Effects of thermal processing on sorghum phenolics and their potential for human health foods

by

Jaymi Marie Peterson

B.S., Kansas State University, 2019
M.S., Kansas State University, 2022

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Food, Nutrition, Dietetics, and Health
College of Health and Human Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2024

Abstract

Recent literature highlights the health benefits of pigmented grains such as rice, corn, and sorghum which are rich in polyphenols. These compounds offer numerous health advantages including antioxidant activity, anti-cancer, anti-diabetic, anti-hypertensive, and anti-obesity effects. This potential makes pigmented grains valuable as natural food additives or bio colorants in foods.

Chapter 1 introduces sorghum as a whole and covers topics in sorghum nutrition, sorghum phenolic classifications, reported health benefits, and both traditional food uses for sorghum as well as current sorghum phenolic applications in foods. Finally, a gap in knowledge is presented and subsequently examined in the following chapters The objective of this dissertation is to investigate the effect of processing on sorghum phenolics to determine their potential for future functional food applications.

Chapter 2 of this dissertation explores the phenolic profiles and bioactivities of various pigmented and non-pigmented rice, corn, and sorghum varieties. Sorghum was significantly higher in total phenolic content (TPC) by 67% in comparison to corn and 76% in comparison to rice. Sorghum was also significantly higher in antioxidant activity (AA) by 50% in comparison to corn and 45% higher than rice. Using the vanillin-HCl analysis, only sorghum had detectable condensed tannin contents (CTC). After further investigation using normal phase high pressure liquid chromatography (NP-HPLC), Sorghum was higher in total proanthocyanidin (condensed tannin) content by 96% in comparison to corn and 84% in comparison to rice.

Using reverse phase HPLC, the phenolic profile of grains was examined. Rice and corn samples contained unique cyanidin and anthocyanidin compound while sorghum contained unique 3-deoxyanthocyanidins.

Chapter 3 of this dissertation evaluates the effect of high moisture heat treatments at different pH levels on sorghum starch digestibility, phenolic profile and bioactivity. Brown whole grain sorghum flour was cooked with various buffers (pH 3, 4, 5, 7, 8) for 0, 10, 30, 60, or 120 minutes and then freeze dried for analysis. The results indicated that cooking for 10 minutes significantly ($p<0.05$) increased digestible starch. A 10-minute cooking duration did not significantly alter TPC but reduced condensed tannin content. Although 3-deoxyanthocyanidins decreased, some flavonoids remained stable or increased after processing. Cooking sorghum for 10 minutes did not significantly reduce cancer cell inhibition in HCT116 cells, except for pH 8. Overall, a 10-minute cooking time increased starch digestibility without significantly compromising health benefits. This further supports sorghum's potential as a health food ingredient.

Chapter 4 of this dissertation further evaluates the effect of various heat processing methods on sorghum phenolics to better understand the potential for sorghum as a health food ingredient. Toasting or dry heat methods increased phenolic content or had no negative affect on phenolic content while extrusion also increased phenolic content or did not affect phenolic content. The bioactivity of sorghum polyphenols was significantly decreased after heat processing regardless of processing method. More Studies are needed to understand the specific effect of each heat processing method on sorghum phenolics to fully utilize this grain for function food formulations.

Chapter 5 of this dissertation discusses the implications of these studies and the future studies to be undertaken to promote sorghum as a health food for human populations.

Effects of thermal processing on sorghum phenolics and their potential for human health foods

by

Jaymi Marie Peterson

B.S., Kansas State University, 2019
M.S., Kansas State University, 2022

A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Food, Nutrition, Dietetics, and Health
College of Health and Human Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2024

Approved by:

Co-Major Professor
Dmitriy Smolensky

Approved by:

Co-Major Professor
Weiqun Wang

Copyright

Abstract

Recent literature highlights the health benefits of pigmented grains such as rice, corn, and sorghum which are rich in polyphenols. These compounds offer numerous health advantages including antioxidant activity, anti-cancer, anti-diabetic, anti-hypertensive, and anti-obesity effects. This potential makes pigmented grains valuable as natural food additives or bio colorants in foods.

Chapter 1 introduces sorghum as a whole and covers topics in sorghum nutrition, sorghum phenolic classifications, reported health benefits, and both traditional food uses for sorghum as well as current sorghum phenolic applications in foods. Finally, a gap in knowledge is presented and subsequently examined in the following chapters The objective of this dissertation is to investigate the effect of processing on sorghum phenolics to determine their potential for future functional food applications.

Chapter 2 of this dissertation explores the phenolic profiles and bioactivities of various pigmented and non-pigmented rice, corn, and sorghum varieties. Sorghum was significantly higher in total phenolic content (TPC) by 67% in comparison to corn and 76% in comparison to rice. Sorghum was also significantly higher in antioxidant activity (AA) by 50% in comparison to corn and 45% higher than rice. Using the vanillin-HCl analysis, only sorghum had detectable condensed tannin contents (CTC). After further investigation using normal phase high pressure liquid chromatography (NP-HPLC), Sorghum was higher in total proanthocyanidin (condensed tannin) content by 96% in comparison to corn and 84% in comparison to rice.

Using reverse phase HPLC, the phenolic profile of grains was examined. Rice and corn samples contained unique cyanidin and anthocyanidin compound while sorghum contained unique 3-deoxyanthocyanidins.

Chapter 3 of this dissertation evaluates the effect of high moisture heat treatments at different pH levels on sorghum starch digestibility, phenolic profile and bioactivity. Brown whole grain sorghum flour was cooked with various buffers (pH 3, 4, 5, 7, 8) for 0, 10, 30, 60, or 120 minutes and then freeze dried for analysis. The results indicated that cooking for 10 minutes significantly ($p<0.05$) increased digestible starch. A 10-minute cooking duration did not significantly alter TPC but reduced condensed tannin content. Although 3-deoxyanthocyanidins decreased, some flavonoids remained stable or increased after processing. Cooking sorghum for 10 minutes did not significantly reduce cancer cell inhibition in HCT116 cells, except for pH 8. Overall, a 10-minute cooking time increased starch digestibility without significantly compromising health benefits. This further supports sorghum's potential as a health food ingredient.

Chapter 4 of this dissertation further evaluates the effect of various heat processing methods on sorghum phenolics to better understand the potential for sorghum as a health food ingredient. Toasting or dry heat methods increased phenolic content or had no negative affect on phenolic content while extrusion also increased phenolic content or did not affect phenolic content. The bioactivity of sorghum polyphenols was significantly decreased after heat processing regardless of processing method. More Studies are needed to understand the specific effect of each heat processing method on sorghum phenolics to fully utilize this grain for function food formulations.

Chapter 5 of this dissertation discusses the implications of these studies and the future studies to be undertaken to promote sorghum as a health food for human populations.

Table of Contents

List of Figures

List of Tables

Acknowledgements

I would like to thank my Major professor, Dr. Weiqun (George) Wang, for accepting me as his doctoral student and allowing me to focus my research on sorghum phenolics, food processing, and health benefits of polyphenols. I will forever be grateful for the opportunity to reach my educational and research goals under your guidance and leadership. I am truly appreciative for your kindness, support, and patience. I would also like to thank my Co-Major professor and USDA-ARS supervisor, Dr. Dmitriy Smolensky, whose guidance, insight, and unwavering support have been instrumental in my academic and scientific career. Your mentorship has not only shaped my research but has also inspired me to strive for excellence and honesty in all that I do. Thank you for believing in my potential when no one else did. Thank you for your endless patience and for encouraging me to explore new limits. I admire your mentorship and aspire to be half as an effective mentor as you are. I also want to thank Dr. Scott Bean, Dr. Xiaorong (Shawn) Wu, and Dr. Kaliramesh Siliveru for agreeing to serve on my committee. I am profoundly grateful for the guidance and support I have received from my committee members, whose example has deeply influenced my path and inspired my growth as a scientist.

I would like to express my heartfelt gratitude to the following collaborators for their contribution to this dissertation. Their insights, dedication, and support have enriched my work and made this journey enjoyable, educational, and rewarding. Thank you all for your commitment and for the countless hours of work and discussions that helped shape this dissertation. I want to thank Dr. Adina Santana for her major contributions to the HPLC and the glycation work done in chapters 2, 3, and 4. In addition, I thank Dr. Santana for her insights and dedication which has made a significant impact on my work. I deeply appreciate her

companionship and kindness in the laboratory. Thank you for being an excellent colleague and friend. Dr. Snehasis Chakraborty for their contributions in chapter 2. Mayra Perez-Fajardo for her contributions in chapter 3 as well as her continued support and friendship throughout this academic journey. I am thankful for her kindness, friendship, and constant encouragement during my academic experience. I also thank Dr. Jose Covarrubias for their contributions in chapter 3. Dr. Ramasamy Perumal for their contributions, support, and guidance, on chapters 2 and 3. Without whom, the sorghum research conducted in this dissertation would not have been as meaningful or impactful. Dr. Shannon Pinson and Dr. Paul Scott for their support and collaboration in chapter 2. Dr. Kathleen Yeater for their mentorship and guidance on statistical analyses. Sarah Cox for their contributions in chapter 3. Joaquin De Leon for their support and guidance during my academic journey. Thank you for setting a great example of what a scientist should be.

I would also like to acknowledge the Grain Quality and Structure Research Unit and the Center for Grain and Animal Health Research for their support. I genuinely appreciate their ability to collaborate, support, and encourage one another. The positive work environment and friendships have made a significant impact on my research experience. Thank you to the USDA-ARS for supporting this research. I also acknowledge my former supervisor and professor whose unconventional approaches to statistical analysis and personnel management have impressed upon me invaluable lessons for my future career. Thank you for inspiring me to seek better methods.

Thank you to my family and friends for your continued support and for encouraging me to continue my degree when the journey was unbearable. It is not possible to name every person who has made a significant impact on my life leading up to this point, however, I am beyond

grateful for the support, friendship, and guidance I have received during this process. The love and support from family and friends played an instrumental part in my success.

Thank you to my father, Dr. Johnny Lawrence, for teaching me the value of hard work and dedication. My memories of you staying up late to write your own dissertation served as a constant source of inspiration during all the late nights. Thank you to my mother, Janea Lawrence, for teaching me how to advocate for and believe in myself. My parents have supported me from my first steps as child all the way to the completion of this dissertation. Their sacrifices and dedication have shaped my values and aspirations, providing me with a strong sense of right and wrong, and the foundation to strive for excellence in all that I do. Thank you for raising me to believe I can do anything I set my mind to.

Thank you to my spouse, Cole Peterson, for always supporting me during this journey. Your patience during the late nights and understanding during times of stress have made this dissertation not only possible, but enjoyable. I am blessed by your partnership and unwavering commitment. Thank you for walking beside me every step of the way.

Thank you to my siblings, Justin Lawrence, Shelbie Lawrence, Jeremy Lawrence (nephew), and Jeana Lawrence, for having my back and encouraging me to prioritize my own needs and well-being. Your support and guidance have encouraged me in ways you can never know.

Thank you, Peterson Family (Dale Peterson, Lori Peterson, Gerri Mann, T.J Peterson, Dana Peterson, Jaxon Peterson, Rylie Peterson, Jace Peterson, & Greyson Peterson) for your unwavering enthusiasm and support in celebrating my accomplishments. Your encouragement and genuine excitement have made a significant impact on my ability to finish my dissertation.

Lastly, thank you to my dogs, Franklin, Zeus, Juno, Korra, & Titan for never letting me forget to enjoy the little things.

Dedication

I dedicate this dissertation to my family. “Never give up, never quit.”

Preface

This dissertation explores the fascinating world of sorghum phenolics, compounds that play a crucial role in plant protection and human health benefits. Sorghum, as a versatile and drought resistant crop, offers significant potential for enhancing food security and promoting sustainable agriculture.

The aim of this dissertation is to investigate the diverse phenolic profiles of various sorghum varieties, their antioxidant properties, and their implications for future functional food applications. By understanding the effects of processing on sorghum phenolics, this work contributes to the broader knowledge of sorghum as a potential functional food ingredient for health foods.

I hope this dissertation will inspire further research and innovation in sorghum. Additionally, I hope this research prompts continued investigation on processing effects on sorghum polyphenols for functional food applications, ultimately increasing sorghum's value and role in global food systems.

Chapter 1 – Review of Literature

1.1 Introduction

Sorghum is the fifth highest produced cereal grain worldwide and ranked third most produced cereal grain in the United States (US). The United States Department of Agriculture – Foreign Agricultural Service (USDA-FAS) reported sorghum production to be 62.106 thousand metric tons as of September 2024. The USDA-FAS further reported that the US is the largest producer of sorghum in the world, producing around 7.67 thousand metric tons (USDA-FAS, 2023). Around 83% of sorghum produced in the US is exported (Foreign Agricultural Service, 2021). Sorghum that is not exported, is mostly used for ethanol production and animal feed. Very little is used for food consumption in the US (Kulamarva et al., 2009).

Sorghum is an agronomically advantageous crop due to the grain's ability to thrive in a variety of conditions. The cereal grain is drought and heat tolerant, making it a hardy grain that can grow in environments with less rainfall than other grains. Secondly, the grain can be grown in a variety of soil conditions, namely saline-alkaline soil or barren soil and can grow in various altitudes (Xiong et al., 2019c). Lastly, the grain contains no protective coverings, but rather, some varieties are coated in tannins to aid the plant in defense against pests and disease (Awika and Rooney, 2004). These features allow sorghum to grow in less-than-ideal conditions while still producing favorable yields.

Other than its agronomical advantages, sorghum has gained recent interest for its polyphenolic composition. Depending on the variety, sorghum has higher phenolic concentrations than fruits and other cereal grains. It is also of note that 3-doexyanthocyanidins, a class of polyphenols reported in sorghum, are not known to exist in any other edible plants (Awika et al., 2004). The diverse and abundant sources of phenolics in sorghum are known to

offer many health benefits like reduced inflammation, cancer prevention, improved cardiovascular health, and reduced oxidative stress. Based on their genetic diversity, unique phenolic profile, and high yield, sorghum will be an increasingly relevant cereal crop in addressing climate changes and disease prevention in humans. Additionally, understanding food processing effects on sorghum phenolics will be as equally important for sorghum to be used as a functional food or nutraceutical. In this review, current research investigating processing effects on sorghum polyphenols, their potential food applications, and challenges will be discussed.

1.2 Sorghum

1.2.1 Sorghum Nutrition

Nutritional composition of sorghum differs among varieties. Like most other cereal grains, sorghum consists of carbohydrates, lipids, proteins, and minerals.

In sorghum, starch is stored in the endosperm and is the most abundant carbohydrate found in sorghum. Starch content will vary between cultivars, generally between 68.4 - 71.4% (Sang et al., 2008). Sorghum is notorious for its limited starch digestibility. Starch granules are surrounded by protein bodies within a protein matrix, which function as a barrier by limiting the gelatinization of starch during cooking. The lack of starch gelatinization in sorghum during cooking may affect overall starch digestibility. Depending on cooking parameters, starch digestibility of sorghum may decrease after cooking. This also allows the grain to maintain high levels of resistant starch, which may be useful for high glycemic index food formulations.

The second major component of sorghum is protein. Protein content and amino acid composition is reported to vary based on the variety and growing condition of the sorghum (Kulamarva et al., 2009). The protein content of sorghum has been reported to be in the range of 7.3-15.6% on a dry basis matter (Emmambux and Taylor, 2003). Amino acid composition of

sorghum is like other cereal grains but is limited in lysine, histidine, and sulfur containing amino acids (Virupaksha and Sastry, 1968). The limiting amino acid of sorghum, like many other cereals, is lysine (Pontieri and Del Giudice, 2016).

Sorghum protein can generally be divided into two classifications: prolamins and non-prolamin proteins. The main prolamin protein found in sorghum is Kafirin and makes up 77-82% of the proteins found in the sorghum endosperm. The non-prolamin proteins, which consist of albumins, globulins, and glutelins, make up around 30% of the protein in sorghum (de Mesa-Stonestreet et al., 2010). When classifying kafirin, there are four types: α-, β-, γ -, and δ-kafirin. It is important to note that while they are the same protein, the type of kafirin protein will play a crucial role in food formulations based on their structural differences. For example, α-kafirin is the least cross-linked and, therefore, the easiest to digest, while β- and γ- kafirin have the highest amounts of cross-linking and are not as readily digested. Furthermore, it has also been reported that condensed tannins have a higher binding affinity to γ- kafirin over any other type of kafirin due to its structural differences (Taylor et al., 2007). This is due to the previously mentioned extensive disulfide bond cross-linkages. These interactions between phenolics and sorghum proteins will further hinder overall protein digestibility, increase enzymatic resistance, and prevent protein hydrolysis (Taylor et al., 2007).

Factors affecting sorghum protein digestibility may be classified as exogenous and endogenous. Exogenous factors involve interactions between proteins and non-proteins (such as polyphenols, lipids, starches, etc.). Endogenous factors entail only protein-protein interactions. Exogenous factors that may affect protein digestibility include cooking, presence of pericarp, starch content, type of phenolics, and the presence of water. All these factors have separate, individual effects and may change with the addition or subtraction of one or more factors. For

example, Duodu, Nunes, and others (2002) reported that when cooking sorghum, the pericarp, endosperm, gelatinized starch, and non-tannin polyphenols seemed to have minor effects on digestibility. This inferred that the decrease in protein digestibility from cooking must come from structural changes within the proteins during cooking. It was hypothesized that the formation of oligomers bound through disulfide bonding might be the reason protein digestibility decreased. This process is mirrored in cooked maize, however, to a lesser extent and further supports the claim of disulfide bound oligomers decreasing digestibility in cooked sorghum (Duodu et al., 2002)). Other groups reported that cooking enhanced the interactions between kafirin and condensed tannins (Duodu et al., 2003). This was further supported by another study that saw a 50% reduction of protein digestibility after the addition of condensed tannins to cooked sorghum (Taylor et al., 2007). It is important to note that the type of phenolic compound plays an important role in terms of limiting protein digestibility. For example, phenolic acids and flavonoid-type phenolics do not bind with kafirin (Emmambux and Taylor, 2003).

In addition, the effect of sorghum digestibility and moisture content under cooking has also been studied to understand protein polymerization mechanisms. Kafirin proteins' hydrophobicity increases upon heating at high moisture levels (de Mesa-Stonestreet et al., 2010). This may be due to the previously mentioned increase in disulfide bonding, which may produce polymerized kafirin monomers that realign the kafirin into β-sheets. This change in structure will then inhibit swelling and water absorbance that reduce proteolysis (Emmambux and Taylor, 2003). In contrast, cooking sorghum at low water concentrations resulted in protein digestibility that was reported to be either greater than or like that of native sorghum.

Sorghum has also been found to be a good source of fiber, with most of the fiber content containing insoluble fibers (75-90%) and soluble fibers (10-25%) (Xiong et al., 2019c). Lipid

composition in sorghum consists of unsaturated and polyunsaturated fatty acids. Polyunsaturated fatty acids are the most common. Primary fatty acids include oleic, linoleic, palmitic, linolenic. The composition of fatty acids is like that of maize, with more unsaturated fatty acids.

In addition to sorghum's general nutrition profile, sorghum is also gluten free (GF). This feature may be highly favorable for food producers, given the rising occurrence of Celiac Disease (Kulamarva et al., 2009). Consumer demands for GF products are bringing about the use of alternative cereal grains to replace gluten in baked products traditionally made with wheat (breads, pastas, etc.). Sorghum further improves its attractiveness for food uses given that it is not a genetically modified organism (GMO). In recent years, consumers have shown preference for non-GMO foods and food products. These distinctive characteristics of sorghum show promise for the cereal grain's application in potential gluten-free or non-GMO food products.

1.2.2 Varieties

Sorghum is highly diverse in genotype with varieties ranging in size, shape, and color. The genetic diversity of sorghum includes more than 40,000 accessions (Rooney and Awika, 2004). The genotype determines the nutrient quality as well as the type and quantity of phenolic compounds. Although somewhat subjective, sorghum can also be classified by color and extractable phenolics. For example, white food sorghums are reported to have no detectable anthocyanins or tannins and extremely low levels of total extractable phenols. Although uncommon, there are some varieties of white sorghum that contain detectable tannins in their testa layer via the bleach test (Duodu and Awika, 2019). Yellow sorghum is known to have slightly higher total extractable phenols than white sorghum and is high in flavanones (Dykes et al., 2011); brown varieties contain significant levels of tannins and varying levels of pericarp pigmentation; red varieties contain no tannins but contain significant levels of extractable total

phenols (Awika and Rooney, 2004). Black varieties of sorghum contain mostly anthocyanins (Taleon et al., 2012). The potential applications of sorghum for food formulations or added value foods may therefore be dependent on sorghum genotype.

Other than physical appearance, sorghum varieties can be classified into three main types based on their total condensed tannin extractability in specific solvent systems. Type I sorghums have no extractable tannins. Type II sorghum have small amounts of extractable tannins (based on 1% HCl-Methanol solvent) while type III sorghum have noticeable extractable tannin contents in both acidified (1% HCL) and non-acidified methanol solvent systems (Awika and Rooney, 2004). This classification method does not account for the use of other solvent systems (i.e., ethanol, acetone, or their acidified versions) or other extractable phenolic compounds. Another common classification of sorghum is dependent on their induvial end usage. For instance, grain sorghum will be used for food applications, forage sorghum for animal feeds, biomass sorghum for biofuel production and sweet sorghum for sorghum syrups.

