (post hoc) LSD test of germination parameters for ultrasound processed sorghum sprouts

Treatment I	Treatment J	Significance levels					
		G	RL	SL	RL/SL	TW	SVI
C	US_1	.000	.237	.196	.966	.002	.000
	US_2	.003	.149	.011	.260	.000	.000
	US_3	.000	.021	.023	.851	.000	.000
	US_4	.000	.013	.006	.947	.000	.000
US_1	C	.000	.237	.196	.966	.002	.000
	US_2	.001	.021	.002	.276	.000	.000
	US_3	.000	.003	.003	.818	.000	.000
	US_4	.000	.002	.001	.913	.000	.000
US_2	C	.003	.149	.011	.260	.000	.000
	US_1	.001	.021	.002	.276	.000	.000
	US_3	.000	.237	.651	.197	.000	.000
	US_4	.000	.149	.651	.236	.000	.000
US_3	C	.000	.021	.023	.851	.000	.000
	US_1	.000	.003	.003	.818	.000	.000
	US_2	.000	.237	.651	.197	.000	.000
	US_4	.000	.758	.375	.904	.140	.000
US_4	C	.000	.013	.006	.947	.000	.000
	US_1	.000	.002	.001	.913	.000	.000
	US_2	.000	.149	.651	.236	.000	.000
	US_3	.000	.758	.375	.904	.140	.000

Based on observed means. The mean difference is significant at the .05 level.

I & J both indicate control and different processed treatments (C, US_1, US_2, US_3, US_4), we are comparing different treatments with each other but I≠J.

C: control; US_1, US_2, US_3, US_4: different ultrasound processed germinated treatments; US_1: (5 min for 40%); US_2: (5 min for 60%); US_3: (10 min for 40%); US_4: (10 min for 60%).

G: germination %; RL: root length; SL: shoot length; RL/SL: ratio of root length & Shoot length; TW: total weight; SVI: seedling vigor index

4.1.2. Effect of microwave processing

Mean squares for germination parameters of microwave treated sprouts are given in table 4. The results showed highly significant ($p \leq 0.01$) changes in germination %, RL & SVI while non-significant ($p > 0.05$) changes occurred in SL, RL/SL ratio & TW. According to presented data of means regarding germination parameters given in table 5, it could observe that both exposure time and microwave power had influence on germination frequency. For untreated control treatment, the percentage of germination frequency was 78.00±2.00 %. Microwave seed treatment for 15 sec time at different power levels 450 watt (MW_1) & 700 watt (MW_2), resulted in significant increase of this parameter as 92.00±1.25% and 95.00±3.00%, respectively. Germination capacity decreased significantly for MW_3 (450W) (72.00±0.90%) & MW_4 (700W) (67.00±1.50%) when the same power levels were used for 30 sec. In general, microwave seed treatment (MW_4) for time 30 sec at 700 watts, negatively affected this parameter. Samples exposed to radiation for 15 sec (MW_1 & MW_2) demonstrate the biggest stem and root length while higher exposure times 30 sec (MW_3 & MW_4) again lead to decrease of these parameters. For exposure 15 sec at 700W (MW_2), SL was 2.06±0.45 cm and RL was 2.90±0.50 cm longer than the control. For the samples exposed for 30 sec (MW_4) gave shorter SL (1.20±0.60 cm) and RL (1.60±0.20 cm). It can be noticed that at higher radiation amplitude and longer exposure times, the positive effect of microwave stimulation is weaker. Similarly, total weight for the samples treated with 450 W (MW_1) at 15 sec was 2.53±0.50 g, and for those treated with 700W (MW_2) was 2.55±0.30 g, so higher than the control (2.42±0.20 g). While RL/SL ratio was higher only in treatment MW_1 (1.50±0.05) as compared to control (1.27±0.22). It is concluded that for 700 W the exposure at 15 sec (MW_2) is more effective in later stages of development than the exposure at 30 sec. The presented results regarding the vigor index (SVI) showed output powers of 450 W & 700 W (MW_1 & MW_2) as stimulation of the growth (SVI: 414.41±51.62 % & 473.73±105.10 %, respectively) at 15 sec. The exposure time of 15 sec is a noticeable growth stimulation compared with controls. Microwave radiation power of 700 W for 30 sec (MW_4: 188.40±57.80 %) showed a decrease in SVI than control (335.53±21.02 %). The results showed that the microwave processing of sorghum grains with different power and time levels used in these investigations caused significant changes in the germination trend, RL & SVI ($p \leq 0.05$) while non-significantly affected effect on SL, TW & RL/SL ratio ($p > 0.05$). According to LSD test

regarding pair wise multiple comparison given in table 6 for germination % the pair wise comparisons of every treatment with other treatments showed significantly different effect on response ($p \leq 0.05$). Regarding RL treatment MW_3, MW_4 and control showed significantly different effect ($p \leq 0.05$) in pair wise comparisons with every other treatment. While SL, TW and RL/SL ratio showed non-significant effect in pair wise comparisons ($p > 0.05$) with other treatments. Long time duration of sonication (10 min) decreased germination performance.

The results obtained in this investigation correspond well with previous investigations on perennial crops (Aladjadjiyan, 2002) and lentils (Aladjadjiyan, 2010). Regarding sorghum grains no significant work was found about microwave application influencing germination parameters. In a study by Naeem et al. (2013), the effect of microwave radiations on okra and corn seed germination and plant growth were studied. Seeds were exposed to microwave radiation (2450 MHz) for 0 (control), 1, 2 and 3 sec and showed decrease germination percentage 80% 40% and 25% respectively as compared to control (100%). Effects of microwave treatment (850 watts) on China aster seed germination was studied in dry conditions and in water for 10, 20, 30, 40, 50, 60, 90, 120 and 180 sec (Han, 2010). Dry microwave seed treatment for time longer than 20 sec, as well as treating seeds in water for 30, 60, 90, 120 and 180 sec, resulted in significant decrease of germination percentage. Barley seeds exposed to microwave treatment 400 W and 720 W with a frequency of 2.45 GHz for 0, 10 and 20 sec showed best results for the treatment with output microwaves power of 400W for 20s (Crețescu et al., 2013). The influence of microwave irradiation (400 & 720 W) treatment on the development of barley seeds with a frequency of 2.45 GHz for the exposure time 0, 30, 60 and 90 sec was studied. Seeds exposed to irradiation for shorter period 30 sec and lower microwave power 400 W showed higher germination capacity (Iuliana et al., 2013). It was observed that radiation affected germination, growth and other morphological character of plants.

Nagy et al. (2005) stated that longer time exposure abandoned the stimulating effects of electromagnetic on wheat and sunflower seeds. The combination of exposure time with amplitude has the major role regarding seed enhancement. Low frequency of microwave radiation accelerates the growth while high frequency reduced plant growth (Murakami et al., 2001). Results can be described as effect of absorbed energy. Radiations with high output power for long exposure time, resulted more energy absorption by the object. The stimulated

germination might be due to the disruption of seed coat due application of microwave treatment, which enabled water diffusion into the seeds inducing higher rate of enzymatic reactions and the start of the development consequently resulted in fast and effective germination (Burtebayeva et al., 2003; Iuliana et al., 2013). The core phenomenon responsible for enhanced germination examined by several researchers is increased water absorption by seeds (Fischer et al., 2004). In this study to improve germination and growth parameters significantly ($p \leq 0.05$) (except SL, TW & RL/SL ratio), optimal microwave power level was 700W for 15 sec (MW_2) having positive effect, while the power of 700W for 30 sec (MW_4) resulted in the significant reduction of growth parameters and germination % (negative effect).

Table 4.

Mean squares of germination parameters for microwave processed sorghum sprouts

SOV	df	G	RL	SL	RL/SL	TW	SVI
Treatment	4	452.100**	0.83100**	0.3557NS	0.03470NS	0.02031NS	40455**
Replicate	2	12.305**	0.39200**	0.1127NS	0.01898NS	0.06758NS	9323NS
Error	8	1.329	0.02200	0.1652	0.18233	0.05618	2128
Total	14						

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

G: germination %; RL: root length; SL: shoot length; RL/SL: ratio of root length & shoot length; TW: total weight; SVI: seedling vigor index

Table 5.

Germination parameters for microwave processed sorghum sprouts

Treatments	G (%)	RL (cm)	SL (cm)	RL/SL	TW (g)	SVI (%)
C	78.00 ± 2.00^{abcd}	2.40 ± 0.30^{abcd}	1.90 ± 0.10^{ns}	1.27 ± 0.22^{ns}	2.42 ± 0.20^{ns}	335.53 ± 21.02^{ab}
MW_1	92.00 ± 1.25^{abcd}	2.70 ± 0.30^{abc}	1.80 ± 0.20^{ns}	1.50 ± 0.05^{ns}	2.53 ± 0.50^{ns}	414.41 ± 51.62^{ab}
MW_2	95.00 ± 3.00^{abcd}	2.90 ± 0.50^{abc}	2.06 ± 0.45^{a}	1.41 ± 0.07^{ns}	2.55 ± 0.30^{ns}	473.73 ± 105.10^{abc}
MW_3	72.00 ± 0.90^{abcd}	2.00 ± 0.10^{abcd}	1.50 ± 0.40^{ns}	1.41 ± 0.26^{ns}	2.38 ± 0.01^{ns}	251.82 ± 18.45^{ab}
MW_4	67.00 ± 1.50^{abcd}	1.60 ± 0.20^{abcd}	1.20 ± 0.60^{a}	1.55 ± 0.19^{ns}	2.38 ± 0.53^{ns}	188.40 ± 57.80^{abc}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; MW_1, MW_2, MW_3, MW_4: different microwave processed germinated treatments

MW_1: (15 sec for 450W); MW_2: (15 sec for 700W); MW_3: (30 sec for 450W); MW_4: (30 sec for 700W)

G: germination %; RL: root length; SL: shoot length; RL/SL: ratio of root length & shoot length; TW: total weight; SVI: seedling vigor index

Table 6.

Multiple comparisons (post hoc) LSD test of germination parameters for microwave processed sorghum sprouts

Treatment I	Treatment J	Significance levels					
		G	RL	SL	RL/SL	TW	SVI
C	MW_1	.000	.038	.771	.528	.585	.070
	MW_2	.000	.003	.629	.692	.521	.006
	MW_3	.000	.011	.262	.692	.841	.057
	MW_4	.000	.000	.068	.440	.841	.005
MW_1	C	.000	.038	.771	.528	.585	.070
	MW_2	.013	.137	.445	.810	.920	.154
	MW_3	.000	.000	.392	.810	.461	.003
	MW_4	.000	.000	.108	.882	.461	.000
MW_2	C	.000	.003	.629	.692	.521	.006
	MW_1	.013	.137	.445	.810	.920	.154
	MW_3	.000	.000	.126	1.000	.405	.000
	MW_4	.000	.000	.031	.699	.405	.000
MW_3	C	.000	.011	.262	.692	.841	.057
	MW_1	.000	.000	.392	.810	.461	.003
	MW_2	.000	.000	.126	1.000	.405	.000
	MW_4	.001	.011	.392	.699	1.000	.131
MW_4	C	.000	.000	.068	.440	.841	.005
	MW_1	.000	.000	.108	.882	.461	.000
	MW_2	.000	.000	.031	.699	.405	.000
	MW_3	.001	.011	.392	.699	1.000	.131

Based on observed means. The mean difference is significant at the .05 level.

I & J both indicate control and different processed treatments (C, MW_1, MW_2, MW_3, MW_4), we are comparing different treatments with each other but I≠J.

C: control; MW_1, MW_2, MW_3, MW_4: different microwave processed germinated treatments

MW_1: (15 sec for 450W); MW_2: (15 sec for 700W); MW_3: (30 sec for 450W); MW_4: (30 sec for 700W)

G: germination %; RL: root length; SL: shoot length; RL/SL: ratio of root length & shoot length; TW: total weight; SVI: seedling vigor index

4.1.3. Effect of combined processing

Table 7 presenting mean square values of germination parameters for combined application of both ultrasonic and microwave treatments. It is indicated through results that combined treatment application significantly ($p \leq 0.01$) affected the germination % along with root length, shoot length, total weight and seed vigor index while root length/shoot length ratio wan not significantly ($p > 0.05$) changed. Sorghum grains were treated with both ultrasonic and microwave application to check that their effect on germination is either synergistic or antagonistic. According to means values (table 8), germination frequency showed maximum value of 75.00±2.50 % at UM_2 (US: 60% for 5min & MW: 700W for 15sec) higher than UM_1, UM_3 and UM_4 but lower as compared to control (78.00±2.00 %). Similarly, as compared to other treatments (UM_1, UM_3 & UM_4), UM_2 showed high values of other growth parameters like SVI (224.66±7.50 %), RL (1.50±0.10 cm), SL (1.50±0.10 cm), TW (2.16±0.06 g) and RL/SL (1.00±0.05) but all these values were lower than control. Combined application of ultrasound and microwave negatively affected the germination and growth parameters because all the values were significantly decreased as compared to control. In combination both techniques caused more intense effects which may negatively affect metabolic processes and cause alterations in endogenous hormonal balance thus negatively reduced the germination. According to LSD test regarding pair wise multiple comparison given in table 9 for germination % (except UM_2) and TW, the pair wise comparisons of every treatment with other treatments showed significantly different effect on response ($p \leq 0.05$). Regarding RL only control and for SVI control and UM_2 showed significantly different effect ($p \leq 0.05$) in pair wise comparisons with every other treatment. While RL/SL ratio showed non-significant effect in pair wise comparisons ($p > 0.05$) with other treatments.

Microwave heating and ultrasonic waves are among the most simple, inexpensive, and valuable tools. Besides saving energy, these green techniques promote faster and more selective transformations. They are of a basically different nature (quantum and non-quantum fields) so they could be combined to enhance their effects (Cravotto & Cintas, 2007). Still further research is required to analyze the optimum levels for combined applications of both techniques to improve germination. Up till now there is not any prominent study about combined effect of microwave and sonication on sorghum seed germination. But there are different other priming techniques used in combination affecting germination process and

showing positive or negative results on different seeds. In a study, *Melia dubia* seeds treated with combined application of microbial consortia and microwave energy. Seeds were exposed to microwave energy (2450 MHz) for 7.5 minutes followed by seed pelletization with selected microbial consortia (Ravi et al., 2012). It was recorded the highest germination percentage of 68% over control as compared to 5 min, 10 min, 2.5 min & 20 min of microwave energy. Even though many methods like stratification with sand paper, acid and hot water treatments remove hard seededness by cracking the seed coat and allowing imbibition, the methods did not work with the study material. In fact, many authors reported that the low seed germination in hard seed coated and physiologically dormant seeds could be overcome by various physical and chemical treatments. Effect of priming with gibberellic acid and chilling stratification on improving germination and growth of Eastern black walnut seeds was studied. The highest percentage of seed germination (69.27 %) was recorded with the combined treatment of two months chilling and gibberellic acid (400 ppm) (Parvin et al., 2015). The combination between a suitable and effective techniques can considerably affect seed germination. Every pre-treatment has some positive and negative impact on the morphological changes of sprouts. Thus, choice of suitable pre-treatment approach can help to attain improved seed germination. Among all the processed treatments, UM_2 (US: 60%:5min & MW: 700W:15sec) showed better results for germination and growth parameters (except ratio of root length and shoot length) but have significantly ($p \leq 0.05$) decreased values compared to control.

Table 7.

Mean squares of germination parameters for combined application processed sorghum sprouts

SOV	df	G	RL	SL	RL/SL	TW	SVI
Treatment	4	725.100**	1.01400**	0.39900*	0.03156^{NS}	0.697650**	31128.1**
Replicate	2	1.658^{NS}	0.03200^{NS}	0.24200^{NS}	0.27651^{NS}	0.000320^{NS}	1857.5^{NS}
Error	8	2.583	0.14200	0.09700	0.26043	0.001570	608.4
Total	14						

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

G: germination %; RL: root length; SL: shoot length; RL/SL: ratio of root length & shoot length; TW: total weight; SVI: seedling vigor index

Table 8.

Germination parameters for combined application processed sorghum sprouts

Treatments	G (%)	RL (cm)	SL (cm)	RL/SL	TW (g)	SVI (%)
C	78.00 ± 2.00^{abc}	2.40 ± 0.30^{abcd}	1.90 ± 0.10^{abc}	1.27 ± 0.22^{ns}	2.42 ± 0.20^{abcd}	335.53 ± 21.02^{abcd}
UM_1	63.00 ± 0.70^{abcd}	1.30 ± 0.40^{a}	1.20 ± 0.30^{a}	1.07 ± 0.06^{ns}	1.57 ± 0.40^{abcd}	157.17 ± 42.35^{abc}
UM_2	75.00 ± 2.50^{abc}	1.50 ± 0.10^{a}	1.50 ± 0.10^{ns}	1.00 ± 0.05^{ns}	2.16 ± 0.06^{abcd}	224.66 ± 7.50^{abcd}
UM_3	52.00 ± 1.00^{abcd}	1.10 ± 0.30^{a}	1.10 ± 0.60^{a}	1.14 ± 0.40^{ns}	1.42 ± 0.01^{abcd}	113.80 ± 44.60^{ab}
UM_4	41.00 ± 0.50^{abcd}	0.90 ± 0.50^{a}	1.00 ± 0.40^{a}	1.17 ± 0.24^{ns}	1.33 ± 0.03^{abcd}	77.86 ± 3.15^{abc}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; UM_1, UM_2, UM_3, UM_4: different combined processed germinated treatments

UM_1: (5 min for 40% & 15 sec for 450W); UM_2: (5 min for 60% & 15 sec for 700W); UM_3: (10 min for 40% & 30 sec for 450W); UM_4: (10 min for 60% & 30 sec for 700W)

G: germination %; RL: root length; SL: shoot length; RL/SL: ratio of root length & shoot length; TW: total weight; SVI: seedling vigor index

Table 9.

Multiple comparisons (post hoc) LSD test of germination parameters for combined application processed sorghum sprouts

Treatment I	Treatment J	Significance levels					
		G	RL	SL	RL/SL	TW	SVI
C	UM_1	.000	.007	.025	.644	.000	.000
	UM_2	.052	.019	.154	.535	.000	.001
	UM_3	.000	.003	.014	.763	.000	.000
	UM_4	.000	.001	.008	.822	.000	.000
UM_1	C	.000	.007	.025	.644	.000	.000
	UM_2	.000	.534	.272	.871	.000	.010
	UM_3	.000	.534	.704	.871	.002	.063
	UM_4	.000	.230	.454	.810	.000	.004
UM_2	C	.052	.019	.154	.535	.000	.001
	UM_1	.000	.534	.272	.871	.000	.010
	UM_3	.000	.230	.154	.746	.000	.001
	UM_4	.000	.087	.085	.688	.000	.000
UM_3	C	.000	.003	.014	.763	.000	.000
	UM_1	.000	.534	.704	.871	.002	.063
	UM_2	.000	.230	.154	.746	.000	.001
	UM_4	.000	.534	.704	.938	.024	.112
UM_4	C	.000	.001	.008	.822	.000	.000
	UM_1	.000	.230	.454	.810	.000	.004
	UM_2	.000	.087	.085	.688	.000	.000
	UM_3	.000	.534	.704	.938	.024	.112

Based on observed means. The mean difference is significant at the .05 level.

I & J both indicate control and different processed treatments (C, UM_1, UM_2, UM_3, UM_4), we are comparing different treatments with each other but I≠J.

C: control; UM_1, UM_2, UM_3, UM_4: different combined processed germinated treatments

UM_1: (5 min for 40% & 15 sec for 450W); UM_2: (5 min for 60% & 15 sec for 700W); UM_3: (10 min for 40% & 30 sec for 450W); UM_4: (10 min for 60% & 30 sec for 700W)

G: germination %; RL: root length; SL: shoot length; RL/SL: ratio of root length & shoot length; TW: total weight; SVI: seedling vigor index

4.2. Phytochemicals profile

In past two decades plant, derived phytochemicals have attained recognition regarding treatment and management of several diseases. All the processed treatment with control were quantitatively analyzed for various phytochemicals to ascertain changes in their levels as given in the following sections.

4.2.1. Alkaloids content

Alkaloid is an important parameter to see the bitterness of the grains that help in resistance to the bird's attack. The results of control and treated germinated sorghum seeds having application of different pre-germination techniques are presented here. Mean squares pertaining to alkaloid contents showed significant difference ($p \leq 0.01$) among all ultrasonic treatments (table 10) and combined application treatments (table 16) while in case of microwave treatments the difference was non-significant ($p > 0.05$) (table 13). The high stimulated germination caused a clear decrease of total alkaloid contents in each processed group as compared to control. In the current study, different pre-germination treatments enhancing germination can reduce the level of total alkaloids as presented by their means in tables 11, 14 & 17. Alkaloid contents of ultrasonic treated, microwave treated, and combined application treated group were reduced from 0.062±0.001 to 0.035±0.002 mg/100 g DM, 0.054±0.015 to 0.033±0.002 mg/100 g DM and 0.065±0.002 to 0.046±0.001 mg/100 g DM respectively. In each group, the treatment having highest germination rate showed the maximum decrease of total alkaloids such as US_1: 0.035±0.002 mg/100 g (5 min & 40% amplitude), MW_2: 0.033±0.002 mg/100 g DM (15 sec & 700W) and UM_2: 0.046±0.001 mg/100 g DM (US: 60%:5min & MW: 700W:15sec) as compared to control treatment (0.051±0.004 mg/100 g DM). So, the processing techniques increasing the germination rate decreased the amount of alkaloid as compared to control treatment (0.051±0.004 mg/100 g DM). In each processed group treatments with high germination rate gave lowest level of alkaloids and treatments with low germination rate has highest alkaloid contents. In ultrasonic treated and combined application treated group, the alkaloid contents decreased significantly ($p \leq 0.05$) while in microwave treated group the reduction was insignificant ($p > 0.05$). According to LSD test regarding pair wise multiple comparison given for alkaloids content the pair wise comparisons of treatment US_1 and US_4 of ultrasound processed group (table 12) showed significantly different effect ($p \leq 0.05$) on response in pair wise comparisons with

every other treatment. In microwave group (table 15) the pair wise comparisons of all treatments showed non-significant ($p > 0.05$) effect while in combined application treated group (table 18) treatment UM_3 and UM_4 gave significantly different effect ($p \leq 0.05$) in pair wise comparisons with every other treatment.

Investigation by Chilomer et al. (2010) reported remarkable reduction in alkaloids contents of lupin sprouts during germination. The current study was in agreement with the work of Zheng et al. (2005) who studied germinated beans, lentils and peas along with cotyledons of germinated mung bean (*Phaseolus aureus*) seeds for their alkaloid contents. No significant study regarding germination affecting alkaloids content in sorghum grains was found. Seeds of black cumin (*Nigella sativa*) during germination were investigated by Kamal and Ahmad (2014) and time dependent decrease of alkaloid content was reported in this study. In another study conducted by Sharara (2017) revealed a decrease in concentration of alkaloids in germinated fenugreek seeds (1.16±0.11 %). Similar results were also reported in another research conducted by Hooda and Jood (2003). The current study was also in agreement with another investigation conducted by Villacrés et al. (2015) who observed the reduction of total alkaloids as germination time increased. Results of current investigation showed compliance with results of Adekanmi et al. (2009) who observed up to 13% reduction in alkaloids in tiger nut (*Cyprus esculents*. L.) due to soaking of grains.

The difference in the residual concentration of alkaloids, depends upon the condition of the grain and the water used. Large contents of alkaloids were removed in bulk with stirred water during soaking. While in the sprouted grain, breaking of husk facilitate the removal of alkaloids (Bilbao et al., 2000). As the alkaloids are soluble in water therefore, these compounds are easily released in water from grains during soaking process. Generally, alkaloid contents in sprouts are dependent on the species, temperature, time and rate of germination (Villacrés et al., 2015). Among all the processed treatments, US_1 (5 min & 40% amplitude), MW_2 (15 sec & 700W) and UM_2 (US: 60%:5min & MW: 700W:15sec) showed better results regarding decreased alkaloid contents as compared with control while maximum reduction was given by treatment MW_2. For microwave processed group the reduction was insignificant ($p > 0.05$) but for ultrasonic processed and combined processed group reduction was significant ($p \leq 0.05$).

4.2.2. Saponins content

Saponin contents are linked with bitter taste, foaming properties and these compounds can cause injuries to the digestive mucosa. Mean squares of saponin contents given in tables 10, 13 & 16 present that saponin contents significantly ($p \leq 0.05$) changed in ultrasonic and microwave processed treatments of germinated sorghum while treatment having combined techniques application showed a non-significant change ($p > 0.05$). In the present study, different pretreatments affecting the germination rate also affected saponins amount as presented in tables 11, 14 & 17. There was a difference between three groups of germinated treatments i.e. microwave treated, ultrasonic treated and groups having application of both treatments in combination. In each group treatment with maximum germination rate showed reduced saponin contents. Reduction in saponins content of ultrasonic treated, microwave treated and combined treated group were ranged from 0.17±0.05 to 0.09±0.02 mg/100 g DM, 0.17±0.03 to 0.07±0.01 mg/100 g DM and 0.18±0.01 to 0.14±0.02 mg/100 g DM respectively. The maximum decrease of saponins was observed in microwave processed treatment at 15 sec & 700W (MW_2: 0.07±0.01 mg/100 g DM), ultrasonic processed treatment at 5 min & 40% amplitude (US_1: 0.09±0.02 mg/100 g DM) as compared to control sprouts (0.15±0.01 mg/100 g DM). While combined application processed treatment UM_2 (US: 60%:5min & MW: 700W:15sec) also showed reduced saponin contents (0.14±0.02 mg/100 g DM) compared to control (0.15±0.01 mg/100 g DM). According to LSD test regarding pair wise multiple comparison given for saponins content the pair wise comparisons of all treatments in combined application processed group (table 18) with other treatments gave non-significant effect ($p > 0.05$) on response. In microwave group (table 15) treatment MW_1 and MW_2 with all other treatments showed significantly ($p \leq 0.05$) different effect on response in pair wise comparisons. In ultrasonic processed group (table 12) treatment US_1 showed significantly ($p \leq 0.05$) different effect on response in pair wise comparisons with treatment US_3, US_4 and C (control).

In a study conducted by Arab et al. (2010) reported 58.17% reduction of saponins in *J. curcus* seeds after germination process. No significant study regarding germination affecting saponins content in sorghum grains was found. The present study is in agreement with some of earlier reports. For instance, Olawoye and Gbadamosi (2017) reported a significant difference of saponins in processed amaranth flour from 4.962 to 2.94 mg/100 g. Reduction of

saponins was observed in *Chenopodium album* from 4.82 to 0.93 g/100 g with the increase in germination time and temperature. Different processing techniques such as hydration, autoclaving, germination, cooking and their combinations were investigated for their effect on the reduction/elimination of antinutrients of three selected *Phaseolus vulgaris* varieties (Shimelis & Rakshit, 2007). More than 50% of the saponin contents were degraded through germination in all three bean varieties. Different processing methods (germination, boiling, pressure cooking and roasting) employed to chickpea caused a reduction of saponin contents and other anti-nutritional factors (Mittal et al., 2012). Saponins has few nutritional and pharmacological properties but also exert anti-nutritional effects and decrease the bioavailability of some nutrients (Stuardo & San Martín, 2008). The reduction of saponin contents during germination linked with solubilization and subsequent their leaching in soaking water. Another reason behind reduction is degradation of saponins due to enzymes but this has not been established yet (Mittal et al., 2012). In each group treatment with maximum germination rate showed reduced saponin contents. However, maximum reduction of saponins content was observed in microwave processed treatment MW_2 (15 sec at 700W), ultrasonic processed treatment US_1 (5 min with 40% amplitude) and UM_2 (US: 60%:5min & MW: 700W:15sec).

Table 10.

Mean squares of different phytochemicals for ultrasound processed sorghum sprouts

SOV	df	Alkaloids	Saponins	Tannins	Phytates	TFAAs	Sterols
Treatment	4	0.000281**	0.002940*	0.000386NS	63.6759**	0.306240**	0.003097**
Replicate	2	0.000013NS	0.001220NS	0.000142NS	28.0852**	0.000260NS	0.000387NS
Error	8	0.000008	0.000695	0.000132	0.0729	0.001585	0.000119
Total	14						

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

TFAAs: total free amino acids

Table 11.

Phytochemicals profile for ultrasound processed sorghum sprouts

Treatments	Alkaloids (mg/100 g DM)	Saponins (mg/100 g DM)	Tannins (mg CE/100 g DM)	Phytates (mg SPE/100 g DM)	TFAAs (mg AE/ g DM)	Sterols (mg CHE/g DM)
C	0.051 ± 0.004^{ab}	0.15 ± 0.01^{a}	0.147 ± 0.001^{ns}	146.05 ± 2.25^{abcd}	7.85 ± 0.03^{abc}	0.655 ± 0.025^{ab}
US_1	0.035 ± 0.002^{abcd}	0.09 ± 0.02^{abc}	0.131 ± 0.004^{a}	143.25 ± 2.32^{abcd}	8.03 ± 0.06^{abcd}	0.697 ± 0.002^{abcd}
US_2	0.040 ± 0.003^{ab}	0.12 ± 0.01^{a}	0.144 ± 0.005^{ns}	145.37 ± 2.51^{abcd}	7.91 ± 0.02^{abc}	0.674 ± 0.015^{abc}
US_3	0.052 ± 0.004^{ab}	0.15 ± 0.03^{a}	0.152 ± 0.001^{ns}	151.14 ± 2.03^{abcd}	7.55 ± 0.01^{abcd}	0.637 ± 0.001^{abc}
US_4	0.062 ± 0.001^{abcd}	0.17 ± 0.05^{ab}	0.162 ± 0.025^{a}	154.46 ± 2.74^{abcd}	7.24 ± 0.04^{abcd}	0.614 ± 0.003^{abcd}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; US_1, US_2, US_3, US_4: different ultrasound processed germinated treatments

US_1: (5 min for 40%); US_2: (5 min for 60%); US_3: (10 min for 40%); US_4: (10 min for 60%)

TFAAs: total free amino acids

Table 12.

Multiple comparisons (post hoc) LSD test of different phytochemicals for ultrasound processed sprouts

Treatment I	Treatment J	Significance levels					
		Alkaloids	Saponins	Tannins	Phytates	TFAAs	Sterols
C	US_1	.000	.024	.126	.000	.001	.002
	US_2	.421	.201	.757	.015	.102	.066
	US_3	.683	1.000	.608	.000	.000	.078
	US_4	.002	.380	.148	.000	.000	.002
US_1	C	.000	.024	.126	.000	.001	.002
	US_2	.000	.201	.203	.000	.006	.033
	US_3	.000	.024	.055	.000	.000	.000
	US_4	.000	.006	.011	.000	.000	.000
US_2	C	.421	.201	.757	.015	.102	.066
	US_1	.000	.201	.203	.000	.006	.033
	US_3	.239	.201	.418	.000	.000	.003
	US_4	.001	.049	.091	.000	.000	.000
US_3	C	.683	1.000	.608	.000	.000	.078
	US_1	.000	.024	.055	.000	.000	.000
	US_2	.239	.201	.418	.000	.000	.003
	US_4	.003	.380	.317	.000	.000	.033
US_4	C	.002	.380	.148	.000	.000	.002
	US_1	.000	.006	.011	.000	.000	.000
	US_2	.001	.049	.091	.000	.000	.000
	US_3	.003	.380	.317	.000	.000	.033

Based on observed means. The mean difference is significant at the .05 level.

I & J both indicate control and different processed treatments (C, US_1, US_2, US_3, US_4), we are comparing different treatments with each other but I≠J.

C: control; US_1, US_2, US_3, US_4: different ultrasound processed germinated treatments;

US_1: (5 min for 40%); US_2: (5 min for 60%); US_3: (10 min for 40%); US_4: (10 min for 60%).

TFAAs: total free amino acids

4.2.3. Tannins content

Tannin effects the palatability and also reduce feed intake due to having stringent taste. Tables 10, 13 & 16 presenting the mean squares of tannin contents showed that difference among all treatments of each group was non-significant ($p > 0.05$). From all the mean results of tannin contents achieved (tables 11, 14 & 17), it was noted that processed germination of the sorghum grain decreased the tannin content. The processed germinated treatments also showed different ranges of tannin contents as 0.162±0.025 to 0.131±0.004 mg CE/100 g DM (ultrasonic processing), 0.149±0.002 to 0.128±0.001 mg CE/100 g DM (microwave processing) and 0.162±0.045 to 0.148±0.025 mg CE/100 g DM (combined processing) as compared to control treatment (0.147±0.001 mg CE/100 g DM). In each processed group, tannin contents reduced insignificantly ($p > 0.05$). The maximum reduction of tannin contents occurred as germination percentage increased in microwave processed treatment MW_2: 15 sec at 700W (0.128±0.001 mg CE/100 g DM) and ultrasonic processed treatment US_1: 5 min with 40% amplitude (0.131±0.004 mg CE/100 g DM). These processed germinated treatments showed a clear decrease of tannin contents as compared to control (0.147±0.001 mg CE/100 g DM). Among the treatments of combined processed group, all the treatments showed higher tannins content as compared to control (0.147±0.001 mg CE/100 g DM). According to LSD test given in table 12, 15 & 18 about pair wise multiple comparison given for alkaloids content the pair wise comparisons of treatments US_1 & US_4 showed significantly ($p \leq 0.05$) different effect on response, while in microwave processed and combined application processed group all the pair wise comparison of every treatment showed a non-significant effect ($p > 0.05$) on response.

Raihanatu et al. (2011) documented similar trends and observed a significant reduction ranged 2.08% to 14.58% of tannin content in five local varieties of sorghum after application of combined processing technologies (sprouting and fermentation). Results of current study are also in agreement with investigation of Nour et al. (2015) who reported that raw sorghum flour contain 0.15 mg/100 g of tannin, which was insignificantly ($p \geq 0.05$) reduced after sprouting up to 0.13 mg/100 g. In another study, sprouting of lotus seeds significantly reduced the tannins concentration from 4.9 mg/g to as low as 0.7 mg/g (Sathithon & Yan-bin, 2012). Similar results were also reported by Khandelwal et al. (2010) as they observed significant reduction in tannin contents in germinated green gram compared to other verities (Bengal

gram, red gram and lentil). Up to 96% reduction in tannin contents was also observed in germinated kidney bean by Shimelis and Rakshit (2007). While in many other studies various processing combinations were investigated for tannin content, and it was observed that tannin content of all processed wheat samples was decreased in processed samples as compare to the raw sample (8.65 mg CAE/ 100g), however highest reduction (85.1%) in tannin contents were reported in fermentation after germination (Gunashree et al., 2014). Change in tannins contents during cereal grain processing was reported by several investigators, results of few studies revealed that tannin contents decreased after processing (Osman & Gassem, 2013).

Hydrophobic association of tannins with seed protein and enzymes is the main reason behind reduction in tannins along with their leaching process in water during germination (Gunashree et al., 2014; Shimelis & Rakshit, 2007). While it was also reported that washing during germination and binding of polyphenols with other organic substances (carbohydrate or protein) caused reduction in tannins (Saharan et al., 2002). During sprouting breakdown of protein-tannin and release of free tannins in soaking water mainly triggers reduction in tannin contents (Megat Rusydi & Azrina, 2012). While activation of peroxidase enzyme may also be the reason behind the reduction of tannin after processing (Hibberd et al., 1982). Reduction in tannin through germination contents enhanced the nutritional and functional quality of sorghum varieties (Osman & Gassem, 2013). Digestibility of sorghum protein was reduced due to presence of tannin because they bind with proteins and inhibit enzymes (Scalbert et al., 2000). Results showed that difference among all treatments of each processed group was non-significant ($p > 0.05$). Reduction of tannin contents in each group with processed germination was insignificant ($p > 0.05$). However, maximum reduction of tannins content was observed in microwave processed treatment MW_2 (15 sec at 700W) and ultrasonic processed treatment US_1 (5 min with 40% amplitude).

4.2.4. Phytates content

Generally, cereals have been regarded as the major source of dietary phytate. Due to their anti-nutritional properties, they are expected to decrease during various processing strategies. Mean squares of phytate contents of differently processed germinated sorghum treatments are given in tables 10, 13 & 16. The result showed a significant difference ($p \leq 0.01$) among different treatments of all processed germinated groups. Means of phytate contents of the processed sprouts showed different range of phytate contents depending upon the rate of

germination (tables 11, 14 & 17). The reduction of phytate contents of ultrasonic treated, microwave treated and combined treated group were ranged from 143.25±2.32 to 154.46±2.74 mg SPE/100 g DM, 142.27±2.47 to 150.34±2.00 mg SPE/100 g DM and 147.24±2.11 to 158.19±2.51 mg SPE/100 g DM respectively. Lowest contents were showed by ultrasound processed treatment for 5 min at 40% amplitude (143.25±2.32 mg SPE/100 g DM: US_1), and microwave processed treatment for 15 sec at 700W (142.27±2.47 mg SPE/100 g DM: MW_2). In both groups treatment with high germination rate give reduced amount of phytate contents. Among the treatments of combined processed group, all the treatments showed higher phytates content as compared to control (146.05±2.25 mg SPE/100 g DM). The results of processed germinated sorghum treatments showed that phytate contents of ultrasound and microwave treated sprouts were significantly ($p \leq 0.05$) decreased as compared to control germinated treatment (146.05±2.25 mg SPE/100 g DM). In contrast, the combined application treated group showed opposite results because there was no reduction in phytate contents in this group in comparison to control. According to LSD test regarding pair wise multiple comparison given for phytates content the pair wise comparisons of every treatment of ultrasound processed group (table 12) showed significantly different effect ($p \leq 0.05$) on response in pair wise comparisons with every other treatment. In microwave processed group (table 15) the pair wise comparisons of treatment MW_1 and MW_2 gave significantly different effect ($p \leq 0.05$) with treatments MW_3 and MW_4. Treatment UM_4 of combined application processed group (table 18) showed significantly ($p \leq 0.05$) different effect on responses with other treatments in pair wise comparisons.

Reduction in phytate contents during seed germination was reported in the literature (Ghavidel & Prakash, 2007; Ibrahim et al., 2002). But there is no significant work on this particular parameter in sorghum grain. Our results are in agreement with a study conducted by Megat Rusydi and Azrina (2012) as they showed that phytate contents of germinated soybean and peanut were significantly reduced 21.74% and 38.11% respectively as compared to non-germinated ones. Two varieties of soybean Giza 21 and Giza 35 containing 0.625% and 0.463% phytate contents respectively showed a significant reduction after various processing treatments such as soaking, germination and cooking methods (Ramadan, 2012). A research conducted on mung bean also reported significant reduction in phytate from 214.33a ± 0.5 to 188.93b ± 1.102 after sprouting (Grewal & Jood, 2006). Results of our investigation showed

compliance with results of Mohamed et al. (2011), who reported 11.0%, 15.4% and 18.5 % reduction of phytate in soybean, mung bean and kidney bean, respectively and also observed that losses of phytate was increased by increasing the germination period. After the 5^{th} day of germination, phytate content of soybean, mung bean and kidney bean gradually reduced from 35.1 to 23.70 mg/ g, 5.2 to 3.03 mg/ g and 5.4 to 3.5 mg/ g, respectively (Mohamed et al., 2011). Shimelis and Rakshit (2007) also reported similar results for three varieties of kidney beans after 4^{th} day of germination.

It was also reported in the literature that various processing treatments such as cooking, dehulling, soaking, fermentation and germination reduced the phytate and therefore improve the nutritional quality of food products (Shah et al., 2011). When phytate bound with protein, resultantly it reduces the solubility and functionality of the bounded protein therefore, reduce its availability (El-Adawy, 2002). Mineral availability greatly depends on the absence of phytate in grains (Khattak et al., 2007; Shimelis & Rakshit, 2007). Higher phytase activity was the main reason behind the reduction of phytate in the germinated grains and it was varied depending upon the variety of grains (Khattak et al., 2007; Shimelis & Rakshit, 2007; Kumar et al., 2010). This decrease in phytate might be linked with leaching of acids during soaking process under the concentration gradient (Liang et al., 2009; Vadivel et al., 2011). Results showed that difference among all treatments of each processed group was significant ($p \leq 0.05$). Reduction of phytates content in each group with processed germination was significant ($p \leq 0.05$). However, maximum reduction of tannins content was observed in microwave processed treatment MW_2 (15 sec at 700W) and ultrasonic processed treatment US_1 (5 min with 40% amplitude).

Table 13.

Mean squares of different phytochemicals for microwave processed sorghum sprouts

SOV	df	Alkaloids	Saponins	Tannins	Phytates	TFAAs	Sterols
Treatment	4	0.000207^{NS}	0.005160^{**}	0.000325^{NS}	35.552^{*}	0.124800^{**}	0.002380^{**}
Replicate	2	0.000049^{NS}	0.001580^{NS}	0.000442^{NS}	2.339^{NS}	0.000620^{NS}	0.000289^{NS}
Error	8	0.000212	0.000380	0.000253	6.779	0.002245	0.000253
Total	14						

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

TFAAs: total free amino acids

Table 14.

Phytochemicals profile for microwave processed sorghum sprouts

Treatments	Alkaloids (mg/100g DM)	Saponins (mg/100g DM)	Tannins (mg CE/100 g DM)	Phytates (mg SPE/100 g DM)	TFAAs (mg AE/ g DM)	Sterols (mg CHE/g DM)
C	0.051 ± 0.004^{ns}	0.15 ± 0.01^{ab}	0.147 ± 0.001^{ns}	146.05 ± 2.25^{ns}	7.85 ± 0.03^{abcd}	0.655 ± 0.025^{ab}
MW_1	0.043 ± 0.025^{ns}	0.11 ± 0.04^{abcd}	0.131 ± 0.035^{ns}	143.05 ± 3.00^{ab}	8.02 ± 0.05^{abc}	0.697 ± 0.005^{abc}
MW_2	0.033 ± 0.002^{ns}	0.07 ± 0.01^{abcd}	0.128 ± 0.001^{ns}	142.27 ± 2.47^{ab}	8.05 ± 0.03^{abc}	0.699 ± 0.025^{abc}
MW_3	0.049 ± 0.005^{ns}	0.16 ± 0.02^{ab}	0.149 ± 0.015^{ns}	148.44 ± 2.30^{ab}	7.71 ± 0.02^{abcd}	0.649 ± 0.003^{ab}
MW_4	0.054 ± 0.015^{ns}	0.17 ± 0.03^{ab}	0.149 ± 0.002^{ns}	150.34 ± 2.00^{ab}	7.57 ± 0.07^{abcd}	0.639 ± 0.004^{ab}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; MW_1, MW_2, MW_3, MW_4: different microwave processed germinated treatments

MW_1: (15 sec for 450W); MW_2: (15 sec for 700W); MW_3: (30 sec for 450W); MW_4: (30 sec for 700W)

TFAAs: total free amino acids

Table 15.

Multiple comparisons (post hoc) LSD test of phytochemicals for microwave processed sorghum sprouts

Treatment I	Treatment J	Significance levels					
		Alkaloids	Saponins	Tannins	Phytates	TFAAs	Sterols
C	MW_1	.520	.036	.253	.196	.002	.012
	MW_2	.168	.001	.182	.113	.001	.010
	MW_3	.870	.547	.882	.293	.007	.656
	MW_4	.807	.244	.882	.078	.000	.253
MW_1	C	.520	.036	.253	.196	.002	.012
	MW_2	.424	.036	.823	.723	.460	.881
	MW_3	.627	.014	.203	.035	.000	.006
	MW_4	.381	.005	.203	.009	.000	.002
MW_2	C	.168	.001	.182	.113	.001	.010
	MW_1	.424	.036	.823	.723	.460	.881
	MW_3	.215	.000	.145	.020	.000	.005
	MW_4	.115	.000	.145	.005	.000	.002
MW_3	C	.870	.547	.882	.293	.007	.656
	MW_1	.627	.014	.203	.035	.000	.006
	MW_2	.215	.000	.145	.020	.000	.005
	MW_4	.685	.547	1.000	.398	.007	.463
MW_4	C	.807	.244	.882	.078	.000	.253
	MW_1	.381	.005	.203	.009	.000	.002
	MW_2	.115	.000	.145	.005	.000	.002
	MW_3	.685	.547	1.000	.398	.007	.463

Based on observed means. The mean difference is significant at the .05 level.

I & J both indicate control and different processed treatments (C, MW_1, MW_2, MW_3, MW_4), we are comparing different treatments with each other but I≠J.

C: control; MW_1, MW_2, MW_3, MW_4: different microwave processed germinated treatments

MW_1: (15 sec for 450W); MW_2: (15 sec for 700W); MW_3: (30 sec for 450W); MW_4: (30 sec for 700W)

TFAAs: total free amino acids

4.2.5. Total free amino acids content (TFAAs)

Nutritional qualities of cereals and legumes can also be judged on the basis of their amino acid profile. Concentration of total free amino acids (TFAA) can be improved through germination and this may be due to activity of proteolytic enzymes. Mean squares of TFAA contents in germinated treatments among all processed groups are presented in tables 10, 13 & 16. These results showed highly significant ($p \leq 0.01$) difference among all germinated sorghum treatments in each group. The germination process improved the TFAA amount as clear from the means of TFAA amount of the all processed germinated treatments given in tables 11, 14 & 17. The TFAA values were ranged between 7.24±0.04 to 8.03±0.06 mg AE/g DM for the ultrasonic group, 7.57±0.07 to 8.05±0.03 mg AE/g DM for the microwave group and 7.16±0.07 to 7.73±0.06 mg AE/g DM for combined application treated group as compared to control treatment (7.85±0.03 mg AE/g DM). The highest TFAA amount was observed for the microwave treatment MW_2: 8.05 mg AE/g DM (15 sec & 700W) and ultrasonic treatment US_1:8.03 mg AE/g DM (5 min & 40% amplitude) as compared to control (7.85 mg AE/g DM). Among combined application processed group all the treatments showed TFAA contents comparatively lower than control (7.85±0.03 mg AE/g DM). So, in microwave processed and ultrasound processed group, TFAA contents increased significantly while in combined application treated group decreased significantly ($p \leq 0.05$). According to LSD test regarding pair wise multiple comparison given for TFAA contents the pair wise comparisons of treatments US_1, US_3, US_4 in ultrasonic processed group (table 12) with other treatments gave significantly different effect ($p \leq 0.05$) on response. In microwave processed group (table 15) treatment MW_3, MW_4 and control while in combined application processed group (table 18) treatment UM_1, UM_2 and control gave significantly different effect ($p \leq 0.05$) on response in pair wise multiple comparison with other treatments.

Results of our study are in agreement with the previous work conducted by Afify et al. (2012c) who subjected sorghum varieties (Dorado, Shandaweel-6 and Giza-15) to germination for 72 h. The obtained results showed that free amino acids in raw sorghum varieties ranged from 0.66 to 1.03 mg/g, in respective varieties while same varieties after treatment gave in the range of 8.78 to 9.94 mg/g. In another research conducted on sorghum investigated 8.2±0.01 mg/g of TFAA in raw sorghum which increased up to 16.6±0.07 mg/g after 72 h of germination (Yang et al., 2016). The study presented that concentration of free amino acids was increased

by 2 times at 72 h of germination. The TFAA concentration after 48 h of germination of wheat was 7.9 mg/g flour, which was remarkably higher than that of the non-germinated wheat i.e. 2.2 mg/g flour (Hung et al., 2012). *Ceiba pentandra* seed was investigated for its increment in free amino acids by Kiran et al. (2012) from 0.78 mg/g of tissue in non-germinating seeds to 2.20 mg/g of tissue in germinating seedlings. After 3rd day of germination, higher TFAA was observed in broad beans in attached cotyledons (Kırmızı & Güleryüz, 2006). Raw lentils seeds contain 1.86 mg/g DW of free amino acids, while 15.20 mg/g DW was reported after 6 days of germination (Fouad & Rehab, 2015). Similar results were reported by other researchers in other products mainly particularly cereals and legumes (Egli et al., 2004; Gernah et al., 2011).

It was also reported that longer time of germination produce higher amounts of the total free amino acids as indicated through research work conducted by Van Hung et al. (2015) who reported high-amylose wheat contained 1.3, 1.8, 2.4, 2.8 and 3.1 mg/g of grains (db) total free amino acids after 6, 12, 24, 36, and 48 hrs of germinated high-amylose wheats, respectively. And these values were significantly higher when compared to the un-germinated high-amylose wheats which was 1.3 mg/g of grains. Similar results were obtained during germination of oats seeds, in which the concentration of crude protein increased while protein was broken down to increase the soluble protein and concentration of free amino acids (Tian et al., 2010). During the germination process, hydrolytic enzymes (protease) degrade the protein into free amino acids to fulfill the embryo growth and seed requirements, while synthesis of new enzymes also helped to generate the free amino acids (Moongngarm & Saetung, 2010). In cellular biosynthesis and homeostasis and as nitrogen transporters free amino acids plays essential role in humans (Mapelli et al., 2001). As maximum germination rate showed increased TFAA contents. Therefore, maximum TFAA contents were observed in microwave processed treatment MW_2 (15 sec at 700W) and ultrasonic processed treatment US_1 (5 min with 40% amplitude).

4.2.6. Sterols content

Sterol is another bioactive compound found in plant oil having cholesterol lowering characteristics along with anticancer and antioxidant properties. In this study, sterol contents in the oil of processed germinated sorghum were analyzed. Mean squares of all the germinated treatments in each group are given in tables 10, 13 & 16, and results showed a highly significant ($p \leq 0.01$) difference in sterols contents among all the treatments of different processed groups.

Means of sorghum sprouts processed through different techniques are given in tables 11, 14 & 17. Ultrasonic processed and microwave processed sprouts showed ranges of 0.614±0.003 to 0.697±0.002 mg CHE/g DM and 0.639±0.004 to 0.699±0.025 mg CHE/g DM respectively. Highest amount was found in microwave processed sprouts at 15 sec & 700W (MW_2: 0.699±0.025 mg CHE/g DM) followed by ultrasonically processed sprouts at 5 min & 40% amplitude (US_1: 0.697±0.002 mg CHE/g DM) as compared to controlled sprouts (0.655±0.025 mg CHE/g DM). Sprouts treated through combined application of microwave and sonication ranged 0.553±0.005 to 0.619±0.025 mg CHE/g DM, which is even lower than the controlled sprouts (0.655±0.025 mg CHE/g DM). Treatments having high germination rate in microwave treated and ultrasonic treated group increased the sterol contents. Both groups showed significant ($p \leq 0.05$) increase while there was a significant ($p \leq 0.05$) decrease in treatments of combined application treated group. According to LSD test regarding pair wise multiple comparison given for sterols content the pair wise comparisons of treatments US_1, US_4 in ultrasonic processed group (table 12) with other treatments gave significantly different effect ($p \leq 0.05$) on response. In microwave processed group (table 15) the pair wise comparisons of treatment MW_1, MW_2 with treatment MW_1, MW_2 and control showed significantly different effect ($p \leq 0.05$) on response while in combined application processed group (table 18) treatment UM_1, UM_2 and control gave significantly different effect ($p \leq 0.05$) on response in pair wise multiple comparison with other treatments.

Several investigations are reported in literature showing effect of different pre-germination treatments on cereal grains. But regarding effect of germination on sterols content in sorghum a significant work was not found. Australian sweet lupin (ASL) was investigated for its total sterols and the concentration of sterols in germinated ASL flour and found higher (800%) as compared to non-germinated ASL flour (300%) (Jayasena & James, 2013). Shi et al. (2010) also investigated that the concentration of total sterols was in the range of 1004 to1987μg/g after 7^{th} day of germination. It was observed that amount of sterol increased 2 times from day 1 to day 3, and then stayed at an almost constant. Another research conducted on total sterols of soybean, sunflower seeds and rapeseeds reported 319.9, 338.7 and 751.6 mg/100 g seeds (dry basis) sterols, respectively (Rossell & Pritchard, 1991).

Actually, in newly growing plant tissues, the membrane biosynthesis is more intensive as compared to mature plant tissues. Resultantly, most of the sterols are produced during the

seed development and germination and therefore, sterols will deliver a supply for the newly growing cells and young shoots (Gül & Amar, 2006). During germination process, decomposition of triglycerides occurred and polar lipids were converted to simpler compounds, which cause the modification in lipids (Youssef et al., 1985). Sterols effect intestinal cholesterol absorption therefore, act as hypo-cholesterolemic (Pollak & Kritchevsky, 1981). They also act as competitor of cholesterol and promote the hypo-cholesterolemic effect (Sautier et al., 1990). Hypo-cholesterolemic properties and antioxidant activities are linked with sterols. It was reported in literature that sterols act as antioxidant due to their ability to restrict oil polymerization and to inhibit peroxide formation (Wang et al., 2002). With increasing germination rate sterols contents increased. Among all processed group maximum sterols content were observed in microwave processed treatment MW_2 (15 sec at 700W) and ultrasonic processed treatment US_1 (5 min with 40% amplitude) while treatments of combined processed group showed significantly decrease contents of sterols.

Table 16.

Mean squares of different phytochemicals for combined application processed sorghum sprouts

SOV	df	Alkaloids	Saponins	Tannins	Phytates	TFAAs	Sterols
Treatment	4	0.000166**	0.000690NS	0.000113NS	69.3740**	0.281940**	0.004749**
Replicate	2	0.000019NS	0.001940NS	0.001156NS	0.5249NS	0.001040NS	0.001584*
Error	8	0.000009	0.000690	0.000382	7.9907	0.002515	0.000229
Total	14						

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

TFAAs: total free amino acids

Table 17.

Phytochemicals profile for combined application processed sorghum sprouts

Treatments	Alkaloids (mg/100g DM)	Saponins (mg/100g DM)	Tannins (mg CE/100 g DM)	Phytates (mg SPE/100 g DM)	TFAAs (mg AE/ g DM)	Sterols (mg CHE/g DM)
C	0.051 ± 0.004^{ab}	0.15 ± 0.01^{ns}	0.147 ± 0.001^{ns}	146.05 ± 2.25^{ab}	7.85 ± 0.03^{abcd}	0.655 ± 0.025^{abcd}
UM_1	0.052 ± 0.003^{abc}	0.15 ± 0.04^{ns}	0.154 ± 0.003^{ns}	151.04 ± 3.00^{a}	7.53 ± 0.01^{abcd}	0.609 ± 0.035^{abc}
UM_2	0.046 ± 0.001^{abc}	0.14 ± 0.02^{ns}	0.148 ± 0.025^{ns}	147.24 ± 2.11^{a}	7.73 ± 0.06^{abcd}	0.619 ± 0.025^{abc}
UM_3	0.059 ± 0.005^{abcd}	0.16 ± 0.05^{ns}	0.156 ± 0.005^{ns}	152.47 ± 2.77^{ab}	7.21 ± 0.04^{abc}	0.574 ± 0.001^{abc}
UM_4	0.065 ± 0.002^{abcd}	0.18 ± 0.01^{ns}	0.162 ± 0.045^{ns}	158.19 ± 2.51^{abcd}	7.16 ± 0.07^{abc}	0.553 ± 0.005^{abc}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; UM_1, UM_2, UM_3, UM_4: different combined processed germinated treatments

UM_1: (5 min for 40% & 15 sec for 450W); UM_2: (5 min for 60% & 15 sec for 700W);

UM_3: (10 min for 40% & 30 sec for 450W); UM_4: (10 min for 60% & 30 sec for 700W)

TFAAs: total free amino acids

Table 18.