1.2.3 Traditional Food Applications

1.2.3.1 Introduction

Sorghum is an important cereal crop in many Asian and African countries where it is commonly consumed as food in the forms of tortillas, porridges, pastas and baked goods (Kulamarva et al., 2009). The development of white sorghum lines have led to the production of some flours produced from sorghum (Taylor et al., 2006). This has been especially helpful in food products as it does not impart any off colors or bitter flavors (Waniska and Rooney, 2003) Dark colors from black or tannin containing varieties may be useful in the health market where darker colors are generally associated as health and widely more accepted in darker baked products such as rye (Taylor et al., 2006). Other reported uses of sorghum in foods include

pastas, noodles, gluten free breads, snack foods non-fermented beverages and fermented beverages (Khan et al., 2013), (Taylor et al., 2006, Yousif et al., 2012)

1.2.3.2 Baked Goods

Wheat is the main source of flour used in the baking industry wherein the global market value for baked goods was 234.5 billion in 2022. The baked goods market is expected to grow by 4% by the year 2032 (Global Market Insights, 2023). Baked goods made from non-wheat grains offer a gluten free flour alternative and have become an area of research interest as the concern for celiac disease continues to increase among consumers. Sorghum is gluten and therefore may serve as an adequate wheat replacer in some gluten free baked good formulations.

In one study wheat flour was partially substituted with white sorghum to understand the effects on rheological properties and bread making quality. It was reported that replacing just 20% of wheat flour with sorghum flour decreased the size of the bread loaf. The authors hypothesized that the decrease in gluten content most likely affected the rheological properties of the dough. The sorghum addition increased dough resistance and decreased elasticity which caused a decrease in gas retention, thus decreasing the size of the loaf. Given the negative effects on the bread quality however, panelists from this study still found the sorghum and wheat mixture breads to be satisfactory in texture and flavor (Ncube et al., 2015). The negative effects of the bread quality could be combatted with increased water during formulation. It has been reported that larger bread volumes from sorghum flours can be achieved with the addition of more water and the addition of corn starch during formulation (Schober et al., 2005). Another technique to overcome changes in bread quality would be to alter the particle size of the flours. Altering the particle size of sorghum flour based breads can increase loaf size, crumb firmness, and crumb structure of breads (Trappey et al., 2014).

Other common baked good applications include sourdough formulations. In these formulations, however, the use of hydrocolloids or other starches (i.e., corn starch) may be necessary to overcome adverse effects on bread quality characteristics. However, recent research suggests that the use of specific bacterial strains, like *Pediococcus pentosaceus* or *Weissella confusa* can be added to aid in sourdough development (Olojede et al., 2022). These specific strains produce exopolysaccharides (EPS) that can improve the properties of sorghum bread and nay serve as hydrocolloid replacers (Galle et al., 2012).

Although it is not common in the US, other countries extensively use sorghum for bread applications. For example, roti an unleavened bread made with sorghum or millet is often consumed in western and central India and Kisra bread is commonly consumed in Sudan (Ari Akin et al., 2022). Sorghum tortilla formulations have also been investigated for their increased extensibility when compared to corn and their gluten free properties (Winger et al., 2014).

1.2.3.3 Other Food Products

Aside from the baking industry, sorghum is starting to make its appearance in extruded products like pastas, noodles, snack chips, and cereal foods. These formulations are likewise made with white sorghum grain varieties to reduce differences in color between sorghum and other grain flours (i.e., wheat, corn, etc.). However, one study investigated the use of sorghum in pasta noodles, white, brown, and red sorghum varieties were evaluated for their pasta noodle application potential. Noodles were formulated with sorghum flour, potato starch, rice flour, eggs, water, and psyllium to create their noodles. From their analyses, the most acceptable pasta formulation was made with the white sorghum flour while the brown and red varieties were ranked lower for their off flavor and appearance (de Oliveira et al., 2022). In another study both red and white sorghum were used to replace portions (20, 30, and 40%) of durum wheat in pasta

noodles. This study found that all levels of sorghum replacement decreased starch digestibility while the 20 and 30% replacements did not have adverse effects on pasta quality parameters. It was noted however, that color and hardness were noticeable impacted but still acceptable to consumers during sensory testing. This study demonstrates the use of sorghum to formulate a higher fiber pasta than durum wheat-based formulations (Khan et al., 2014).

Other than pasta, sorghum has also shown product applications for Chinese egg noodles, wherein a research group produced acceptable egg noodles from sorghum flours by altering particle size and monitoring starch damage. It was reported that smaller particle size flours with increased starch damage resulted in the best noodle texture using sorghum flours (Liu et al., 2012).

Another recent sorghum food application of note is the use of sorghum in chips and cereals. One research group formulated sorghum chips with soy protein to produce a higher protein and lower glycemic index chip product (Jiang et al., 2018). Other commercial cereal products are becoming more relevant as producers try to meet consumers' needs for whole grain, high fiber, or gluten free breakfast cereals. Commercially available examples of sorghum cereals present at the time of this review include Grain Berry Cereal, Multi-Bran Flakes with Onyx Sorghum. Other traditional uses of sorghum in foods include porridges. Although not yet common in the US, African countries use sorghum for porridges and more recently instant soft porridges (Taylor, 2003).

1.2.3.4 Alcoholic Beverages

In the US, the production of fermented beverages with sorghum is less common. These cereal grains are most often used to produce opaque, gluten free or pale ale beers (Taylor et al., 2014). In other African or Asian countries sorghum is already used for the malting and brewing

of beverages. For example, Nigeria has been commercially producing beer with sorghum and millet grains since the 1980s. In Africa, the use of sorghum to produce beer can be traced back as early as the 6th and 7th centuries in Africa Depending on the process and location, sorghum beer will have different names, the most common being kaffir in southern Africa (Konfo et al., 2015). In China sorghum is the most often used grain to create Baijiu, a colorless and clear spirit. The distinctive flavor and high alcohol content (38-65%) is produced by solid-state fermentation (Tan et al., 2023). Given that sorghum is cheap to import, contains 70% starch content (which is necessary for microorganisms during fermentation) and has a low fat content, sorghum is often the preferred and superior grain to produce this iconic Chinese beverage (Zong et al., 2022).

1.2.3.5 Non-Alcoholic Beverages

Given the rise in dairy alternative beverages, for example almond, soy, rice and oat, hazelnut, and coconut milks sorghum has gained little attention in this area of commercialization. Currently, there are no commercially available sorghum grain "milk" beverages in the US. Additionally, there are also no sorghum based, non-fermented beverages commonly consumed in the US. In other African countries sorghum is used to produce Mahewu, a non-fermented sorghum beverage (Bvochora et al., 1999, Kudita et al., 2023). Interestingly some sorghum grains contain phytochemicals similar to those found in tea leaves and could show promise as a tea beverage (Xiong et al., 2019c). In the US, sorghum is not commonly used for tea consumption.

1.2.3.6 Films

Edible films have been known to improve the safety, quality, and shelf life of food by inhibiting microbiological damage, preventing mechanical damage, moisture loss, fat migration, and gas movement. Within sorghum, the major protein of interest is the prolamin storage protein,

kafirin. The unique hydrophobic and disulfide-rich characteristics of kafirin make it a strong contender for protein-based films or coatings. It has been reported by Xiao and others (2017) that films formed from kafirin exhibited higher tensile strength and lower extensibility when compared to zein-based films. This was believed to be due to the high amount of disulfide bonding within kafirin as compared to zein proteins. The kafirin-based films also had poorer water barrier properties as compared to zein-based films. This is thought to be due to the increased thickness of kafirin films or low flexibility. If the flexibility were low enough, microcracks or damage to the film surface could occur and decrease water barrier properties of the kafirin films. It was further reported that these qualities combined with kafirin's low water vapor barrier and solvent resistance make the applications of kafirin as an edible film less than ideal when compared to currently used polymers (Xiao et al., 2017). These reported qualities have limited the protein's potential in food packaging applications. As a result, research involving kafirin in films has changed from edible films or coatings to bioactive packing through the incorporation of bioactive compounds.

In one study by Giteru and others (2015), kafirin films were created with essential oils to encapsulate polyphenols (well known for their antioxidant potential) to improve the overall antimicrobial activity of kafirin-based films and extend their shelf-life. The results of their findings showed that kafirin-based films with phenolic incorporation had significant antimicrobial inhibition against listeria monocytogenes, campylobacter jejuni, and pseudomonas fluorescens (Giteru et al., 2015). In addition, it was reported that oxygen permeability and tensile strength were significantly lowered (Giteru et al., 2015). This study showed the potential for kafirin-based films to be used for bioactive packaging through phytochemical incorporation.

1.3 Phenolics in Sorghum

1.3.1 Overview

Polyphenols are a growing area of interest in research for their reported health benefits including lowering the risk for some cancers, decreasing the chance for diabetes, improving gut and colon health, decreasing cardiovascular disease, and lowering the occurrence of obesity (Dykes and Rooney, 2007). Phenolic compounds are a group of secondary metabolites found in many plant sources, including sorghum. Other common characteristics of phenolics include their antioxidant and antimicrobial capabilities as well as their astringent taste and dark color (Xiong et al., 2019c). The use of polyphenols, whether it be for functional food formulation, beverages, or nutraceutical uses, will be dependent on their size, structure, and polarity (Xianli Wu, 2004).

Sorghum contains various phytochemicals that aid the grain in defense of pests and disease (Dykes and Rooney, 2006). Sorghum polyphenols are often divided into two basic groups: non-flavonoids and flavonoids. Polyphenols can further be separated into subgroups based on the number of phenol units, substituent group, and the linkage between phenol units (Singla et al., 2019).

1.3.2 Non-Flavanoids

Non-flavonoids compounds consist of phenolic acids, stilbenes, and lignans. Their structure is more basic than flavonoids and generally consists of a single aromatic ring that may contain several hydroxyl groups on its ring (Singla et al., 2019).The main subclass of non-flavonoids consists of phenolic acids. The two main groups of phenolic acids include benzoic and cinnamic acid derivatives. Common hydroxybenzoic derivatives include protocatechuic, syringic, gallic, and vanillic acids (Dykes and Rooney, 2007). Common hydroxycinnamic acids include chlorogenic, caffeic, ferulic acids and p-coumaric acids (Sytar, 2012). Stilbenes are

characterized by two phenols linked together by a two-carbon methylene group. The most known stilbene is resveratrol, which can be found in peanuts, grapes and berries (Dykes and Rooney, 2007). Other stilbenes reported in sorghum include the trans-resveratrol and trans-piceid in red sorghum grains (Bröhan et al., 2011). Lignans are composed of two propyl benzene units lined together on the C8 position of propane side chains (Singla et al., 2019). Due to their wide availability for substitutions on the C9 positions, there are a wide range of structural forms. Some lignan has been reported in sweet sorghum bagasse (Yan et al., 2014).

1.3.3 Flavanoids

Several group of phenolics have been classified based on their basic carbon back bone .For example, flavonoids, the largest group of plant phenols, contain those compounds with a C6-C3-C6 structure (Sytar, 2012). Flavonoids may be further classified based on differences in hydroxylation pattern, oxidation rate, and the absence or presence of a double bond between the C2 and C3 potions with the formation of a carbonyl group on the C4 (Singla et al., 2019). The flavonoid family can be broken down further into subgroups like flavones, flavanols, flavanones, flavanonols, flavonols, isoflavones, anthocyanidins, anthocyanins, and proanthocyanidins.

Flavones consist of two phenolic rings linked to a heterocyclic pyran ring. Flavones will have a carbonyl group on the C4 position (like most flavonoids) and are attached to the heterocyclic ring at the C2 position. There is a hydrogen atom on the C3 position. The most common flavones found in sorghum grain are luteolin and apigenin. The main difference between flavones and flavonols is the placement of the hydroxyl group on the C3 position in flavonols. Flavonols are considered the 3-hyrdroxy derivative of flavanones (Singla et al., 2019). Common flavonols include kaempferol and quercetin. Flavanones differ from flavones and

flavonols from the lack of a hydroxyl group present on the C3 position and have a saturated three carbon chain in the heterocyclic ring (Kyselova, 2011). Common Flavanones detected in sorghum include naringenin and eriodictyol. Flavanols, commonly referred to as flavan-3-ols, contain a saturated heterocyclic ring and no double bond between C2 and C3 like flavanones but contain a hydroxyl group at the C3 position (Singla et al., 2019) and no carbonyl group on the C4 position (Chobot et al., 2016). Flavanols are only found in foods as aglycones and may be found in their monomeric units. Examples of monomeric flavanols (flavon-3-ols) found in sorghum are catechin and epicatechin. Flavanonols are the 3-hydroxy derivatives of flavanones. This class of flavonoids is highly diverse given their ability for substitution (Panche et al., 2016). The most common flavanonol in sorghum is taxifolin.

Anthocyanins and anthocyanidins differ from other flavonoid groups due to their double bond in their heterocyclic rings. Anthocyanins are the glycosylated form of anthocyanidins, meaning they contain a glucose or sugar unit on their C3 position where anthocyanidins will have a hydroxyl group. The term proanthocyanidins, better known as condensed tannins or non-hydrolysable tannins are heavily abundant in some colored sorghum varieties. They are classified based on their ability to convert to anthocyanins under oxidation (Singla et al., 2019).

1.4 Methods of phenolic determination and quantification

1.4.1 Spectrophotometric

Spectrophotometric techniques are some of the most basic methods used for quantification of phenolics. These methods are useful for determining relative quantities among samples. The Folin-Ciocalteu (FC) method is one of the most common methods used to quantify total phenolic content (TPC) for sorghum grains. This method is based on a chemical redox reaction, much like antioxidant assays. However, the reaction between the FC reagent and

phenolic compounds takes place around pH 10 (Sánchez-Rangel et al., 2013). The increased pH limits interference from other compounds like ascorbic acid are limited at high pH levels pH > 3. The product of this reaction will turn blue in the presence of phenolics and will have a broad light absorption around 765 nm (Khoddami et al., 2013). Another assay commonly used to determine phenolic content is the Prussian blue assay. Although Both the FC and Prussian blue assays quantify the amount of total phenolics, they will not be able to discern between tannin and non-tannin phenolics (Ann E. Hagerman, 1989).

Other spectrophotometric techniques to determine total condensed tannin content (CTC) in sorghum are the vanillin-HCl or modified vanillin-HCl method which provides relative tannin values in sorghum samples (Dykes, 2019). The vanillin assay is based on tannin polymer's ability to bind with vanillin and form colored complexes that have a light absorption around 500 nm. Although the vanillin-HCl method is quick and can determine relative tannin content, non-tannin phenolics may interfere with this assay and cause underestimation in some Type 1 sorghums (Dykes et al., 2005). Another condensed tannin quantification assay is the butanol-HCl method. In this method, butanol depolymerizes the terminal condensed tannin unit and is catalyzed by hydrochloric acid to produce a colored pigment which is detectable using a spectrophotometer (Schofield et al., 2001). In addition to the previously mentioned methods, there is the protein precipitation method. The protein precipitation assay is based on tannins' ability to precipitate proteins (Hagerman and Butler, 1978). In this assay, the crude methanolic tannin extract is combined with a protein solution and then centrifuged. If a pellet is formed, it is then separated from the supernatant and dissolved in SDS- triethanolamine solution and combined with a ferric chloride reagent. The absorbance of the reaction is then read for quantification. An additional method of detection worth mentioning is the 4-

dimethylaminocinnamaldehyde (DMAC) assay (Ashley et al., 2019). This method is used to determine total proanthocyanidin content (TAC). The term proanthocyanidin (PAC) is often synonymous with the term condensed tannin (Ferreira et al., 1999). The DMAC assay is less common in sorghum condensed tannin analyses as it was created for determining soluble PAC content in cranberry products. The DMAC reagent will react with PAC to produce a green color, thus limiting detection of endogenous red anthocyanin pigment already present within samples.

1.4.2 Chromatographic

Apart from spectrophotometric techniques, chromatographic methods may also be used to quantify or identify the amount and type of phenolics in sorghum. Chromatographic techniques are a direct method of measuring phenolics. Reverse phase high performance liquid chromatography (RP-HPLC) can be used to determine phenolic quantity (Lee et al., 2022). The reverse phase method requires the use of a non-polar mobile phase to move the analytes through the machine until they are eluted out and their peaks can be identified with standards. Normal phase HPLC (NP-HPLC) can also be run to determine the degree of polymerization (DP). The degree of polymerization method will help determine what portion of detectable phenolic compounds are monomeric (DP=1), dimeric (DP=2), trimeric (DP=3), or polymeric (DP>3). The NP-HPLC relies on fluorescence. Tannins, regardless of their degree of polymerization, will fluoresce. Another chromatographic technique is liquid chromatography-mass spectrometry (LC-MS). This method has been used to successfully identify a wide range of phenolics in many sorghum varieties (Kang et al., 2016, Rao et al., 2018).

1.5 Antioxidant Activity

Oxidation refers to the chemical reaction in which one substance loses one or more electrons. The greater the loss of electrons, the greater the amount of oxidation within a

substance. An antioxidant is any substance that deters oxidation. Antioxidants are free-radical scavengers that neutralize free radicals within the body or food system. The antioxidant capacity of polyphenols is dependent on the arrangement of functional groups in relation to the chemical structure. Furthermore, both the number and position of hydrogen donating hydroxyl groups are the main features of the phenolic structure that influence a compound's antioxidant potential (Soobrattee et al., 2005). Antioxidants are often used in food systems to aide in the stabilization of foods. There are both synthetic and organic antioxidants, however, recent consumer trends have shown an overall rejection over the use of synthetic antioxidants. Thus, the need for naturally occurring, abundant, and low-cost antioxidants are needed. As food preservatives, sorghum tannins could serve as an optimal solution for high fatty foods. Since tannins are hydrophobic in nature, they would be ideal additions for food formulations with high susceptibility to lipid oxidation (Girard and Awika, 2018). In fact, one study reported sausages formulated with high tannin sorghum bran had lower lipid oxidation rates than those formulated without due to the hydrophobic and strong antioxidant potential of the tannins (Luckemeyer et al., 2015).

In addition to the use of phenolics in food formulations, there may also be some use for phenolics in human health. The presence of free radicals in the body is highly associated with an increased risk of some diseases. Consuming foods rich in antioxidants could decrease the amount of damage from oxidative stress in the body. Likewise, the over consumption of antioxidants can cause adverse health consequences (Sen and Chakraborty, 2011). In some instances, like physical exercise, reactive oxygen species are increased and play an important role in signaling pathways to improve cell proliferation and adaptation (Pesta and Roden, 2017). The overconsumption of antioxidants during exercise may inhibit reactive oxygen species' ability to

improve cell proliferation and may increase muscle fatigue and delay post workout recovery (Close et al., 2006). Sorghum phenolics may serve as natural source of antioxidants, however, literature is limited on the direct effect sorghum phenolics have in vivo on the prevention of oxidative damage from free radicals and should be explored further. A strong step towards understanding the mechanisms of antioxidants in the body would be to first detect and analyze antioxidant capacities in vitro using appropriate methods.

1.6 Methods of antioxidant determination

The many ways to determine the amount of antioxidant activity fall into three general categories: spectrometric, chromatographic, and in vitro/in vivo techniques. The largest of the three categories being spectrometric with nine commonly used methods. There are also additional in vitro methods of determination.

1.6.1 Spectrophotometric

Spectrometric methods depend on the ability of a radical, radical cation or complex with an antioxidant molecule capable to donate a hydrogen atom (Pisoschi and Negulescu, 2012). Common examples of spectrometric methods to determine total antioxidant capacity include 2,2-diphenyl-1-picrylhydrazyl (DPPH), ABTS, ferric reducing antioxidant power (FRAP), potassium ferricyanide reducing power (PFRAP), and the cupric reducing antioxidant power (CUPRAC) assays. All of which are colorimetry-based methods, meaning the concentration of a compound can be determined by its measured absorbance. There are other methods in the spectrometric category that depend on the fluorimetry rather than the colorimetry of a sample, such as oxygen radical absorption (ORAC) and hydroxyl radical absorption (HORAC) assays. Fluorimetry is based on fluorescence which is an emission of light by a substance that has absorbed light or other electromagnetic radiation of a different wavelength (Pisoschi and Negulescu, 2012).

Fluorescence emission occurs when an electron of a molecule relaxes to its ground state and emits a photon of light after being excited by some other type of energy (Pisoschi and Negulescu, 2012).

1.6.2 Chromatographic

Chromatographic methods are often applied to antioxidant separation and detection and include commonly used chromatography methods such as gas chromatography and high-performance liquid chromatography (HPLC). Gas chromatography is conducted between a liquid stationary phase and a gas mobile phase. It is often used to separate and analyze compounds that can be vaporized without decomposition (Pisoschi and Negulescu, 2012). HPLC is another common method used to separate, identify, and analyze a compound, however, this method is more often used for liquids rather than gaseous samples as the name implies. Samples for HPLC are moved through a tightly packed column where the compounds can be eluted out for the detector to collect retention times for characterization later. Reverse phase-HPLC is the most popular technique for the analysis of polyphenols from different foods (Proestos et al., 2005).