Multiple comparisons (post hoc) LSD test of phytochemicals for combined application processed sorghum sprouts

Treatment I	Treatment J	Significance levels					
		Alkaloids	Saponins	Tannins	Phytates	TFAAs	Sterols
C	UM_1	.695	1.000	.673	.063	.000	.006
	UM_2	.077	.653	.952	.620	.019	.020
	UM_3	.012	.653	.588	.024	.000	.000
	UM_4	.000	.199	.375	.001	.000	.000
UM_1	C	.695	1.000	.673	.063	.000	.006
	UM_2	.041	.653	.717	.138	.001	.442
	UM_3	.022	.653	.903	.553	.000	.022
	UM_4	.001	.199	.630	.015	.000	.002
UM_2	C	.077	.653	.952	.620	.019	.020
	UM_1	.041	.653	.717	.138	.001	.442
	UM_3	.001	.378	.630	.053	.000	.007
	UM_4	.000	.099	.406	.001	.000	.001
UM_3	C	.012	.653	.588	.024	.000	.000
	UM_1	.022	.653	.903	.553	.000	.022
	UM_2	.001	.378	.630	.053	.000	.007
	UM_4	.041	.378	.717	.038	.257	.128
UM_4	C	.000	.199	.375	.001	.000	.000
	UM_1	.001	.199	.630	.015	.000	.002
	UM_2	.000	.099	.406	.001	.000	.001
	UM_3	.041	.378	.717	.038	.257	.128

Based on observed means. The mean difference is significant at the .05 level.

I & J both indicate control and different processed treatments (C, UM_1, UM_2, UM_3, UM_4), we are comparing different treatments with each other but I≠J.

C: control; UM_1, UM_2, UM_3, UM_4: different combined processed germinated treatments

UM_1: (5 min for 40% & 15 sec for 450W); UM_2: (5 min for 60% & 15 sec for 700W);

UM_3: (10 min for 40% & 30 sec for 450W); UM_4: (10 min for 60% & 30 sec for 700W)

TFAAs: total free amino acids

4.3. Proximate composition

Food grade sorghum flour proved to be a much interesting and nutritional product. Proximate analysis of food is the determination of the major components of food. So, the processed germinated treatments of each group along with control were subjected to proximate analysis i.e. protein, carbohydrate and lipids.

4.3.1. Protein contents

Protein is the integral part of the body, it is used as a building material for various parts of the body such as muscle, brain, blood, skin, hair, nails, bones and body fluids. In this study protein contents were analyzed in differently processed germinated sorghum treatments. The mean squares from different treatments are given in tables 19, 22 & 25. From the result, it showed a highly significant change ($p \leq 0.01$) in protein contents among different treatments of processed germinated sorghum in each group. There was difference between the three groups of processed germinated treatments and control as the means are given in tables 20, 23 & 26. The processed sprouts showed different range of protein depending upon the germination rate as protein contents of ultrasonic treated, microwave treated, and combined application treated group were ranged from 10.23±0.07 to 11.96±0.01%, 10.50±0.04 to 11.84±0.05% and 10.21±0.05 to 10.73±0.03% respectively. Protein contents were increased with increasing rate of germination as the highest content was in ultrasonic processed treatment US_1 at 5 min & 40% amplitude (11.96±0.01%) followed by microwave processed treatment MW_2 at 15 sec & 700W (11.84±0.05%) as compared to control (10.83±0.05%). The results of germinated sorghum treatments showed that protein contents of microwave treated, and ultrasound treated sprouts were significantly higher ($p \leq 0.05$). Among combined application treated group the protein contents were not changed as compared to control treatment (10.83±0.05%). According to LSD test regarding pair wise multiple comparison (tables 21, 24 & 27) given for protein contents, the pair wise comparisons of all treatments in each processed group with other treatments gave significantly different effect ($p \leq 0.05$) on response.

Our study is in agreement with a study in which protein content of both white and yellow sprouted sweet maize was investigated by Oluwalana (2014) and it was reported that both varieties showed significantly higher protein contents as compared to control. Similarly, in many other studies increment in protein content was observed after sprouting (Ijarotimi & Keshinro, 2011; Sade, 2009). Effect of germination was investigated on brown rice after

various time intervals and higher protein contents were observed as compared to non-germinated rice. The protein contents were increased from 6.12 ± 0.04 to 8.14 ± 0.21 after 12 hr of germination then decreased from 8.01 ± 0.08 to 7.81 ± 0.12 after 24 hrs and 48 hrs respectively nevertheless still the protein content was higher as compared to non-germinated rice (Cornejo et al., 2015). African yam bean showed significantly higher protein contents in germinated seeds. On the other hand, fluted pumpkin seeds showed significantly higher protein after germination as compared to non-germinated ones (Onwuka et al., 2009). Various cereals, legumes and other seeds crops also showed the similar results (Abu El Gasim et al., 2008; Inyang & Zakari, 2008). Raw Australian sweet lupin seeds containing 44% of protein showed increase in protein contents up to 61.0 ± 2.0% (P = 0.05) after 9 days of germination as compared to raw seeds (Rumiyati et al., 2012). Data obtained from other studies revealed that the protein contents in wheat, maize, mung bean and groundnut flour was increased up to 10.02 ± 1.10 to 14.10±1.83%, 5.64 ± 0.15 to 7.30±1.54%, 21.9± 1.60 to 31.83±2.83% and 29.12 ± 1.26 to 31.60±2.83%, respectively (Kavitha & Parimalavalli, 2014). Protein contents of soaked and germinated soybean, sorghum and barley significantly increased from 29.09 % to 34.99 %, 7.25 % to 9.85 % and 11.25% to 13.85%, respectively (Sonone, 2014). It was reported by Tatsadjieu et al. (2004) that prolonged germination increased the protein contents of sorghum. Soybean also showed higher protein contents after germination up to 29.09 % to 34.99 % (Warle et al., 2015).

Mobilization of the stored protein cause increment in protein content. Synthesis of enzymes or a compositional change following the degradation may also cause the increment in the protein content (Oluwalana, 2014). Synthesis of enzymatic protein after germination was the reason reported behind this increment (Nzelibe & Nwasike, 1995). Other researchers reported that the increase in protein on germination was due to mobilization of stored nitrogen producing the nutritionally high-quality proteins for the young plant needs for its development and due to the degradation of stored protein and synthesis of new protein and other materials (Onwuka et al., 2009). Protein contents increased with increasing germination rate in each group. The results of germinated sorghum treatments showed that maximum protein contents were observed in ultrasonic processed treatment US_1 (5 min with 40% amplitude) and microwave processed treatment MW_2 (15 sec at 700W).

Table 19.

Mean squares of proximate composition for ultrasound processed sorghum sprouts

SOV	df	Proteins	Carbohydrates	Lipids
Treatment	4	1.72551^{**}	1.1567^{**}	0.544800^{**}
Replicate	2	0.00050^{NS}	24.1560^{**}	0.169280^{**}
Error	8	0.00225	0.0120	0.002180
Total	14			

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

Table 20.

Proximate composition for ultrasound processed sorghum sprouts

Treatments	Proteins (%)	Carbohydrates (%)	Lipids (%)
C	10.83 ± 0.05^{abcd}	75.05 ± 2.15^{abcd}	6.28 ± 0.21^{abcd}
US_1	11.96 ± 0.01^{abcd}	75.87 ± 2.27^{abcd}	6.53 ± 0.12^{abcd}
US_2	11.65 ± 0.04^{abcd}	75.55 ± 2.30^{abcd}	6.65 ± 0.15^{abcd}
US_3	10.42 ± 0.02^{abcd}	74.64 ± 2.24^{abcd}	7.15 ± 0.21^{abcd}
US_4	10.23 ± 0.07^{abcd}	74.37 ± 2.03^{abcd}	7.29 ± 0.23^{abcd}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; US_1, US_2, US_3, US_4: different ultrasound processed germinated treatments

US_1: (5 min for 40%); US_2: (5 min for 60%); US_3: (10 min for 40%); US_4: (10 min for 60%)

Table 21.

Multiple comparisons (post hoc) LSD test of proximate composition for ultrasound processed sorghum sprouts

Treatment I	Treatment J	Significance levels		
		Protein	Carbohydrate	Lipids
C	US_1	.000	.000	.000
	US_2	.000	.001	.000
	US_3	.000	.002	.000
	US_4	.000	.000	.000
US_1	C	.000	.000	.000
	US_2	.000	.007	.014
	US_3	.000	.000	.000
	US_4	.000	.000	.000
US_2	C	.000	.001	.000
	US_1	.000	.007	.014
	US_3	.000	.000	.000
	US_4	.000	.000	.000
US_3	C	.000	.002	.000
	US_1	.000	.000	.000
	US_2	.000	.000	.000
	US_4	.001	.016	.006
US_4	C	.000	.000	.000
	US_1	.000	.000	.000
	US_2	.000	.000	.000
	US_3	.001	.016	.006

Based on observed means. The mean difference is significant at the .05 level. I & J both indicate control and different processed treatments (C, US_1, US_2, US_3, US_4), we are comparing different treatments with each other but I≠J.

C: control; US_1, US_2, US_3, US_4: different ultrasound processed germinated treatments; US_1: (5 min for 40%); US_2: (5 min for 60%); US_3: (10 min for 40%); US_4: (10 min for 60%)

4.3.2. Carbohydrate contents

In this study carbohydrate contents were analyzed in differently processed germinated sorghum treatments as their mean square values are given in tables 19, 22 & 25. These tables are indicating a clear significant difference ($p \leq 0.01$) of carbohydrate contents among all treatments. Similarly, the processed sprouts showed different range of carbohydrate contents depending upon the rate of germination as clear from their means given in tables 20, 23 & 26. Carbohydrate contents of ultrasonic treated, microwave treated and combined treated group were ranged from 74.37±2.03 to 75.87±2.27%, 74.68±2.00 to 75.87±2.34% and 74.3±2.00 to 74.8±2.21%, respectively. The results of germinated sorghum treatments showed that carbohydrate contents increased with increasing germination rate. In microwave processed group treatment MW_2 (15 sec & 700W) and ultrasound processed treatment US_1 (5 min & 40% amplitude) increase was up to 75.87% as compared to control germination treatment (75.05±2.15%). Among combined application processed group, all treatments showed lower protein contents compared to control treatment (75.05±2.15%). So, the results showed that carbohydrate contents of microwave treated, and ultrasound treated sprouts were increased significantly while decreased for combined treated group as compared to control treatment. According to LSD test regarding pair wise multiple comparison (tables 21, 24 & 27) given for carbohydrate contents, the pair wise comparisons of all treatments in ultrasonic processed group with other treatments gave significantly different effect ($p \leq 0.05$) on response. For microwave processed group treatment MW_2, with MW_3, MW_4 and control showed significantly different effect ($p \leq 0.05$) on response in pair wise comparisons. While in combined application treated group control with treatment UM_1, UM_3 and UM_4 gave significantly different effect ($p \leq 0.05$) on response.

Results of our study are also in agreement with other study conducted by Banja et al. (2016) who noted surprisingly higher carbohydrate contents after soaking and germination of Black Beni sesame seeds as compared to the raw seeds. Concentration of carbohydrates was in the range of 14.6% - 39.5% in germinated and soaked seeds. Carbohydrate contents of germinated seeds were 39.5% which were significantly higher at $p < 0.05$ compared to the raw seeds which was 24.1%. Carbohydrates of sorghum starches after 5 to 7 days of sprouting were in the range of 79.39% and 78.64% (Otutu et al., 2014). In another research, continues increment in carbohydrate contents were observed in sorghum grains as sprouted days

increased (Mbaeyi & Onweluzo, 2010). Concentrations of carbohydrate of wheat, maize, mung bean and groundnut flour after germination were noted in the range of 80.32±7.26 - 87.60±6.83%, 78.02 ± 3.26-97.60±4.83 %, 61.24±1.32 - 65.12 ± 3.26% and 76.21±4.83 - 79.01± 5.21%, respectively (Kavitha & Parimalavalli, 2014). These findings showed compliance with results of Torres et al. (2007) who quantified that carbohydrate content of the raw and germinated wheat flour samples ranged 82.13 ± 0.49 - 84.63±0.43%. Similar results were given by Adetunde et al. (2012) as sorghum sprouted for 5 days having the highest carbohydrate content.

It was observed that stored lipids extensively converted to soluble carbohydrate after germination leading to increased carbohydrate contents (Eastmond & Graham, 2001). Germination process enhances enzymatic reactions in germinated grains, resulting breakdown of complex carbohydrate into simpler ones (Nout & Ngoddy, 1997). Research works conducted in various cereal grains examined that hydrolytic enzymes become triggered during germination leading to breakdown of starch and non-starch polysaccharides. The combined effect of α- and β-amylases, debranching enzyme and α-glucosidase leads to starch breakdown thus increasing sugars and short chain carbohydrates (Hung et al., 2012). So, the results showed that microwave processed treatment MW_2 (15 sec & 700W) and ultrasound processed treatment US_1 (5 min & 40% amplitude) due to high germination rate gave maximum protein contents as compared to control germination treatment.

Table 22.

Mean squares of proximate composition for microwave processed sorghum sprouts

SOV	df	Proteins	Carbohydrates	Lipids
Treatment	4	1.11531^{**}	0.8947^{*}	0.63816^{**}
Replicate	2	0.00288^{NS}	30.0615^{**}	0.08450^{NS}
Error	8	0.00118	0.1774	0.05025
Total	14			

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

Table 23.

Proximate composition for microwave processed sorghum sprouts

Treatments	Proteins (%)	Carbohydrates (%)	Lipids (%)
C	10.83 ± 0.05^{abcd}	75.05 ± 2.15^{a}	6.28 ± 0.21^{abcd}
MW_1	11.71 ± 0.03^{abcd}	75.79 ± 3.00^{ab}	6.77 ± 0.22^{abc}
MW_2	11.84 ± 0.05^{abcd}	75.87 ± 2.34^{abc}	7.02 ± 0.24^{ab}
MW_3	10.73 ± 0.01^{abcd}	74.86 ± 2.77^{ab}	7.24 ± 0.25^{ab}
MW_4	10.50 ± 0.04^{abcd}	74.68 ± 2.00^{ab}	7.48 ± 0.27^{abc}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; MW_1, MW_2, MW_3, MW_4: different microwave processed germinated treatments

MW_1: (15 sec for 450W); MW_2: (15 sec for 700W); MW_3: (30 sec for 450W); MW_4: (30 sec for 700W)

Table 24.

Multiple comparisons (post hoc) LSD test of proximate composition for microwave processed sorghum sprouts

Treatment I	Treatment J	Significance levels		
		Proteins	**Carbohydrates**	**Lipids**
C	MW_1	.000	.064	.028
	MW_2	.000	.044	.004
	MW_3	.007	.596	.001
	MW_4	.000	.313	.000
MW_1	C	.000	.064	.028
	MW_2	.002	.822	.209
	MW_3	.000	.027	.033
	MW_4	.000	.012	.005
MW_2	C	.000	.044	.004
	MW_1	.002	.822	.209
	MW_3	.000	.019	.264
	MW_4	.000	.009	.036
MW_3	C	.007	.596	.001
	MW_1	.000	.027	.033
	MW_2	.000	.019	.264
	MW_4	.000	.615	.226
MW_4	C	.000	.313	.000
	MW_1	.000	.012	.005
	MW_2	.000	.009	.036
	MW_3	.000	.615	.226

Based on observed means. The mean difference is significant at the .05 level. I & J both indicate control and different processed treatments (C, MW_1, MW_2, MW_3, MW_4), we are comparing different treatments with each other but I≠J.

C: control; MW_1, MW_2, MW_3, MW_4: different microwave processed germinated treatments. MW_1: (15 sec for 450W); MW_2: (15 sec for 700W); MW_3: (30 sec for 450W); MW_4: (30 sec for 700W)

4.3.3. Lipid contents

Mean squares of total lipid contents are presented in tables 19, 22 & 25. The results showed that all treatments are significantly ($p \leq 0.01$) different in lipid contents. Means of oil extraction yield after germination processed through applying different processing pre-treatments have been presented in tables 20, 23 & 26. The pre-germination processing treatments increased the lipid contents in all treatments as compared to control (6.28±0.21%). The lipid contents of ultrasonic treated, microwave treated, and combined application treated sprouts were ranged between 6.53±0.12 to 7.29±0.23%, 6.77±0.22 to 7.48±0.27% and 6.81±0.16 to 7.57±0.26%, respectively. The optimum condition for microwave pretreatment was 700 W for 30 sec (MW_4) which resulted in the highest oil yield of 7.48±0.27%. Ultrasonic amplitude of 60% (US_4) for 10 min showed highest oil yield of 7.29±0.23% for samples as compared to control (6.28±0.21%). Both treatment amplitude and exposure duration had an encouraging influence on extracted oil yield from germinated sorghum grains. The combined application of microwave and ultrasound treatment exhibited significant difference in oil yield as compared to control. Maximum oil yield 7.57±0.26% (UM_4) was detected by combined application of microwave power (700 W) and ultrasound amplitude (60%) for 30s and 10 min, respectively, as compared to control (6.28±0.21%). According to LSD test regarding pair wise multiple comparison (tables 21, 24 & 27) given for lipids contents, the pair wise comparisons of all treatments in ultrasonic processed group with other treatments gave significantly different effect ($p \leq 0.05$) on response. For microwave processed group and combined application treated group, control showed significantly different effect ($p \leq 0.05$) on response in pair wise comparisons with all other treatments.

Oil yield is not in direct correlation with germination rate like other parameters while it depends upon amplitude and pretreatment time. Many studies proved that oil yield increased by increasing the level of ultrasound (Hashemi et al., 2015) and this increment linked with cavitation process which produced due to ultrasound treatment. Membrane of cells in the hot spot ruptured by the physical effects of cavitation process result in release of material. These released material pours out into the solvent and get dissolved in it (Feng et al., 2008; Wang et al., 2006). Due to the microwave treatment cell membrane of oil seeds can readily ruptured therefore, higher yield of oil and increased mass transfer coefficients can be obtained. Microwave treatment also create permanent pores which increase the permeability of oil hence,

increase the oil yield (Azadmard-Damirchi et al., 2011). Pretreatment of oil seeds with other physical methods before ultrasound process weaken the particle surface bounds and increase the oil yield effectively (Arabani et al., 2015). The results of oil yield are in compliance with earlier results (Chung et al., 2011; Hamad, 2007; Uquiche et al., 2008). Results showed with increased levels of microwave and ultrasound treatments the oil yield also increased significantly. Among all the processed groups highest oil yield was observed in treatments UM_4 (60% for 10 min; 700 W for 30 sec), MW_4 (700 W for 30 sec) and US_4 (60% for 10 min) as compared to control (6.28±0.21%).

Table 25.

Mean squares of proximate composition for combined application processed sorghum sprouts

SOV	df	Proteins	Carbohydrates	Lipids
Treatment	4	0.212160^{**}	0.2758^{**}	0.75801^{**}
Replicate	2	0.005120^{**}	24.3322^{**}	0.03302^{NS}
Error	8	0.000320	0.0350	0.04720
Total	14			

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

Table 26.

Proximate composition for combined application processed sorghum sprouts

Treatments	Proteins (%)	Carbohydrates (%)	Lipids (%)
C	10.83 ± 0.05^{abcd}	75.05 ± 2.15^{abc}	6.28 ± 0.21^{abcd}
UM_1	10.41 ± 0.01^{abcd}	74.6 ± 2.51^{a}	6.81 ± 0.16^{abc}
UM_2	10.73 ± 0.03^{abcd}	74.8 ± 2.21^{ab}	7.14 ± 0.19^{ab}
UM_3	10.33 ± 0.02^{abcd}	74.4 ± 2.16^{ab}	7.34 ± 0.22^{ab}
UM_4	10.21 ± 0.05^{abcd}	74.3 ± 2.00^{ab}	7.57 ± 0.26^{abc}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; UM_1, UM_2, UM_3, UM_4: different combined processed germinated treatments

UM_1: (5 min for 40% & 15 sec for 450W); UM_2: (5 min for 60% & 15 sec for 700W);

UM_3: (10 min for 40% & 30 sec for 450W); UM_4: (10 min for 60% & 30 sec for 700W)

Table 27.

Multiple comparisons (post hoc) LSD test of proximate composition for combined application processed sorghum sprouts

Treatment I	Treatment J	Significance levels		
		Proteins	**Carbohydrates**	**Lipids**
C	UM_1	.000	.019	.017
	UM_2	.000	.298	.001
	UM_3	.000	.003	.000
	UM_4	.000	.002	.000
UM_1	C	.000	.019	.017
	UM_2	.000	.104	.100
	UM_3	.001	.227	.017
	UM_4	.000	.140	.003
UM_2	C	.000	.298	.001
	UM_1	.000	.104	.100
	UM_3	.000	.014	.292
	UM_4	.000	.008	.042
UM_3	C	.000	.003	.000
	UM_1	.001	.227	.017
	UM_2	.000	.014	.292
	UM_4	.000	.752	.231
UM_4	C	.000	.002	.000
	UM_1	.000	.140	.003
	UM_2	.000	.008	.042
	UM_3	.000	.752	.231

Based on observed means. The mean difference is significant at the .05 level. I & J both indicate control and different processed treatments (C, UM_1, UM_2, UM_3, UM_4), we are comparing different treatments with each other but I≠J.

C: control; UM_1, UM_2, UM_3, UM_4: different combined processed germinated treatments. UM_1: (5 min for 40% & 15 sec for 450W); UM_2: (5 min for 60% & 15 sec for 700W); UM_3: (10 min for 40% & 30 sec for 450W); UM_4: (10 min for 60% & 30 sec for 700W)

4.4. Fatty acids profile

4.4.1. Saturated fatty acids

Mean squares regarding saturated fatty acids contents including palmitic, stearic and arachidic acid are presented in tables 28, 31 & 34. The treatments difference was non-significant ($p > 0.05$) in term of saturated fatty acids content. The difference in the fatty acid composition of sorghum oil was observed between control treatment and all processed sample as given in tables 29, 32 & 35. Range of palmitic acid was 14.49±0.10 to 14.54±0.09%, 14.50±0.09 to 14.55±0.13% and 14.50±0.10 to 14.56±0.12% for ultrasonic treated, microwave treated and combine application treated sprouts, respectively, compared to control (14.51±0.12%). For stearic acid, the ranges were 1.74±0.05 to 1.78±0.10% (ultrasonic group), 1.73±0.10 to 1.77±0.11% (microwave group) and 1.74±0.05 to 1.79±0.12% (combined application) in comparison to control (1.73±0.05%). Similarly, as compared to control (0.18±0.02%), arachidic acid ranges were 0.17±0.02 to 0.20±0.03%, 0.18±0.02 to 0.21±0.01% and 0.17±0.03 to 0.20±0.04% for ultrasonic, microwave and combined application group respectively. Palmitic, stearic and arachidic acids were observed at their highest levels (14.55±0.13%, 1.77±0.11% and 0.21±0.01%, respectively) for microwave treatment MW_4 of 700W for 30 sec. Similarly, maximum fatty acid % for palmitic, stearic and arachidic acid (14.54±0.09%, 1.78±0.10% and 0.20±0.03%, respectively) for ultrasound treatment (US_4) was at 60% US amplitude for 10 min. The highest value in palmitic acid, stearic and arachidic acid (14.56±0.12%, 1.79±0.12% and 0.20±0.04%, respectively) was analyzed for combined application of microwave and sonication UM_4 (US: 60% for 10 min & MW: 700W for 30 sec). The results presented improved composition of saturated fatty acid in treated germinated grain. Regarding saturated fatty acids, palmitic acid was the most leading fatty acids (14.56 ± 0.12%), while least one was arachidic acid (0.21 ± 0.01%). So, in each group saturated fatty acids increase insignificantly ($p > 0.05$) as compared to control. According to LSD test regarding pair wise multiple comparison (tables 30, 33 & 36) given for saturated fatty acids, the pair wise comparisons of treatment US_1 with US_4 for arachidic acid in ultrasonic processed group gave significantly different effect ($p \leq 0.05$) on response. In microwave group pair wise comparisons of treatment MW_4 with C, MW_I, MW_2 for palmitic acid, showed significantly different effect ($p \leq 0.05$), while in combine application group all treatments gave non-significant ($p > 0.05$) effect for all saturated fatty acids in pair wise comparison with other treatments.

Reported composition of sorghum grain is exactly alike with previously reported composition (Mehmood et al., 2008; Pontieri et al., 2012). Effect of microwave treatment on peanut seeds (*Arachis hypogaea* L.) was investigated by Yoshida et al. (2005), on sunflower seed (*Heliantus annuus* L.) by Anjum et al. (2006) and on pumpkin seeds (*Cucurbita spp.*) by Yoshida et al. (2006). The composition of extracted oil was significantly changed due the effect of microwave treatment. Similar changes were also reported by the other authors in previous investigations, for instance, effect of MW on peanut oil was conducted by the Yoshida et al. (2003) and significant changes were observed in composition of oil. During germination increase in oil was also reported which linked with non-conversion of fatty acids into carbohydrates (Afam-Anene & Onuoha, 2006). Actually, the increase in treatment time improved fatty acid composition. Among all the processed groups highest contents of saturated fatty acids were observed in treatments UM_4 (60% for 10 min; 700 W for 30 sec), MW_4 (700 W for 30 sec) and US_4 (60% for 10 min) as compared to control.

Table 28.

Mean squares of saturated fatty acids for ultrasound processed sorghum sprouts

SOV	df	Palmitic acid	Stearic acid	Arachidic acid
Treatment	4	0.001050^{NS}	0.001110^{NS}	0.000360^{NS}
Replicate	2	0.021860^{*}	0.009500^{NS}	0.003980^{**}
Error	8	0.004510	0.004500	0.000230
Total	14			

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

Table 29.

Saturated fatty acids for ultrasound processed sorghum sprouts

Treatments	Palmitic acid (%)	Stearic acid (%)	Arachidic acid (%)
C	14.51 ± 0.12^{ns}	1.73 ± 0.05^{ns}	0.18 ± 0.02^{ns}
US_1	14.49 ± 0.10^{ns}	1.74 ± 0.05^{ns}	0.17 ± 0.02^{a}
US_2	14.50 ± 0.05^{ns}	1.75 ± 0.10^{ns}	0.18 ± 0.04^{ns}
US_3	14.51 ± 0.07^{ns}	1.76 ± 0.05^{ns}	0.18 ± 0.04^{ns}
US_4	14.54 ± 0.09^{ns}	1.78 ± 0.10^{ns}	0.20 ± 0.03^{a}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; US_1, US_2, US_3, US_4: different ultrasound processed germinated treatments

US_1: (5 min for 40%); US_2: (5 min for 60%); US_3: (10 min for 40%); US_4: (10 min for 60%)

Table 30.

Multiple comparisons (post hoc) LSD test of saturated fatty acids for ultrasound processed sorghum sprouts

Treatment I	Treatment J	Significance levels		
		Palmitic acid	Stearic acid	Arachidic acid
C	US_1	.725	.860	.443
	US_2	.860	.724	1.000
	US_3	1.000	.599	1.000
	US_4	.599	.388	.145
US_1	C	.725	.860	.443
	US_2	.860	.860	.443
	US_3	.725	.724	.443
	US_4	.388	.486	.042
US_2	C	.860	.724	1.000
	US_1	.860	.860	.443
	US_3	.860	.860	1.000
	US_4	.487	.599	.145
US_3	C	1.000	.599	1.000
	US_1	.725	.724	.443
	US_2	.860	.860	1.000
	US_4	.599	.724	.145
US_4	C	.599	.388	.145
	US_1	.388	.486	.042
	US_2	.487	.599	.145
	US_3	.599	.724	.145

Based on observed means. The mean difference is significant at the .05 level. I & J both indicate control and different processed treatments (C, US_1, US_2, US_3, US_4), we are comparing different treatments with each other but I≠J.

C: control; US_1, US_2, US_3, US_4: different ultrasound processed germinated treatments; US_1: (5 min for 40%); US_2: (5 min for 60%); US_3: (10 min for 40%); US_4: (10 min for 60%)

Table 31.