1.6.3 In Vivo/In Vitro

Determination of antioxidant activity can further be determined from both in vitro and in vivo chemical assays. Traditionally, in vitro studies are performed outside of a living organism, such as a petri dish, test tube, etc. Common chemical in vitro assays for antioxidant determinations include DPPH, ABTS, HORAC, and super oxide radical scavenging activity (SOD) (Alam et al., 2013). In contrast, in vivo studies are administered to a live organism according to a protocol and after a certain amount of time, samples are then taken from the organism and used for antioxidant assays. The most common in vivo assay used to determine antioxidant potential is the lipid peroxidation assay (LPO). This is likely due to the fact that

lipids are a major component in cell membranes making the peroxidation level easy to correlate to cell damage in vivo. (Alam et al., 2013).

In conclusion, there are many ways to measure antioxidant activity of a given sample. The proper method for determining antioxidant activity is dependent upon sample characteristics and only comparable on a same by sample basis (Pisoschi and Negulescu, 2012). Experimental parameters should also be considered when selecting an appropriate method to measure antioxidants. In general, spectrometric methods are the least complex of the three categories and only require a UV-vis spectrophotometer. Using spectrometric methods provide simple and rapid determinations, making them a popular choice in the screening for antioxidant content (Pisoschi and Negulescu, 2012).

1.7 Potential health benefits of sorghum phenolics

Recent literature from chemical, clinical, biochemical, and epidemiological studies suggests that phenolics may be used as a preventative against many oxidative stress derived diseases and disorders (Soobrattee et al., 2005). Phenolic compounds are most often consumed orally and absorbed withing the body during digestion. Antioxidant activity, and therefore their predicted health benefit, is dependent on the phenolic derivative present in the gastrointestinal tract. Phenolics are metabolized in the small intestine by colon microflora and in the liver. Metabolization of the phenolics may alter their chemical structure and therefore their functionality and may reduce their redox potential.

The phytochemicals found in sorghum have gained recent attention due to their reported antioxidant properties, cholesterol lowering capabilities, anti-cancer effects, ability to decrease cardiovascular disease, decrease obesity, and lower chronic inflammation caused by oxidative

stress (Dykes and Rooney, 2007). Furthermore, it has been reported that the antiviral activity of sorghum may play an important role in slowing the effects of HIV and AIDS (Chiremba et al., 2009b).

1.7.1 Obesity

According to the World Health Organization (WHO), obesity may be defined as an abnormal or excessive fat accumulation that increases the risk of health problems (WHO, 2024). Obesity may increase the likeliness of cardiovascular disease, diabetes, osteoarthritis, and some cancers. In one study, various sorghum varieties were evaluated for their anti-adipogenic activity in pre adipocytes. It was reported that the high phenolic sorghum varieties reduced lipid accumulation intracellularly and repressed the formation of reactive oxygen species (ROS) in preadipocytes. Additionally, the high phenolic sorghum varieties reduced glucose uptake and insulin signaling in cells. All of which served as positive cellular and molecular indications of obesity prevention in the body (Lee et al., 2022). In animal models, sorghum has shown promise to reduce obesity in mice models. One study from (Andressa Rodrigues de Sousaa, 2018) reported that extruded sorghum fed to obese rats showed a significant decrease in body mass index (BMI) and increased lipid oxidation. Both changes in BMI and lipid oxidation are considered positive indicators for decreasing obesity.

In addition, human clinical trials have been performed to study the effects of sorghum treatments on obesity-related parameters. In one human study by Stefoska-Needham et al. (2016) the effect of whole grain sorghum formulated biscuits compared to wheat biscuits on healthy individuals was evaluated. It was reported that individuals fed sorghum formulated biscuits reported higher levels of satiety than individuals fed wheat biscuits (Anita Stefoska-Needham and Tapsell1, 2016, Stefoska-Needham et al., 2016). Increasing levels of satiety, often through

the consumption of high fiber foods, is a common regulation tool for controlling weight and obesity.

1.7.2 Diabetes

Diabetes can be described as the condition in which the body's ability to produce or response to insulin in impaired, which causes abnormal metabolism and increased levels of glucose in the blood (Chung et al., 2011). Diabetes affects around 4% of the world's population may cause other health concerns like, hypertension, heart attack or stroke (Chung et al., 2011). Sorghum studies related to the prevention of diabetes and their corresponding mechanisms have been extensively studied using animal models. (Nguyen et al., 2014) reported the use of flavonoids from sorghum extracts to have a 40% increased inhibition of α-amylase. The inhibition of α-amylase from sorghum extracts was found to be comparable to acarbose, a commonly used antidiabetic drug (Nguyen et al., 2014). Increasing inhibition of α-amylase prevents the hydrolysis of starch to simple sugars and is a common method used to manage diabetes effectively. Sorghum may therefore be a more cost-effective and just as potent method of controlling glucose levels in diabetic populations.

Controlling glucose levels in the blood may also prevent the onset of diabetes. One animal study from (Moraes et al., 2018) investigated the potential of sorghum as a diabetic disease prevention. Sorghum fractions improved glucose tolerance, insulin sensitivity, and insulin secretion restoration rate. These results support the use of sorghum as a prediabetic preventative by controlling glucose/insulin levels and lowering liver fat. In addition, sorghum has also been shown to be comparable to aminoguanidine, a diabetic prevention medicine, at limiting the formation of advanced glycation end-products (AGE)(Ofosu et al., 2020). AGE is

linked to the onset of diabetes and has shown to be inhibited by polyphenolic compounds. These findings show the potential of sorghum phenolics as a diabetic treatment and prevention tool.

1.7.3 Cardiovascular disease prevention

Cardiovascular diseases (CVD) are diseases related or pertaining to heat and blood vessel disorders (WHO, 2021). The Center for Disease control and Prevention reports that 1 in every 5 deaths every year in the US is from heart disease (CDC, 2022). The use of sorghum as a CVD preventative has been studied using both human cells and animal models. Francis et al. (2019) reported the use of black sorghum extracts to prevent CVD. Their study reported sorghum significantly decreased the expression of nicotinamide adenine dinucleotide phosphate oxidase 4, which is a precursor for the thickening or hardening of plaque formation in arteries (Francis et al., 2019). Diabetic rats fed diets formulated with sorghum extracts showed reduced plasma cholesterol and triglycerides, which may improve overall heart health (Chung et al., 2011).

1.7.4 Anticancer

As previously mentioned, the phytochemicals found in sorghum may aid in the prevention of many diseases and as well as some cancers. Many other cereal grains have been studied for their phytochemical profile and compositions. It is well reported that phytochemicals may be used for cancer prevention. Since sorghum contains many phytochemicals, its use as chemo preventative has been studied in recent years. In one study from Lee et al. (2020) polyphenolic extracts were derived from the bran of a high polyphenol sorghum variety and used as a cancer preventative against human colorectal cancer cells. It was reported that phenolic extracts could potentially cause cancer cell cycle arrest while not causing further cell damage to normal tissues (Lee et al., 2020). Extracts showed anticancer effects via the same pathways as

other commonly used cancer medicines. This showed the potential for sorghum to be used as a natural alternative to other cancer drugs.

1.7.5 Antimicrobial activity

Antimicrobial activity can be defined as a collective term for all agents that inhibit the growth of bacteria, prevent the growth of microbial colonies, and may destroy microorganisms. Controlling the growth of microorganisms can play an important role in food borne illnesses, which are still a major concern to consumers and producers alike. The use of preservatives can help mitigate the growth of microorganisms known to cause some foodborne illnesses, however, there is a growing concern from consumers over the use of synthetic preservatives in foods (Al-Zoreky, 2009). Therefore, the need for antimicrobial compounds from natural sources is needed. Polyphenols are derived naturally in plant materials, making them a suitable option to replace some synthetic antimicrobials.

While phenolics from a wide range of food bases have been studied for their potential health benefits and antioxidant effects, less is known about their antimicrobial abilities (Periera et al., 2006, Periera et al., 2007). It is often difficult to identify all of the phenolic compounds in a given sample, and to test each compound against various microorganisms individually (Proestos et al., 2005). Furthermore, it is common that the crude extracts are not as effective in inhibiting microbial growth as their pure active compound counterparts (Periera et al., 2006). These reasons make testing the antimicrobial activity somewhat difficult and may explain why some research is limited in this area.

Research has been conducted on the antimicrobial capabilities of some phenolic compounds. In general, it has been reported that antimicrobial activity is dependent on the concentration of the phenolics and on the types of processes used (Periera et al., 2006). Meaning

that if the processing effects the phenolic compound adversely, it will likewise have adverse effects on the ability of the phenolic compound's ability to inhibit microbial growth. Other literature also suggests that antioxidants are effective against a wide range of microorganisms and fungi (Zhu et al., 2004).

Despite having high levels of polyphenols and a wide variety of phenolic compounds, research on sorghum as an added health food is lacking when compared to that of other foods such as vegetables and fruits. For this reason, the use of sorghum as a health additive is underutilized (Awika and Rooney, 2004).

1.8 Effects of processing on sorghum phenolics

As previously mentioned, sorghum is commonly consumed and considered a food staple in many Asian and African countries. In the US however, sorghum is mostly produced for animal feed and ethanol production. Recently, consumer demands for gluten free, natural, and healthy foods in the US have led product developers to produce value added functional food products. A functional food is a food that contains an added health benefit beyond its original nutrients. Since sorghum is gluten free and contains beneficial phytochemicals, using sorghum for value added or function food formulations has gained recent attention from scientists and food product developers alike. To use sorghum in functional food formulations, however, it is imperative to understand how processing conditions may change phenolic content, structure, and bioactivity. The following section will discuss comprehensive food processes that may affect the sorghum phenolics in foods.

1.8.1 Soaking

Soaking can increase the overall water content in a samples system and cause starch granules to swell. Once the plant cell is swollen cellular contents are releasees and the leaching

of water soluble compounds is accelerated (Wu et al., 2013). There are many published works investigating the effect of soaking on sorghum nutrition (Afify et al., 2011, Afify et al., 2012b) as well as sorghum flour functionality (AFIFY et al., 2015). There are fewer studies investigating the direct effect of soaking on sorghum polyphenols currently published in literature. In addition to the lack of knowledge on this subject, the published papers available that investigate soaking on sorghum polyphenols use either white or red sorghum varieties that are low in phenolic content prior to the soaking treatments. For example, one research group investigated the effect of soaking on biochemical changes in phenolic compounds and antioxidant activity of three white sorghum varieties. The authors reported the total phenolic content of raw sorghum varieties ranged from 1.09 to 1.17 mg/g dwt (Afify et al., 2012a). Although the authors report a decrease in total phenolic content and condensed tannin content, the effect of soaking on sorghum polyphenols could be better observed in higher tannin content sorghum varieties. The low level of total phenolics and condensed tannins presented in the study make the results difficult to interpret. In a different study, both red and white sorghum varieties were studied. Sorghum samples were soaked for 2 hours and then dried. The authors reported that the soaked red sorghum was the highest in phenolic content while the unsoaked red sorghum had the highest antioxidant activity (Indrianingsih et al., 2023). Although there are reported differences in phenolic and condensed tannin content from the sorghum used in the study, it may be useful for future research to examine the effect of soaking on high polyphenol and high tannin containing sorghum varieties

1.8.2 Malting

Malting is a controlled process consisting of three major steps: steeping, germinating, and kilning. The initial step steeping, or soaking, raises the initial moisture content and activates the enzymes present within the grain. During the germination process peak enzymatic activity is reached and the grain starts to sprout. After germinating, grains are kilned or dried to stop the enzymatic process. The increased moisture content, enzyme activity, and heat treatment are all predicted to affect the total phenolic content or phenolic composition in sorghum (Vingrys et al., 2022). One study examined the effect of malting on phenolic composition in eight sorghum varieties harvested in Australia. The results showed that malting decreased the total phenolic content while 3-deoxyanthocyanidin content increased in all eight samples (Khoddami et al., 2017). While the results are valuable for potential sorghum-based food products, it should be noted that all eight sorghum varieties were tannin-free and already low in total phenolic content. The sensitivity of the Folin-Ciocalteu assay might be better suited for a sorghum variety that is higher in total phenolic content prior to malting as well as higher in condensed tannin content. In another study investigating the effects of malting on phenolic content in cereal grains (Sorghum, what, barely, and breakfast cereals) all grains saw a significant increase in total phenolic content after malting (Vingrys et al., 2022).

The direct effect of malting on phenolic composition in sorghum grain was discussed in an older study that compared malting on two tannin and two non-tannin containing sorghum varieties. The four sorghum varieties were malted, ground and then later extracted into three different fractions (F1, F2, and F3) to understand changes in phenolic content after malting. It was reported by the authors that the root and shoot development period increased the simple phenolic acids content in the first fraction (F1) wherein no condensed tannins were detected.

Malting greatly altered the elution behavior of the tannins in the third fraction. For example, before malting, the two main tannins eluted separately. After malting, the tannins eluted together (McGrath et al., 1982). This may be due to the increased enzymatic activity within the grain. For example, phenolic acids and tannins bind with other macronutrients (starches and proteins) in raw grains and the enzymatic activity in the grain may break these linkages and improve hydroxyl group exposure of the tannins, making them more detectable after malting. Given little research has been done to investigate the exact phenomena described by the authors, more research is needed to verify their hypothesis. The increase in phenolic content after germination is also reported in another germination study using Nigerian sorghum varieties where the total phenolic content increased seven fold after germination (Nwanguma and Eze, 1996). The change in tannin content reported by McGrath et al. (1982) is further supported by Elmaki et al. (1999) who examined the effect of soaking and germination time on tannin content in both a low and high tannin containing sorghum variety. In their study, soaking did not affect tannin content while germination time (seed sprouting stage) decreased tannin content by up to 98% after 72 hours (Elmaki et al., 1999). Given the focus of the paper was on tannin content and starch and protein digestibility, phenolic content was not evaluated. While the decrease in tannin content is significant, it would be of interest to examine changes in phenolic content as well as tannin content of the sorghum after soaking and germination.

1.8.3 Fermentation

Fermentation is a natural process in which microorganisms like bacteria or yeast convert sugars into acids or alcohols. Fermented foods may undergo spontaneous or culture dependent fermentation processes. An example of spontaneous fermentation would include sauerkraut and kimchi whereas an example of cultured fermentation processing would be kefir, kombucha or

sourdough (Dimidi et al., 2019). The fermentation process is full of variables from the nutrition of the starting material to the microorganism used, and the environmental conditions during fermentation. Changes in any of these variables may affect the quality of foods and therefor the phenolic content and composition of sorghum phenolics. It is therefore important to understand how fermentation affects phenolic compounds to create a possible fermented sorghum food.

To better understand how sorghum phenolics are altered during fermentation, one research group created six sour dough formulation out of white and red wheat, white and yellow sorghum, and white and brown teff flours. The flours were fermented for 48-96 h and changes in phenolic profiles were monitored using UPLC-tandem quadripole mass spec (MS). The authors of the study report that flavonoid O-glycosides were metabolized during the 48-hour fermentation resulting in a 66% reduction in content. It was hypothesized that the flavonoid O-glycosides were converted into aglycones, which was reported to increase by 2.5-fold after 48 hours. Phenolic acids were reportedly more metabolized than other flavonoids detected in sorghum. Additionally, in comparison to wheat and teff, sorghum phenolics were more susceptible to microbe metabolism during fermentation. This is most likely due to differences in cell wall structure in the grains (Ravisankar et al., 2021). This study gives insight into specific phenolic compound changes during the fermentation process in sourdough breads. This shows promise for high phenolic sorghum grains to be used for value added health food formulations as the fermentation process enhanced total antioxidant activity in sourdoughs for all grain flours.

An additional research group studied changes in phenolic content of red sorghum from Botswanan during sourdough fermentation with *Lactobacillus* strains. In this study a control sorghum slurry was prepared with water acidified with lactic acid and acetic acids to account for the effect of acids and not microbial activity during fermentation. The authors reported a

decrease in phenolic acids in the fermented sorghum sourdough samples when compared to the unfermented flours. Additionally, the fermented sourdoughs had lower total phenolic contents than the chemically acidified sorghum slurry controls. This indicates there is a pH and microbial relationship and phenolic content during fermentation. The authors further investigated each of the four *Lactobacillus* strains used for fermentation on phenolic compounds to understand the metabolism of phenolic acids during fermentation. It was further reported that each strain of *Lactobacillus* metabolized different phenolic acids using different phenolic and flavonoid metabolism pathways (Svensson et al., 2010). Unlike the previously discussed project, the antioxidant activity of sourdough breads was not recorded. This research is still useful however as it helps to further understand the conversion of specific phenolic acids and flavonoids in sorghum during fermentation using specific strains of *Lactobacillus*.

Additional studies focused primarily on high tannin containing sorghum varieties rather than low polyphenol or no polyphenol sorghum varieties may be useful to understand the effect of fermentation on all sorghum phenolic compounds. There are some studies that examine the effect of fermentation on tannin containing sorghum varieties from a nutritional aspect (Towo et al., 2006) but not from a change in tannin concentration point of view. It may therefore be beneficial to investigate this relationship with specific microbial strains to understand how microbes metabolize condensed tannins, if at all.

1.8.4 Thermal Applications

1.8.4.1 Roasting

Roasting is a dry-heat cooking process where the food is surrounded by hot air and is cooked evenly on all sides at higher temperatures (≥150 °C). In one study a non-tannin white sorghum variety was soaked, steamed, and then roasted before analyzing differences in total

phenolic content and antioxidant activity. After roasting, total phenolic content and condensed tannins content significantly increased while antioxidant activity remained unchanged (Xiong et al., 2019a). The lack of change could be due to the low phenolic content of the white sorghum used in the study. The authors mentioned that the use of low tannin white sorghum may not be a preferred model to understand changes in phenolic content in sorghum polyphenols for tea processing and later address this in a follow up paper. In their second paper, the authors compared the same roasting conditions on black and brown colored sorghum types wherein the total phenolic content and condensed tannin contents decreased (Xiong et al., 2020).

1.8.4.2 Baking

Baking is a common food application that usually uses dry heat in an enclosed space. Given the use of heat, it is expected to degrade phenolics (Blanch and Ruiz Del Castillo, 2021). The effect of baking on phenolic content in cookies formulated with various sorghum varieties were evaluated. In this study, condensed tannin sorghum and tannin-free sorghum and compared to wheat-based cookies. It was reported that baking slightly decreased the total phenolic content, condensed tannin content and antioxidant activity of sorghum phenolics. The red tannin-containing sorghum still retained more phenolics than the tannins free and white sorghum and showed the highest antioxidant activity (Chiremba et al., 2009a). Although there was a decrease in phenolic content and antioxidant activity when compared to the raw sorghum flours, there were still significant amounts of phenolics remaining after the baking process when compared to the wheat-based cookies. This shows the potential for high polyphenol containing sorghum varieties to be used for functional food applications in some baked goods.

1.8.4.3 Microwaving

Microwave processing uses electromagnetic waves to pass through food. The electromagnetic waves create vibrations in the water molecules in foods that create heat in the food. To apply sorghum phenolics in functional food applications understanding the effect of multiple heating methods is pertinent information. One research group investigated the effect of microwaving on sorghum phenolics, antioxidant activity, and in vitro bioaccessibility of white and red sorghum phenolics. In their study, samples heated for 40 s increased total phenolic and total flavonoid content. Although total phenolic and flavonoid content increased, it was noted that longer heating times decreased overall total phenolic and flavonoid contents (Li et al., 2022a). Notably, the total phenolic content reported in the red sorghum variety was not significantly different than the white variety. As previously mentioned, white sorghum varieties are generally low in phenolic content and do not contain condensed tannins. When compared to other brown or black sorghum varieties, the total phenolic content of type I white and red sorghum is much lower and does not contain condensed tannins in raw grains. It may be possible to microwave sorghum longer if the starting phenolic content was higher in raw grains, however, further research is needed to investigate this hypothesis.

In a different study, a tannin containing sorghum variety was evaluated for condensed tannin and total phenolic content before and after microwaving at various levels. The condensed tannin content significantly decreased after microwaving for 15 s at both 350 and 500 W. Microwaving for longer periods of time continued to decrease total condensed tannin content. Additionally, total phenolic content significantly increased after 30 and 45 s at both wattages when compared to the microwaved grain (Almaiman et al., 2021). It is possible that the phenolic content increases as bound phenolics are released from the cell wall during the microwave

treatments. However, further research is needed to understand why condensed tannins decrease and phenolic compounds increase.