Mean squares of saturated fatty acids for microwave processed sorghum sprouts

SOV	df	Palmitic acid	Stearic acid	Arachidic acid
Treatment	4	0.001110^{NS}	0.001200^{NS}	0.000450^{NS}
Replicate	2	0.060500^{**}	0.011180^{NS}	0.000020^{NS}
Error	8	0.000350	0.008880	0.000670
Total	14			

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

Table 32.

Saturated fatty acids for microwave processed sorghum sprouts

Treatments	Palmitic acid (%)	Stearic acid (%)	Arachidic acid (%)
C	14.51 ± 0.12^{a}	1.73 ± 0.05^{ns}	0.18 ± 0.02^{ns}
MW_1	14.51 ± 0.12^{a}	1.73 ± 0.10^{ns}	0.18 ± 0.02^{ns}
MW_2	14.50 ± 0.09^{a}	1.75 ± 0.10^{ns}	0.19 ± 0.03^{ns}
MW_3	14.52 ± 0.09^{ns}	1.77 ± 0.11^{ns}	0.19 ± 0.03^{ns}
MW_4	14.55 ± 0.13^{abc}	1.77 ± 0.11^{ns}	0.21 ± 0.01^{ns}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; MW_1, MW_2, MW_3, MW_4: different microwave processed germinated treatments

MW_1: (15 sec for 450W); MW_2: (15 sec for 700W); MW_3: (30 sec for 450W); MW_4: (30 sec for 700W)

Table 33.

Multiple comparisons (post hoc) LSD test of saturated fatty acids for microwave processed sorghum sprouts

Treatment I	Treatment J	Significance levels		
		Palmitic acid	Stearic acid	Arachidic acid
C	MW_1	1.000	1.000	1.000
	MW_2	.531	.801	.649
	MW_3	.531	.617	.649
	MW_4	.031	.617	.194
MW_1	C	1.000	1.000	1.000
	MW_2	.531	.801	.649
	MW_3	.531	.617	.649
	MW_4	.031	.617	.194
MW_2	C	.531	.801	.649
	MW_1	.531	.801	.649
	MW_3	.227	.801	1.000
	MW_4	.011	.801	.372
MW_3	C	.531	.617	.649
	MW_1	.531	.617	.649
	MW_2	.227	.801	1.000
	MW_4	.085	1.000	.372
MW_4	C	.031	.617	.194
	MW_1	.031	.617	.194
	MW_2	.011	.801	.372
	MW_3	.085	1.000	.372

Based on observed means. The mean difference is significant at the .05 level. I & J both indicate control and different processed treatments (C, MW_1, MW_2, MW_3, MW_4), we are comparing different treatments with each other but I≠J.

C: control; MW_1, MW_2, MW_3, MW_4: different microwave processed germinated treatments. MW_1: (15 sec for 450W); MW_2: (15 sec for 700W); MW_3: (30 sec for 450W); MW_4: (30 sec for 700W)

Table 34.

Mean squares of saturated fatty acids for combined application processed sorghum sprouts

SOV	df	Palmitic acid	Stearic acid	Arachidic acid
Treatment	4	0.001740^{NS}	0.001710^{NS}	0.000390^{NS}
Replicate	2	0.023120^{NS}	0.014820^{NS}	0.003440^{*}
Error	8	0.005720	0.006195	0.000490
Total	14			

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

Table 35.

Saturated fatty acids for combined application processed sorghum sprouts

Treatments	Palmitic acid (%)	Stearic acid (%)	Arachidic acid (%)
C	14.51 ± 0.12^{ns}	1.73 ± 0.05^{ns}	0.18 ± 0.02^{ns}
UM_1	14.50 ± 0.10^{ns}	1.74 ± 0.05^{ns}	0.17 ± 0.03^{ns}
UM_2	14.52 ± 0.06^{ns}	1.76 ± 0.09^{ns}	0.18 ± 0.03^{ns}
UM_3	14.54 ± 0.06^{ns}	1.77 ± 0.11^{ns}	0.19 ± 0.04^{ns}
UM_4	14.56 ± 0.12^{ns}	1.79 ± 0.12^{ns}	0.20 ± 0.04^{ns}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; UM_1, UM_2, UM_3, UM_4: different combined processed germinated treatments

UM_1: (5 min for 40% & 15 sec for 450W); UM_2: (5 min for 60% & 15 sec for 700W);

UM_3: (10 min for 40% & 30 sec for 450W); UM_4: (10 min for 60% & 30 sec for 700W)

Table 36.

Multiple comparisons (post hoc) LSD test of saturated fatty acids for combined application processed sorghum sprouts

Treatment I	Treatment J	Significance levels		
		Palmitic acid	Stearic acid	Arachidic acid
C	UM_1	.875	.880	.595
	UM_2	.875	.653	1.000
	UM_3	.640	.551	.595
	UM_4	.442	.378	.301
UM_1	C	.875	.880	.595
	UM_2	.754	.764	.595
	UM_3	.535	.653	.301
	UM_4	.360	.459	.136
UM_2	C	.875	.653	1.000
	UM_1	.754	.764	.595
	UM_3	.754	.880	.595
	UM_4	.535	.653	.301
UM_3	C	.640	.551	.595
	UM_1	.535	.653	.301
	UM_2	.754	.880	.595
	UM_4	.754	.764	.595
UM_4	C	.442	.378	.301
	UM_1	.360	.459	.136
	UM_2	.535	.653	.301
	UM_3	.754	.764	.595

Based on observed means. The mean difference is significant at the .05 level. I & J both indicate control and different processed treatments (C, UM_1, UM_2, UM_3, UM_4), we are comparing different treatments with each other but $I \neq J$.

C: control; UM_1, UM_2, UM_3, UM_4: different combined processed germinated treatments. UM_1: (5 min for 40% & 15 sec for 450W); UM_2: (5 min for 60% & 15 sec for 700W); UM_3: (10 min for 40% & 30 sec for 450W); UM_4: (10 min for 60% & 30 sec for 700W)

4.4.2 Unsaturated fatty acids

Tables 37, 40 & 43 presents the mean squares of sorghum unsaturated fatty acids after germination by applying different pre-germination processing. Among all studied unsaturated fatty acids, results showed a significant difference ($p \leq 0.05$) in linoleic acid and eicosenoic acid for ultrasonic treatments, eicosenoic acid ($p \leq 0.01$) for microwave treatments while oleic acid, linoleic acid ($p \leq 0.05$) and eicosenoic acid ($p \leq 0.01$) in combined application treatments. Means of unsaturated fatty acids in tables 38, 41 & 44 showed that fatty acid compositions of oils did not change much with microwave and ultrasound treatment. Range of palmitoleic acid was 0.47±0.01 to 0.48±0.04%, 0.47±0.01 to 0.49±0.05% and 0.47±0.01 to 0.49±0.05% for ultrasonic treated, microwave treated and combine application treated sprouts, respectively, compared to control (0.48±0.02%). For oleic acid, the ranges were 34.08±0.12 to 34.15±0.13% (ultrasonic group), 34.04±0.10 to 34.12±0.14% (microwave group) and 34.12±0.13 to 34.15±0.11% (combined application) in comparison to control (34.10±0.11%). Linoleic acid and linolenic acids were ranged between 43.42±0.21 to 43.48±0.25% & 1.86±0.05 to 1.96±0.12% (ultrasonic group); 43.48±0.24 to 43.57±0.31% & 1.85±0.05 to 1.93±0.111% (microwave group); 43.45±0.22 to 43.57±0.31% & 1.87±0.06 to 1.96±0.12% (combined application) respectively, as compared to control (linoleic acid: 43.45±0.22% & linolenic acid: 1.89±0.07%). Similarly, eicosenoic acid ranges were 0.30±0.01 to 0.33±0.03 %, 0.34±0.02 to 0.38±0.03% and 0.33±0.02 to 0.39±0.04% for ultrasonic, microwave and combined application group, respectively, in comparison to control (0.34±0.01%). The results showed that the highest values for linoleic acid and oleic acid in microwave treatment (MW_4: 30 sec & 700W) were 43.57±0.31% and 34.12±0.14%, respectively. The ultrasound processed sorghum samples showed good results for linolenic acid 1.96±0.12% (US_4: 10 min & 60%) while amount of eicosenoic acid decreased from 0.34±0.01% to 0.30±0.01%. The eicosenoic acid increased from 0.34±0.01% to 0.39±0.04% (UM_3, UM_4) as a result of applying microwave and sonication combined treatments while there was no significant change in other fatty acids. According to LSD test regarding pair wise multiple comparison (tables 39, 42 & 45) given for un-saturated fatty acids, the pair wise comparisons of mostly treatments in each processed group showed non-significant ($p > 0.05$) effects on response in pair wise comparisons with other treatments. In ultrasound processed group in pair wise comparisons for linoleic acid treatment US_1, US_2 with US_3, US_4 showed significantly ($p \leq 0.05$) different effect on response,

for linolenic acid treatment US_1, US_2 with US_4 showed significantly ($p \leq 0.05$) different effect and for eicosenoic acid C (control), US_4 showed significantly ($p \leq 0.05$) different effect in pair wise comparison with treatment US_1, US_2. In pair wise comparisons among microwave processed group treatment MW_4 with all other treatments showed significantly ($p \leq 0.05$) different effect for eicosenoic acid. For combined processed group treatment UM_3 and UM4 in pair wise comparisons with other treatments showed significantly ($p \leq 0.05$) different effect for linoleic acid and eicosenoic acid.

Concentration of unsaturated fatty acids evaluated in sorghum grains are in compliance with results of previous studies (Mehmood et al., 2008; Pradeep & Guha, 2011). Chemical and physical properties of seed oils changed when oil interact with other food items and atmosphere. Nutritional value, physical properties and stability of oil is indicated by its fatty acid composition. Many food processing technologies change the fatty acid composition of the treated oil (Lee et al., 2004; Yoshida et al., 2005). On the other hand, effect of ultrasound on seaweed oil was evaluated by Cravotto et al. (2008) and non-significant modifications in polyunsaturated fatty acids of oil was reported. Effect of germination was determined in buckwheat seeds by Kang et al. (2003) and after 7 days of germination it was observed that concentration of oleic acid was reduced up to 50%, while concentration of linoleic and linolenic acid was increased by 1.3 and 5.4 times, respectively. It was reported that germinated derooted sesame was rich in linolenic acid (Hahm et al., 2009). On the other hand, prevention of cardiovascular disease by the intake of linolenic acid also reported in the literature (Geleijnse et al., 2010; Harris, 2008; Wang et al., 2006). Results showed among all processed germinations, marked changes were observed for palmitoleic acid ($0.49 \pm 0.05\%$), linolenic acid ($1.96 \pm 0.12\%$) and eicosenoic acid ($0.39 \pm 0.04\%$). Maximum reduction in unsaturated fatty acid was detected for oleic acid ($34.04 \pm 0.10\%$) which might be due to effect of lipolytic enzymes causing its decomposition.

Table 37.

Mean squares of un-saturated fatty acids for ultrasound processed sorghum sprouts

SOV	df	Palmitoleic Acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid
Treatment	4	0.000090^{NS}	0.001950^{NS}	0.002310^{*}	0.006360^{NS}	0.000960^{*}
Replicate	2	0.002420^{**}	0.051740^{**}	0.259920^{**}	0.034820^{**}	0.000980^{*}
Error	8	0.000170	0.004015	0.000420	0.001945	0.000155
Total	14					

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

Table 38.

Un-saturated fatty acids for ultrasound processed sorghum sprouts

Treatments	Palmitoleic acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	Eicosenoic acid (%)
C	0.48 ± 0.02^{ns}	34.10 ± 0.11^{ns}	43.45 ± 0.22^{ns}	1.89 ± 0.07^{ns}	0.34 ± 0.01^{ab}
US_1	0.47 ± 0.01^{ns}	34.08 ± 0.12^{ns}	43.42 ± 0.21^{ab}	1.86 ± 0.05^{a}	0.30 ± 0.01^{ab}
US_2	0.47 ± 0.01^{ns}	34.11 ± 0.12^{ns}	43.42 ± 0.21^{ab}	1.86 ± 0.08^{a}	0.30 ± 0.01^{ab}
US_3	0.48 ± 0.03^{ns}	34.11 ± 0.10^{ns}	43.47 ± 0.25^{ab}	1.94 ± 0.12^{ns}	0.32 ± 0.02^{ns}
US_4	0.48 ± 0.04^{ns}	34.15 ± 0.13^{ns}	43.48 ± 0.25^{ab}	1.96 ± 0.12^{ab}	0.33 ± 0.03^{ab}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; US_1, US_2, US_3, US_4: different ultrasound processed germinated treatments

US_1: (5 min for 40%); US_2: (5 min for 60%); US_3: (10 min for 40%); US_4: (10 min for 60%)

Table 39.

Multiple comparisons (post hoc) LSD test of un-saturated fatty acids for ultrasound processed sorghum sprouts

Treatment I	Treatment J	Significance levels				
		Palmitoleic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid
C	US_1	.375	.709	.111	.429	.004
	US_2	.375	.852	.111	.429	.004
	US_3	1.000	.852	.266	.202	.085
	US_4	1.000	.362	.111	.088	.354
US_1	C	.375	.709	.111	.429	.004
	US_2	1.000	.578	1.000	1.000	1.000
	US_3	.375	.578	.017	.057	.085
	US_4	.375	.213	.007	.024	.018
US_2	C	.375	.852	.111	.429	.004
	US_1	1.000	.578	1.000	1.000	1.000
	US_3	.375	1.000	.017	.057	.085
	US_4	.375	.462	.007	.024	.018
US_3	C	1.000	.852	.266	.202	.085
	US_1	.375	.578	.017	.057	.085
	US_2	.375	1.000	.017	.057	.085
	US_4	1.000	.462	.567	.594	.354
US_4	C	1.000	.362	.111	.088	.354
	US_1	.375	.213	.007	.024	.018
	US_2	.375	.462	.007	.024	.018
	US_3	1.000	.462	.567	.594	.354

Based on observed means. The mean difference is significant at the .05 level. I & J both indicate control and different processed treatments (C, US_1, US_2, US_3, US_4), we are comparing different treatments with each other but I≠J.

C: control; US_1, US_2, US_3, US_4: different ultrasound processed germinated treatments; US_1: (5 min for 40%); US_2: (5 min for 60%); US_3: (10 min for 40%); US_4: (10 min for 60%)

Table 40.

Mean squares of un-saturated fatty acids for microwave processed sorghum sprouts

SOV	df	Palmitoleic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid
Treatment	4	0.000150^{NS}	0.002940^{NS}	0.007290^{NS}	0.003840^{NS}	0.000810^{**}
Replicate	2	0.002880^{**}	0.019220^{NS}	0.111380^{NS}	0.015680^{NS}	0.001620^{**}
Error	8	0.000230	0.010620	0.056480	0.004080	0.000070
Total	14					

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

Table 41.

Un-saturated fatty acids for microwave processed sorghum sprouts

Treatments	Palmitoleic acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	Eicosenoic acid (%)
C	0.48 ± 0.02^{ns}	34.10 ± 0.11^{ns}	43.45 ± 0.22^{ns}	1.89 ± 0.07^{ns}	0.34 ± 0.01^{a}
MW_1	0.47 ± 0.01^{ns}	34.04 ± 0.10^{ns}	43.48 ± 0.24^{ns}	1.85 ± 0.05^{ns}	0.34 ± 0.02^{a}
MW_2	0.48 ± 0.02^{ns}	34.10 ± 0.10^{ns}	43.52 ± 0.26^{ns}	1.85 ± 0.05^{ns}	0.35 ± 0.01^{a}
MW_3	0.48 ± 0.02^{ns}	34.11 ± 0.10^{ns}	43.55 ± 0.26^{ns}	1.91 ± 0.10^{ns}	0.35 ± 0.02^{a}
MW_4	0.49 ± 0.05^{ns}	34.12 ± 0.14^{ns}	43.57 ± 0.31^{ns}	1.93 ± 0.111^{ns}	0.38 ± 0.03^{abcd}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; MW_1, MW_2, MW_3, MW_4: different microwave processed germinated treatments

MW_1: (15 sec for 450W); MW_2: (15 sec for 700W); MW_3: (30 sec for 450W); MW_4: (30 sec for 700W)

Table 42.

Multiple comparisons (post hoc) LSD test of un-saturated fatty acids for microwave processed sorghum sprouts

Treatment I	Treatment J	Significance levels				
		Palmitoleic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid
C	MW_1	.443	.496	.881	.465	1.000
	MW_2	1.000	1.000	.728	.465	.181
	MW_3	1.000	.908	.620	.711	.181
	MW_4	.443	.818	.553	.465	.000
MW_1	C	.443	.496	.881	.465	1.000
	MW_2	.443	.496	.842	1.000	.181
	MW_3	.443	.430	.728	.283	.181
	MW_4	.145	.370	.655	.164	.000
MW_2	C	1.000	1.000	.728	.465	.181
	MW_1	.443	.496	.842	1.000	.181
	MW_3	1.000	.908	.881	.283	1.000
	MW_4	.443	.818	.803	.164	.002
MW_3	C	1.000	.908	.620	.711	.181
	MW_1	.443	.430	.728	.283	.181
	MW_2	1.000	.908	.881	.283	1.000
	MW_4	.443	.908	.920	.711	.002
MW_4	C	.443	.818	.553	.465	.000
	MW_1	.145	.370	.655	.164	.000
	MW_2	.443	.818	.803	.164	.002
	MW_3	.443	.908	.920	.711	.002

Based on observed means. The mean difference is significant at the .05 level. I & J both indicate control and different processed treatments (C, MW_1, MW_2, MW_3, MW_4), we are comparing different treatments with each other but I≠J.

C: control; MW_1, MW_2, MW_3, MW_4: different microwave processed germinated treatments. MW_1: (15 sec for 450W); MW_2: (15 sec for 700W); MW_3: (30 sec for 450W); MW_4: (30 sec for 700W)

Table 43.

Mean squares of un-saturated fatty acids for combined application processed sorghum sprouts

SOV	df	Palmitoleic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid
Treatment	4	0.000210^{NS}	0.001410^{*}	0.012300^{*}	0.003750^{NS}	0.002610^{**}
Replicate	2	0.003920^{*}	0.047360^{NS}	0.317520^{**}	0.027380^{*}	0.002880^{**}
Error	8	0.000270	0.003960	0.001970	0.001930	0.000230
Total	14					

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

Table 44.

Un-saturated fatty acids for combined application processed sorghum sprouts

Treatments	Palmitoleic acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	Eicosenoic acid (%)
C	0.48 ± 0.02^{ns}	34.10 ± 0.11^{ns}	43.45 ± 0.22^{ab}	1.89 ± 0.07^{ns}	0.34 ± 0.01^{ab}
UM_1	0.47 ± 0.01^{ns}	34.12 ± 0.13^{ns}	43.45 ± 0.22^{ab}	1.87 ± 0.06^{a}	0.33 ± 0.02^{ab}
UM_2	0.48 ± 0.02^{ns}	34.14 ± 0.10^{ns}	43.46 ± 0.22^{ab}	1.90 ± 0.01^{ns}	0.34 ± 0.01^{ab}
UM_3	0.49 ± 0.04^{ns}	34.15 ± 0.11^{ns}	43.57 ± 0.29^{abc}	1.93 ± 0.11^{ns}	0.39 ± 0.04^{abc}
UM_4	0.49 ± 0.05^{ns}	34.15 ± 0.11^{ns}	43.57 ± 0.31^{abc}	1.96 ± 0.12^{a}	0.39 ± 0.04^{abc}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; UM_1, UM_2, UM_3, UM_4: different combined processed germinated treatments

UM_1: (5 min for 40% & 15 sec for 450W); UM_2: (5 min for 60% & 15 sec for 700W);

UM_3: (10 min for 40% & 30 sec for 450W); UM_4: (10 min for 60% & 30 sec for 700W)

Table 45.

Multiple comparisons (post hoc) LSD test of un-saturated fatty acids for combined application processed sorghum sprouts

Treatment I	Treatment J	Significance levels				
		Palmitoleic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid
C	UM_1	.477	.707	1.000	.592	.443
	UM_2	1.000	.459	.790	.787	1.000
	UM_3	.477	.359	.011	.297	.004
	UM_4	.477	.359	.011	.087	.004
UM_1	C	.477	.707	1.000	.592	.443
	UM_2	.477	.707	.790	.427	.443
	UM_3	.174	.575	.011	.133	.001
	UM_4	.174	.575	.011	.036	.001
UM_2	C	1.000	.459	.790	.787	1.000
	UM_1	.477	.707	.790	.427	.443
	UM_3	.477	.851	.016	.427	.004
	UM_4	.477	.851	.016	.133	.004
UM_3	C	.477	.359	.011	.297	.004
	UM_1	.174	.575	.011	.133	.001
	UM_2	.477	.851	.016	.427	.004
	UM_4	1.000	1.000	1.000	.427	1.000
UM_4	C	.477	.359	.011	.087	.004
	UM_1	.174	.575	.011	.036	.001
	UM_2	.477	.851	.016	.133	.004
	UM_3	1.000	1.000	1.000	.427	1.000

Based on observed means. The mean difference is significant at the .05 level. I & J both indicate control and different processed treatments (C, UM_1, UM_2, UM_3, UM_4), we are comparing different treatments with each other but I≠J.

C: control; UM_1, UM_2, UM_3, UM_4: different combined processed germinated treatments. UM_1: (5 min for 40% & 15 sec for 450W); UM_2: (5 min for 60% & 15 sec for 700W); UM_3: (10 min for 40% & 30 sec for 450W); UM_4: (10 min for 60% & 30 sec for 700W)

4.5. Phenolic profile

4.5.1. Total phenolic contents (TPC)

A phenol is defined as any compound containing a benzene ring with one or more hydroxyl groups. Sorghum grain is good source of number of phenolic compounds therefore, it is highly important for human health. The total phenolic contents of different processed germinated sorghum treatments were studied in this study as compared to control. The mean squares are presented in tables 46, 49 & 52, showing significant difference ($p \leq 0.05$) in TPC among only ultrasonic processed treatments while treatments of microwave treated, and combined application treated group gave non-significant changes ($p > 0.05$). Means of all processed treatments given in tables 47, 50 & 53 are showing that TPC ranged between 1.13±0.04 to 1.26±0.01 mg GAE/g DM for the ultrasonic group, 1.19±0.04 to 1.27±0.03 mg GAE/g DM for the microwave group and 1.14±0.06 to 1.18±0.02 mg GAE/g DM for combined application treated group against the control treatment (1.18±0.07 mg GAE/g DM). The highest phenolic content (1.27±0.03 mg GAE/g DM) was observed for the microwave treatment MW_2 (15 sec & 700W), followed by ultrasonic treatment US_1 (5 min & 40%) having 1.26±0.01 mg GAE/g DM as compared to control (1.18±0.07 mg GAE/g DM). In fact, treatments with highest germination rate gave highest TPC. Among treatments of combined application processed group TPC was insignificantly different and all treatments showed lower contents compared to control treatment (1.18±0.07 mg GAE/g DM). According to LSD test regarding pair wise multiple comparison (tables 48, 51 & 54) given for TPC, the pair wise comparisons of all treatments in microwave processed and combined application processed group showed non-significant ($p > 0.05$) effects on response. In ultrasonic group, some comparison i.e. pair wise comparisons of treatment US_1 with C, US_3, US_4 showed significant ($p \leq 0.05$) different effect on responses.

The present study agreed with the findings of Phattayakorn et al. (2016) who reported significantly ($p < 0.05$) higher total phenolic content in two varieties of rice, black waxy rice and white non-waxy rice from 416.16 to 437.16 mg GAE/g when compared with control. A research conducted on buckwheat by Zhang et al. (2015), showed that by increasing the germination time significant increment was observed in the TPC ($p < 0.05$). After 72 h of germination in buckwheat the TPC of control increased from 3.03±0.09 to 8.42±0.44 mg GAE/g. There is no significant study found on this parameter in sorghum grains. In Ecuadorian

brown rice sprouting increased the total phenolic content and also antioxidant activity (Ti et al., 2014). In another research, 2-folds increment in TPC was observed in quinoa sprouts after 82 h of germination (Alvarez-Jubete et al., 2010). TPC of rice at various germination durations (GR2, GR3 and GR4) were remarkably increased from 62.32 mg $100g^{-1}$ before germination to 98.56, 101.19 and 105.84 mg $100g^{-1}$, respectively, after germination (Moongngarm & Khomphiphatkul, 2011). In another research, significantly higher amount of TPC in quinoa seeds was achieved during the whole germination period. After 72 h of germination remarkable increase of TPC i.e. 101.2% (79.04 ±1.18 mg GAE/100 g) was observed, as compare to non-germinated seeds (39.3 ± 0.9 mg GAE/100 g) (Carciochi et al., 2014). Wheat sprouts gave higher values of total phenolic compounds and showed increase in TPC from 41,458 mg GAE /100 g to 197,083 mg GAE /100 g after germination (Elzamzamy, 2014). Khang et al. (2016) investigated different legumes for their TPC, and higher values of TPC were reported after germination in various legumes including black beans, mung beans, peanuts, adzuki beans, soybeans and white cowpeas. The values of TPC were increased significantly after 5 days of germination.

Breakdown of the cell wall during the germination process and increment in the concentration of free form of TPC due to hydrolysis were main reasons reported in the literature which cause higher TPC after germination. Therefore, germinated grains contain higher concentration of hydrolysable phenolic compounds as compared to non-germinated grains (Khyade & Jagtap, 2016). Through germination contents of bioactive compounds and their activities intensely changed. Concentration of TPC can be influenced by different factors like method of extraction, grain variety, germination conditions and time (Ti et al., 2014). Phenolic compounds are known for their number of advantages for human health such as decreasing cholesterol levels, keep capillaries and arteries strong and flexible, and also assist in preventing high blood pressure (Qiao et al., 2013). Antioxidant activity is directly associated with phenolic composition and contents (Kwee & Niemeyer, 2011). This could be due to the release and biosynthesis of phenolic compounds. Cell wall-degrading enzymes are active during germination, and they contribute to modification of the cell wall structure of the grain. The significance of this lies in the fact that phenolic compounds such as hydroxycinnamates (e.g., ferulic and p-coumaric acids) are bound to non-starch polysaccharides in grain cell walls through associations such as ester and ether bonds (Perales-Sánchez et al., 2014). The action

of cell wall-degrading enzymes (mainly esterases) on these bonds contributes to the release of bound phenolic compounds. On the other hand, activation of phenylalanine ammonia lyase (key enzyme in phenolic biosynthesis) during germination of seeds also has been previously reported to contribute in the augmentation of phenolic compounds (Duodu, 2014). As results showed with increase germination rate total phenolic contents were also increase. The highest phenolic contents were observed for the microwave treatment MW_2 (15 sec & 700W), followed by ultrasonic treatment US_1 (5 min & 40%) as compared to control.

4.5.2. Total flavonoid contents (TFC)

Flavonoids, a class of secondary plant metabolites with significant antioxidant and chelating properties were found to increase significantly on sprouting. The mean squares of total flavonoid content of all germinated sorghum treatments presented in table 46, 49 & 52 are showing a significant change ($p \leq 0.01$) in TFC among treatments of each processed group. Means of TFC content of the all processed germinated treatments are given in table 47, 50 & 53 ranged between 0.78±0.03 to 1.02±0.06 mg QE/g DM for the ultrasonic group, 0.84±0.02 to 1.05±0.01 mg QE/g DM for the microwave group and 0.77±0.04 to 0.83±0.05 mg QE/g DM for combined treated group against the control treatment (0.88±0.04 mg QE/g DM). In this study, a significant increase was showed by ultrasonic and microwave processed treatments of germinated sorghum in terms of total flavonoids content as compared to germinated sample from untreated seeds. Total flavonoid contents increased with increasing germination rate. The highest flavonoids content for the microwave treatment was 1.05±0.01 mg QE/g DM for MW_2 (15 sec & 700W) and among the ultrasonic processed sprouts, treatment US_1 (5 min & 40%) showed highest TFC content of 1.02±0.06 mg QE/g DM against the control treatment (0.88±0.04 mg QE/g DM). In combined application, processed group all treatments showed lower contents of TFC as compared to control treatment (0.88±0.04 mg QE/g DM). According to LSD test regarding pair wise multiple comparison (tables 48, 51 & 54) given for TFC, the pair wise comparisons in ultrasonic group, treatment US_1 with C, US_2, US_3 and US_4 showed significantly ($p \leq 0.05$) different effect on response. In microwave group, treatment MW_4 with MW_1, MW_2 and MW_3 showed significantly ($p \leq 0.05$) different effect while in combined application treated group treatment C with treatment UM_3 and UM_4 showed highly significant effect on response ($p \leq 0.05$) in pair wise comparisons.