1.8.4.4 Boiling

Boiling is a traditional cooking method used to prepare foods wherein foods are submerged in water and heated for a predetermined amount of time. Usual boiling time points for foods include 5 – 15 minutes for rice and porridges or even 1 to 2 hours for stews, soups, and broths. Therefore, it is important to study changes in sorghum phenolics after boiling for a wide variety of time points and temperatures. One study that evaluated changes in phenolic content in various sorghum grains reported a significant increase in total soluble phenolic acid content after 12 minutes of wet cooking. It was reported that the increase in soluble phenolic acids was caused by the increased solubilization of p-coumaric and ferulic acids. The total phenolic content and 3-deoxyanthocyanidin content, however, decreased and caused a significant decrease in antioxidant capacity (N'Dri et al., 2013). These results are in line with another group who investigated boiling effects on phenolic content in Indian sorghum wherein a decrease in total phenolic content was also observed (Hithamani and Srinivasan, 2014). Additionally, the researchers used a tannin-containing sorghum variety and reported a decrease of 22% in total condensed tannins content. Changes in phenolic and condensed tannin content are likely caused by changes in other macronutrients like starch and protein which may inhibit phenolic extraction.

1.8.4.5 Steaming

Steaming is a common cooking method in food applications. It is predicted to be extremely disruptive to the cellular structures in grains (Randhir et al., 2008). In one study a non-tannin white sorghum variety was soaked, steamed, and then roasted before analyzing

differences in total phenolic content and antioxidant activity. After the steaming at 100°C for 50 minutes, total phenolic content, condensed tannin content, and antioxidant activity were not significantly affected when compared to the raw sorghum grain (Xiong et al., 2019a). The lack of change in total phenolic content, condensed tannin content, and antioxidant activity reported by the authors could be due to the low amount of phenolics present in white grains. Given that the authors used a white, non-tannin sorghum, measuring total phenolic content, condensed tannin content and antioxidant activity may be difficult given the grain will be inherently low in phenolic and tannic compounds as compared to other sorghum varieties. The authors justified their selection of the white, non-tannin sorghum grain due to its popularity in consumption, however, if one were to investigate the effects of steaming on specifically sorghum polyphenols it would be ideal to select a grain high in phenolic content.

1.8.4.6 Extrusion

Extrusion is a process in which food material is forced through a series of dies to create preferred shapes. Common extruded products include chips, cereals, and pet foods. During this process food matrices are exposed to extreme shear forces, heat, and pressure, all of which may affect the phenolic profile of sorghum phenolics.

To examine the effect of extrusion on sorghum phenolics on research group extruded sorghum bran to evaluate phenolic compounds and antioxidant activity. They reported an increase in total phenolic content in sorghum bran when extruded at 180°C with 20% moisture content (Salazar Lopez et al., 2016). It is thought that bound phenolics are released from the bran matrix during the extrusion process thus increasing total phenolic content and their potential bioactivities (Ortiz-Cruz et al., 2020).

In another study three specific sorghum grains were examined before and after extrusion. One sorghum variety was high in 3-deoxyanthocyanidins and condensed tannins, the second was also high in 3-doexy anthocyanidins but low in condensed tannins while the third was low in both phenolic compound groups. The authors reported that all 3-deoxyanthocyanidins decreased after the extrusion process, retaining on average 16% of compounds (de Morais Cardoso et al., 2015). It is thought that the extrusion process reduced the extractability of these compounds through unknown mechanisms. The condensed tannin content was not measured in this study using spectrophotometric techniques. The authors did, however, measure degrees of polymerization using NP-HPLC. In the higher condensed tannin containing sorghum variety, monomer, dimers, trimers, and oligomer content increased after extrusion while polymer content decreased. Overall proanthocyanidin content (condensed tannin content) decreased after extrusion (de Morais Cardoso et al., 2015).

Depending on the phenolic compound of interest, extrusion may increase or decrease specific phenols. It is imperative that further studies be done by examining various extrusion parameters (like moisture content, feed rate, and temperature) on phenolic profiles using RP-HPLC. The work of these papers does show the potential for high polyphenol sorghum to be used as a functional ingredient in extruded products.

1.8.4.7 Nixtamalization

Nixtamalization is an alkaline cooking method generally associated with corn to produce corn tortillas. In this cooking method corn kernels are usually soaked and cooked in alkaline solution and then hulled before being ground into masa (flour). This application can also be performed on other grains, like sorghum, to remove the pericarp. Given that a large portion of sorghum phenolics are present in the pericarp, removing this layer will likely cause a decrease in

total phenolic content in sorghum. One study used nixtamalization in red and white sorghum varieties to reduce condensed tannin content by 74% (Luzardo-Ocampo et al., 2020). In another study the authors attempted to find an optimal level where condensed tannins content was reduced while still maximizing antioxidant content. They were able to reduce condensed tannin content by 27% in white sorghum and 90% in red sorghum. The author reported that the most optimal nixtamalization parameter to reduce condensed tannin content while maintaining antioxidant activity was 1.13% lime and 31.11 minutes of cooking (Gaytán-Martínez et al., 2017). These studies suggest that while nixtamalization does reduce condensed tannin content, products with high antioxidant activity can be produced.

1.8.5 Irradiation

Irradiation is a food safety process that decreases the risk for food borne illness caused by microorganisms using ionizing radiation. The use of irradiation for food safety has been approved in the US was well as 60 other countries that have approved irradiation for foods (Sanaei Nasab et al., 2023). The irradiation of cereal grains and flour products is often employed as a non-thermal microbiological control for food safety purposes. Therefore, understanding the effect of irradiation on sorghum phenolics is necessary to use sorghum phenolics for food formulations.

A recent study investigates the effect of gamma irradiation on sorghum flour from two varieties during Sudanese asida production. For both raw sorghum flours irradiation increased total phenolic content and antioxidant activity (Mohamed et al., 2022). Additionally, when the gamma radiation was increased from 1.0 kGy to 2.0 kGy, phenolic content and antioxidant activity increased. The condensed tannin content was inversely affected by irradiation in this study. Although the authors do not examine the cause for decreasing condensed tannin content

directly, the authors suggest the possible reason for the increase in total phenolic content or decrease in condensed tannin content, is due to the rearrangement of the matrix structure, causing bound phenolic to be more detectable.

In another recent study, sorghum sprouts were irradiated with UV-A LED lights and milled into a flour for cookie formulations and evaluated for antioxidant activity and phenolic content. Six major phenolics were identified in phenolics, gallic acid, ferulic acid, protocatechuic acid, p-Coumaric acid, sinapic acid, and catechin. Of the six, only gallic acid concentration increased after sprouting and irradiation treatments (Ruiz-Hernández et al., 2023). These results indicated that not all types of irradiations will increase all phenolic acids. Although irradiation was investigated on the sprouted sorghum flour, it would have also been ideal to irradiate regular sorghum in the study to observe difference due to radiation and differences due to sprouting, especially since the authors used a non-sprouted or irradiated sorghum flour and wheat flour for controls. In general, irradiation increases phenolic content up to a specific level before phenolic content and antioxidant activity are decreased. Regardless of irradiation level, condensed tannin content decreases after irradiation. This information may be useful for product developers looking to produce high phenolic sorghum flours. Based on the currently published literature, food producers could market a food safe sorghum flour high in phenolic content using irradiation techniques.

1.8.6 Extraction Methods

The detection of phenolics comes down to their extractability. Therefore, there are many published methods using various solvents and technologies extracting sorghum phenolics. There are also many reviews examining different extraction techniques and methods. The next section

of the review will therefore briefly list and examine the effects of various extraction methods on sorghum phenolics.

1.8.6.1 Solvent system

The solvent system during extraction makes a noticeable difference on phenolic content detection. For example Cox et al. (2019) examined different ethanol based extractions on sorghum phenolic content and their antiproliferative activity in human cancer cells. Other groups have examined alkaline extraction conditions on sorghum phenolics (Blackwell et al., 2012) while other groups have investigated phenolic extractions under acidic conditions (Shelembe et al., 2014). Choosing an appropriate solvent will depend on end uses and goals for the research.

1.8.6.2 Conventional method

For sorghum polyphenol extractions, the solid-liquid extraction technique is the most commonly used method and often referred to as the conventional method. The conventional extraction technique consists of mixing ground grain with either alcohols (methanol or ethanol), acetone, or ethyl acetate and mixing for a predefined time. However, very polar phenolics like cinnamic or benzoic acids, may need a mixture of alcohols and water or acetone and water solutions to be extracted (Stalikas, 2007). Other examples include the use of 70% acetone to extract sorghum phenols and evaluating antioxidant activity (Awika et al., 2005) while 1%HCl-Methanol has been used to extract tannins (Ann E. Hagerman, 1998) and 3-deoxyanthocyanidins (Awika and Rooney, 2004). The conventional method then uses centrifugation to separate the soluble phenolics from the pellet. After centrifugation the supernatant is collected and analyzed (Speranza et al., 2021). Differences in conventional methods include the length of shake or the inclusion or absence of an overnight sit (Lee et al., 2020). This method is simple, cost effective and does not require extra equipment. It can be conducted in most labs that have the appropriate

solvents. Other alteration in this method include the use of filters over centrifuges to separate the soluble phenolics from the flour (Ardoin et al., 2023).

1.8.6.3 Sonication

Sonication is another technology that might increase the yield of phenolics. During sonication high intensity ultrasonic waves are applied to the liquid wherein they create small vacuum bubbles. As the bubbles burst, they collapse and produce a high-pressure cycle that cleaves the phenolics from the matrix making them more extractable. In one study conventional solvent extraction techniques were compared to ultrasonic assisted extraction methods. This study found that ultrasonic assisted extraction was more efficient in extracting polyphenolic compounds than conventional solvent extraction (Luo et al., 2018).

1.8.6.4 Microwave Assisted

Microwave assisted extraction like ultrasonic assisted extractions are another technique used to create used to extract used to optimize sorghum phenolic extractions. Microwave assisted extraction works by using microwave energy to heat the solvent and help cleave phenolics from the sample matrix. This method is rapid and the main advantage of this technique. Many studies have been conducted using microwave assisted extractions to optimize sorghum phenolic extraction yields (Duke, 2021, Izza et al., 2023). The drawback of this method would include overheating the sample. If the sample is overheated, phenolics may experience thermal degradation, thus limiting the quality of extracted phenolic compounds.

1.8.6.5 Accelerated solvent extraction

Perhaps one of the more technical techniques to extract phenolics is with accelerated solvent or pressurized solvent extraction methods. This method requires the most equipment to run that consists of a nitrogen source, pump valve, oven for heating, extraction cell, static valve,

and collection vial. Altering the flow rate, temperature and pressure can alter the total phenolic content yield and quality (Santana et al., 2023). In one study, accelerated solvent extraction was used and compared to conventional extraction techniques for a variety of solvent systems. When the ASE is used at 60°C, it is comparable to the conventional extracts. However, as temperature increased so did the total phenolic content for citric acid in water, water, 50% ethanol and 70%ethannol (Barros et al., 2013). The advantages of this method include its short extraction times, low solvent usage, and can process multiple samples per cycle. Some drawbacks for consideration include the cost and upkeep of the equipment as well as the propensity of the equipment to produce inconsistent extraction volumes, regardless of consistency in method adherence (Giergielewicz-Mozajska et al., 2001).

1.9 Potential uses for sorghum phenolics

1.9.1 Baked goods

Common examples of baked goods with phenolic additives include breads, cookies, cakes, brownies, tortillas, and other snack foods. There are plenty of studies using sorghum as a gluten replacer in baked goods formulations. There are even studies published about using sorghum for its increased starch resistance to impart a health benefit (Poquette et al., 2014). There are far fewer studies examining sorghum inclusion in baked goods with phenolic composition changes in mind. One study investigated the effect of sorghum phenolics in biscuits on human satiety. In this study, subjects were required to fast for 12 hours prior to consuming a biscuit formulated with either; white sorghum, red sorghum, brown sorghum, or wheat. Subjects reported significantly high satiety ratings in sorghum blend biscuits compared to wheat biscuits. Additionally, the post prandial GLP-1 levels were higher in subjects that consumed sorghum biscuits as compared to wheat (Anita Stefoska-Needham and Tapsell1, 2016).

These results indicate that pigmented sorghum may be a useful food ingredient for foods targeting satiety or weight control. An additional randomized controlled study was performed by the same research group that investigated the effectiveness of an energy restricted meal plan in sixty-four overweight adults. The two groups were given a red sorghum biscuit or a diet containing white wheat flaked biscuits. After 12 weeks, both groups lost weight and there was no effect specifically related to sorghum consumption reported from the study (Stefoska-Needham et al., 2017).

1.9.2 Other Foods

With concerns from consumers like celiac disease, obesity, and cardiovascular disease many producers are turning to value added cereals. For example, cheerios' "heart healthy" claims, or other breakfast cereal brands producing whole grain, keto friendly, and gluten free cereals to appeal to the multitude of rising health concerns. For these reasons, breakfast cereals made from high phenolic cereal grains (such as sorghum and millet) are becoming a growing area of research interest to producers. In some instances, high phenolic breakfast cereals have already tested for consumer acceptance. For example, the sensory acceptance of sorghum cereals was compared against whole wheat cereals. In this study it was found that the acceptability of the high phenolic cereal was higher than the whole wheat cereal (Anunciacao et al., 2017). This study could lead to the development of other breakfast cereal products such as breakfast, granola, or oatmeal bars. Furthermore, as sorghum is already commonly consumed in African countries as porridge, the development of a porridge or oatmeal product that is high in phenolic content could be another viable food product if sensory attributes are found to be acceptable.

Extruded tannin containing sorghum breakfast blends were analyzed and compared to extruded maize breakfast cereals. The extruded sorghum tannin breakfast cereal was found to be

higher in fiber than the extruded maize. Additionally, when combined with non-fermented milk, it alleviated inflammation and reduced oxidative stress in patients with chronic kidney disease (Lopes et al., 2018).

Other food formulation examples include the use of sorghum to create pasta noodles. It has been demonstrated that pasta noodles can be formulated with sorghum and produce pasta high in polyphenol and antioxidant activity. It is hypothesized that the increased antioxidant activity will therefore reduce markers of oxidative stress in vivo. A clinical trial examining the effect of sorghum phenolics in pasta noodles on oxidative stress markers in vivo was therefore conducted using red and white sorghum varieties. The authors of the study reported that pasta formulated with red whole grain sorghum flour enhanced antioxidant status and improved oxidative stress markers (Khan et al., 2015).

Another reported use of sorghum polyphenols in foods was reported in sorghum formulated churros. The authors of this study wanted to replace gluten with tannin-containing sorghum and create an antioxidant rich food. From the study, it was reported that both the cooking and frying processing did not entirely diminish the phenolic content of the sorghum formulated churros. Additionally, the consumer acceptability of the sorghum churros was not affected by the presence of phenolics (Queiroz et al., 2021).

1.9.3 Biocolorants

Sorghum also shows great promise as a source for biocolorants. Biocolorants not only act as food colorings, but also as antimicrobials, antioxidants, and may prevent several diseases in humans (Rymbai et al., 2011). Recently, the use of sorghum leaves and leaf sheaths as a biocolorant has been developed in Kentucky. The company, RedLeaf, has manufactured sorghum leaf extracts for health, wellness, cosmetics, food, and beverage applications. Their

extracts are reported to be higher in flavonoid content than other typical botanical extracts, GMO-free, water soluble and highly stable. The success of their product demonstrates the ability of sorghum to be used as food colorants with value added functions (increased antioxidant activity, etc.).

A phenolic compound specific to sorghum is 3-deoxyanthocyanidins (3-DA). these compounds are more stable than their anthocyanin counterpart but are more difficult to extract from plant materials. With the help of microwaves assisted extraction, 3-DAs have demonstrated their potential for stable food biocolorants (Herrman et al., 2020). Additional studies examining ultrasound and microwaves assisted extraction techniques on 3-DA extraction have been performed using response surface analysis (Shukla et al., 2022). The ideal settings for ultrasound and microwave assisted extraction are outlined in the study and could be used to produce bioactive 3-DA colorants from sorghum for other food applications. It is important to note that the presence of tannins significantly affect the color and yield of extractable 3-DAs during microwaves assisted extraction in sorghum grains (Brantsen et al., 2021). The condensed tannin containing sorghum was higher in relative pigment yield than non-tannin containing sorghum. This is thought to be due to the protective effects of tannins on 3-DAs through pigmentation. However, in tannin-containing samples, extreme depolymerization occurred during microwave assisted extraction. The presence of condensed tannins may therefore lead to unstable color attributes in food and beverage applications (Brantsen et al., 2021).

1.9.4 Fermented foods

The use of sorghum flour in sourdough bread has been employed to not only replace gluten but increase antioxidant activity. During sourdough fermentation, microorganisms

metabolize phenolic compounds present in sorghum, producing metabolites that increase overall antioxidant activity in the sourdough breads (Ravisankar et al., 2021).

1.9.5 Fermented beverages

The use of sorghum to produce beers in African countries or Baijiu in China is already well reported. There are few studies examining the health benefits of consuming sorghum beers formulated with polyphenols as most sorghum beers are formulated with white sorghum varieties. One study examined the changes in phenolic content for opaque beers produced from peal millet and a white and red sorghum. The white variety contained no detectable condensed tannins until after malting. The total phenolic content and antioxidant activity of both sorghum varieties significantly decreased after malting (Embashu and Nantanga, 2019). The sorghum varieties were the only samples with detectable condensed tannin contents after malting. This shows the potential for sorghum to be used for fermented beverage applications as a way of delivering phenolics into the human diet.

1.9.6 Non-fermented beverages

Lastly, other common food products rich in polyphenols and antioxidants are teas and coffees. Specific examples of teas containing catechins and other antioxidants include black and green teas (Van Buiten et al., 2018, Kirtil et al., 2017, Dag and Oztop, 2017, Ho, 1992). Common examples of antioxidants present in coffee include caffeic and ferulic acids (Ho, 1992). Sorghum based teas are not commonly consumed in the US. Despite their lack of consumption, there are sorghum leaf-based tea products available to consumers on the market. Waakye for example, is a powdered sorghum leaf product intended to be used for sorghum tea beverages. Other beverage applications include the use of extruded sorghum in drink formulations to decrease glycemic response in subjects. For instance, tannin and 3-DA sorghum, no tannin, and

3-DA sorghum and low 3-Da and no tannin sorghum or no sorghum drinks were given to subjects after a 12 h overnight fasting period. After consuming the formulated drinks, subjects were given glucose solutions, and their glycemic response was monitored over a period of 120 minutes. It was reported that the tannin and 3-DA sorghum drink minimized glycemic peak (Anunciação et al., 2018). This demonstrates the use of high tannin and 3-DA sorghum varieties to be used in drink formulation for controlling blood sugar levels. One last study evaluated consumer acceptance of an acidified cold brewed sorghum bran beverage (sweetened and unsweetened). Generally after hearing the potential health benefits of phenolics and antioxidants consumer purchase intent of the sweetened beverage increased to 56% (Ardoin et al., 2023). This shows that positive product messaging can enhance consumer acceptance of novel sorghum food applications.

1.10 Conclusion

Although sorghum and its phenolics may be a quality candidate for value-added food applications, there are some limitations to its application. The most obvious challenge in using sorghum phenolics in functional foods are the changes in appearance and sensory. It is already well reported that off flavors and colors may occur in foods formulated with phenolic compounds (Taylor et al., 2007, Chiremba et al., 2009b, de Camargo et al., 2014, Marston et al., 2016, Yousif et al., 2012). Additionally, condensed tannins have also been found to impart astringent or bitter sensory qualities and may even cause overall product color changes (Xiong et al., 2019c). Furthermore, CT's have been shown to decrease protein and starch digestibility (Emmambux and Taylor, 2003, Duodu et al., 2002).

Other obstacles of using sorghum phenolics as a value-added food is the improper release of the phenolic compounds after ingestion. This is in part due to the phenolic compound's

hydrophilic nature, which allows the interaction of other compounds in the gastrointestinal (GI) tract and deactivates the phenolic compound before it is intended release. Improper release of phenolic compounds could adversely affect the bioavailability of the phenolics in the GI tract (McClements, 2015). When considering phenolic applications in foods, the delivery target (i.e., colon, blood, plasma, small intestine, stomach, etc.) must be considered to ensure potential health benefits are delivered.

Other challenges include labelling and regulatory requirements. Under the dietary supplement health and education acts of 1994, (DSHEA) manufacturers of dietary supplements are responsible for the safety and labelling of their products before distribution to ensure they meat DSHEA and FDA requirements. To do so, sorghum food applications with a potential health claim will need clinical trials at the minimum to validate their proposed health benefit. Additionally, clinical trials will need to be carefully designed and executed to ensure adherence among subjects.

In summary, sorghum has the potential to be used for more than just a gluten free alternative in food formulations. Given the extensive and well reported health benefits of sorghum phenolics, there is a possibility for sorghum to enter the value added or functional foods market. It is imperative that processing conditions on phenolics be examined to understand their changes in structure and bioactivity. Doing so will not only add value to consumers looking for healthy food sources but create value for sorghum grain beyond an ethanol and feed grain. If sorghum grain value is increased, there is room for sorghum production to increase, adding monetary value to sorghum farmers. Sorghum has the potential to outproduce other cereal grains given its low input requirement and its drought tolerance.