In a research conducted by Zhang et al. (2015) showed that amount of TFC in buckwheat increased by increasing germination duration (4.17±0.11 to 11.69± 0.87 mg RE/g). It was also observed that concentration of these active compounds significantly changed after germination for 48 to 72 h. The present study agreed with the findings of Phattayakorn et al. (2016) who observed remarkable higher TFC in germinated Hang rice (92.66 mg CE/100g) when compared with non-germinated rice (3.66 mg CE/100g). There is no significant work found regarding germination affecting TFC in sorghum grains. Another study conducted on quinoa seed after 72 hrs of germination, an increase in total flavonoid up to 59.6% was reported as compared to non-germinated seed. The concentration of these compounds was increased from 11.06 ± 0.42 mg QE/100 g (raw) to 17.65 ± 0.45 mg QE/100 g (germinated) (Carciochi et al., 2014). In another research, higher contents of total flavonoids (128%) were reported in cotyledons and sprouts of peanut seeds after 5 days of germination (Li et al., 2014). A research conducted on the extracts of germinated yam bean (68.31 mg/100 g of dry weight) were remarkably higher than that of extracts of non-germinated yam bean (59.64 mg/100 g) (de Mira et al., 2009). Another study conducted by the Sharma et al. (2015) reported that total flavonoids of foxtail millet significantly ($p < 0.05$) increased 27.10 to 57.72 mg RU/g after germination. Additionally, similar results were reported by Perales-Sánchez et al. (2014) in amaranth for TFC during germination. Kim et al. (2012) also observed that the amount of TFC of germinated mung bean was three times higher when compared with non-germinated seeds.

Pająk et al. (2014) also reported that the TFC of mung bean was 13.7 mg QE/g on 5^{th} day of germination, which was almost 2 times higher than un-germinated seeds. TFC of sprouts of soybean, mung bean & black bean were also analyzed and showed significantly higher values during germination. In that study mung bean showed highest value (5.58 mg RE/g FW) followed by black bean (4.25 mg RE/g FW) and soybean (2.81 mg RE/g FW) on the fifth day of germination (Xue et al., 2016). In another research work, germination resulted in higher amounts of total flavonoids in chickpea, fenugreek, lentil, faba bean, white bean (Salem et al., 2014) and Phaseolus vulgaris (Kiyani-Sam et al., 2015). It was proved through various studies that flavonoids show antioxidant activity in human nutrition and exert considerable impacts on human health. These compounds caused scavenging and chelating activity (Kessler et al., 2003). More secondary metabolites (flavonoids) were resulted due to bio-chemical metabolism of seed during germination (Randhir et al., 2004). During seed sprouting a multitude of

biochemical processes take place, leading to radical changes in primary and secondary metabolites composition, which could result in a change of intrinsic phenolic compounds profile, and antioxidant activity (Xu et al., 2009). Germination significantly affected the TFC contents. TFC contents increased significantly in microwave treatment (MW_2: 15 sec & 700W) and ultrasonic treatment (US_1: 5 min & 40%) but decreased significantly in all treatments of combined application treated group.

Table 46.

Mean squares of phenolic and antioxidant profile for ultrasound processed sorghum sprouts

SOV	df	TFC	TPC	DPPH	FRAP	ORAC
Treatment	4	0.025560^{**}	0.007140^{*}	54.9430^{**}	0.000011^{NS}	7.23441^{**}
Replicate	2	0.000260^{NS}	0.003500^{NS}	16.8545^{**}	0.000034^{**}	0.00512^{NS}
Error	8	0.001585	0.001100	0.4257	0.000003	0.01942
Total	14					

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

TFC: total phenolic contents; TPC: total flavonoid contents; DPPH: 1,1-diphenyl-2-picrylhydrazyl; FRAP: ferric reducing antioxidant power; ORAC: oxygen radical absorbance capacity

Table 47.

Phenolic and antioxidant profile for ultrasound processed sorghum sprouts

Treatments	TFC (mg QE/g DM)	TPC (mg GAE/g DM)	DPPH (%)	FRAP (mmol FE/g DM)	ORAC (µmol TE/g DM)
C	0.88 ± 0.04^{ab}	1.18 ± 0.07^{a}	83.76 ± 1.50^{abcd}	0.029 ± 0.001^{ns}	25.38 ± 0.13^{abc}
US_1	1.02 ± 0.06^{abcd}	1.26 ± 0.01^{abc}	89.11 ± 2.25^{abcd}	0.031 ± 0.004^{ab}	27.06 ± 0.12^{abcd}
US_2	0.91 ± 0.01^{abc}	1.22 ± 0.02^{a}	86.35 ± 1.00^{abcd}	0.029 ± 0.005^{ns}	25.64 ± 0.11^{abc}
US_3	0.82 ± 0.02^{ab}	1.18 ± 0.03^{a}	81.24 ± 2.68^{abcd}	0.027 ± 0.002^{a}	23.11 ± 0.15^{abcd}
US_4	0.78 ± 0.03^{abc}	1.13 ± 0.04^{ab}	78.14 ± 1.75^{abcd}	0.026 ± 0.001^{a}	23.87 ± 0.13^{abcd}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; US_1, US_2, US_3, US_4: different ultrasound processed germinated treatments

US_1: (5 min for 40%); US_2: (5 min for 60%); US_3: (10 min for 40%); US_4: (10 min for 60%)

TFC: total phenolic contents; TPC: total flavonoid contents; DPPH: 1,1-diphenyl-2-picrylhydrazyl; FRAP: ferric reducing antioxidant power; ORAC: oxygen radical absorbance capacity

Table 48.

Multiple comparisons (post hoc) LSD test of phenolic and antioxidant profile for ultrasound processed sorghum sprouts

Treatment I	Treatment J	Significance levels				
		TFC	TPC	DPPH	FRAP	ORAC
C	US_1	.003	.018	.000	.214	.000
	US_2	.383	.178	.001	1.000	.052
	US_3	.102	1.000	.001	.214	.000
	US_4	.015	.102	.000	.078	.000
US_1	C	.003	.018	.000	.214	.000
	US_2	.010	.178	.001	.214	.000
	US_3	.000	.018	.000	.027	.000
	US_4	.000	.001	.000	.010	.000
US_2	C	.383	.178	.001	1.000	.052
	US_1	.010	.178	.001	.214	.000
	US_3	.024	.178	.000	.214	.000
	US_4	.004	.010	.000	.078	.000
US_3	C	.102	1.000	.001	.214	.000
	US_1	.000	.018	.000	.027	.000
	US_2	.024	.178	.000	.214	.000
	US_4	.253	.102	.000	.519	.000
US_4	C	.015	.102	.000	.078	.000
	US_1	.000	.001	.000	.010	.000
	US_2	.004	.010	.000	.078	.000
	US_3	.253	.102	.000	.519	.000

Based on observed means. The mean difference is significant at the .05 level. I & J both indicate control and different processed treatments (C, US_1, US_2, US_3, US_4), we are comparing different treatments with each other but I≠J.

C: control; US_1, US_2, US_3, US_4: different ultrasound processed germinated treatments; US_1: (5 min for 40%); US_2: (5 min for 60%); US_3: (10 min for 40%); US_4: (10 min for 60%)

TFC: total phenolic contents; TPC: total flavonoid contents; DPPH: 1,1-diphenyl-2-picrylhydrazyl; FRAP: ferric reducing antioxidant power; ORAC: oxygen radical absorbance capacity

4.6. Radical scavenging activity

Radical scavenging activity is protective factor against the oxidative damage in the human body. So**,** another parameter studied in this study was the radical scavenging assay. All the processed germinated sorghum treatment and control treatment were subjected to analyze the DPPH, FRAP & ORAC assay.

4.6.1. DPPH assay

Antioxidant activity of the foods can be determined by analyzing the DPPH scavenging activity. In this study, DPPH radical scavenging activity of all processed germinated sorghum treatments was analyzed, and their mean squares presented in tables 46, 49 & 52 are giving a highly significant change ($p \leq 0.01$) in DPPH activity among all treatments of each processed group. The means of the all processed germinated treatments as given in tables 47, 50 & 53 ranged between 78.14±1.75 to 89.11±2.25% for the ultrasonic group, 81.24±1.00 to 89.73±2.11% for the microwave group and 77.57±2.25 to 79.09±1.50% for combined application treated group against the control treatment (83.76±1.50%). The highest DPPH activity observed for the microwave treatment and ultrasonic treatment was 89.73±2.11% and 89.11±2.25% for MW_2 (15 sec & 700W) and US_1 (5 min & 40%), respectively, as compared to control (83.76±1.50%). DPPH radical scavenging activity increased with high germination rate. Among the combined application processed group all treatments showed DPPH value lower as compared to control treatment (83.76±1.50%). According to LSD test regarding pair wise multiple comparison (tables 48, 51 & 54) given for DPPH radical scavenging activity, the pair wise comparisons of every treatment with almost all other treatments showed significantly ($p \leq 0.05$) different effect on response.

Germination process increased the scavenging activity in components of sprouts, so providing sprouts an important physiological role to stabilize the free radicals and reducing the risk of degenerative diseases (Khyade & Lonkar, 2013). The present study agreed with the findings of Khyade and Jagtap (2016) who observed that germination increased DPPH inhibition percentage up to 14.81% in yellow mustard, 11.23% in chickpea and 48.42 % in cowpea seeds. Similarly steeping and germination also caused increment in the DPPH radical-scavenging activity of oat samples (Oksman-Caldentey et al., 2001). Regarding effect of germination on DPPH assay in sorghum grains, no significant study was found. Effect of germination was studied by using buckwheat for 72 h and reported the highest free radical-

scavenging activity against DPPH (IC50 = 1.34 mg/mL). Similarly, the trolox equivalent (TE) value also increased from 6.32 to 24.50 mmol TE/kg in the DPPH scavenging assay (Zhang et al., 2015). DPPH activity in paddy rice after germination was in the range of 0.2712 to 0.3035 mg Trolox/g and DPPH activity (antioxidant activity) was increased by increasing the germination time during 24 to 60 h (Maisont & Narkrugsa, 2010). While in another study it was reported that all germinated varieties of 'Hang' rice showed greater antioxidant activity (63.26±0.09, 73.60±1.63 & 89.23±0.99%) as compared to non-germinated one (37.93±1.56, 69.10±2.57 & 84.73±0.42%) in DPPH analysis (Phattayakorn et al., 2016). Wheat and barley were also investigated for their antioxidant activity and higher DPPH scavenging activity (49.08% & 79.75%, respectively) observed as compared to non-germinated which gave only 21.78% & 35.58%, respectively (Elzamzamy, 2014). According to Oksman-Caldentey et al. (2001), no change in the DPPH radical-scavenging activity of oat was observed during the latter part of the germination, hence it could be concluded that longer germination didn't showed the higher antioxidant activity in oat. Number of biological functions (antimutagenicity, anti-carcinogenicity, antiaging etc.) originate from antioxidant action and this property of the food products is highly important for human as it prevents the incidence of many maladies through improving the antioxidant potential of human diets (Holasova et al., 2002). After germination, enzyme synthesis can increase the inherent phytochemical compounds and resultantly higher antioxidant activity is observed (Phattayakorn et al., 2016). Combined application of both techniques drastically reduced the DPPH value. So, in case of ultrasonic and microwave processing DPPH activity was increased significantly with increasing germination rate while decreased in combine treatment group.

Table 49.

Mean squares of phenolic and antioxidant profile for microwave processed sorghum sprouts

SOV	df	TFC	TPC	DPPH	FRAP	ORAC
Treatment	4	0.031050^{**}	0.004440^{NS}	38.352^{**}	0.000016^{NS}	9.51276^{**}
Replicate	2	0.002940^{NS}	0.002480^{NS}	9.194^{*}	0.000010^{NS}	0.03200^{NS}
Error	8	0.001640	0.002755	1.804	0.000016	0.01675
Total	14					

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

TFC: total phenolic contents; TPC: total flavonoid contents; DPPH: 1,1-diphenyl-2-picrylhydrazyl; FRAP: ferric reducing antioxidant power; ORAC: oxygen radical absorbance capacity

Table 50.

Phenolic and antioxidant profile for microwave processed sorghum sprouts

Treatments	TFC (mg QE/g DM)	TPC (mg GAE/g DM)	DPPH (%)	FRAP (mmol FE/g DM)	ORAC (µmol TE/g DM)
C	0.88 ± 0.04^{ab}	1.18 ± 0.07^{ns}	83.76 ± 1.50^{ab}	0.029 ± 0.001^{ns}	25.38 ± 0.13^{abcd}
MW_1	1.03 ± 0.07^{abc}	1.25 ± 0.06^{ns}	88.41 ± 2.00^{abc}	0.030 ± 0.003^{ns}	27.52 ± 0.14^{abcd}
MW_2	1.05 ± 0.01^{abc}	1.27 ± 0.03^{ns}	89.73 ± 2.11^{abc}	0.033 ± 0.006^{ns}	28.03 ± 0.16^{abcd}
MW_3	0.85 ± 0.05^{ab}	1.23 ± 0.05^{ns}	83.57 ± 2.17^{ab}	0.028 ± 0.002^{ns}	24.32 ± 0.12^{abc}
MW_4	0.84 ± 0.02^{abc}	1.19 ± 0.04^{ns}	81.24 ± 1.00^{ab}	0.027 ± 0.005^{ns}	24.24 ± 0.15^{abc}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; MW_1, MW_2, MW_3, MW_4: different microwave processed germinated treatments

MW_1: (15 sec for 450W); MW_2: (15 sec for 700W); MW_3: (30 sec for 450W); MW_4: (30 sec for 700W)

TFC: total phenolic contents; TPC: total flavonoid contents; DPPH: 1,1-diphenyl-2-picrylhydrazyl; FRAP: ferric reducing antioxidant power; ORAC: oxygen radical absorbance capacity

Table 51.

Multiple comparisons (post hoc) LSD test of phenolic and antioxidant profile for microwave processed sorghum sprouts

Treatment I	Treatment J	Significance levels				
		TFC	TPC	DPPH	FRAP	ORAC
C	MW_1	.002	.141	.003	.769	.000
	MW_2	.001	.069	.001	.260	.000
	MW_3	.391	.277	.867	.769	.000
	MW_4	.261	.821	.051	.561	.000
MW_1	C	.002	.141	.003	.769	.000
	MW_2	.562	.653	.263	.389	.001
	MW_3	.001	.653	.002	.561	.000
	MW_4	.000	.199	.000	.389	.000
MW_2	C	.001	.069	.001	.260	.000
	MW_1	.562	.653	.263	.389	.001
	MW_3	.000	.378	.001	.168	.000
	MW_4	.000	.099	.000	.106	.000
MW_3	C	.391	.277	.867	.769	.000
	MW_1	.001	.653	.002	.561	.000
	MW_2	.000	.378	.001	.168	.000
	MW_4	.770	.378	.066	.769	.471
MW_4	C	.261	.821	.051	.561	.000
	MW_1	.000	.199	.000	.389	.000
	MW_2	.000	.099	.000	.106	.000
	MW_3	.770	.378	.066	.769	.471

Based on observed means. The mean difference is significant at the .05 level. I & J both indicate control and different processed treatments (C, MW_1, MW_2, MW_3, MW_4), we are comparing different treatments with each other but I≠J.

C: control; MW_1, MW_2, MW_3, MW_4: different microwave processed germinated treatments MW_1: (15 sec for 450W); MW_2: (15 sec for 700W); MW_3: (30 sec for 450W); MW_4: (30 sec for 700W)

TFC: total phenolic contents; TPC: total flavonoid contents; DPPH: 1,1-diphenyl-2-picrylhydrazyl; FRAP: ferric reducing antioxidant power; ORAC: oxygen radical absorbance capacity

4.6.2. FRAP assay

Total antioxidant activity is directly related to ability of phytochemicals to scavenge the free radicals. Ferric reducing ability power (FRAP) is used to determine the antioxidant ability of a substance in the reaction medium. In this study, FRAP value of all processed germinated sorghum treatments analyzed as mean squares are given in tables 46, 49 & 52. All treatments in each group showed a non-significant change ($p > 0.05$) in FRAP activity of germinated sorghum. The means of FRAP values for all processed germinated treatments as given in tables 47, 50 & 53 ranged between 0.026±0.001 to 0.031±0.004 mmol FE/g DM for the ultrasonic group, 0.027±0.005 to 0.033±0.006mmol/g DM for the microwave group and 0.026±0.001 to 0.028±0.005 mmol FE/g DM for combined treated group against the control treatment (0.029±0.001 mmol FE/g DM). The highest FRAP value was observed for the microwave treatment (MW_2: 15 sec & 700W) and ultrasonic treatment (US_1: 5 min & 40%) at 0.033±0.006 mmol FE/g DM and 0.031±0.004 mmol FE/g DM, respectively, as compared to control (0.029±0.001 mmol/g DM) but combined application of both techniques reduced the FRAP activity. In this group, treatment UM_2 (US: 60% for 5min & MW: 700W for 15sec) showed 0.028±0.005 mmol FE/g DM high as compared to other treatments but lower in comparison to control (0.029±0.001 mmol FE/g DM). Insignificant ($p > 0.05$) increase in antioxidant activity was observed by increasing germination rate in case of FRAP analysis. According to LSD test regarding pair wise multiple comparison (tables 48, 51 & 54) given for FRAP assay, in microwave processed and combined application processed group the pair wise comparisons of every treatment with all other treatments showed non-significantly ($p > 0.05$) different effect on responses. Only in ultrasonic group pair wise comparison of treatment US_1 with US_3, US_4 gave significantly different effect ($p \leq 0.05$) on response.

In an investigation conducted by Maisont and Narkrugsa (2010) who analyzed FRAP of the germinated paddy rice within the range of 0.6888 to 0.8570 mg Trolox/g. During 24 to 60 h of germination significant increment in FRAP was observed. Phattayakorn et al. (2016) studied three different varieties of rice for their antioxidant activity by FRAP methods and it was reported that all germinated varieties (252.66±41.15, 372.33±18.56, 352.33±18.85µmol $FeSO_4$/g) showed higher antioxidant activity as compared to the non-germinated (99.00±32.66, 232.66±25.30, 279.66±27.81µmol $FeSO_4$/g, respectively). Germinated wheat and barley showed higher FRAP values (68.561 & 98.030 mg ascorbic acid /100g) as compared to non-

germinated (63.561 & 38.385 mg ascorbic acid /100g, respectively) (Elzamzamy, 2014). Similarly, higher values were reported for FRAP assay in germinated buckwheat. After 72 h of germination the FRAP value were increased from 18.07 ± 0.93 to 63.94 ± 4.22 mmol Fe^{2+} equivalents/kg (Zhang et al., 2015). Sorghum seed also showed higher FRAP values after germination i.e from 4.26 to 4.88 mmol Fe^{2+} equiv/100 g DM. Therefore, it can be concluded that antioxidant capacity depends upon both germination and time (Kayodé et al., 2013). FRAP value of African yam bean (98.60±0.04µmol/g) was higher as compared to non-germinated bean (96.11±1.13µmol/g) (Uchegbu & Amulu, 2015). Up to 89% increment in FRAP values were reported in germinated quinoa seeds (Kaur et al., 2016). Higher FRAP values were obtained after germination of quinoa (79%) (Alvarez-Jubete et al., 2010). Similar results were obtained after 6 days germination of soybeans seeds i.e. 293.60% higher FRAP values were reported as compared to those germinated for 2 days and the non-germinated seeds (Guzmán-Ortiz et al., 2017). FRAP values were reportedly higher for the germinated finger millet (2.44±0.02 µ/g) and pearl millet (2.36±0.12 µg/g) and lower in the un-germinated raw finger millet and pearl millet 1.53±0.03 µ/g & 2.02±0.02 µ/g, respectively. Up to 59.47% and 16.83% increment of FRAP values were obtained after germination of finger millet flour and pearl millet flour, respectively (Chauhan & Sarita, 2017). Wang et al. (2015) also reported that antioxidant activity increased significantly as the germination time increased. It was observed that extracts from soybean germinated for 6 days showed the highest capacity to transfer electron and hydrogen atom to complete the oxidation process. Antioxidant activity increased during germination as enzyme synthesis enhance the inherent phytochemical compounds (Ti et al., 2014). Increase germination affected the FRAP assay non-significantly. DM). The highest FRAP value was observed for the microwave treatment (MW_2: 15 sec & 700W) and ultrasonic treatment (US_1: 5 min & 40%) as compared to control (0.029±0.001 mmol/g DM).

4.6.3. ORAC assay

ORAC assay was also used to evaluate the antioxidant activity of samples. In this method, ORAC assay of processed germinated sorghum treatments against control treatment was analyzed and their mean squares are given in tables 46, 49 & 52. Results showed highly significant difference ($p \leq 0.01$) between germinated sorghum treatments of each processed group. The means of ORAC assay values for the all processed germinated treatments as given in tables 47, 50 & 53 having values ranged between 23.11±0.15 to 27.06±0.12 µmol TE/g DM

for the ultrasonic group, 24.24±0.15 to 28.03±0.16 µmol TE/g DM for the microwave group and 22.27±0.12 to 24.67±0.11 µmol TE/g DM for combined treated group against the control treatment (25.38±0.13 µmol TE/g DM). The highest ORAC assay values at 28.03±0.16 µmol TE/g DM and 27.06±0.12 µmol TE/g DM were observed for the microwave treatment (MW_2: 15 sec & 700W) and ultrasonic treatment (US_1: 5 min & 40%), respectively, as compared to control (25.38±0.13 µmol TE/g DM) but combined application of both techniques reduced the ORAC assay values. Continuous increase in ORAC assay was observed with increasing germination rate. In combined application group, all treatments gave lower ORAC values in comparison to control (25.38±0.13 µmol TE/g DM). According to LSD test regarding pair wise multiple comparison (tables 48, 51 & 54) given for ORAC assay, in all processed groups the pair wise comparisons of every treatment with all other treatments showed significantly ($p \leq 0.05$) different effect on response.

ORAC is affected by increase in phenolic content along with germination. In a study conducted by Wang et al. (2016), antioxidant activity of raw flaxseed determined via ORAC was 28.17 ± 0.55 µmol TE/g DW, which was increased to 192.51 ± 7.38 µmol TE/g DW after germination and caused 6.8 folds increase in the ORAC value of the 10 days flax sprouts. Results of current investigation are also in agreement with other studies showing similar effects after germination of edible seeds (Aguilera et al., 2014; Cevallos-Casals & Cisneros-Zevallos, 2010). But there is no significant finding on this parameter in sorghum grains. Antioxidant activity of germinated buckwheat gave highest ORAC value (256.67 mmol TE/kg) after 72 h and it was 3-folds higher when compared with the control (Zhang et al., 2015). Value of antioxidant activity through ORAC assays of extracts from raw quinoa seeds when compared with the results achieved from sprouts after 4 days of germination gave remarkable difference. ORAC assay of 4 days quinoa sprouts resulted an increase about 2- and 2.8-fold considerably higher than whole flour (Laus et al., 2017). Antioxidant activity of brown rice is directly affected by the germination time. Higher values of antioxidant were observed at higher germination time ($P \leq 0.05$). Analyzed antioxidant values (ORAC) were 2-folds higher after 48 h while 4 folds higher after 9 h of after sprouting of brown rice (Cáceres et al., 2014). Similar research was conducted on lentil, broccoli and red cabbage by using ORAC assay which showed 0.7 µmol Trolox/g DW in lentil and 2.4 µmol Trolox/g DW in broccoli and red cabbage (Aguilera et al., 2015). Effect of germination on antioxidant activity was also studied by using

ORAC assay on lentil (1.0 μmol Trolox/g DW), broccoli (3.4 μmol Trolox/g DW), red cabbage (3.3 μmol Trolox/g DW) and radish seeds (3.3 μmol Trolox/g DW) showing 3-fold increase after germination (Duenas et al., 2007; 2009). Similarly, amaranth flour after germination revealed a remarkable effect in ORAC activity therefore, values increased up to 21.56±0.30 mmol TE/100 g DW when compared to unprocessed seed flour 5.40±0.13 mmol TE/100 g DW (Perales-Sánchez et al., 2014). The antioxidative capacity by ORAC assay increased after the caryopses were fully sprouted. Sprouting alters the phenolic acids and other free phytochemicals might be present, leading to an improved antioxidative capacity in fully sprouted grain (Engert et al., 2012). As germination increased the ORAC activity increased significantly in microwave processed and ultrasonic processed group but decreased in combined application group. Highest values were observed in microwave treatment MW_2 (15 sec & 700W) and ultrasonic treatment US_1 (5 min & 40%).

Table 52.

Mean squares of phenolic and antioxidant profile for combined application processed sorghum sprouts

SOV	Df	TFC	TPC	DPPH	FRAP	ORAC
Treatment	4	0.005310^{*}	0.000840^{NS}	18.356^{**}	0.000005^{NS}	5.61429^{**}
Replicate	2	0.005580^{*}	0.009260^{*}	15.1728^{**}	0.000013^{NS}	0.08450^{**}
Error	8	0.000955	0.001160	0.2091	0.000010	0.00035
Total	14					

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

TFC: total phenolic contents; TPC: total flavonoid contents; DPPH: 1,1-diphenyl-2-picrylhydrazyl; FRAP: ferric reducing antioxidant power; ORAC: oxygen radical absorbance capacity

Table 53.

Phenolic and antioxidant profile for combined application processed sorghum sprouts

Treatments	TFC (mg QE/g DM)	TPC (mg GAE/g DM)	DPPH (%)	FRAP (mmol FE/g DM)	ORAC (µmol TE/g DM)
C	0.88 ± 0.04^{abc}	1.18 ± 0.07^{ns}	83.76 ± 1.50^{abcd}	0.029 ± 0.001^{ns}	25.38 ± 0.13^{abcd}
UM_1	0.82 ± 0.01^{a}	1.17 ± 0.07^{ns}	79.09 ± 1.50^{abc}	0.027 ± 0.004^{ns}	23.07 ± 0.16^{abcd}
UM_2	0.83 ± 0.05^{a}	1.18 ± 0.02^{ns}	79.57 ± 1.25^{abc}	0.028 ± 0.005^{ns}	24.67 ± 0.11^{abcd}
UM_3	0.79 ± 0.06^{a}	1.16 ± 0.01^{ns}	77.93 ± 2.21^{abc}	0.026 ± 0.003^{ns}	22.54 ± 0.13^{abcd}
UM_4	0.77 ± 0.04^{ab}	1.14 ± 0.06^{ns}	77.57 ± 2.25^{abc}	0.026 ± 0.001^{ns}	22.27 ± 0.12^{abcd}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; UM_1, UM_2, UM_3, UM_4: different combined processed germinated treatments

UM_1: (5 min for 40% & 15 sec for 450W); UM_2: (5 min for 60% & 15 sec for 700W); UM_3: (10 min for 40% & 30 sec for 450W); UM_4: (10 min for 60% & 30 sec for 700W)

TFC: total phenolic contents; TPC: total flavonoid contents; DPPH: 1,1-diphenyl-2-picrylhydrazyl; FRAP: ferric reducing antioxidant power; ORAC: oxygen radical absorbance capacity

Table 54.

Multiple comparisons (post hoc) LSD test of phenolic and antioxidant profile for combined application processed sorghum sprouts

Treatment I	Treatment J	Significance levels				
		TFC	TPC	DPPH	FRAP	ORAC
C	UM_1	.045	.728	.000	.456	.000
	UM_2	.083	1.000	.000	.706	.000
	UM_3	.007	.492	.000	.274	.000
	UM_4	.002	.188	.000	.274	.000
UM_1	C	.045	.728	.000	.456	.000
	UM_2	.702	.728	.235	.706	.000
	UM_3	.269	.728	.015	.706	.000
	UM_4	.083	.312	.004	.706	.000
UM_2	C	.083	1.000	.000	.706	.000
	UM_1	.702	.728	.235	.706	.000
	UM_3	.152	.492	.002	.456	.000
	UM_4	.045	.188	.001	.456	.000
UM_3	C	.007	.492	.000	.274	.000
	UM_1	.269	.728	.015	.706	.000
	UM_2	.152	.492	.002	.456	.000
	UM_4	.451	.492	.363	1.000	.000
UM_4	C	.002	.188	.000	.274	.000
	UM_1	.083	.312	.004	.706	.000
	UM_2	.045	.188	.001	.456	.000
	UM_3	.451	.492	.363	1.000	.000

Based on observed means. The mean difference is significant at the .05 level. I & J both indicate control and different processed treatments (C, UM_1, UM_2, UM_3, UM_4), we are comparing different treatments with each other but I≠J.

C: control; UM_1, UM_2, UM_3, UM_4: different combined processed germinated treatments UM_1: (5 min for 40% & 15 sec for 450W); UM_2: (5 min for 60% & 15 sec for 700W); UM_3: (10 min for 40% & 30 sec for 450W); UM_4: (10 min for 60% & 30 sec for 700W)

TFC: total phenolic contents; TPC: total flavonoid contents; DPPH: 1,1-diphenyl-2-picrylhydrazyl; FRAP: ferric reducing antioxidant power; ORAC: oxygen radical absorbance capacity

4.7. HPLC profile of phenolic compounds

In this study, all the processed germinated treatments were analyzed for their individual phenolic compounds as gallic acid, ferulic acid, catechin and quercetin. Mean squares regarding gallic acid and ferulic acid are given in tables 55, 58 & 61. Results showed highly significant ($p \leq 0.01$) difference of gallic acid among the treatments of all treated groups. While ferulic acid showed a highly significant ($p \leq 0.01$) difference among the treatments of ultrasonic treated group but for microwave treated group and combine treated group the difference was non-significant ($p > 0.05$). Means values given in table 56, 59 & 62 showed ranges of gallic acid between 15.11±0.13 to16.29±0.09 µg/g, 15.39±0.08 to 16.31±0.11 µg/g and 14.02±0.12 to 14.57±0.07 µg/g for ultrasonic treated, microwave treated and combined treated sprouts, respectively. Means values of ferulic acid contents for ultrasonic treated, microwave treated and combined treated sprouts ranged between 117.25±2.08 to 120.33±2.07 µg/g, 117.93±2.00 to 120.36±2.14 µg/g and 116.75±2.10 to 117.87±2.11 µg/g, respectively. Highest amount of gallic acid and ferulic acid in ultrasonic group was for treatment US_1 processed for 5 min & 40% amplitude (gallic acid: 16.29±0.09 µg/g; ferulic acid: 120.33±2.07 µg/g). While in microwave group treatment, MW_2 (15 sec & 700W) showed highest amount of gallic acid (16.29±0.09 µg/g) and ferulic acid (120.36±2.14 µg/g). So, the ultrasonic and microwave processing increased the ferulic acid and gallic acid contents as compared to control (gallic acid: 15.65±0.10 µg/g; ferulic acid: 118.45±2.05 µg/g). Combined application of both techniques decreased gallic acid and ferulic acid contents as compared to control. The treatment having highest germination rate showed the maximum level of gallic acid and ferulic acid contents for ultrasonic group and microwave group as compared to control treatment. According to LSD test regarding pair wise multiple comparison (table 57, 60 & 63) given for gallic acid and ferulic acid, in every processed groups the pair wise comparisons of every treatment with all other treatments showed significantly ($p \leq 0.05$) different effect on gallic acid. For ferulic acid in ultrasonic group the pair wise comparisons of every treatment with all other treatments showed significantly ($p \leq 0.05$) different effect on response while in microwave treated and combined treated group the pair wise comparison for all treatments showed non-significant ($p > 0.05$) effect.

Various benefits were associated with gallic acid such as it was used against fungal and viral attacks and also used as antioxidant and helps to protect our cells from oxidative damage

(Zucca et al., 2013). No significant work was found regarding effect of germination on phenolic compound in sorghum grains. Higher concentration of gallic acid was obtained after providing stress conditions to germinated seeds. It was proved through various studies that germinated seeds gave highest amount of gallic acids. Germinated seeds of *Vitis californica* contain 42.40 to 204.00 µg/g of fresh weight (FW) of gallic Acid which is its highest concentration in seeds (Weidner et al., 2014). Generally, phenolic acids are bound to hydrolysable tannins, lignins, cellulose and proteins which are mainly structural components of bran, building a protective layer to inner parts of the caryopsis (Engert et al., 2012). Specific synthesis pathway was operated during the germination for few individual phenolic which linked with the observed variations in content of a few individual phenolic compounds. Large number of simple molecules were produced during the primary metabolic pathways, like shikimic acid, acetates and amino acids. These molecules constitute the starting materials for the biosynthetic pathways of many secondary metabolites (García, 2004; Prakash et al., 2007). Un-treated grains contain less amount of free ferulic acid which increased and become healthier after various treatments such as fermentation, germination, and cooking (Singh et al., 2015). It was reported in literature that germination affect differently and concentration of ferulic acids were changed after the germination process (Hübner & Arendt, 2013). Wheat grain were examined for its phenolic acid content after germination process. It was observed that after steeping the wheat grain for 24 to 48 h, ferulic acid considerably increased (932.4mg/g) during germination process (Yang & Ooraikul, 2001). The largest group of antioxidants in wheat sprout are phenolic acids. After 5 days of germination, higher amounts of ferulic acid were observed. This increment is linked with the synthesis of phenolic compounds and hydrolysis of polyphenolic compounds which were bound to cell walls of the wheat grain (Hatcher & Kruger, 1997). The key enzyme for the biosynthesis of these compounds is phenylalanine ammonia lyase (PAL) (Maillard & Berset, 1995). Fermentation and germination caused an increase in various compounds. It was observed that bioavailability of ferulic acid was increased after processing the cereal bran (Napolitano et al., 2009). Up to 75 to 130 % increment in free ferulic acid was observed after fermentation (48 h) process in pizza dough (Moore et al., 2009). The increment in ferulic acid during pizza dough fermentation is linked with enzymatic hydrolysis of insoluble bound or soluble conjugated ferulic acid by different enzymes produced from yeast or other microorganisms and enzymes

present in the dough, additionally, similar results were obtained in fermentation of rye bran by using yeast (Katina et al., 2007). It was reported in previous investigation that major phenolic compound in un-germinated rice was ferulic acid which was reputedly increased (1 to 2 time) after germination process (Tian et al., 2004).

Table 55.

Mean squares of HPLC profile of phenolic compounds for ultrasound processed sorghum sprouts

SOV	df	Gallic acid	Ferulic acid	Catechin	Quercetin
Treatment	4	0.609300^{**}	4.1330^{**}	0.065910^{**}	0.272760^{**}
Replicate	2	0.027380^{NS}	21.7987^{**}	0.001280^{NS}	0.084500^{**}
Error	8	0.008530	0.0010	0.001730	0.000250
Total	14				

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

Table 56.

HPLC profile of phenolic compounds for ultrasound processed sorghum sprouts

Treatments	Gallic acid (µg/g)	Ferulic acid (µg/g)	Catechin (µg/g)	Quercetin (µg/g)
C	15.65 ± 0.10^{abcd}	118.45 ± 2.05^{abcd}	5.58 ± 0.04^{ab}	21.43 ± 0.14^{abcd}
US_1	16.29 ± 0.09^{abcd}	120.33 ± 2.07^{abcd}	5.89 ± 0.02^{abcd}	21.86 ± 0.12^{abcd}
US_2	15.86 ± 0.11^{abcd}	118.81 ± 2.11^{abcd}	5.71 ± 0.01^{abcd}	21.61 ± 0.13^{abcd}
US_3	15.39 ± 0.12^{abcd}	117.79 ± 2.13^{abcd}	5.60 ± 0.06^{abc}	21.25 ± 0.15^{abcd}
US_4	15.11 ± 0.13^{abcd}	117.25 ± 2.08^{abcd}	5.51 ± 0.05^{abc}	21.09 ± 0.11^{abcd}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; US_1, US_2, US_3, US_4: different ultrasound processed germinated treatments

US_1: (5 min for 40%); US_2: (5 min for 60%); US_3: (10 min for 40%); US_4: (10 min for 60%)

Table 57.

Multiple comparisons (post hoc) LSD test of HPLC profile of phenolic compounds for ultrasound processed sorghum sprouts

Treatment I	Treatment J	Significance levels			
		Gallic acid	Ferulic acid	Catechin	Quercetin
C	US_1	.000	.000	.000	.000
	US_2	.024	.000	.005	.000
	US_3	.009	.000	.572	.000
	US_4	.000	.000	.073	.000
US_1	C	.000	.000	.000	.000
	US_2	.000	.000	.001	.000
	US_3	.000	.000	.000	.000
	US_4	.000	.000	.000	.000
US_2	C	.024	.000	.005	.000
	US_1	.000	.000	.001	.000
	US_3	.000	.000	.012	.000
	US_4	.000	.000	.000	.000
US_3	C	.009	.000	.572	.000
	US_1	.000	.000	.000	.000
	US_2	.000	.000	.012	.000
	US_4	.006	.000	.029	.000
US_4	C	.000	.000	.073	.000
	US_1	.000	.000	.000	.000
	US_2	.000	.000	.000	.000
	US_3	.006	.000	.029	.000

Based on observed means. The mean difference is significant at the .05 level. I & J both indicate control and different processed treatments (C, US_1, US_2, US_3, US_4), we are comparing different treatments with each other but I≠J.

C: control; US_1, US_2, US_3, US_4: different ultrasound processed germinated treatments US_1: (5 min for 40%); US_2: (5 min for 60%); US_3: (10 min for 40%); US_4: (10 min for 60%)

Table 58.

Mean squares of HPLC profile of phenolic compounds for microwave processed sorghum sprouts

SOV	df	Gallic acid	Ferulic acid	Catechin	Quercetin
Treatment	4	0.535800^{**}	3.874^{NS}	0.094110^{**}	0.22035^{**}
Replicate	2	0.058320^{**}	8.064^{NS}	0.000320^{NS}	0.03872^{NS}
Error	8	0.000670	3.343	0.001070	0.01117
Total	14				

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

Table 59.

HPLC profile of phenolic compounds for microwave processed sorghum sprouts

Treatments	Gallic acid (µg/g)	Ferulic acid (µg/g)	Catechin (µg/g)	Quercetin (µg/g)
C	15.65 ± 0.10^{abc}	118.45 ± 2.05^{ns}	5.58 ± 0.04^{abc}	21.43 ± 0.14^{ab}
MW_1	16.29 ± 0.15^{abc}	120.21 ± 2.10^{ns}	5.93 ± 0.03^{abc}	21.80 ± 0.10^{abc}
MW_2	16.31 ± 0.11^{abc}	120.36 ± 2.14^{ns}	5.93 ± 0.01^{abc}	21.86 ± 0.13^{abc}
MW_3	15.61 ± 0.10^{abc}	118.35 ± 2.06^{ns}	5.65 ± 0.02^{abc}	21.33 ± 0.12^{ab}
MW_4	15.39 ± 0.08^{abcd}	117.93 ± 2.00^{ns}	5.60 ± 0.04^{ab}	21.28 ± 0.15^{ab}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; MW_1, MW_2, MW_3, MW_4: different microwave processed germinated treatments. MW_1: (15 sec for 450W); MW_2: (15 sec for 700W); MW_3: (30 sec for 450W); MW_4: (30 sec for 700W)

Table 60.

Multiple comparisons (post hoc) LSD test of HPLC profile of phenolic compounds for microwave processed sorghum sprouts

Treatment I	Treatment J	Significance levels			
		Gallic acid	Ferulic acid	Catechin	Quercetin
C	MW_1	.000	.272	.000	.003
	MW_2	.000	.237	.000	.001
	MW_3	.095	.948	.031	.280
	MW_4	.000	.737	.475	.120
MW_1	C	.000	.272	.000	.003
	MW_2	.372	.922	1.000	.507
	MW_3	.000	.248	.000	.001
	MW_4	.000	.165	.000	.000
MW_2	C	.000	.237	.000	.001
	MW_1	.372	.922	1.000	.507
	MW_3	.000	.215	.000	.000
	MW_4	.000	.142	.000	.000
MW_3	C	.095	.948	.031	.280
	MW_1	.000	.248	.000	.001
	MW_2	.000	.215	.000	.000
	MW_4	.000	.786	.098	.578
MW_4	C	.000	.737	.475	.120
	MW_1	.000	.165	.000	.000
	MW_2	.000	.142	.000	.000
	MW_3	.000	.786	.098	.578

Based on observed means. The mean difference is significant at the .05 level. I & J both indicate control and different processed treatments (C, MW_1, MW_2, MW_3, MW_4), we are comparing different treatments with each other but I≠J.

C: control; MW_1, MW_2, MW_3, MW_4: different microwave processed germinated treatments. MW_1: (15 sec for 450W); MW_2: (15 sec for 700W); MW_3: (30 sec for 450W); MW_4: (30 sec for 700W)

Mean squares regarding quercetin and catechin are given in tables 55, 58 & 61. Mean squares regarding quercetin and catechin showed a significant ($p \leq 0.05$) difference among the treatments of all treated groups. Means of quercetin contents given in tables 56, 59 & 62 showed ranges between 21.09±0.11 to 21.86±0.12 µg/g, 21.28±0.15 to 21.86±0.13 µg/g and 21.04±0.12 to 21.33±0.14 µg/g for ultrasonic treated, microwave treated, and combined application treated sprouts, respectively. Means values of catechin for ultrasonic treated, microwave treated and combined treated sprouts were ranges between 5.51±0.05 to 5.89±0.02 µg/g, 5.60±0.04 to 5.93±0.03 µg/g and 5.51±0.04 to 5.62±0.01 µg/g, respectively. So, the ultrasonic and microwave processing increased the quercetin and catechin contents as compared to control (quercetin: 21.43±0.14 µg/g; catechin: 5.58±0.04 µg/g). While combined application of both techniques decreased the quercetin contents but slightly increased the catechin contents as compared with control. The treatment having highest germination rate showed the maximum level of quercetin and catechin as for treatment US_1:5 min & 40% (quercetin: 21.86±0.12 µg/g; catechin: 5.89±0.02 µg/g) and MW_2: 15 sec & 700W (quercetin: 21.86±0.13 µg/g; catechin: 5.89±0.02 µg/g) as compared to control treatment. According to LSD test regarding pair wise multiple comparison (table 57, 60 & 63) given for quercetin and catechin, in every processed groups the pair wise comparisons of every treatment with all other treatments showed significantly ($p \leq 0.05$) different effect on quercetin. In case of catechin in ultrasonic and microwave groups the pair wise comparisons of every treatment with all other treatments showed significantly ($p \leq 0.05$) different effect on response. While in combined treated group the pair wise comparisons of treatment UM_2 with UM_3, UM_4 and treatment UM_4 with UM_2, UM_3 showed significantly different ($p \leq 0.05$) different effect on catechin.

Buck wheat contain 0.18-0.47 g kg-1 of quercetin and after germination process it was increased to 0.49-2.53 g kg-1. Furthermore, it was reported that Bifidobacterium breve BV-B gave the greatest amount of quercetin i.e 2.53 g kg-1 after fermentation of germinated buckwheat (Ratha & Jhon, 2017). Similarly, buckwheat hull, bran, and flour were studied for their quercetin concentration and reported values were 0.3, 1.2 and 0.2 g kg-1, respectively (Cho et al., 2014), and buckwheat noodle contain 4.3 g kg-1 (Yoo et al., 2012). Kim et al. (2007) studied sprouted buckwheat for its flavonoid content including the C-glucoside flavons (orientin, iso-orientin, vitexin, isovitexin) as well as rutin and quercetin for a period of 1-10 days in a glass house under low light conditions. Obtained result showed that the amount of

the flavonoids including morin, myricetin, quercetin and kaempferol, for the radish and alfalfa sprouts was significantly higher. They were kept in dark than when they were treated with UV or IR radiation (Janicki et al., 2005). Modifications in the conditions of seed sprouting were also investigated and change in the flavonol content reported. For example, the highest myricetin, morin, quercetin and kaempferol content in the radish and lucerne sprouts was detected after sprouting in dark at 20°C (Janicki et al., 2005). Quercetin originally not noticed in the raw black bean and surprisingly improved to 0.97 ± 0.21mg/100g and this concentration increased (2.24 ± 0.24 mg/100g) after 4^{th} day of germination (Guajardo-Flores et al., 2012). Quercetin in soybean sample was also improved after germination of 6^{th} days from 523.82 to 955.29μmol/100 g db (Guzmán-Ortiz et al., 2017). Similarly, the catechin content of soybean were also increased along with germination from 38.38 μmol/100 g db to 42.6 and 159.95 μmol/100 g db after 2 and 6 days of germination (Guzmán-Ortiz et al., 2017). Synthesis of new hydroxycinamic acids (cumaric acid) during germination was the main reason behind the increment of quercetin, catechin and gallic acid (Dueñas et al., 2004). Both, barley and malt contain higher amount of catechin, varying from 20.8 to 70.4 mg/kg DW and 64 to 604 mg/kg DW, respectively. This increment in catechins after germination process is linked with the release of free phenolic content by enzymes synthesized during germination of the grain (Carvalho et al., 2015). With increasing germination rate level of gallic acid, ferulic acid, quercetin and catechin contents improved for ultrasonic treatment (US_1) and microwave treatment (MW_2) as compared to control. But for catechin and quercetin the change was very slight.

Table 61.

Mean squares of HPLC profile of phenolic compounds for combined application processed sorghum sprouts

SOV	df	Gallic acid	Ferulic acid	Catechin	Quercetin
Treatment	4	1.26384^{**}	1.708^{NS}	0.006510^{*}	0.075660^{**}
Replicate	2	0.02738^{NS}	8.141^{NS}	0.001460^{NS}	0.079380^{**}
Error	8	0.00673	3.564	0.001585	0.000280
Total	14				

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

Table 62.

HPLC profile of phenolic compounds for combined application processed sorghum sprouts

Treatments	Gallic acid (µg/g)	Ferulic acid (µg/g)	Catechin (µg/g)	Quercetin (µg/g)
C	15.65 ± 0.10^{abcd}	118.45 ± 2.05^{ns}	5.58 ± 0.04^{ns}	21.43 ± 0.14^{abcd}
UM_1	14.27 ± 0.09^{abc}	117.79 ± 2.15^{ns}	5.60 ± 0.06^{a}	21.25 ± 0.10^{abcd}
UM_2	14.57 ± 0.07^{abcd}	117.87 ± 2.11^{ns}	5.62 ± 0.01^{ab}	21.33 ± 0.14^{abcd}
UM_3	14.21 ± 0.13^{abc}	116.73 ± 2.17^{ns}	5.53 ± 0.03^{a}	21.11 ± 0.13^{abcd}
UM_4	14.02 ± 0.12^{abcd}	116.75 ± 2.10^{ns}	5.51 ± 0.04^{ab}	21.04 ± 0.12^{abcd}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; UM_1, UM_2, UM_3, UM_4: different combined processed germinated treatments

UM_1: (5 min for 40% & 15 sec for 450W); UM_2: (5 min for 60% & 15 sec for 700W);

UM_3: (10 min for 40% & 30 sec for 450W); UM_4: (10 min for 60% & 30 sec for 700W)

Table 63.

Multiple comparisons (post hoc) LSD test of phenolic compounds for combined application processed sorghum sprouts

Treatment I	Treatment J	Significance levels			
		Gallic acid	Ferulic acid	Catechin	Quercetin
C	UM_1	.000	.680	.555	.000
	UM_2	.000	.716	.253	.000
	UM_3	.000	.297	.163	.000
	UM_4	.000	.302	.063	.000
UM_1	C	.000	.680	.555	.000
	UM_2	.002	.960	.555	.000
	UM_3	.397	.511	.063	.000
	UM_4	.006	.519	.024	.000
UM_2	C	.000	.716	.253	.000
	UM_1	.002	.960	.555	.000
	UM_3	.001	.481	.024	.000
	UM_4	.000	.488	.010	.000
UM_3	C	.000	.297	.163	.000
	UM_1	.397	.511	.063	.000
	UM_2	.001	.481	.024	.000
	UM_4	.022	.990	.555	.001
UM_4	C	.000	.302	.063	.000
	UM_1	.006	.519	.024	.000
	UM_2	.000	.488	.010	.000
	UM_3	.022	.990	.555	.001

Based on observed means. The mean difference is significant at the .05 level. I & J Both indicate control and different processed treatments (C, UM_1, UM_2, UM_3, UM_4), we are comparing different treatments with each other but I≠J.

C: control; UM_1, UM_2, UM_3, UM_4: different combined processed germinated treatments UM_1: (5 min for 40% & 15 sec for 450W); UM_2: (5 min for 60% & 15 sec for 700W); UM_3: (10 min for 40% & 30 sec for 450W); UM_4: (10 min for 60% & 30 sec for 700W)

4.8. Bio-accessibility of phytochemicals

Physicochemical accessibility of nutrients from the food matrix can be improved by using different strategies which can also reduce the content of anti-nutrients. In this study, all the processed germinated treatments were subjected to different bio-accessibility tests i.e. in vitro protein digestibility and polyphenol bio-accessibility.

4.8.1. *In-vitro* protein digestibility (IVPD)

Bio-accessibility of sorghum protein is indicated by digestibility and affected by multiple factors. Mean squares regarding effect of seed germination on IVPD value of all sorghum treatments are presented in tables 64, 67 & 70. There is a highly significant difference ($p \leq 0.01$) in IVPD efficiency among germinated treatments of each processed group. The means of IVPD values presented in tables 65, 68 & 71 are showing that different pretreatments can improve the level of IVPD. There was a significant difference between the three groups of germinated treatments i.e. microwave treated, ultrasonic treated and groups having application of both treatments in combination. IVPD % for ultrasonic treated, microwave treated and combined treated groups were ranged from 64.70±0.50 to 74.40±0.20%, 66.70±0.60 to 74.40±0.20% and 64.40±0.10 to 69.60±0.60%, respectively. In each group, the treatment having highest germination rate showed the maximum level of IVPD% such as US_1 (5 min & 40%) at 74.40±0.20%, MW_2 (15 sec & 700W) at 74.40±0.20% & UM_2 (US: 60% for 5 min & MW: 700 W for 15 sec) at 69.60±0.60% as compared to control treatment (71.30±0.50%). So, the ultrasonic and microwave treatments increasing the germination rate significantly increased the IVPD% as compared to control (71.3%). There was a significant difference ($P \leq 0.01$) among the treatments of each group. But combined application of both techniques significantly decreased the IVPD% as compared to control. According to LSD test regarding pair wise multiple comparison (table 66, 69 & 72) given for IVPD, in every processed groups the pair wise comparisons of every treatment with all other treatments showed significantly ($p \leq 0.05$) different effect on response.

Results of current investigation showed compliance with results of Afify et al. (2012c), who observed in vitro protein digestibility in raw sorghum ranged 50.94 to 52.09%, additionally, germination increased the protein digestibility from 70 to 78%. The germination caused a clear increase of IVPD % in all germinated treatments. In another study conducted by Nour et al. (2015), sprouting effected the IVPD% of raw sorghum flour (51.2%) which

improved to 65.03% after sprouting. Degradation of anti-nutrients is generally occurred after processing which ultimately improves the IVPD of sorghum. IVPD of legume sprouts were investigated after soaking and germination which significantly improved it (Uppal & Bains, 2012). Germination caused 15 to 25% increase in mung bean, 6 to 17% in chickpea and 6 to 17% of IVPD in cowpea. During different germination periods, significant improvement in IVPD was noted in three legumes. Therefore, it can be concluded that longer germination period improves the IVPD. Progressive improvement in protein digestibility was observed as the germination period increased (Punia, 2000; Trugo et al., 2000). Germination of millet grain significantly increased the protein digestibility of two varieties as compared to non-germinated samples. Protein digestibility increased from 45.5% to 88.2% for K variety and 49.3% to 78.9% for MRB variety (Pushparaj & Urooj, 2011). Samples of amaranth flour were also evaluated for IVPD after germination and improvement in protein digestibility was observed as IVPD increased from 35.84 ± 0.60% to 65.21 ± 0.50% (Olawoye & Gbadamosi, 2017). Significant improvements in the IVPD of raw mucuna bean were observed after germination i.e. from 67.21% to 69.14 ±0.10 (Mugendi & Njagi, 2010). Germination process enhances the activity of endogenous protease enzyme responsible to hydrolyze the stored protein. Endogenous protease increases the protein digestibility by partially hydrolyzing the stored protein, it also increases the soluble protein which linked with increase in IVPD. Thus these partially hydrolyzed proteins become readily available for digestion process (Bhise et al., 1988).

Germinated grains contain protease enzymes which increased the in vitro protein digestibility. Protein digestibility and sensory properties can be improved through germination process (Wedad et al., 2008). Sorghum protein contain high degree of cross linking which reduces its digestibility. Germination activate the number of intrinsic enzymes including amylases, proteases, phytases and fiber-degrading enzymes, thus improved the nutrient digestibility (Correia et al., 2010). Various factors affect the digestibility of sorghum protein and these factors were divided into two categories i.e. exogenous and endogenous. Exogenous factors involving interaction of proteins with non-protein compounds like polyphenols, starch, non-starch polysaccharides, phytates and lipids, on the other hand endogenous factors arise from the sorghum proteins themselves (Baker et al., 2010). Legumes and cereals were evaluated for its protein digestibility and improvement were observed after germination and

fermentation (Taylor & Taylor, 2002). In ultrasonic (US_1) and microwave (MW_2) treatments with increase germination rate IVPD% was significantly increased compared to control.

Table 64.

Mean squares of bio-accessibility profile for ultrasound processed sorghum sprouts

SOV	df	IVPD	Bio-accessible polyphenols	Bio-accessible flavonoids
Treatment	4	59.6310^{**}	0.002250^{NS}	0.000750^{NS}
Replicate	2	0.3920^{*}	0.000980^{NS}	0.003380^{NS}
Error	8	0.0620	0.001530	0.001330
Total	14			

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

IVPD: *in-vitro* protein digestibility

Table 65.

Bio-accessibility profile for ultrasound processed sorghum sprouts

Treatments	IVPD (%)	Bio-accessible polyphenols (mg/g)	Bio-accessible flavonoids (mg/g)
C	71.30 ± 0.50^{abcd}	0.60 ± 0.05^{ns}	0.10 ± 0.03^{ns}
US_1	74.40 ± 0.20^{abcd}	0.64 ± 0.04^{ns}	0.12 ± 0.05^{ns}
US_2	72.80 ± 0.30^{abcd}	0.61 ± 0.02^{ns}	0.11 ± 0.06^{ns}
US_3	65.20 ± 0.10^{abcd}	0.58 ± 0.05^{ns}	0.09 ± 0.04^{ns}
US_4	64.70 ± 0.50^{abcd}	0.57 ± 0.01^{ns}	0.08 ± 0.01^{ns}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; US_1, US_2, US_3, US_4: different ultrasound processed germinated treatments

US_1: (5 min for 40%); US_2: (5 min for 60%); US_3: (10 min for 40%); US_4: (10 min for 60%)

IVPD: *in-vitro* protein digestibility

Table 66.