Chapter 2 – Phenolic profile and bioactivity of pigmented rice, corn, and sorghum grains

2.1 Introduction

Pigmented grains are known to contain health promoting compounds called "polyphenols." Grain polyphenols are reported to have anticancer, antidiabetic, anti-obesity health effects while decreasing the risk for heart disease and type II diabetes. Polyphenols are a diverse class of phytochemicals found in plants that contain numerous functional groups or "families" each consisting of unique compounds. Some groups of phenolics are specific to certain grains. For example, it is reported that sorghum is a unique grain source for 3-deoxyanthocyanidins (3-DA). Rice and corn grains are reported to be rich in anthocyanins whereas sorghum grains are not. Since pigmented grains have unique blends of polyphenols, it is therefore important to study the phenolic profiled and their potential health benefits of each grain to guide future studies on whole grain health food applications.

In addition to the potential health benefits of pigmented grains, there is a rising need for gluten free (GF) food formulations due to the increase in consumer demand for GF foods. GF foods also offer an alternative food source consumers with celiac disease. In the United States (US) the top three GF produced cereal grains are rice, corn, and sorghum (USDA-FAS, 2022). Of the three, sorghum is produced 97% less in comparison to corn and 84% less in comparison to rice.

To date, there are many studies and reviews that report the phenolic content and antioxidant activity of pigmented rice, corn, and sorghum grains. There is a current trend in literature to report the results of one study and compare to another study with similar methods and results. Many of the methods used to measure phenolics and bioactivity are based on spectrophotometric and chromatography techniques. For both techniques, a slight modification in protocol and sample

preparation can alter the results for samples. It is therefore somewhat unreliable to compare total phenolic content or bioactivity of samples across studies. It is more dependable to quantitatively compare samples within the same study. However, due to the size constraints of many studies, this is not always feasible. To accurately compare the phenolic profile of pigmented grains they should be studied all at once using the same laboratory with the same preparation and detection methods. Therefore, the objective of this paper was to determine the different phenolic profile of pigmented rice, corn, and sorghum samples and their bioactivity.

2.2 Materials and methods

2.2.1 Chemicals

The Folin-Ciocalteu (FC), vanillin reagent, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (Milwaukee Wisconsin, USA). The gallic acid, ethanol, sodium bicarbonate, hydrochloric acid, Trolox, acetonitrile, and glacial acetic acid were purchased from Thermo Fischer Scientific (Waltham, Massachusetts, USA). Absolute methanol (ACS reagent grade) and sodium hydroxide were purchased from Ricca Chemical Company (Arlington, Texas, USA). Ultrapure water was obtained from Millipore (Milli-Q®, St. Louis, MO, USA). Sodium azide, fructose, and bovine serum albumin were purchased from Sigma Aldrich (St. Louis, MO, USA). Dibasic anhydrous phosphate was purchased from Fisher Scientific, Pittsburg, PA, USA. The analytical standards used for HPLC analysis included 4-hydroxybenzoic acid, protocatechuic acid, quercetin, naringenin (Sigma-Aldrich, St. Louis, MO, USA), taxifolin (HWI, Ruelzheim, Germany), 7-methoxyapigeniden (Cayman, Ann Harbor, MI, USA), catechin (TCI, Portland, OR, USA), chlorogenic acid, caffeic acid, p-coumaric acid, eriodyctiol, luteolin, apigenin, luteolinidin, and apigenidin (Indofine, Hillsborough, NJ, USA).

2.2.2 Grain material

In this study thirteen rice samples, six corn samples, and eight sorghum samples were obtained from a variety of different vendors, locations, and countries to increase sample pool diversity. Rice samples MNSN1.a2, 242, Tiara, HB1, Sierra, Scarlette, Eclipse, and IAC 600 were provided by the National Rice Research Center (USDA-ARS, Stuttgart, AR, US). US Red and US Black rice samples were purchased from Lundberg Family Farms (Richvalle, CA, US). Other rice samples were purchased from companies that sourced from Italy (Riso Scotti, Pavia, Italy), Thailand (Riviana Foods Inc., TN, USA) and China (Big Green Organic Food, CA, US). These samples were labelled as Italy Black, Thailand Black, and China Black, respectively. Maize Morado was purchased Peruvian Important Co., Inca's Foods (Passiac, NJ, USA). Heirloom Purple corn was purchased from Alma Semillera (Pinole, CA, USA). The white, red, blue, and yellow corn samples were supplied by the Corn Insects and Crop Genetics Research Unit (USDA-ARS, Ames, IA, USA). The white, burgundy, waxy burgundy, sumac and black sorghum samples were purchased from Nu Life Market (Scott City, KS, US). Sorghum varieties SC84, SC991, and PI570481 were grown in Puerto Vallarta, Mexico during the 2018-2019 growing season. The SC84, SC991, and PI570481 sorghum samples were grown by the sorghum breeding program, Kansas State University, Agricultural Research Center (Hays, KS, USA). The location of sourced grains, supplier, and pericarp color of all grains used in the experiment are shown in **Table 1.**

Table 2.1 Grain sources

Grain	Variety	Supplier	Country of Origin	Pericarp Color
Rice	MNSN1a.2	ARS-Rice[1]	AR, US	Purple
Rice	242	ARS-Rice[1]	AR, US	Purple
Rice	Tiara	ARS-Rice[1]	AR, US	Purple

Rice	HB1	ARS-Rice[1]	AR, US	Purple
Rice	Sierra	ARS-Rice[1]	AR, US	Brown
Rice	Scarlette	ARS-Rice[1]	AR, US	Red
Rice	Eclipse	ARS-Rice[1]	AR, US	Brown
Rice	IAC600	ARS-Rice[1]	AR, US	Purple
Rice	US Red	Commercial[2]	CA, US	Red
Rice	US Black	Commercial[2]	CA, US	Black
Rice	Italy Black	Commercial[3]	Italy	Black
Rice	Thailand Black	Commercial[4]	Thailand	Black
Rice	China Black	Commercial[5]	China	Black
Corn	Maize Morado	Commercial[6]	Peru	Purple
Corn	Heirloom Purple	Commercial[7]	CA, US	Purple
Corn	Blue	ARS-Corn[8]	IA, US	Blue
Corn	Red	ARS-Corn[8]	IA, US	Red
Corn	White	ARS-Corn[8]	IA, US	White
Corn	Yellow	ARS-Corn[8]	IA, US	Yellow
Sorghum	White	Commercial[9]	KS, US	White
Sorghum	Burgundy	Commercial[9]	KS, US	Red
Sorghum	Waxy Burgundy	Commercial[9]	KS, US	Red
Sorghum	Black	Commercial[9]	KS, US	Black
Sorghum	Sumac	Commercial[9]	KS, US	Red
Sorghum	SC84	K-State[10]	Mexico	Brown
Sorghum	SC991	K-State[10]	Mexico	Brown
Sorghum	PI570481	K-State[10]	Mexico	Black

[1]National Rice Research Center (AR, US); [2]Lundberg Family Farms (CA, US); [3]Riso Scotti (Pavia, Italy); [4]Thailand Brand, LOCATION; [5]China Brand, LOCATION; [6]Peruvian Import Co. Inc., Inca's Foods (NJ, US); [7]Alma Semillera (CA, US); [8]Corn Insects and Crop Genetics Research Unit, USAD-ARS (IA, US); [9]NuLife Market (KS, US); [10]Kansas State University, Agricultural Research Service (KS, US).

2.2.3 Sample preparation

Whole grain samples were first photographed prior to samples preparation using a Cannon EOS Revel T1I camera (Cannon, NY, USA). All grain samples were then milled using a cyclone mill with a 0.5 mm screen (UDY Corporation Cyclone mill, Fort Collins, Co, USA). After milling, flour samples were photographed before storage at -80 °C until further analysis.

2.2.4 Proximate analysis

Proximate analysis of samples was performed by Eurofins (Des Moines, IA, USA). Ash was determined using the AOAC 942.05 method; Carbohydrates were analyzed using CFR 21-calc; Protein was evaluated using the AOAC 992.15; Crude fat was determined using ethyl ether extraction (AOAC920.39) and moisture was determined using a forced air draft oven method (AOAC 930.15)

2.2.5 Color analysis

Whole grain samples and their flour counterparts were analyzed for color using a MiniScan EZ 45°/0° spectrophotometer/colorimeter (Hunter Associates Laboratory, Inc., Reston, VA, USA). After collecting *L*, a*,* and *b** values, hue was calculated according to equation 1 while chroma was calculated according to equation 2.

$$\text{Hue} = \arctan(b^*/a^*) \quad (1)$$

$$\text{Chroma} = \sqrt{(average\ a\ *)^2 + (average\ b\ *)^2} \quad (2)$$

2.2.6 Total phenolic content

Phenolic extractions were produced by combining one gram of flour with 9 mL of ethanol (70% v/v) and shaking for 2 hours. After an overnight sit at -20°C, samples were centrifuged at 4°C for 10 minutes at 2970 x g. Supernatants were collected for total phenolic content (TPC) using the Folin-Ciocalteu method previously described by Herald et al. (2012).

Sample were measured using a Biotek synergy 2 multi-detection plate reader (Winooski VT, USA). TPC of samples are expressed as milligram gallic acid equivalents per gram of sample (mg GAE/g)

2.2.7 Condensed tannin content

Condensed tannin extractions were conducted by combining 0.1g flour with 1 mL 1%HCl-Methanol and shaking for 20 minutes. Samples were centrifuged for 6 min and supernatants were collected for analysis. The condensed tannin content of samples was analyzed using the modified vanillin-HCl method as previously described by Herald et al., (2014) and modified Lee et al., (2022). Sample were measured using a Biotek synergy 2 multi-detection plate reader (Winooski VT, USA). Sample values were expressed as milligram catechin equivalents per gram of flour (mg CE/g).

2.2.8 Antioxidant activity

Antioxidant activity was determined using the 2,2-diphenyl- 1-picrylhydrazyl (DPPH*) method based on Plank et al. (2012) and modified by Santana et al. (2023). Absorbance was measured using a UV-Vis spectrophotometer (Shanghai Metash Instruments Co., Ltd, Shanghai, China). The Trolox standard curve in 70% ethanol ranged from 0 -100 milligram per milliliter. Sample results were expressed as milligram Trolox equivalents (TE) per gram of sample

2.2.9 RP-HPLC

The detection and quantification of phenolic compounds present in corn, rice, and sorghum samples were determined using reversed-phase high pressure liquid chromatography (RP-HPLC). Phenolic profile determination was determined using previously published methods from (Lee et al., 2022) adapted from Irakli et al. (2012). The 1260 Infinity HPLC device with a diode-array detector (HPLC-DAD, Agilent, Santa Clara, CA, USA) and a C18 column

(Kinetex®, 2.6µm, 100Å, 150mm×4.6mm) coupled with a SecurityGuard™ column (Phenomenex, Torrance, USA) were used for phenolic analysis. Results are expressed in terms of microgram of phenolic compound per gram sample

2.2.10 NP-HPLC

Normal-phase HPLC was used to detect monomers, oligomers and polymeric proanthocyanidins (condensed tannins) present in samples based on their degree of polymerization (DP). Samples were prepared by combining 1 g of sample with 10 mL solvent (acetone:water:acetic acid, 70:29.5:0.5, v/v/v) shaken at 400 rpm (Thermo Scientific, MaxQ 2500, Thermo Fisher, Pittsburgh, PA, USA) for 2 h, followed by centrifugation. The supernatant was filtered and inserted into a sealed glass vial for analysis.

A1290 Infinity II HPLC (Agilent, Santa Clara, CA, USA), with a fluorescence detector at excitation wavelength of 230 nm, and emission wavelength of 320 nm (Langer et al., 2011) in conjunction with a Develosil column (100 Diol, 5µm, 4.6 mm×250 mm, Phenomenex, Torrance, USA) was used to determine DP. The mobile phases consisted of acetonitrile:glacial acetic acid at 98:2, v/v (A) and methanol:water:glacial acetic acid, 95:3:2, v/v/v (B). The elution gradient at 35℃ and 0.6 mL/min of A:B solvents was as follows: 0–3 min of 93:7%; 3-57 min of 62.4:37.6%; 57-60 min of 0:100%; 60-67 min of 0:100%; 67-73 min of 93:7%; and 73-83 min of 93:7%.

Total proanthocyanidins, more precisely procyanidins, in sorghum were quantified using the catechin standard curve (0.00125-0.1 mg/mL, R2=0.999) and the relative response factor previously assigned to each DP value as described by Prior and Gu (2005). Results were expressed in terms of milligram catechin equivalent per gram sample (mg CE/g). To

quantitatively compare the DP of samples, the polymerization index (DPi) was calculated based on equation 3.

$$DP_i = \frac{\sum(C_i \times DP)}{\sum C_i} \quad (3)$$

Where Ci (mg CE/g) is the sum of proanthocyanidins calculated and DP is the degree of polymerization (unitless). This equation underestimates the mean degree of polymerization by assigning a DP value of 11 for peaks larger than a DP of 10 (eluted at or after 64 min). This allows for comparison between samples without the need for additional experiments.

2.2.11 Glycation inhibition

For the inhibition of advanced glycation end products (AGE) assay 1 ml of extract (70% ethanol) was dried at 60°C for 2h (Rocket Synergy 2, Thermo Fisher, Pittsburgh, PA, USA). The dried extract was solubilized in 1 mL sodium phosphate buffer (50 mM and pH 7.4 at 0.02% sodium azide, m/v), with the aid of an ultrasound probe (Fisherbrand, Waltham, MA, USA) at 85% amplitude for up to 2 min.

The ability of sorghum extracts to inhibit the formation of advanced glycation end products was evaluated using the bovine serum albumin-fructose (BSA-FRU) assay from a previously published method(Wang et al., 2011). Sodium phosphate buffer at 50 mM and pH 7.4 (0.02% sodium azide, m/v) was the solvent used to prepare solutions containing the sorghum extracts, fructose, BSA, and the positive control (aminoguanidine, at 122 mg/mL). One milliliter of sorghum extracts was mixed with 1 ml of fructose (1.5M) in capped polypropylene test tubes, incubated in an oven at 37°C for 2 h. Afterwards, 1 mL of BSA (30 mg/mL), was added to the mixture, followed by incubation at 37°C for 6 days. Two hundred microliters of reaction products were inserted into 96-well plates. Fluorescent AGEs were measured using a 96-well plate reader (Synergy 2, Biotek Instruments Inc, Winooski, VT, USA) at excitation wavelength

of 340 nm and emission wavelength of 440 nm. The potential of extracts to inhibit AGE formation (%) was calculated according to equation 4.

$$Inhibition\ (\%) = \left[1 - \left(\frac{F\ treated}{F\ untreated}\right)\right] \times 100 \quad (4)$$

Where $F_{treated}$ the fluorescence emitted by treated BSA, and $F_{untreated}$ is the fluorescence of glycated BSA with no treatment.

2.2.12 Principal component analysis and Pearson's correlations

The data underwent principal component analysis (PCA) to condense the information into a smaller set of components. Origin Pro 2023 software (Origin Lab Corporation, Northampton, MA, USA) was utilized for this purpose, employing multivariate analysis of variance (MANOVA) approach. Pearson's correlation coefficients were used to compare pairs of responses.

2.2.13 Statistical analysis

The data reported is representative of at least three independent experiments. Statistical analysis was conducted using one-way analysis of variance (ANOVA) with Tukey's post hoc testing using GraphPad Prism, version 10.1.0 (GraphPad, San Diego, CA). Significant difference in mean values was determined at p-value < 0.05.

2.3 Results

2.3.1 Proximate analysis

The results for proximate composition are shown in **Table 2.2.** Analysis was performed once per grain sample.

Table 2.2 Proximate analysis of rice, corn, and sorghum samples.

Grain	Variety	Ash	Carbohydrate	Protein	Fat	Moisture
Rice	***MNSN1a.2***	1.62	73.09	9.38	3.90	11.98
	242	1.49	75.34	8.69	2.94	11.54

	Tiara	1.30	73.09	11.00	2.77	11.84
	HB1	1.29	75.26	8.00	3.31	12.14
	Sierra	1.50	73.93	9.81	2.61	12.15
	Scarlette	1.48	72.84	11.06	2.62	12.00
	Eclipse	1.48	73.85	9.38	2.90	12.39
	IAC 600	1.57	72.43	11.69	2.45	11.86
	US Red	1.61	70.46	8.56	6.52	12.85
	US Black	1.47	75.86	7.19	2.61	12.87
	Italy Black	1.65	75.44	8.88	2.18	11.85
	Thailand Black	1.37	74.87	8.38	2.90	12.48
	China Black	1.15	76.88	7.75	2.33	11.91
Corn	***Maize Morado***	1.64	74.52	8.44	4.25	11.15
	Heirloom Purple	1.77	74.99	8.13	5.08	10.03
	Blue	1.24	72.44	10.88	4.37	11.07
	Red	1.18	73.18	10.25	4.21	11.18
	White	1.42	70.63	12.31	3.94	11.7
	Yellow	1.37	72.51	12.06	4.04	10.02
Sorghum	***White***	1.17	71.97	11.31	2.87	12.68
	Burgundy	1.34	73.00	9.81	3.10	12.75
	Waxy Burgundy	1.35	74.27	9.44	3.63	11.31
	Black	1.98	70.97	12.63	2.87	11.55
	Sumac	1.49	74.34	8.25	2.81	13.11
	SC84	1.92	75.07	12.19	3.39	7.43
	SC991	1.96	72.30	13.69	3.71	7.91
	PI570481	1.82	73.81	12.31	2.88	9.18

The average ash content of rice was 1.46% with China black being the lowest and Italy Black being the highest (1.15% and 1.65% respectively). The average ash content of corn was 1.44% and ranged from 1.18%(Red) to 1.77% (Heirloom Purple). The average ash content of sorghum was 1.63% with White being the lowest and Black being the highest (1.17% and 1.98%, respectively). The average ash content of rice, corn, and sorghum grains were not significantly different from one another.

The average carbohydrate content of rice was 74.10% and ranged from 70.46% (US Red) to 76.88% (China Black). The average carbohydrate content of corn was 73.05% and ranged from 70.63% (White) to 74.99% (Heirloom Purple). The average carbohydrate content of sorghum was 73.22% and ranged from 70.97% (Black) to 75.07% (SC84). The rice, corn, and sorghum carbohydrate content were not statistically significant from one another.

The average protein content of rice was 9.21% and ranged from 7.19% (US Black) to 11.69% (IAC600). The protein content of rice was significantly ($p<0.05$) lower than the protein content of corn. The average protein content of corn was 10.35% and ranged from 8.13% (Heirloom Purple) to 12.31% (White). The average protein content of sorghum was 10.35% and ranged from 8.25% (Sumac) to 13.69% (SC991).

The average fat content of rice was 3.08% and ranged from 2.18% (Italy Black) to 6.52% (US Red). The average fat content of corn was 4.32% and ranged from 3.94% (White) to 5.08% (Heirloom Purple). The average fat content of sorghum was 3.16% and ranged from 2.81% (Sumac) to 3.71% (SC991). The fat content of rice and sorghum were significantly lower than the average fat content of corn.

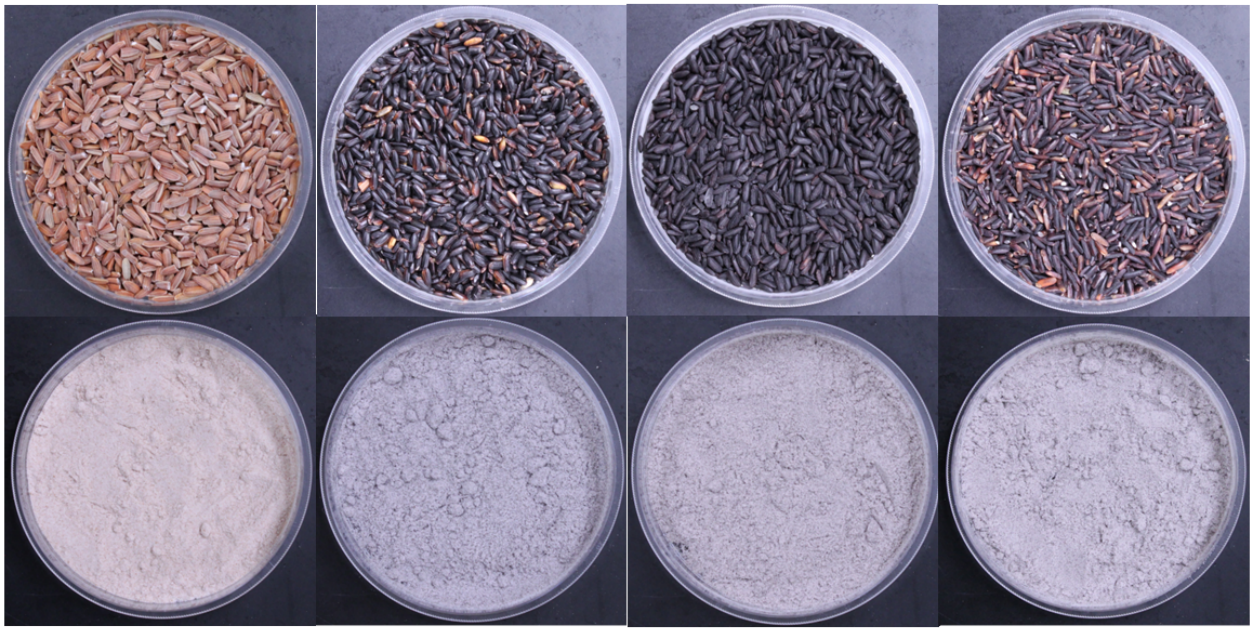
The average moisture content of rice was 12.14% and ranged from 11.54% (242) to 12.87% (US Black). The average moisture content of corn was 10.86% and ranged from 10.02% (yellow) to 11.70 %(white). The average moisture content of sorghum was 10.74% and ranged from 7.43 %(SC84) to 13.11% (Sumac). The average moisture content of rice corn, and sorghum grains was not significantly different from one another.

2.3.2 Color analysis

Photographs of rice, corn, and sorghum grains and their flours are represented in **Figure 2.1** to demonstrate the visual diversity of samples analyzed in the present study.

*MNSN1a *242 *Tiara *HB1

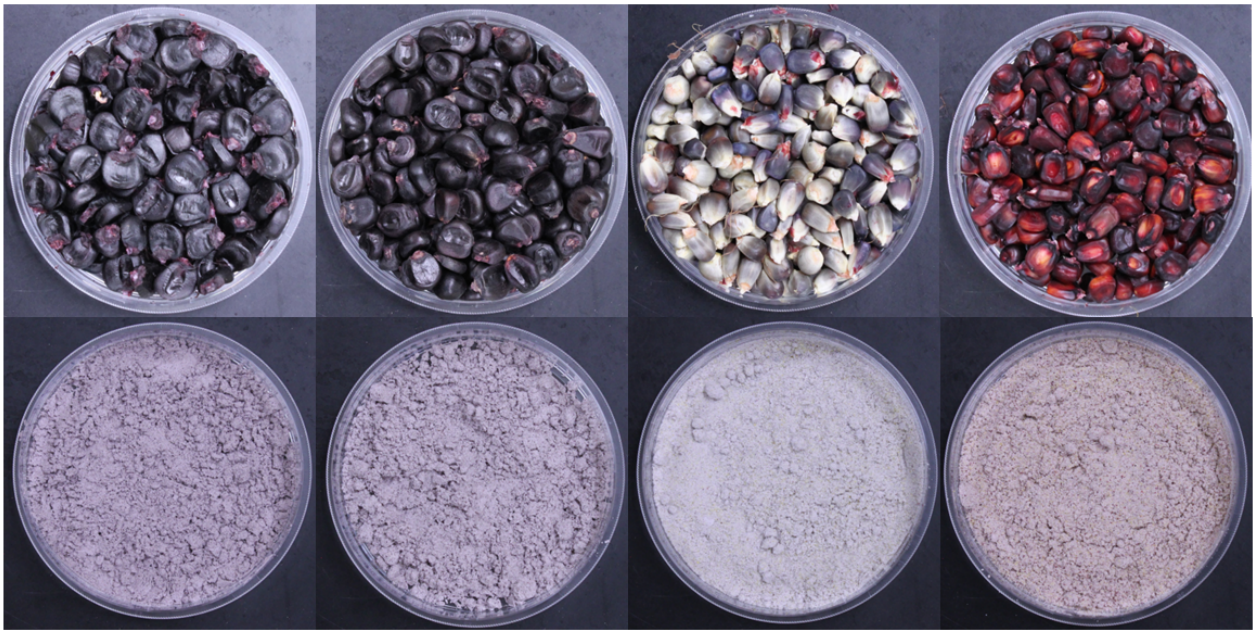
*Sierra *Scarlet *Eclipse *IAC600



[#]US Red [#]US Black [#]Italy Black [#]Thailand Black

[#]China Black

(B)



#Maize Morado #Heirloom Purple *Blue *Red

*White *Yellow

(C)

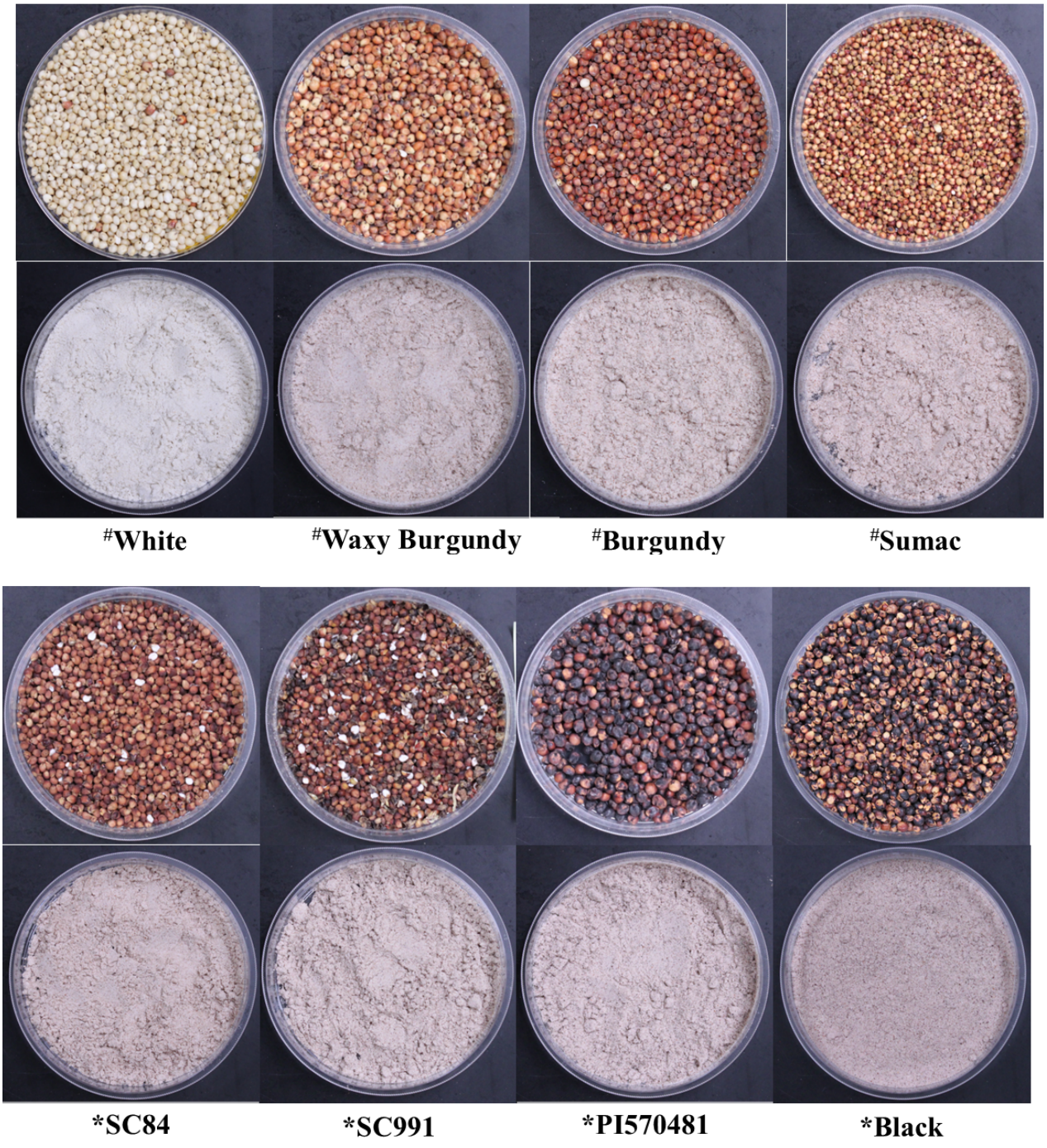


Figure 2.1A Photos of rice (A), corn (B), and sorghum (C) grain and flours.

Samples with (*) denote non-commercial samples while samples with (#) denote commercially available samples.

In literature, pigmented grains are often grouped by color (white, red, black, yellow, purple, etc.). To analyze the effect of grain pigmentation on phenolic profile and bioactivity, color measurements were taken to ensure that accurate color correlations could be made. The results from the color analysis of whole grains can be found in **Table 2.3.** Samples were measured in triplicate.

Table 2.3 Color analysis of whole grain rice, corn, and sorghum samples.

Grain	Sample	L*	a*	b*	Chroma	Hue Angle
Rice	***Lundberg Red***	39.52 ± 0.41 H	14.32 ± 0.36 B	18.10 ± 0.55 F	23.08 ± 0.65 CD	0.90 ± 0.01 F
	MNSN1a.2	32.91 ± 0.01 L	10.27 ± 0.02 E	10.48 ± 0.03 I	14.67 ± 0.01 I	0.80 ± 0.01 G
	242	32.50 ± 0.74 L	13.15 ± 0.38 C	10.69 ± 0.67 I	16.95 ± 0.72 G	0.68 ± 0.02 H
	Tiara	25.78 ± 0.01 NO	4.33 ± 0.03 JK	1.29 ± 0.02 LM	4.52 ± 0.02 MN	0.29 ± 0.01 K
	HB-1	34.91 ± 0.01 K	7.34 ± 0.00 G	6.15 ± 0.01 J	9.58 ± 0.00 K	0.70 ± 0.00 H
	Sierra	66.41 ± 0.77 A	4.99 ± 0.09 I	23.52 ± 0.23 B	24.04 ± 0.24 C	1.36 ± 0.00 B
	Scarlett	41.43 ± 0.16 EF	13.16 ± 0.04 C	17.33 ± 0.02 F	21.76 ± 0.04 E	0.92 ± 0.00 F
	Eclipse	64.01 ± 0.11 B	4.03 ± 0.05 JKL	21.75 ± 0.02 CD	22.12 ± 0.02 DE	1.39 ± 0.00 AB
	IAC600	25.50 ± 0.01 NO	3.33 ± 0.03 MN	0.66 ± 0.08 MN	3.40 ± 0.02 OP	0.20 ± 0.02 L
	Italy Black	20.78 ± 0.41 R	2.73 ± 0.04 O	-0.72 ± 0.03 O	2.83 ± 0.05 P	-0.26 ± 0.01 P
	US Black	23.75 ± 0.34 PQ	4.56 ± 0.02 IJ	1.38 ± 0.17 LM	4.77 ± 0.07 MN	0.30 ± 0.03 JK
	Thailand Black	24.58 ± 1.07 OP	6.33 ± 0.06 H	2.18 ± 0.05 L	6.69 ± 0.07 L	0.33 ± 0.01 J
	China Black	26.39 ± 0.25 N	4.05 ± 0.02 JKL	-0.46 ± 0.01 O	4.08 ± 0.02 NO	-0.11 ± 0.00 O
Corn	***Maize Morado***	20.62 ± 0.52 R	2.81 ± 0.22 NO	-0.68 ± 0.09 O	2.89 ± 0.22 P	-0.24 ± 0.03 P
	Heirloom Purple	22.42 ± 0.39 Q	3.88 ± 0.19 KM	-0.24 ± 0.11 NO	3.88 ± 0.18 NO	-0.06 ± 0.03 N
	Blue	50.07 ± 0.06 C	2.42 ± 0.01 O	4.51 ± 0.01 K	5.11 ± 0.01 M	1.08 ± 0.00 D
	Red	40.62 ± 0.04 EGH	6.93 ± 0.01 G	0.96 ± 0.01 M	6.99 ± 0.01 L	0.14 ± 0.00 M
	White	67.34 ± 0.02 A	3.32 ± 0.02 MN	20.16 ± 0.02 E	20.43 ± 0.01 F	1.41 ± 0.00 A
	Yellow	67.37 ± 0.06 A	7.10 ± 0.05 G	25.75 ± 0.21 A	26.71 ± 0.19 A	1.30 ± 0.01 C
Sorghum	***White***	66.42 ± 0.91 A	3.75 ± 0.06 LM	22.11 ± 0.23 C	22.43 ± 0.23 DE	1.40 ± 0.00 AB
	Waxy	46.86 ± 0.45 D	14.37 ± 0.13 B	22.16 ± 0.26 C	26.42 ± 0.28 AB	0.99 ± 0.01 E
	Waxy Burgundy	39.11 ± 0.34 I	16.52 ± 0.25 A	17.23 ± 0.21 F	23.87 ± 0.22 C	0.81 ± 0.01 G
	Sumac	42.16 ± 0.66 E	14.52 ± 0.38 B	21.01 ± 0.36 DE	25.54 ± 0.51 B	0.96 ± 0.01 E
	SC84	39.88 ± 0.47 GI	14.40 ± 0.12 B	16.00 ± 0.37 G	21.53 ± 0.32 E	0.84 ± 0.01 G
	SC991	37.39 ± 0.12 J	10.95 ± 0.04 D	11.24 ± 0.14 I	15.70 ± 0.09 H	0.80 ± 0.01 G
	PI570481	29.83 ± 0.29 M	9.40 ± 0.31 F	5.51 ± 0.40 J	10.90 ± 0.46 J	0.53 ± 0.02 I
	Black	41.10 ± 0.72 EG	13.61 ± 0.35 C	14.23 ± 0.87 H	19.69 ± 0.86 F	0.81 ± 0.02 G

Samples that do not share a letter within the same column are statistically significant (p<0.05).

The *L** value of rice grains ranged from 20.78 (Italy Black) to 66.41 (Sierra). The *L** value of corn grains from 20.62 (Maize Morado) to 67.37 (White). The *L** value of sorghum grains ranged from 29.83 (PI570481) to 66.42 (White). The *a** value of rice grains ranged from 2.73 (Italy Black) to 14.32 (Red). The *a** value of corn grains ranged from 2.42 (Blue) to 7.10 (Yellow). The *a** value of sorghum grains ranged from 3.75 (White) to 16.52 (Burgundy). The *b** value of rice grains ranged from -0.72 (Italy Black) to 23.52 (Sierra). The *b** value of corn grains ranged from -0.68 (Maize Morado) to 25.75 (Yellow). The *b** value of sorghum grains ranged from 5.510 (PI570481) to 22.16 (Waxy Burgundy). The chroma value for rice grains ranged from 2.83 (Italy Black) to 24.04 (Sierra). The chroma value for corn grains ranged from 2.89 (Maize Morado) to 26.71 (Yellow). The chroma value for sorghum grains ranged from 10.90 (PI570481) to 26.42 (Waxy Burgundy). The hue angle of rice grains ranged from -0.26 (Italy Black) to 1.39 (Eclipse). The hue angle of corn grains ranged from -0.24 (Maize Morado) to 1.41 (White). The hue angle of sorghum grains ranged from 0.53 (PI570481) to 1.40 (White).

The results from the color analysis of whole flours can be found in **Table 2.4**. Samples were measured in triplicate.

Table 2.4 Color analysis of rice, corn, and sorghum flour samples.

Flour	Sample	L*	a*	b*	Chroma	Hue Angle
Rice	***Lundberg Red***	75.52 ± 0.47 D	7.02 ± 0.15 E	11.59 ± 0.18 E	13.55 ± 0.23 E	1.02 ± 0.01 G
	MNSN1a.2	68.43 ± 0.02 G	4.43 ± 0.02 G	5.79 ± 0.01 K	7.29 ± 0.01 N	0.92 ± 0.00 J
	242	69.24 ± 0.17 G	5.99 ± 0.02 F	3.21 ± 0.01 N	6.79 ± 0.02 O	0.49 ± 0.00 Q
	Tiara	66.10 ± 0.14 HI	3.53 ± 0.01 I	2.64 ± 0.02 O	4.41 ± 0.01 RS	0.64 ± 0.00 O
	HB-1	73.47 ± 0.01 E	3.02 ± 0.02 J	3.15 ± 0.01 N	4.36 ± 0.01 ST	0.81 ± 0.01 L
	Sierra	88.08 ± 0.02 AB	0.80 ± 0.01 L	10.35 ± 0.01 G	10.38 ± 0.01 J	1.49 ± 0.00 B
	Scarlett	75.23 ± 0.03 D	7.29 ± 0.02 D	12.79 ± 0.01 D	14.72 ± 0.00 C	1.05 ± 0.00 F
	Eclipse	87.40 ± 0.01 B	0.95 ± 0.02 L	11.13 ± 0.00 F	11.17 ± 0.00 I	1.49 ± 0.01 B
	IAC600	62.24 ± 0.02 KL	3.48 ± 0.01 I	3.09 ± 0.01 N	4.65 ± 0.01 R	0.73 ± 0.01 N
	Italy Black	60.90 ± 0.21 LM	4.26 ± 0.03 G	4.48 ± 0.03 L	6.18 ± 0.05 P	0.81 ± 0.00 L
	US Black	64.04 ± 0.54 J	3.47 ± 0.04 I	2.15 ± 0.02 P	4.08 ± 0.03 T	0.56 ± 0.01 P
	Thailand Black	66.33 ± 0.33 HI	3.87 ± 0.02 H	3.65 ± 0.05 M	5.32 ± 0.04 Q	0.75 ± 0.01 M
	China Black	60.41 ± 0.38 M	4.28 ± 0.03 G	1.25 ± 0.04 Q	4.46 ± 0.03 RS	0.28 ± 0.01 R
Corn	***Maize Morado***	58.65 ± 0.53 N	8.14 ± 0.05 C	1.28 ± 0.04 Q	8.24 ± 0.05 K	0.16 ± 0.01 S
	Heirloom Purple	61.99 ± 0.40 LM	7.26 ± 0.05 D	2.23 ± 0.03 P	7.60 ± 0.06 M	0.30 ± 0.00 R
	Blue	74.15 ± 0.05 DE	3.47 ± 0.01 I	7.09 ± 0.01 J	7.89 ± 0.01 L	1.12 ± 0.01 E
	Red	67.72 ± 0.01 GH	9.08 ± 0.02 B	9.54 ± 0.01 I	13.17 ± 0.01 F	0.81 ± 0.00 L
	White	89.57 ± 0.01 A	0.32 ± 0.01 M	12.74 ± 0.01 D	12.74 ± 0.01 G	1.55 ± 0.01 A
	Yellow	86.70 ± 0.03 B	-1.38 ± 0.02 N	30.28 ± 0.02 A	30.31 ± 0.02 A	-1.53 ± 0.01 T
Sorghum	***White***	80.70 ± 0.08 C	1.52 ± 0.05 K	13.62 ± 0.14 B	13.71 ± 0.14 E	1.46 ± 0.00 C
	Waxy	75.13 ± 0.61 D	5.96 ± 0.12 F	13.11 ± 0.24 C	14.40 ± 0.22 D	1.14 ± 0.02 D
	Waxy Burgundy	73.09 ± 1.17 EF	6.15 ± 0.14 F	12.80 ± 0.08 D	14.20 ± 0.13 D	1.13 ± 0.01 DE
	Sumac	71.61 ± 0.57 F	7.35 ± 0.17 D	12.90 ± 0.09 CD	14.85 ± 0.16 BC	1.05 ± 0.01 F
	SC84	71.82 ± 1.28 F	7.93 ± 0.10 C	12.74 ± 0.10 D	15.01 ± 0.14 B	1.01 ± 0.01 GH
	SC991	64.81 ± 0.71 IJ	7.02 ± 0.05 E	10.99 ± 0.01 F	13.04 ± 0.04 F	1.00 ± 0.01 H
	PI570481	65.71 ± 1.30 I	7.14 ± 0.07 DE	9.93 ± 0.08 H	12.23 ± 0.10 H	0.95 ± 0.00 I
	Black	63.68 ± 0.02 JK	9.38 ± 0.15 A	10.90 ± 0.02 F	14.38 ± 0.08 D	0.86 ± 0.01 K

Samples that do not share a letter within the same column are statistically significant (p<0.05).

The *L** value of rice flours ranged from 60.41 (China Black) to 88.08 (Sierra). The *L** value of corn flours ranged from 58.65 (Maize Morado) to 89.57 (White). The *L** value of sorghum flours ranged from 63.68 (Black) to 80.70 (White). The *a** value of rice flours ranged from 0.80 (Sierra) to 7.29 (Scarlette). The *a** value of corn flours ranged from -1.38 (Yellow) to 9.08 (Red). The *a** value of sorghum flours ranged from 1.52 (White) to 9.38 (Black). The *b** value of rice flours ranged from 1.25 (China Black) to 12.79 (Scarlette). The *b** value of corn flours ranged from 1.28 (Maize Morado) to 30.28 (Yellow). The *b** value of sorghum flours ranged from 9.93 (PI570481) to 13.62 (White). The chroma value of rice flours ranged from 4.08 (US Black) to 14.72 (Scarlette). The chroma value of corn flours ranged from 7.60 (Heirloom Purple) to 30.31 (Yellow). The chroma value of sorghum flours ranged from 12.23 (PI570481) to 15.01 (SAC84). The hue angle of rice flours ranged from 0.28 (China Black) to 1.49 (Sierra). The hue angle of corn flours ranged from -1.53 (Yellow) to 1.55 (White). The hue angle of sorghum flours ranged from 0.86 (Black) to 1.46 (White).

2.3.3 Total phenolic content

The total phenolic content (TPC) of rice, corn and sorghum are represented in **Figure 2.2** Samples were measured in triplicate.