Multiple comparisons (post hoc) LSD test of bio-accessibility profile for ultrasound processed sorghum sprouts

Treatment I	Treatment J	Significance levels		
		IVPD	Bio-accessible polyphenols	Bio-accessible flavonoids
C	US_1	.000	.246	.521
	US_2	.000	.762	.746
	US_3	.000	.549	.746
	US_4	.000	.375	.521
US_1	C	.000	.246	.521
	US_2	.000	.375	.746
	US_3	.000	.097	.343
	US_4	.000	.060	.216
US_2	C	.000	.762	.746
	US_1	.000	.375	.746
	US_3	.000	.375	.521
	US_4	.000	.246	.343
US_3	C	.000	.549	.746
	US_1	.000	.097	.343
	US_2	.000	.375	.521
	US_4	.039	.762	.746
US_4	C	.000	.375	.521
	US_1	.000	.060	.216
	US_2	.000	.246	.343
	US_3	.039	.762	.746

Based on observed means. The mean difference is significant at the .05 level. I & J both indicate control and different processed treatments (C, US_1, US_2, US_3, US_4), we are comparing different treatments with each other but I≠J.

C: control; US_1, US_2, US_3, US_4: different ultrasound processed germinated treatments. US_1: (5 min for 40%); US_2: (5 min for 60%); US_3: (10 min for 40%); US_4: (10 min for 60%). IVPD: *in-vitro* protein digestibility

4.8.2. Bio-accessibility of polyphenols

Sprouting process proved to be beneficial for human health as it could increase the level of bio-accessible polyphenols. The result of mean squares regarding bio-accessible polyphenol contents of germinated sorghum having application of different processing techniques are presented in tables 64, 67 & 70. All treatments showed non-significant ($p > 0.05$) changes in bio-accessible polyphenol contents. The means of bio-accessible polyphenol contents given in tables 65, 68 & 71 showed the bio-accessible polyphenol for ultrasonic treated, microwave treated and combined treated group were ranged from 0.57±0.01 to 0.64±0.04 mg/g, 0.58±0.06 to 0.64±0.01 mg/g and 0.57±0.03 to 0.59±0.05 mg/g, respectively. In each group, the treatment having highest germination rate showed the maximum bio-accessibility of polyphenol i.e. ultrasonic treatment US_1 (5 min & 40%), microwave treatment MW_2 (15 sec & 700W) and combined application treatment UM_2 (US: 60% for 5min & MW: 700W for 15sec). So, the ultrasonic and microwave processing increasing the germination rate insignificantly ($p > 0.05$) increased the bio-accessible polyphenol content as compared to control (0.60±0.05 mg/g). But combined application of both techniques insignificantly ($p > 0.05$) decreased the bio-accessible polyphenols level as compared to control. According to LSD test regarding pair wise multiple comparison given for the bio-accessible polyphenols, in every processed groups the pair wise comparisons of every treatment with all other treatments showed insignificantly ($p > 0.05$) different effect on response (tables 66, 69 & 72).

Various processing methods affect the availability of phenolic compounds. Number of processing methods had been used traditionally including thermal processing, sprouting, and fermentation to enhance the bioavailability of phenolic compounds (Hotz & Gibson, 2007). The results of our study are also in agreement with other findings. Bio-accessibility of polyphenols of wheat was evaluated and no changes in polyphenol bio-accessibility was observed after sprouting and remain same as in un-sprouted wheat (0.96 mg/g). Native bio-accessible polyphenols of sorghum grain were 0.60 mg/g and after the sprouting little improvement was observed as total bio-accessible polyphenols i.e. 0.64mg/g. Effect of sprouting process was studied by using green gram, they contain 2.71 mg/g total bio-accessible polyphenols and after sprouting process no significance changes were observed in total bio-accessible polyphenols. Native bio-accessible polyphenol content of chickpea was 1.49 mg/g and after sprouting significant increment (1.62 mg/g) was observed (Hithamani & Srinivasan,

2014a). Finger millet samples were evaluated for its bio-accessible polyphenol showing reduction as it was observed that native and germinated sample contain 2.65 mg/g and 2.49 mg/g of polyphenols, respectively. Another study conducted on pearl millet contain 1.89 mg/g of bio-accessible polyphenol and after sprouting significant increment was observed in bio-accessible polyphenol (2.27 mg/g) content (Hithamani & Srinivasan, 2014b). The concentration and functionality of polyphenol may also increase after thermal processing and it has different effect on different group of polyphenols. The higher phenolic contents after processing were linked with the degradation of tannin or other food component into simple compounds which act as polyphenol (Oghbaei & Prakash, 2017; Schütz et al., 2004). Germination process is efficient for releasing phenolic compounds by enhancing their surface area ratio, inducing endogenous enzymatic reactions and conversion of the bioactive compounds into more effective ones. During digestion process action of endogenous enzymes like proteases and esterases can facilitate the release of phenolic compounds from the cell wall matrix (Udeh et al., 2017). High germination increased insignificantly increased the bio-accessible polyphenol contents of ultrasound treatment (US_1) and microwave treatment (MW_2) as compared to control.

Table 67.

Mean squares of bio-accessibility profile for microwave processed sorghum sprouts

SOV	df	IVPD	Bio-accessible polyphenols	Bio-accessible flavonoids
Treatment	4	33.6360^{**}	0.001740^{NS}	0.000960^{NS}
Replicate	2	0.1280^{NS}	0.002420^{NS}	0.000080^{NS}
Error	8	0.1730	0.001570	0.001180
Total	14			

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

IVPD: *in-vitro* protein digestibility

Table 68.

Bio-accessibility profile for microwave processed sorghum sprouts

Treatments	IVPD (%)	Bio-accessible polyphenols (mg/g)	Bio-accessible flavonoids (mg/g)
C	71.30 ± 0.50^{abcd}	0.60 ± 0.05^{ns}	0.10 ± 0.03^{ns}
MW_1	73.70 ± 0.40^{abcd}	0.62 ± 0.03^{ns}	0.12 ± 0.03^{ns}
MW_2	74.70 ± 0.20^{abcd}	0.64 ± 0.01^{ns}	0.12 ± 0.05^{ns}
MW_3	68.70 ± 0.10^{abcd}	0.59 ± 0.04^{ns}	0.09 ± 0.02^{ns}
MW_4	66.70 ± 0.60^{abcd}	0.58 ± 0.06^{ns}	0.08 ± 0.01^{ns}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; MW_1, MW_2, MW_3, MW_4: different microwave processed germinated treatments. MW_1: (15 sec for 450W); MW_2: (15 sec for 700W); MW_3: (30 sec for 450W); MW_4: (30 sec for 700W)

IVPD: *in-vitro* protein digestibility

Table 69.

Multiple comparisons (post hoc) LSD test of bio-accessibility profile for microwave processed sorghum sprouts

Treatment I	Treatment J	Significance levels		
		IVPD	Bio-accessible polyphenols	Bio-accessible flavonoids
C	MW_1	.000	.554	.496
	MW_2	.000	.251	.496
	MW_3	.000	.765	.731
	MW_4	.000	.554	.496
MW_1	C	.000	.554	.496
	MW_2	.019	.554	1.000
	MW_3	.000	.381	.316
	MW_4	.000	.251	.192
MW_2	C	.000	.251	.496
	MW_1	.019	.554	1.000
	MW_3	.000	.161	.316
	MW_4	.000	.101	.192
MW_3	C	.000	.765	.731
	MW_1	.000	.381	.316
	MW_2	.000	.161	.316
	MW_4	.000	.765	.731
MW_4	C	.000	.554	.496
	MW_1	.000	.251	.192
	MW_2	.000	.101	.192
	MW_3	.000	.765	.731

Based on observed means. The mean difference is significant at the .05 level. I & J both indicate control and different processed treatments (C, MW_1, MW_2, MW_3, MW_4), we are comparing different treatments With each other but I≠J.

C: control; MW_1, MW_2, MW_3, MW_4: different microwave processed germinated treatments.

MW_1: (15 sec for 450W); MW_2: (15 sec for 700W); MW_3: (30 sec for 450W); MW_4: (30 sec for 700W). IVPD: *in-vitro* protein digestibility

4.8.3. Bio-accessibility of flavonoids

The mean squares presenting bio-accessible flavonoids content of sorghum treatments having application of different processing techniques are presented in tables 64, 67 & 70. There is non-significant ($p > 0.05$) difference of bio-accessible flavonoids content in all the germinated treatments. Means of bio-accessible flavonoid contents given in tables 65, 68 & 71 for ultrasonic treated, microwave treated and combined treated group were ranged from 0.08±0.01 to 0.12±0.05 mg/g, 0.08±0.01 to 0.12±0.05 mg/g and 0.08±0.01 to 0.10±0.03 mg/g, respectively. In each group, the treatment having highest germination rate showed the maximum bio-accessibility of flavonoids. i.e. US_1 (5 min & 40%), MW_2 (15 sec & 700W) and combined application treatment UM_2 (US: 60% for 5 min & MW: 700 W and 15 sec). So, the ultrasonic and microwave treatments increasing the germination rate also increased the bio-accessible flavonoids content insignificantly ($p > 0.05$) while combined application treatments insignificantly ($p > 0.05$) decreased the bio-accessibility of flavonoids as compared to control (0.10±0.03 mg/g). According to LSD test regarding pair wise multiple comparison given for the bio-accessible flavonoids, in every processed groups the pair wise comparisons of every treatment with all other treatments showed insignificantly ($p > 0.05$) different effect on response (tables 66, 69 & 72).

The results of our study are also in agreement with other findings. Finger millet samples were evaluated for its bio-accessible flavonoid contents and reduction in bio-accessible flavonoid content was observed from 1.09 mg/g to 0.81 mg/g after germination process. Another study conducted on pearl millet contain 0.14 mg/g bio-accessible flavonoid and after sprouting significant increment was observed (0.19 mg/g) (Hithamani & Srinivasan, 2014b). Similarly, bio-accessibility of flavonoid content remain same as in non-germinated wheat (0.04 mg/g). Native bio-accessible flavonoids of sorghum grain were 0.09 mg/g and after the sprouting 0.10 mg/g. Effect of sprouting process was studied by using green gram and concentration of total bio-accessible flavonoids in green gram was 0.21 mg/g and about 33% higher concentrations of bio-accessible flavonoids obtained after sprouting process. Native legume contains 0.10 mg/g of bio-accessible total flavonoid content remained unchanged after sprouting (Hithamani & Srinivasan, 2014a). To utilize bioactivity of phenolics, they have to be bio-accessible (released from the food matrix and solubilized after digestion). To evaluate the bioactivity of phenolic compounds an in vitro gastrointestinal model was used to create the

in vivo physiological environment (Hotz & Gibson, 2007). Surprisingly, it was reported that cereals containing highest level of polyphenols were not showed highest bio-accessibility. Different amounts of bio-accessible phenolics contents were determined in different grain, e.g. brown rice, wheat, and oat were 528.99, 308.83, and 443.44 μg FAE/g DW, respectively. After improved extrusion cooking treatment, bio-accessibility of phenolics compounds was reportedly decreased in brown rice, oat while showed minimal effect in wheat (Zeng et al., 2016). Higher bioavailability after treatments might be due to the breakage of interactions due to heat treatment which cause the improvement in bio-accessibility. Effect of germination was studied in horse gram and improvements in nutritional quality, antioxidant characteristics and reduce the anti-nutrients (Pal et al., 2016). Bio-accessible flavonoid contents of ultrasound treatment (US_1) and microwave treatment (MW_2) as compared to control insignificantly increased with increasing germination rate.

Table70.

Mean squares of bio-accessibility profile for combined application processed sorghum sprouts

SOV	df	IVPD	Bio-accessible polyphenols	Bio-accessible flavonoids
Treatment	4	28.9290^{**}	0.000510^{NS}	0.000360^{NS}
Replicate	2	0.0500^{NS}	0.002000^{NS}	0.000080^{NS}
Error	8	0.1750	0.003100	0.001180
Total	14			

* = Significant (≤ 0.05); ** = Highly significant (≤ 0.01); NS= Non-significant (> 0.05)

IVPD: *in-vitro* protein digestibility

Table 71.

Bio-accessibility profile for combined application processed sorghum sprouts

Treatments	IVPD (%)	Bio-accessible polyphenols (mg/g)	Bio-accessible flavonoids (mg/g)
C	71.30 ± 0.50^{abcd}	0.60 ± 0.05^{ns}	0.10 ± 0.03^{ns}
UM_1	65.50 ± 0.30^{abc}	0.58 ± 0.09^{ns}	0.08 ± 0.05^{ns}
UM_2	69.60 ± 0.60^{abcd}	0.59 ± 0.05^{ns}	0.10 ± 0.03^{ns}
UM_3	64.90 ± 0.20^{ab}	0.57 ± 0.02^{ns}	0.08 ± 0.02^{ns}
UM_4	64.40 ± 0.10^{abc}	0.57 ± 0.03^{ns}	0.08 ± 0.01^{ns}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) indicated various letters. ns: nonsignificant; a: one treatment is significantly different from other one treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from other three treatments; abcd: one treatment is significantly different from other four treatments.

C: control; UM_1, UM_2, UM_3, UM_4: different combined processed germinated treatments

UM_1: (5 min for 40% & 15 sec for 450W); UM_2: (5 min for 60% & 15 sec for 700W);

UM_3: (10 min for 40% & 30 sec for 450W); UM_4: (10 min for 60% & 30 sec for 700W)

IVPD: *in-vitro* protein digestibility

Table 72.

Multiple comparisons (post hoc) LSD test of bio-accessibility profile for combined application processed sorghum sprouts

Treatment I	Treatment J	Significance levels		
		IVPD	Bio-accessible polyphenols	Bio-accessible flavonoids
C	UM_1	.000	.672	.496
	UM_2	.001	.831	1.000
	UM_3	.000	.528	.496
	UM_4	.000	.528	.496
UM_1	C	.000	.672	.496
	UM_2	.000	.831	.496
	UM_3	.117	.831	1.000
	UM_4	.012	.831	1.000
UM_2	C	.001	.831	1.000
	UM_1	.000	.831	.496
	UM_3	.000	.672	.496
	UM_4	.000	.672	.496
UM_3	C	.000	.528	.496
	UM_1	.117	.831	1.000
	UM_2	.000	.672	.496
	UM_4	.181	1.000	1.000
UM_4	C	.000	.528	.496
	UM_1	.012	.831	1.000
	UM_2	.000	.672	.496
	UM_3	.181	1.000	1.000

Based on observed means. The mean difference is significant at the .05 level. I & J both indicate control and different processed treatments (C, UM_1, UM_2, UM_3, UM_4), we are comparing different treatments with each other but I≠J.

C: control; UM_1, UM_2, UM_3, UM_4: different combined processed germinated treatments. UM_1: (5 min for 40% & 15 sec for 450W); UM_2: (5 min for 60% & 15 sec for 700W); UM_3: (10 min for 40% & 30 sec for 450W); UM_4: (10 min for 60% & 30 sec for 700W)

IVPD: *in-vitro* protein digestibility

CHAPTER 5

SUMMARY

Sorghum (*Sorghum bicolor* (L.) Moench) having different phytochemicals is a powerhouse of distinct constituents. Nutritionally it is comparable with other cereal grains. Present work was an attempt to study the influence of microwave and sonication processing regarding phytochemicals composition and their bio-accessibility through germination. For this purpose, germination of sorghum grains was carried out by using two different levels of microwave and sonication for two different time intervals.

Sorghum grains were exposed to sonication and microwave separately along with their combined application to observe their effect on germination % and growth parameters (root length, shoot length, total weight, seedling vigor index and RL/SL ration). Morphological changes showed that ultrasonic treatment significantly affected the germination % along with root length (RL), shoot length (SL), total weight (TW) and seed vigor index (SVI) while root length/shoot length ratio (RL/SL) was not significantly affected. The results showed that seed germination percentage was maximum at 40% (US_1: 94.00±3.00 %) amplitude for 5 minutes as compared to control sprouts (78.00±2.00 %). Same treatment (US_1) produced maximum RL (2.80±0.10 cm), SL (2.20±0.30 cm), TW (2.56±0.04 g), RL/SL ratio (1.29±0.22) and SVI % (470.40±33.80 %). Ultrasound treatment can enhance the enzymatic and physiological activities if applied at appropriate amplitude level and duration. But higher amplitude can induce damage to cellular or enzymatic structure. Both time of exposure and power of microwave had influence on growth parameters. Microwave seed treatment for time 15 sec at power level 700 watt (MW_2), resulted in significant increase of germination percentage 95.00±3.00 % as compared to control. Treatment MW_2 showed highest results of other growth parameter such as RL: 2.90±0.50 cm, SL: 2.06±0.45 cm, TW: 2.55±0.30 g, SVI: 473.73±105.10 %). It is concluded that for 700 W the exposure at 15 sec (MW_2) is more effective in later stages of sprout development. Microwave electromagnetic treatment caused disturbance of the seed coat which enabled water diffusion into the seeds inducing higher rate of enzymatic reactions and the start of the initial development stages consequently resulted in faster and more effective germination. For among combined application of both ultrasonic and microwave processed treatments, maximum value of germination 75.00±2.50 % for UM_2 (US:

60%:5min & MW: 700W:15sec) was less as compared to control (78.00±2.00 %). Similarly, treatment UM_2 showed values of other growth parameters like RL (1.50±0.10 cm), SL (1.50±0.10 cm), TW (2.16±0.06 g), SVI (224.66±7.50 %) and RL/SL (1.00±0.05) less than control.

All the processed germinated treatments were quantitatively analyzed for phytochemicals. Regarding alkaloid contents in each group, the treatment having highest germination rate showed the maximum decrease of total alkaloids such as US_1: 0.035±0.002 mg/100 g DM (ultrasonic treatment: 5 min & 40% amplitude), MW_2: 0.033±0.002 mg/100 g DM (microwave treatment: 15 sec & 700W) and UM_2: 0.046±0.001 mg/100 g DM (combined treatment: US: 60% & 5min & MW: 700W & 15sec) as compared to control treatment (0.051±0.004 mg/100 g DM). For tannin contents among the processed treatments of combined application treated group, the treatment UM_2 (US: 60%:5min & MW: 700W:15sec) showed the reduction of tannin (mg CE/100 g DM) which was higher as compared to control treatment (0.147±0.001 mg/100 g). The maximum reduction of tannin contents occurred as germination percentage increased in microwave processed treatment MW_2: 15 sec & 700W (0.128±0.001 mg CE/100 g DM) and ultrasonic processed treatment US_1: 5 min & 40% amplitude (0.131±0.004 mg CE/100 g DM). Lowest phytate contents were showed by ultrasound processed treatment at 5 min & 40% amplitude (143.25±2.32 mg SPE/100 g DM: US_1), and microwave processed treatment at 15 sec & 700W (142.27±2.47 mg SPE/100 g DM: MS_2). In both groups treatment with high germination rate give reduced amount of phytate contents. In combined application treated group among processed treatments, treatment UM_2 (US: 60%:5min & MW: 700W:15sec) (147.24±2.11 mg SPE/100 g DM) has higher amount than control germinated treatment (146.05±2.25 mg SPE/100 g DM). The maximum decrease of saponins was observed in microwave processed treatment at 15 sec & 700W (MW_2: 0.07±0.01 mg/100 g DM), ultrasonic processed treatment at 5 min & 40% amplitude (US_1: 0.09±0.02 mg/100 g DM) as compared to control sprouts (0.15±0.01 mg/100 g DM). While combined application processed treatment UM_2: 0.14±0.02 mg/100 g DM (US: 60%:5min & MW: 700W:15sec) also showed reduced saponin contents compared to control. Germination decreased the level of anti-nutrients up to significant level and also enhanced several bioactive constituents.

Highest level of sterols was found in microwave processed sprouts at 15 sec & 700W (MW_2: 0.699±0.025 mg CHE/g DM) followed by ultrasonically processed sprouts at 5 min & 40% amplitude (US_1: 0.697±0.002 mg CHE/g DM) in comparison to control sprouts (0.655±0.025 mg CHE/g DM). While among processed treatments of combined application group treatment UM_2 (US: 60%:5min & MW: 700W:15sec) has lower sterol contents (0.619±0.025 mg CHE/g DM) as compared to control. Resultantly, most of the sterols are produced during the seed development and germination and therefore, sterols will deliver a supply for the newly growing cells and young shoots. The germination process improved the TFAA amount as the highest TFAA contents were observed for the microwave treatment and ultrasonic treatment MW_2: 8.05 mg AE/g DM (15 sec & 700W) and US_1:8.03 mg AE/g DM (5 min & 40% amplitude) as compared to control (7.85±0.03 mg AE/g DM). Among processed treatments of combined application group treatment UM_2 (US: 60%:5min & MW: 700W:15sec) (7.73±0.06 mg AE/g DM) of TFAA showed comparatively lower content than control. During the germination process, hydrolytic enzymes (protease) degrade the protein into free amino acids to fulfill the embryo growth and seed requirements.

The processed germinated treatments subjected to proximate analysis (protein, carbohydrate and lipids) showed that protein contents were increased with increasing rate of germination as the highest content was in ultrasonic processed treatment US_1 at 5 min & 40% amplitude (11.96±0.01%) followed by microwave processed treatment MW_2 at 15 sec & 700W (11.84±0.05%) as compared to control (10.83±0.05%). Among combined application treated group the treatment UM_2 (US: 60%:5min & MW: 700W:15sec) (10.73±0.03%) showed lower low protein contents compared to control treatment. Mobilization of stored nitrogen during germination was the reason of increased protein content. This mobilization of nitrogen produces the high-quality proteins required by growing plant for its growth and development. Similarly, for carbohydrate increased germination rate increased their content. In microwave processed group treatment MW_2 (15 sec & 700W) showed highest content of 75.87±2.34% and ultrasound processed treatment US_1 (5 min & 40% amplitude) increased 75.87±2.27% as compared to control germination treatment (75.05±2.15%). Among combined application processed group treatment UM_2 (US: 60%:5min & MW: 700W:15sec) (74.8±2.21%) showed decreased contents compared to control treatment. It was observed that stored lipids extensively converted to soluble carbohydrate after germination leading to increased

carbohydrate contents. In contrast to protein and carbohydrate the oil yield increased with increasing treatment intensities rather than higher germination.

Combined application of microwave and ultrasound treatments gave a significant difference regarding oil yield as compared to control. Maximum oil yield 7.57±0.26% (UM_4) was obtained at 700 W microwave power and 60 % ultrasound amplitude applied for 30s and 10 min, respectively as compared to control (6.28±0.21%). The optimum condition for microwave pretreatment was 700 W (MW_4) for 30 sec, which gave maximum oil yield of 7.48±0.27%. Ultrasonic amplitude 60% (US_4) for 10 min showed highest oil yield of 7.29±0.23% as compared to control.

Similar trends were observed for saturated and unsaturated fatty acids. The change of 14.56±0.12% in palmitic acid was studied by giving combined treatment of microwave and sonication (US: 60%:10 min & MW: 700W:30 sec) while 1.79±0.12% and 0.20±0.04% change in stearic and arachidic acid respectively. According to results major fatty acids in sorghum seed oils were linoleic acid (43.57±0.31%) and oleic acid (34.12±0.14%) for microwave treatment (MW_4: 30 sec & 700W). While for ultrasound processed sorghum samples highest level was for linolenic acid 1.96±0.12% (US_4: 10 min & 60%) but contents of eicosenoic acid were reduced from 0.34±0.01% to 0.30±0.01% (US_1, US_2) respectively. Contents of eicosenoic acid were increased from 0.34±0.01% to 0.39±0.04% (UM_3, UM_4) by giving combined treatment of microwave and sonication while showed non-significant change for other fatty acids. Among all treatments processed germination process induced obvious variations regarding percentage of palmitoleic acid (0.49 ± 0.05%), linolenic acid (1.96 ± 0.12%) and eicosenoic acid (0.39 ± 0.04%), respectively. Regarding unsaturated fatty acid more reduced contents were analyzed for oleic acid (34.04±0.10%) which may be caused through decomposition by lipolytic enzymes.

Antioxidant activity (DPPH, FRAP & ORAC) and phenolic profile (TPC, TFC) of all processed germinated sorghum treatment was improved with highest germination rate. The highest total phenolic content was observed for the microwave treatment MW_2: 1.27±0.03 mg GAE/g DM (15 sec & 700W), followed by ultrasonic treatment US_1 (5 min & 40%) 1.26±0.01 mg GAE/g DM as compared to control (1.18±0.07 mg GAE/g DM). Among treatments of combined application processed group treatment UM_2 (US: 60%:5min & MW: 700W:15sec) showed maximum TPC 1.18±0.02 mg GAE/g DM but were insignificantly different from

control treatment. The highest flavonoids content for the microwave treatment was 1.05±0.01 mg QE/g DM (MW_2: 15 sec & 700W), among the ultrasonic sprouts, treatment US_1 (5 min & 40%) showed highest TFC content of 1.02±0.06 mg QE/g DM against the control treatment (0.88±0.04 mg QE/g DM). In combined application processed group treatment UM_2 (US: 60%:5min & MW: 700W:15sec) has decreased content of TFC 0.83±0.05 mg QE/g DM as compared to control treatment. Regarding DPPH assay the value for microwave treatment and ultrasonic treatment was (MW_2: 15 sec & 700W) 89.73±2.11% and (US_1: 5 min & 40%) 89.11±2.25% respectively, as compared to control (83.76±1.50%). Among the combined application processed group DPPH activity of treatment UM_2 (US: 60%:5min & MW: 700W:15sec) was 79.09±1.50% which was lower as compared to control treatment. The FRAP assay gave highest value for the microwave treatment and ultrasonic treatment (MW_2: 15 sec & 700W) 0.029±0.001 mmol FE/g DM and (US_1: 5 min & 40%) 0.031±0.004 mmol FE/g DM as compared to control (0.029±0.001 mmol FE/g DM) but combined application of both techniques reduced the FRAP activity. In this group treatment UM_2 (US: 60%:5min & MW: 700W:15sec) showed 0.028±0.005 mmol FE/g DM as compared to to control. Similarly, highest ORAC assay value was observed for the microwave treatment and ultrasonic treatment (MW_2: 15 sec & 700W) 28.03±0.16 µmol TE/g DM and (US_1: 5 min & 40%) 27.06±0.12 µmol TE/g DM as compared to control (25.38±0.13 µmol TE/g DM) but combined application of both techniques reduced the ORAC assay values. After germination, enzyme synthesis can increase the inherent phytochemical compounds and resultantly higher antioxidant activity was observed with increased germination.

Quantification of various individual phenolic compounds through HPLC showed the maximum level of ferulic acid such as US_1: 120.33±2.07 µg/g (ultrasonic treatment: 5 min & 40%) and MW_2: 120.36±2.14 µg/g (microwave treatment: 15 sec & 700W) as compared to control treatment (118.45±2.05 µg/g). The treatment having highest germination rate showed the maximum level of quercetin such as US_1: 21.86±0.12 µg/g (ultrasonic treatment: 5 min & 40%) and MW_2: 21.86±0.13 µg/g (microwave treatment: 15 sec & 700W) as compared to control treatment (21.43±0.14 µg/g). Highest amount of gallic acid and catechin in ultrasonic group was for treatment US_1 (5 min & 40%) 16.29±0.09 µg/g & 5.89±0.02 µg/g respectively. While in microwave group treatment MW_2 (15 sec & 700W) showed highest amount of 16.31±0.11 µg/g (gallic aid) and 5.93±0.03 µg/g (catechin).