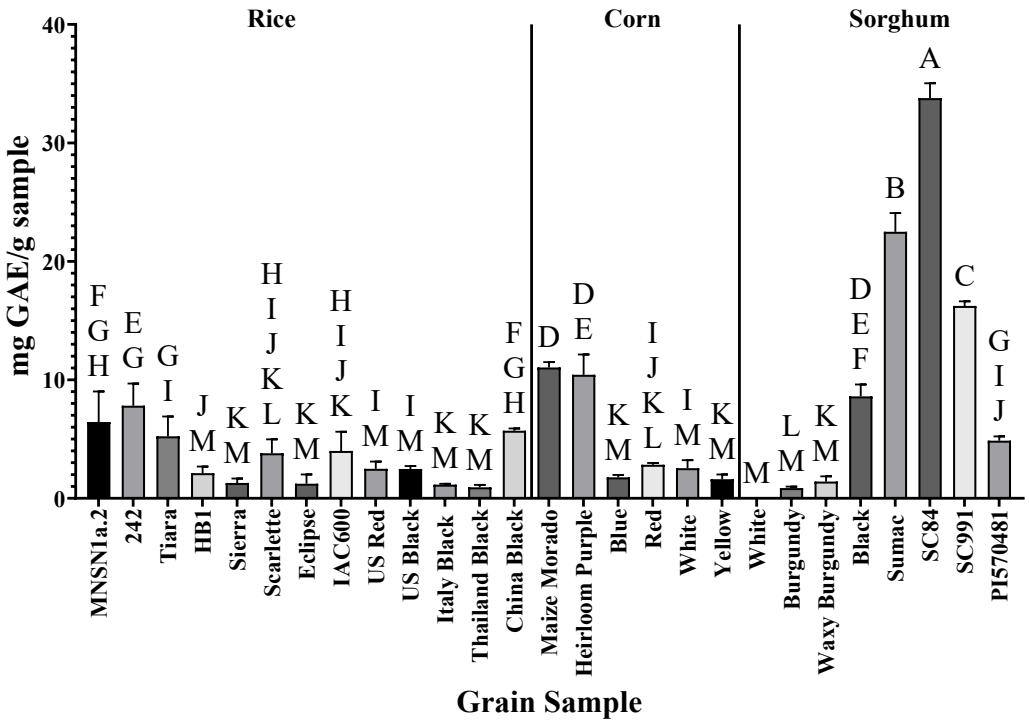


Figure 2.2 Total phenolic content of rice, corn, and sorghum samples.

Total phenolic content expressed as milligram gallic acid equivalents per gram (mg GAE/g). Samples that do not share a letter a statistically significant (p<0.05).

The TPC of rice ranged from 0.92 (Thailand Black) to 7.81 (242) mg GAE/g. The TPC of corn ranged from 1.60 (Yellow) to 11.04 (Maize Morado) mg GAE/g. The TPC of the blue corn (1.76 mg GAE/g) was not significantly different from the yellow or white samples. The TPC of sorghum ranged from 0.00 (White) to 33.77 (Sumac) mg GAE/g. The TPC of Burgundy (0.86 mg GAE/g) and Waxy Burgundy (1.41 mg GAE/g) were not significantly different from White sorghum (0.00 mg GAE/g

2.3.4 Condensed tannin content

The condensed tannin content (CTC) of rice, corn, and sorghum are shown in **Figure 2.3**. Samples were measured in triplicate.

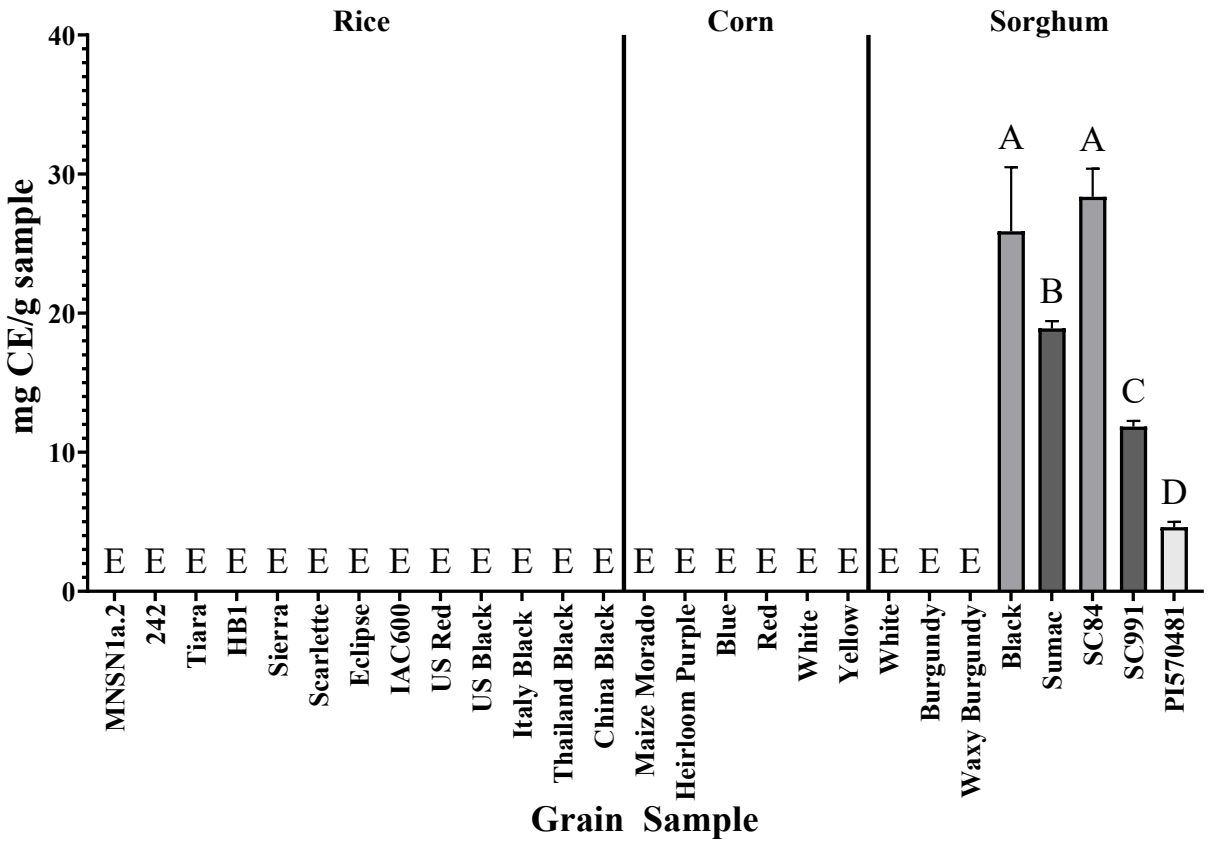


Figure 2.3 Condensed tannin content of rice, corn, and sorghum samples

Condensed tannin content expressed as milligram catechin equivalents per gram (mg CE/g). Samples that do not share a letter a statistically significant (p<0.05).

The condensed tannin content (CTC) of rice and corn was not detectable using the modified vanillin-HCl method. Only the sumac, black, SC84, SC991, and PI570481 sorghum grain samples had detectable condensed tannins. The CTC of sorghum ranged from 4.28 mg CE/g (PI570481) to 26.14 mg CE/g (SC84). Both Black (22.09 mg CE/g) and SC84 (26.14 mg CE/g) sorghum samples were significantly higher in CTC than the other sorghum samples.

2.3.5 Antioxidant activity

The antioxidant activity of rice, corn, and sorghum flours are represented in **Figure 2.4.** Samples were analyzed in triplicate.

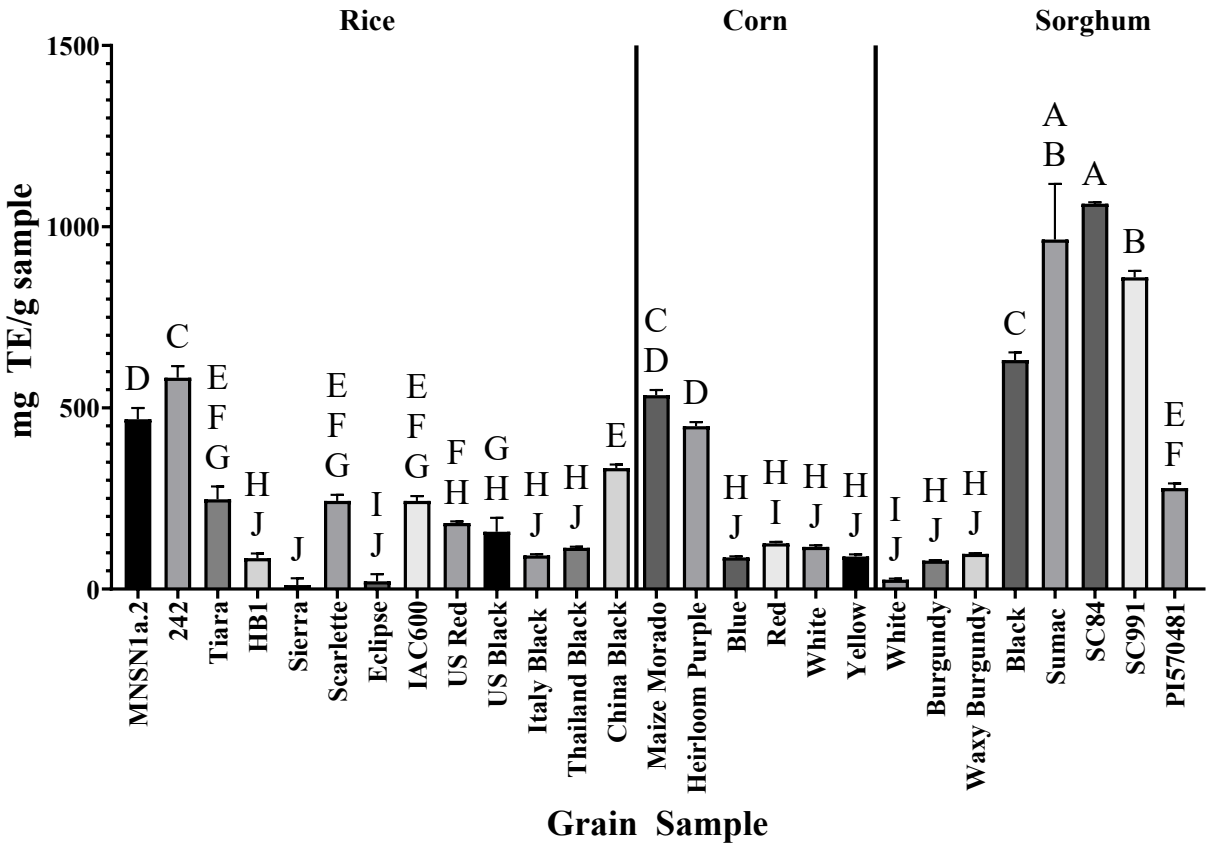


Figure 2.4. The antioxidant activity of rice, corn, and sorghum samples.

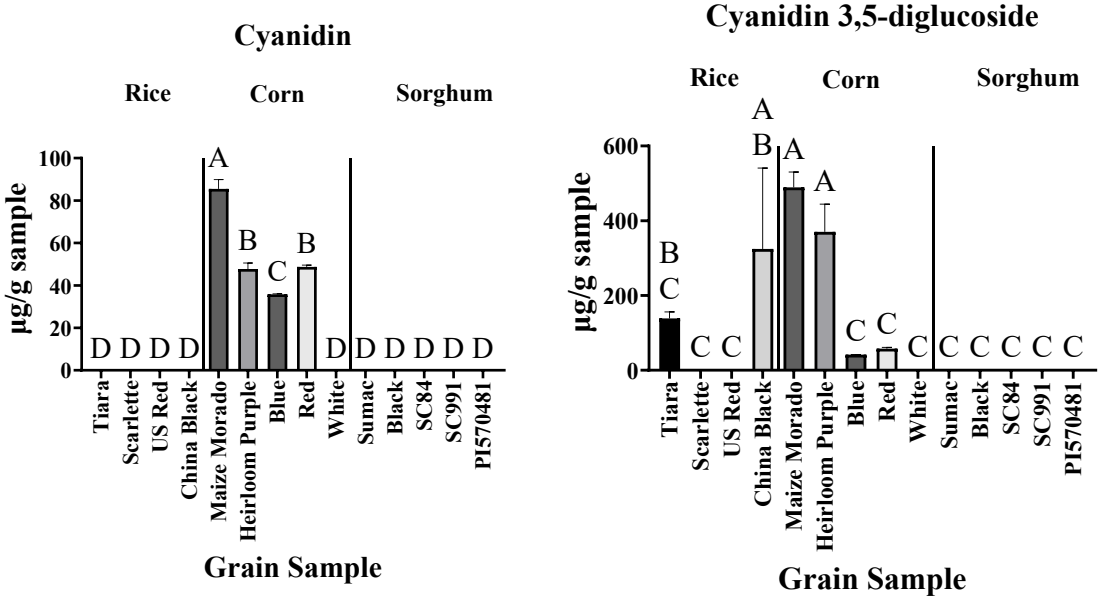
Antioxidant activity is expressed as milligram Trolox equivalents per gram (mg TE/g). Samples that do not share a letter a statistically significant (p<0.05).

The average antioxidant activity (AA) of rice was 214.2 mg TE/g and ranged from 10.98 mg TE/g (Sierra) to 583.2 mg TE/g sample (242). The average AA of corn samples was 234.0 mg TE/g and ranged from 87.4 mg GE/g (Blue) sample to 534.9 mg TE/g (Maize Morado). The average AA of sorghum samples was 500.0 mg TE/g and ranged from 25.97 mg TE/g (White) to 1064 mg TE/g (SC84). Both Sumac (964.3 mg TE/g) and SC84 (1064 mg TE/g) were significantly higher in AA in comparison to all the other rice, corn, and sorghum flours.

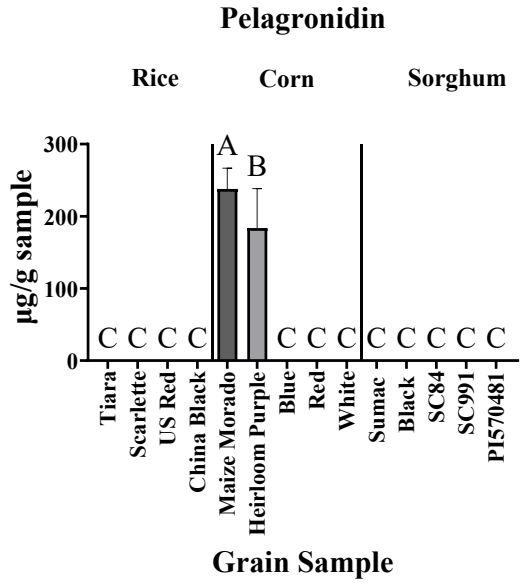
2.3.6 RP-HPLC

The reverse phase high pressure liquid chromatography (RP-HPLC) results of rice are represented in **Figure 2.5.** Samples were measured in triplicate.

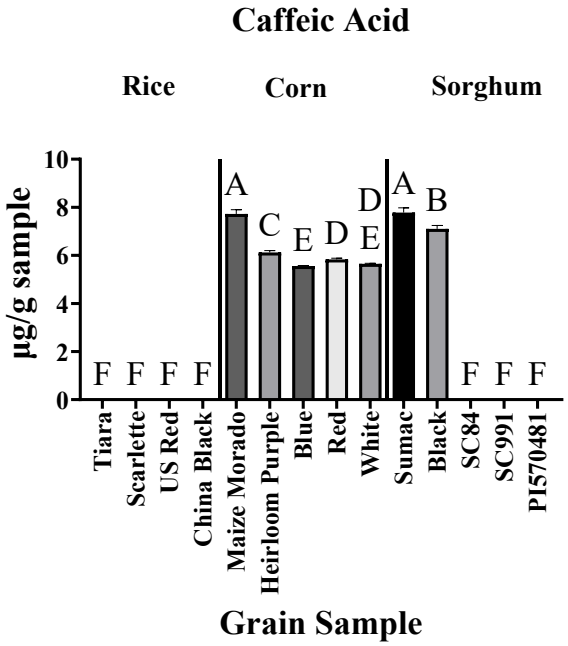
(A)

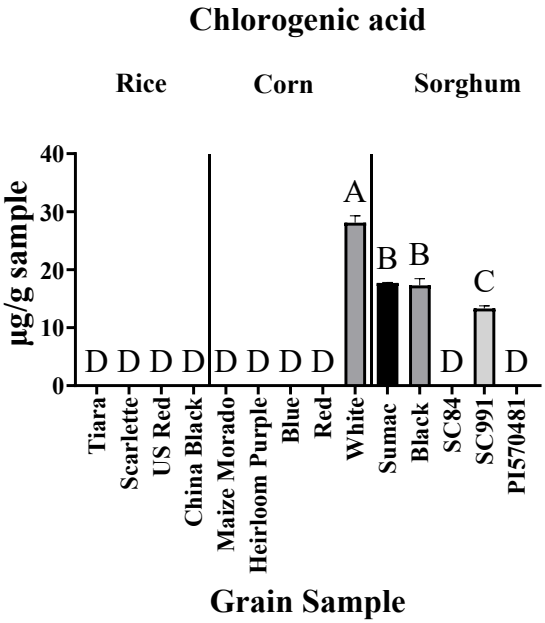


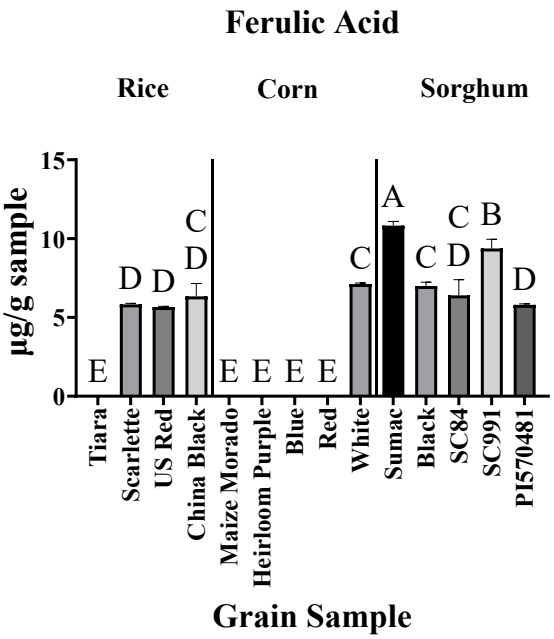
(B)

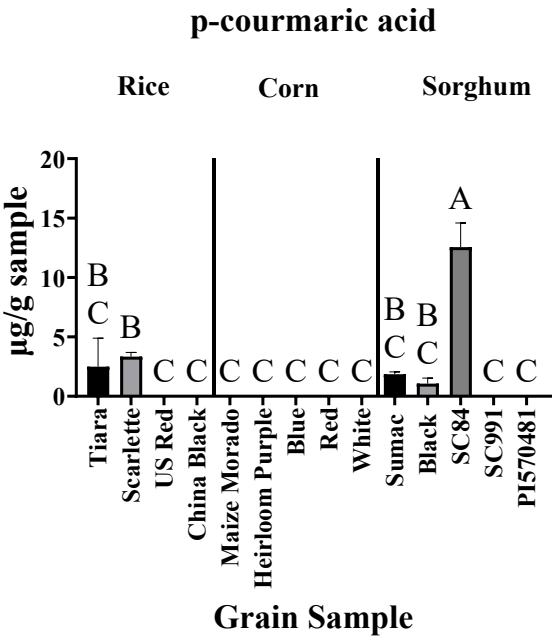


(C)

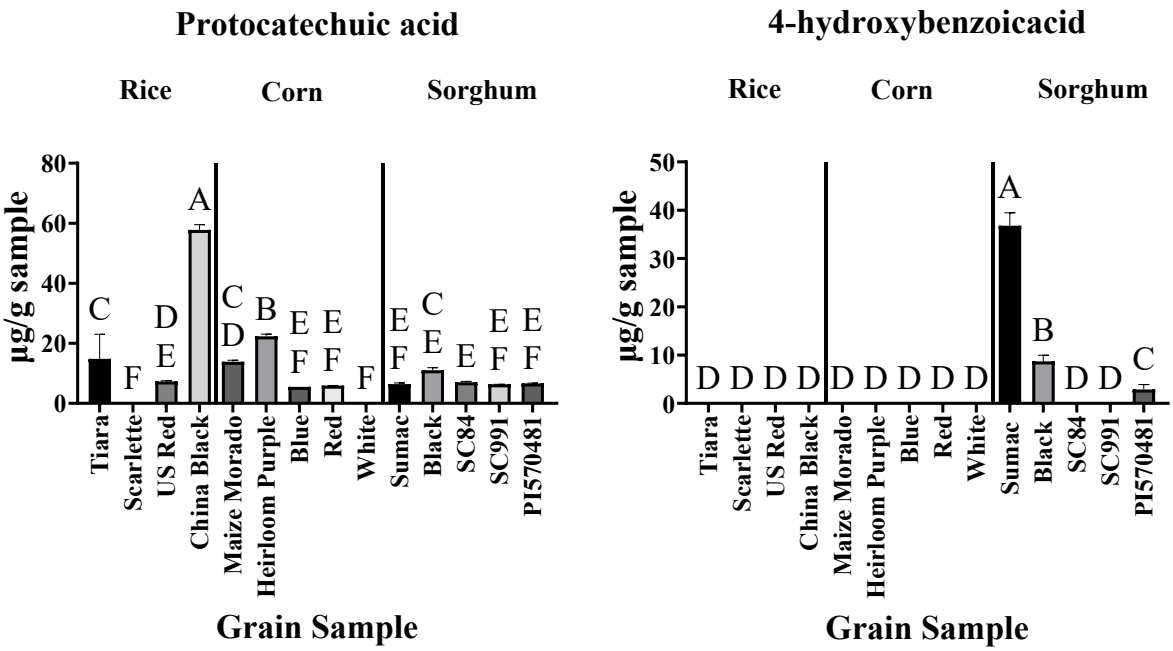

Caffeic Acid
Rice
Corn
Sorghum
µg/g sample
Tiara
Scarlette
US Red
China Black
Maize Morado
Heirloom Purple
Blue
Red
White
Sumac
Black
SC84
SC991
PI570481
Grain Sample