Furthermore, *in vitro* protein digestibility in each group increased with highest germination rate. Results showed the maximum level of IVPD% such as US_1: 74.40±0.20% (ultrasonic treatment: 5 min & 40%), MW_2: 74.40±0.20% (microwave treatment: 15 sec & 700W) while treatment UM_2: 69.60±0.60% (combined treatment: US: 60%:5min & MW: 700W:15sec) showed decreased value compared to control treatment (71.30±0.50%). Germinated grains contain protease enzymes which increased the *in vitro* protein digestibility. Regarding bio-accessible polyphenols in each group, the treatment having highest germination rate showed the maximum bio-accessibility i.e. ultrasonic treatment 0.64±0.04 mg/g (US_1: 5 min & 40%), microwave treatment 0.64±0.01 mg/g (MW_2: 15 sec & 700W) wile combined application treatment 0.59±0.05 mg/g (UM_2: US: 60%:5min & MW: 700W:15sec) decreased the bio-accessibility of polyphenols in comparison to control. Similar trend was observed for bio-accessible flavonoids i.e. ultrasonic treatment 0.12±0.05 mg/g (US_1: 5 min & 40%), microwave treatment 0.12±0.05 mg/g (MW_2: 15 sec & 700W) and combined application treatment 0.10±0.03 mg/g (UM_2: US: 60%:5min & MW: 700W:15sec). While, the activities of different endogenous enzymes during digestion like proteases and esterases might affect release of the phenolic compounds from the cell wall matrix.

As nutritionally sorghum is comparable with other cereal grains and has wide variety of phenolic compounds in substantial levels. Conclusively, the novel processing techniques has the worth for introducing physiological changes and variations in phytochemical profile. Application of ultrasound (US_1) and microwave (MW_2) performed better by enhancing germination along with improved levels of phytochemical profile, proximate analysis, fatty acid profile, antioxidant activities, HPLC quantification IVPD and bio-accessible phenolic compounds.

CONCLUSION

Controlled and improved germination of edible seed is a valued technique to improve the nutritional composition and to decrease different anti-nutritional factors. Physical stimulating technologies are an innovation in the research area of seed invigoration. Novel technologies, such as sonication and microwave could be easily adopted to stimulate germination. Microwave and ultrasonic processing of sorghum grains resulted in significantly improved germination percentage and growth parameters. Processed sprouts showed significant reduction in antinutrients content (alkaloids, saponin, tannin and phytate) while increased the total free amino acids, sterols, protein, carbohydrate contents, oil yield and fatty acid composition. Antioxidant activities and HPLC analysis for individual phenolic compounds have also shown the improved levels which are directly correlated with high germination rates. In-vitro protein digestibility was significantly higher while bio-accessibility of polyphenol and flavonoid was insignificantly affected. From the present investigation, it is concluded that ultrasound and microwave processing improved the composition and bio-accessibility of phytochemicals through enhanced germination.

RECOMMENDATIONS

As the importance of sorghum in terms of its nutritional significance and agronomic advantages especially with reference to developing countries in the semi-arid tropics is immense, therefore,

1. Processing through novel technologies should be introduced as an important tool in terms of value addition for the cereals.
2. There must be research regarding the effect of processing on the nutritional and viscoelastic properties of sorghum.
3. Development of composite flours comprising sorghum with flours from other cereals or legumes should be encouraged to promote utilization of sorghum grains.
4. The development of new foods (complementary food, food blends) from germinated sorghum, having enhanced or enriched bioavailable functional compounds and phytochemicals, should be an interesting option for health and wellbeing.
5. To support the efforts for promoting the utilization of sorghum as food, evaluation of nutritional values and various bioactive fractions regarding its potential health in animal and human models should be performed in future research.

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APPENDICES

Appendix I

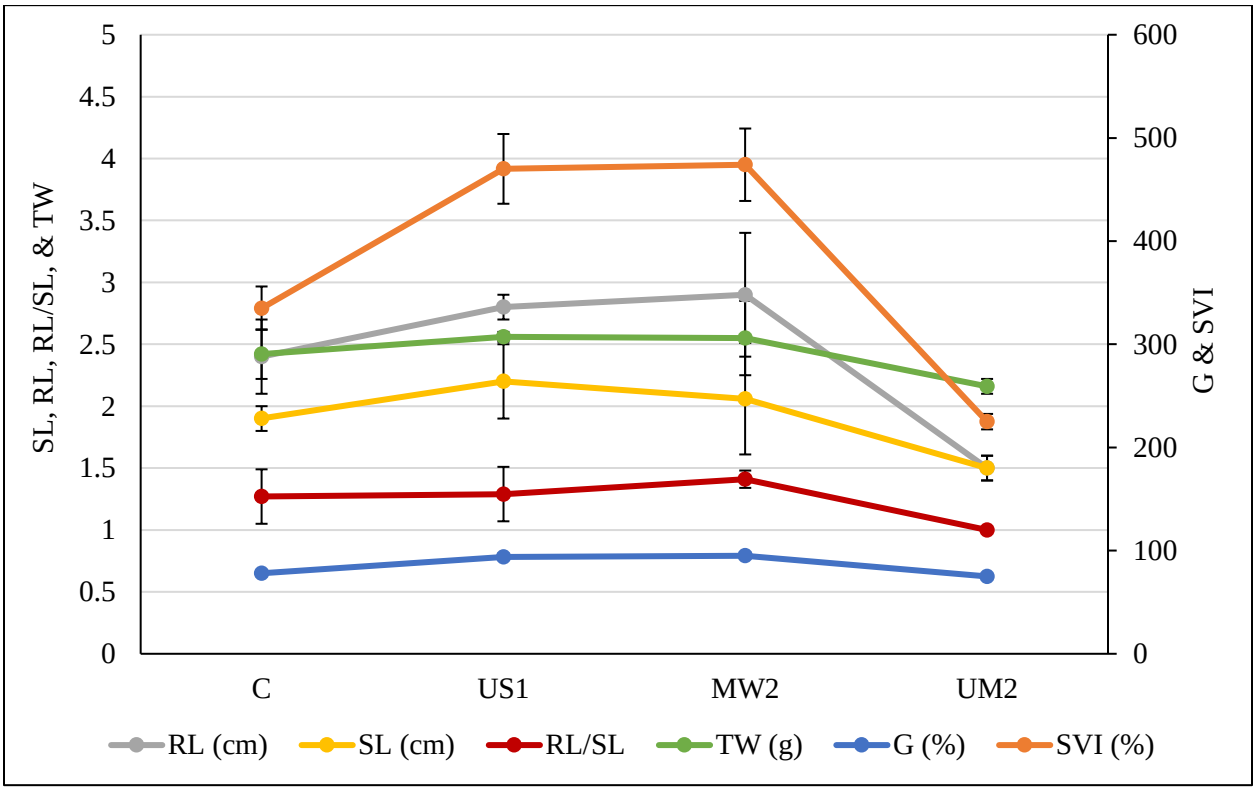

Figure 1. Graphical representation for germination parameters of best processed treatments

Appendix II

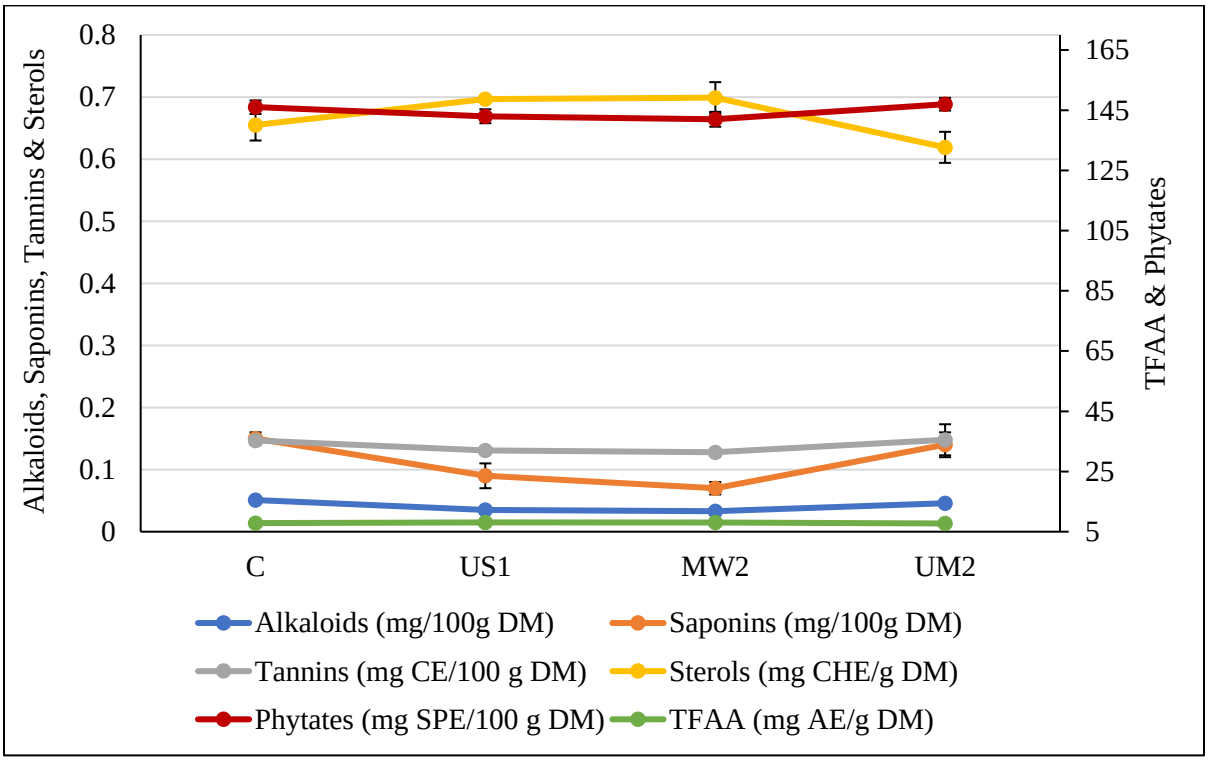

Figure 2. Graphical representation for phytochemicals profile of best processed treatments

Appendix III

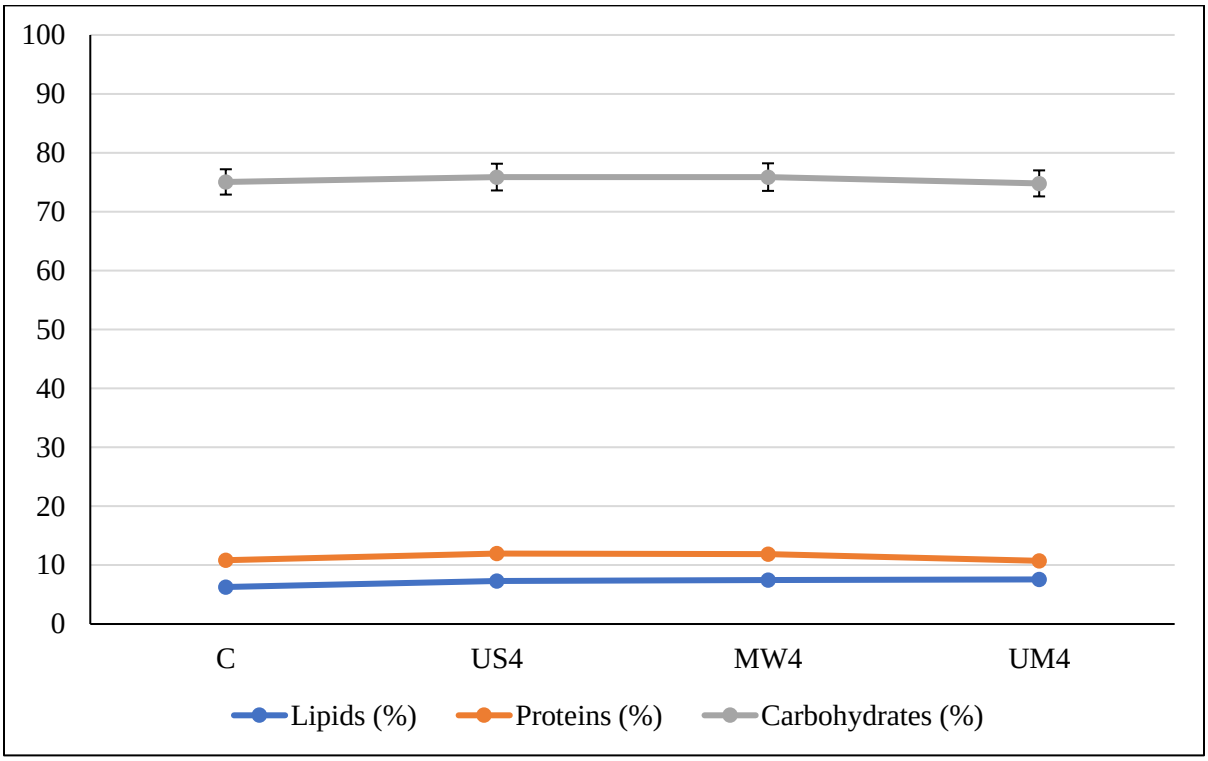

Figure 3. Graphical representation for proximate composition of best processed treatments

Appendix IV

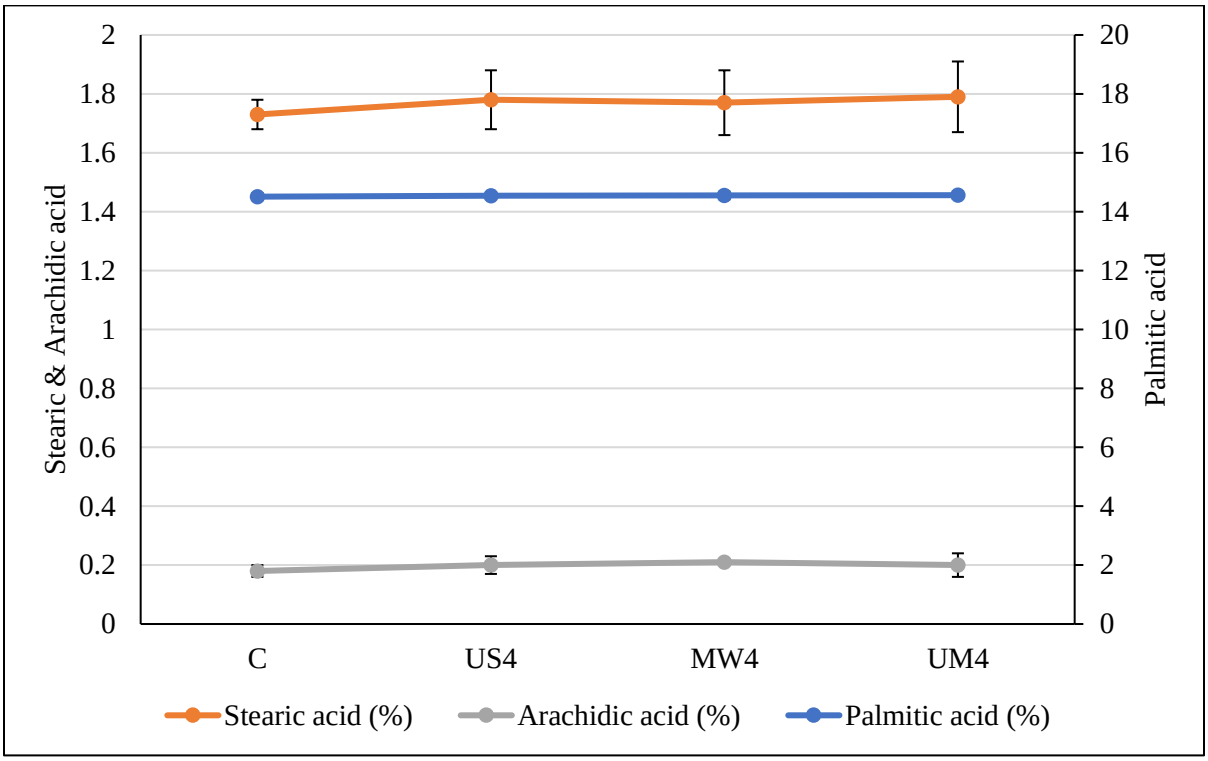

Figure 4. Graphical representation for saturated fatty acids of best processed treatments

Appendix V

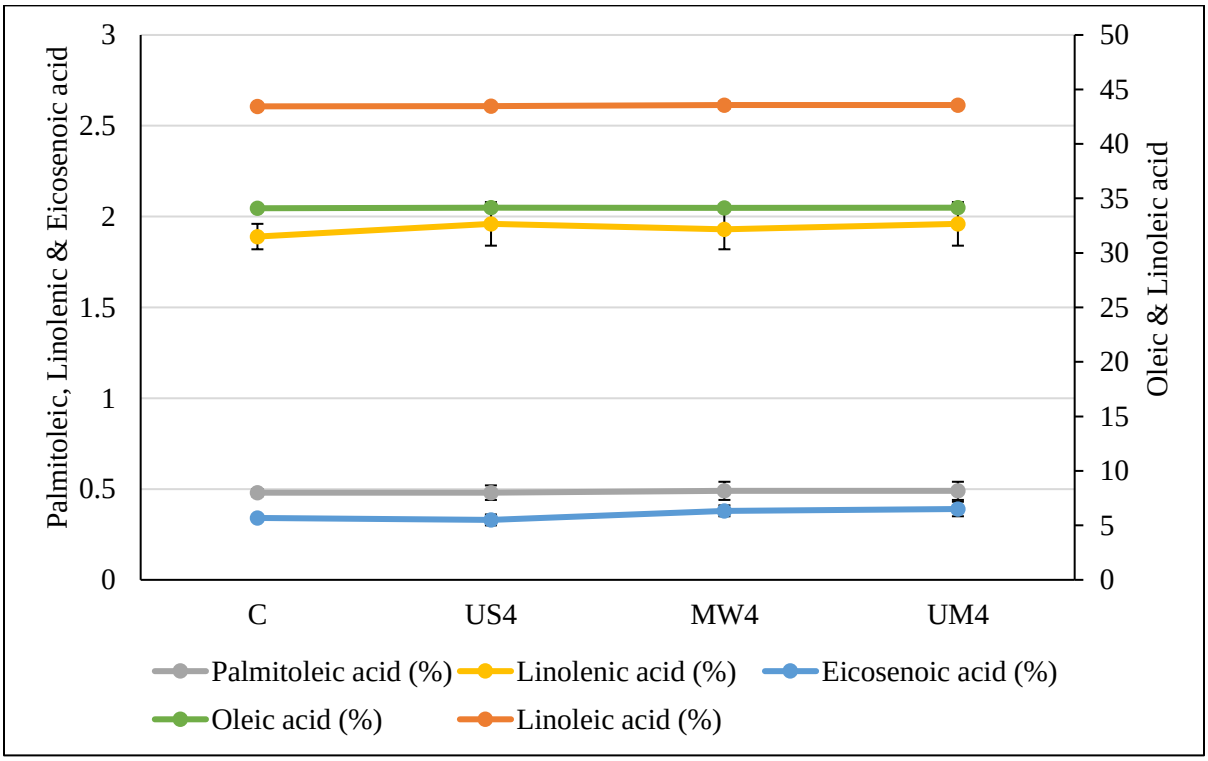

Figure 5. Graphical representation for unsaturated fatty acids of best processed treatments

Appendix VI

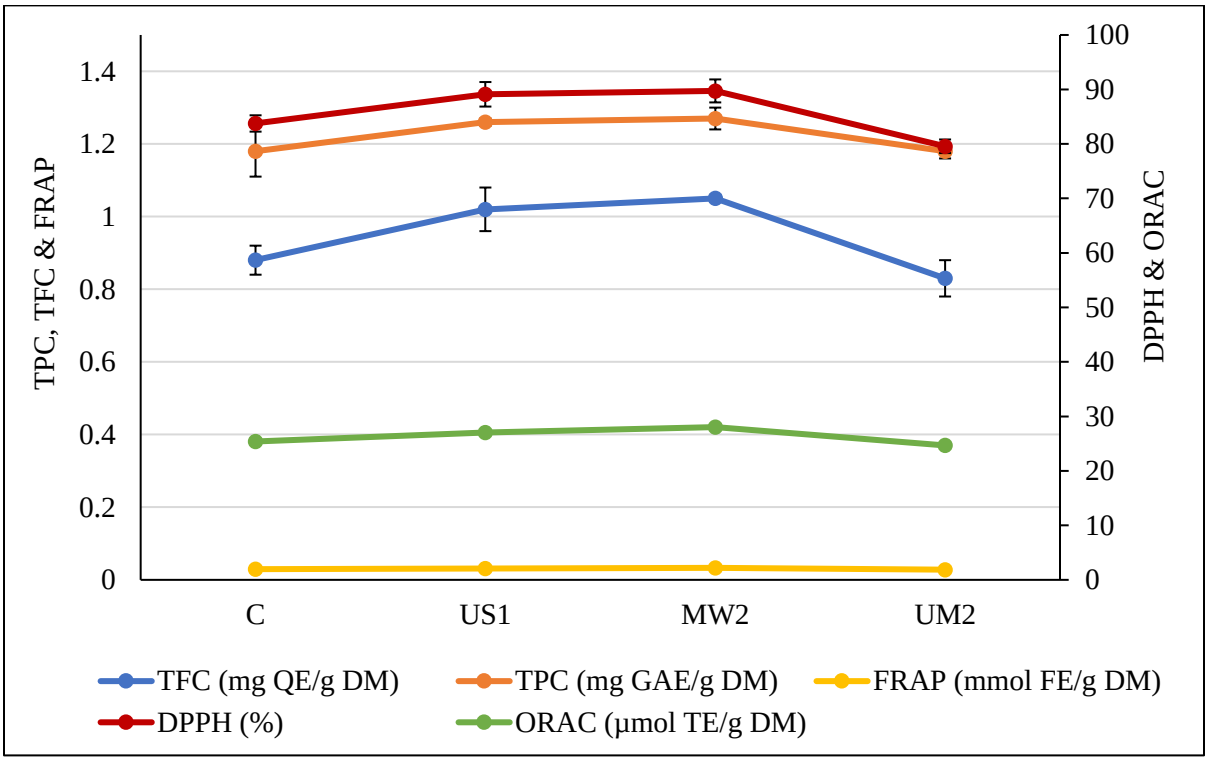

Figure 6. Graphical representation for phenolic profile and radical scavenging activity of best processed treatments

Appendix VII

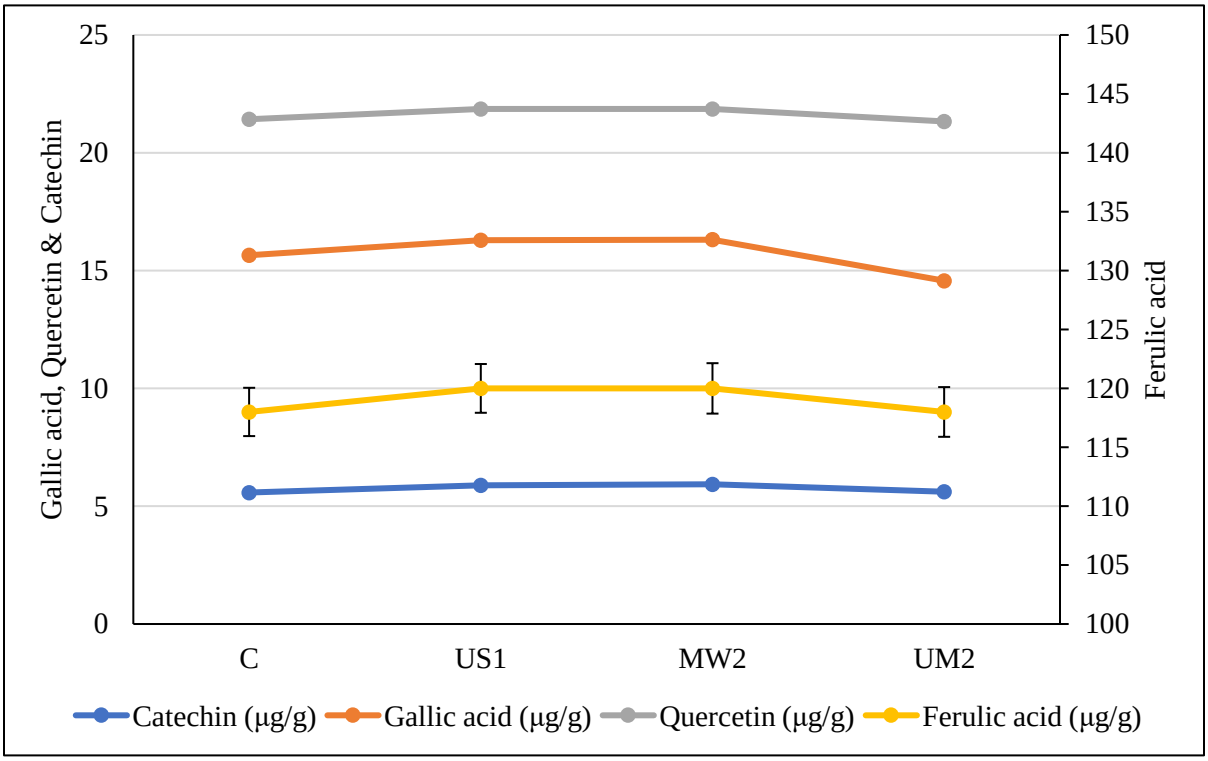

Figure 7. Graphical representation for individual phenolic compounds of best processed treatments

Appendix VIII

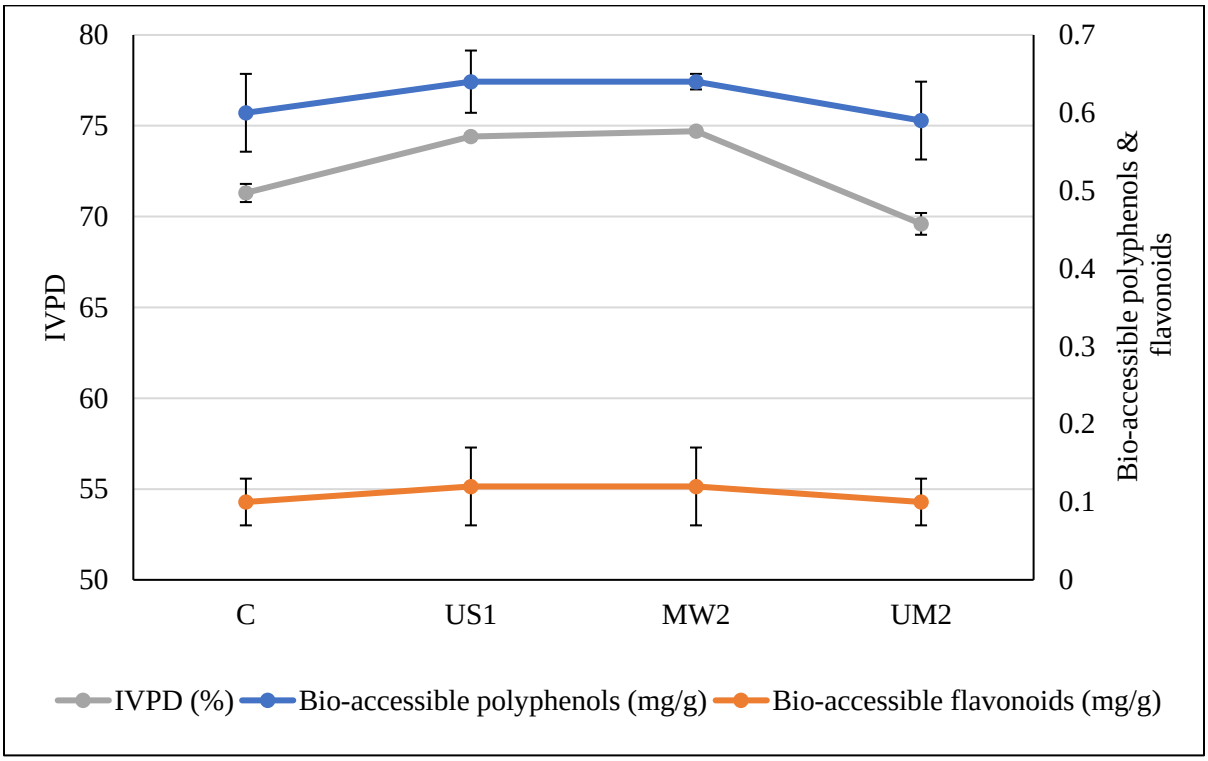

Figure 8. Graphical representation for bio-accessibility and IVPD of best processed treatments