Chlorogenic acid
Rice
Corn
Sorghum
µg/g sample
Grain Sample

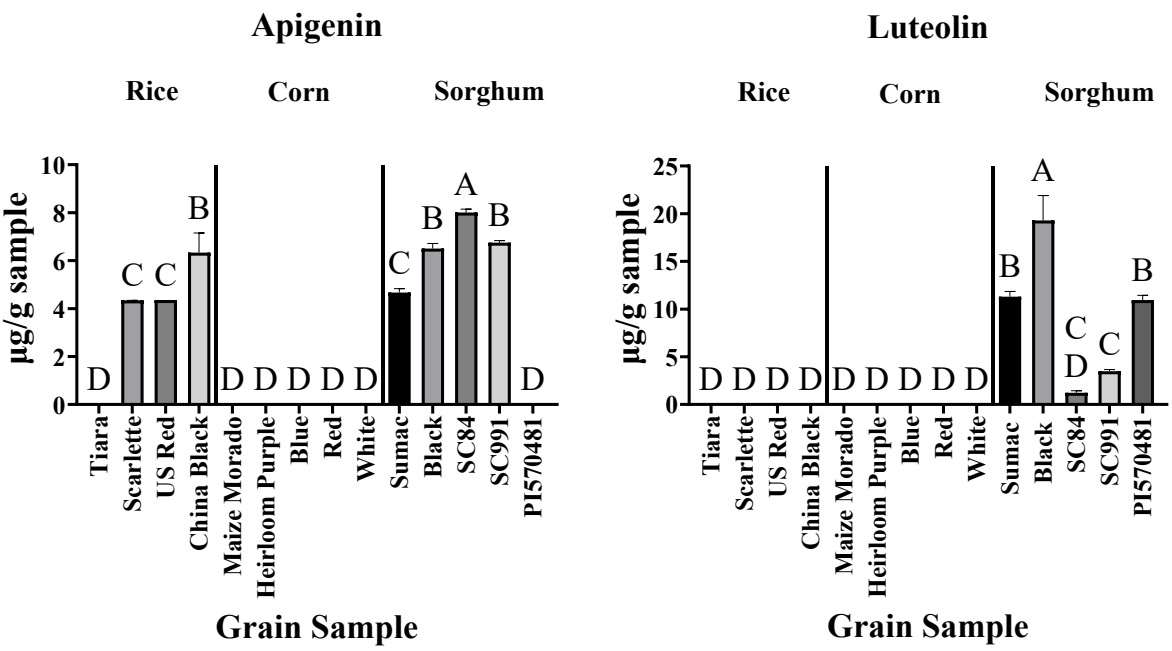

Ferulic Acid
Rice
Corn
Sorghum
µg/g sample
Grain Sample


p-courmaric acid
Rice
Corn
Sorghum
µg/g sample
Grain Sample

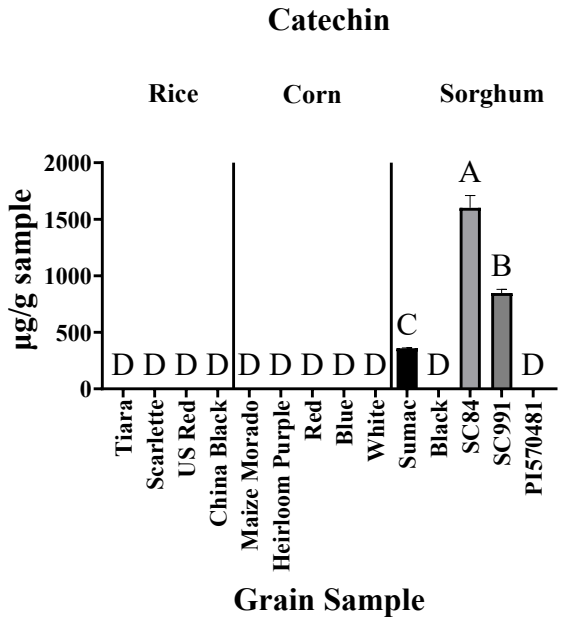
(D)


Protocatechuic acid
Rice
Corn
Sorghum
µg/g sample
80
60
40
20
0
C
F
D
E
A
C
D
B
E
F
E
F
F
E
F
C
E
E
F
E
F
Tiara
Scarlette
US Red
China Black
Maize Morado
Heirloom Purple
Blue
Red
White
Sumac
Black
SC84
SC991
PI570481
Grain Sample
4-hydroxybenzoicacid
Rice
Corn
Sorghum
µg/g sample
50
40
30
20
10
0
D D D D D D D D D A B D D C
Tiara
Scarlette
US Red
China Black
Maize Morado
Heirloom Purple
Blue
Red
White
Sumac
Black
SC84
SC991
PI570481
Grain Sample

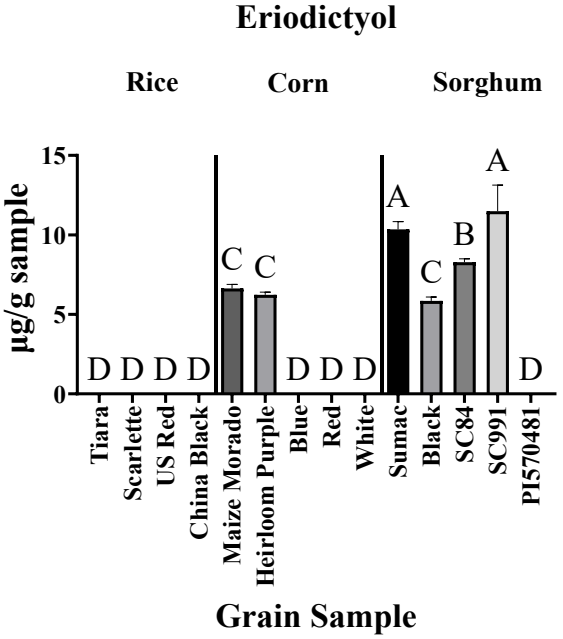
(E)

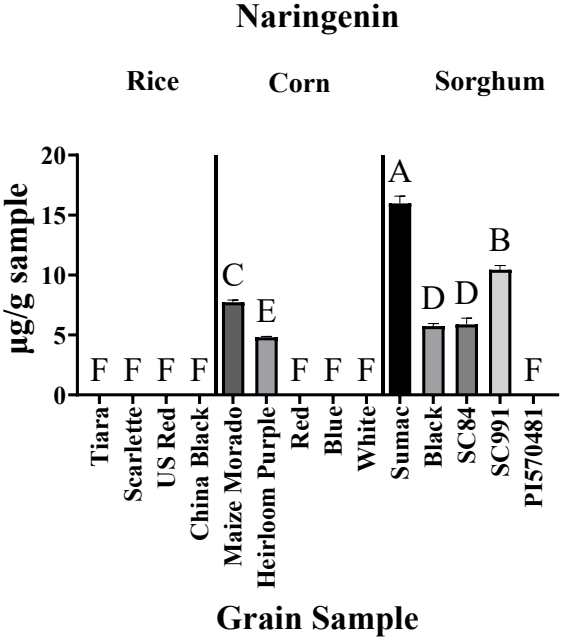

Apigenin
Rice
Corn
Sorghum
µg/g sample
10
8
6
4
2
0
D
C C
B
D D D D D
C
B
A
B
D
Tiara
Scarlette
US Red
China Black
Maize Morado
Heirloom Purple
Blue
Red
White
Sumac
Black
SC84
SC991
PI570481
Grain Sample
Luteolin
Rice
Corn
Sorghum
µg/g sample
25
20
15
10
5
0
D D D D D D D D D B A C D C B
Tiara
Scarlette
US Red
China Black
Maize Morado
Heirloom Purple
Blue
Red
White
Sumac
Black
SC84
SC991
PI570481
Grain Sample

(F)

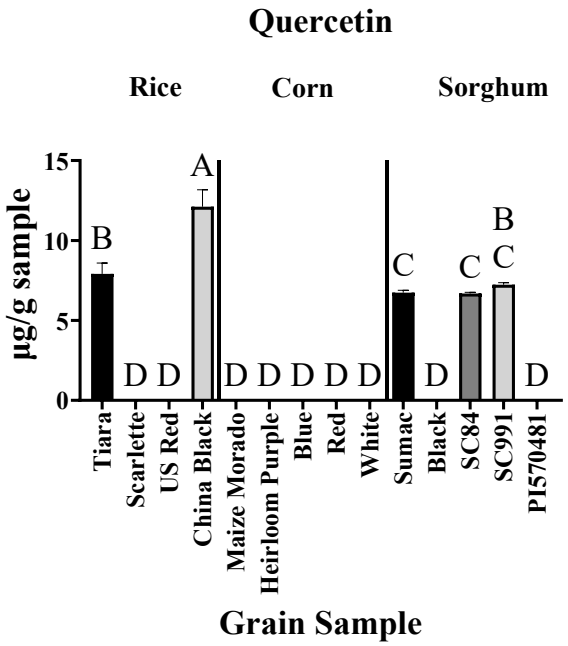

Catechin
Rice
Corn
Sorghum
µg/g sample
2000
1500
1000
500
0
D D D D D D D D D D C D A B D
Tiara
Scarlette
US Red
China Black
Maize Morado
Heirloom Purple
Red
Blue
White
Sumac
Black
SC84
SC991
PI570481
Grain Sample

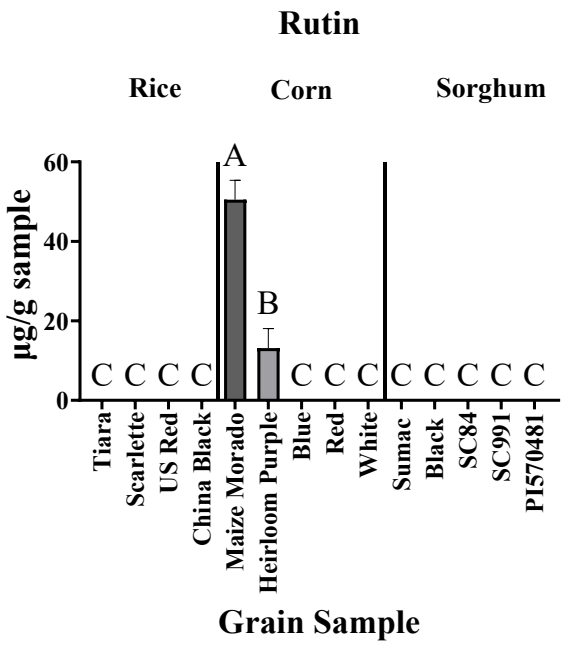
(G)

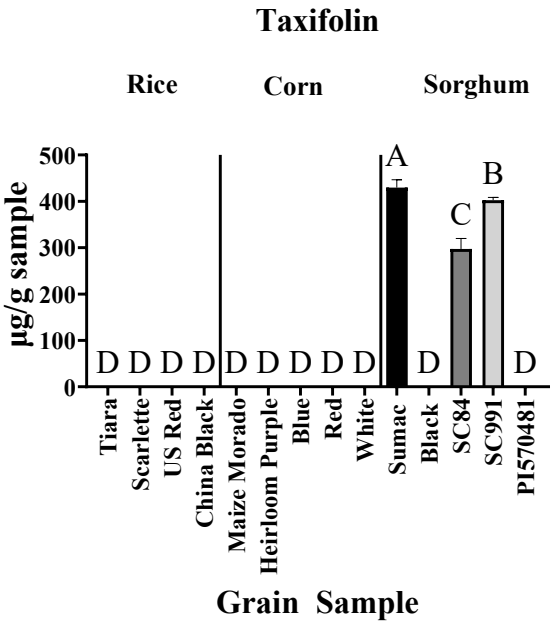

Eriodictyol
Rice
Corn
Sorghum
µg/g sample
15
10
5
0
D D D D C C D D D A C B A D
Tiara
Scarlette
US Red
China Black
Maize Morado
Heirloom Purple
Blue
Red
White
Sumac
Black
SC84
SC991
PI570481
Grain Sample


Naringenin
Rice
Corn
Sorghum
µg/g sample
20
15
10
5
0
F F F F C E F F F A D D B F
Tiara
Scarlette
US Red
China Black
Maize Morado
Heirloom Purple
Red
Blue
White
Sumac
Black
SC84
SC991
PI570481
Grain Sample

(H)


Quercetin
Rice
Corn
Sorghum
µg/g sample
15
10
5
0
B
D D
A
D D D D D
C
D
C
B C
D
Tiara
Scarlette
US Red
China Black
Maize Morado
Heirloom Purple
Blue
Red
White
Sumac
Black
SC84
SC991
PI570481
Grain Sample


Rutin
Rice
Corn
Sorghum
µg/g sample
60
40
20
0
C C C C
A
B
C C C
C C C C C
Tiara
Scarlette
US Red
China Black
Maize Morado
Heirloom Purple
Blue
Red
White
Sumac
Black
SC84
SC991
PI570481
Grain Sample


Taxifolin
Rice
Corn
Sorghum
µg/g sample
500
400
300
200
100
0
D D D D
D D D D D
A
D
C
B
D
Tiara
Scarlette
US Red
China Black
Maize Morado
Heirloom Purple
Blue
Red
White
Sumac
Black
SC84
SC991
PI570481
Grain Sample

(I)

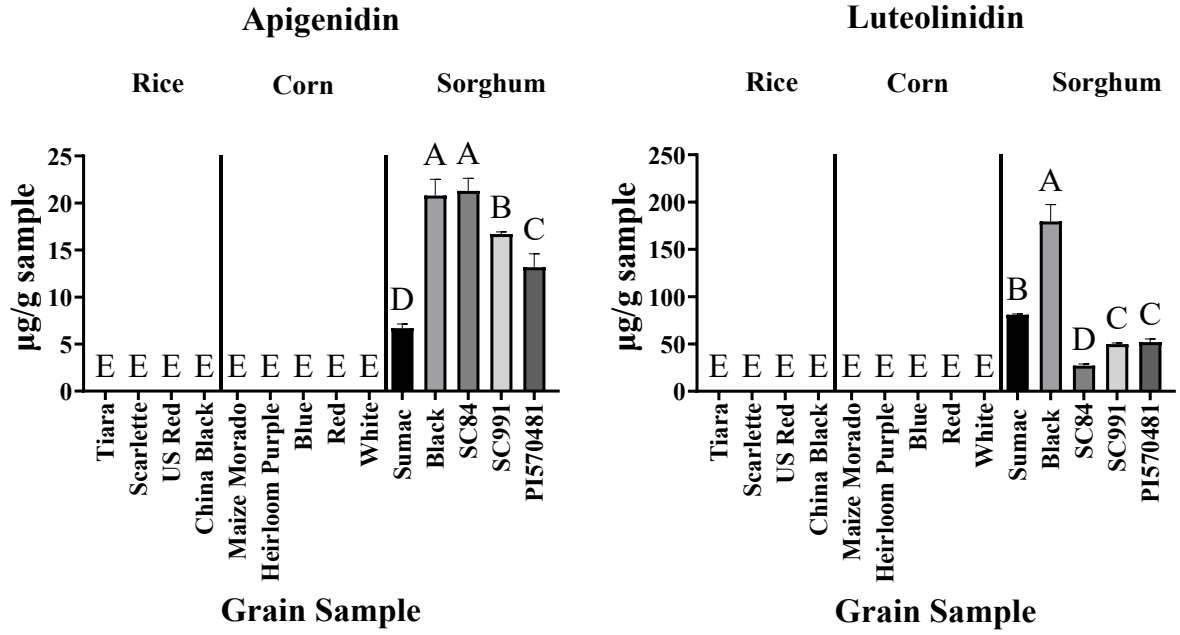


Figure 2.5. The RP-HPLC results of rice, corn, and sorghum grain samples for anthocyanin and anthocyanidin (A), cinnamic acids (B), benzoic acids (C), flavone (D), flavanol (E), flavonone (F), flavononol (G), and 3-deoxy anthocyanidin content (I).

Results represent mean values of at least three experiments. Values are expressed as µg/g sample. Samples that do not share a letter are statistically significant (p<0.05).

The current study was able to detect three anthocyanin compounds, of which, none were detectable in sorghum. The first anthocyanin, cyanidin, was not detected in rice samples or in the white corn sample. The average cyanidin content for the other corn samples ranged from 35.8 ug/g (Blue) to 85.5 ug/g (Maize Morado). The second compounds, 3,5-diglucoside, was detected in two rice samples, Tiara (138.86 ug/g) and China Black (432.67 ug/g). The cyanidin 3,5-diglucoside in corn ranged from 41.6 ug/g (Blue) to 489.48 ug/g (Maize Morado) and was not detected in the white corn sample. The last anthocyanin compound, pelargonidin 3,5-diglucoside, was only detected in Maize Morado (237.92 ug/g) and Heirloom Purple (183.71 ug/g) corn samples.

Caffeic acid, a member of the cinnamic acid group, was not detected in any of the rice samples. In corn, the caffeic acid content ranged from 5.6 ug/g (Blue & White) to 7.7 ug/g (Maize Morado). In sorghum, the average caffeic acid content ranged from 7.1 ug/g (Black) to 7.8 ug/g (Sumac). Chlorogenic acid, also a member of the cinnamic acid group, was only detected in sorghum apart from white corn. The chlorogenic acid of white corn was 28.1 ug/g. The chlorogenic acid content of sorghum ranged from 13.3 ug/g (SC991) to 17.7 ug/g (Sumac). Ferulic acid was detected in Scarlette (5.82 ug/g), US Red (5.66 ug/g), and China Black (6.33 ug/g). Of the corn samples, ferulic acid was only detected in white corn where the concentration was 7.1 ug/g. Ferulic acid was detected in all the sorghum samples and ranged from 5.8 ug/g (PI570481) to 10.8 ug/g (Sumac). Low concentrations of p-coumaric acid were detected in Tiara (2.5 ug/g) and Scarlette (3.3 ug/g). P-coumaric acid was not detected in corn samples. In sorghum, p-coumaric acid ranged from 1.1 ug/g (Black) to 12.6 ug/g (Sumac).

Protocatechuic acid, a member of the benzoic acid family, ranged from 7.3 ug/g (US Red) to 57.8 ug/g (China Black) in rice samples. For corn samples, protocatechuic acid ranged from 5.5 ug/g (Blue) to 22.3 ug/g (Heirloom Purple) and was not detected in White corn. Protocatechuic acid in sorghum ranged from 6.3 ug/g (SC991) to 11.1 ug/g (Black). The other benzoic acid, 4-hydroxybenzoic acid, was only detected in sorghum samples where it ranged from 2.9 ug/g (PI570481) to 36.8 ug/g (Sumac).

The flavone, apigenin, was only detected in rice and sorghum. The apigenin content of rice ranged from 4.3 ug/g (Scarlette) to 6.3 ug/g (China Black) in. The apigenin content in sorghum ranged from 4.7 ug/g (Sumac) to 8.0 ug/g (SC84). The other flavone, luteolin, was only detected in the sorghum samples where it ranged from 1.2 µg/g (SC84) to 19.3 ug/g (Black).

For the rice samples, catechin was only detected in the Scarlette sample (2.6 ug/g). Catechin was not detected in any of the corn samples. In sorghum, catechin ranged from 359.4 ug/g (Sumac) to 1,600.6 ug/g (SC84).

Eriodyctiol was not detected in rice samples. Of the corn samples, eriodyctiol was only detected in Maize Morado (6.6 ug/g) and Heirloom Purple (6.2 ug/g). Eriodyctiol was not detected in the sorghum samples. Naringenin was not detected in rice. In corn, naringenin was only detected in Maize Morado (7.7 ug/g) and Heirloom Purple (4.8 ug/g). Naringenin was detectable in all sorghum samples except PI570481. In sorghum, naringenin ranged from 5.7 ug/g (Black) to 16.0 ug/g (Sumac).

In rice samples quercetin was only detectable in the Tiara and China Black samples at 7.9 ug/g and 12.1 ug/g, respectively. In sorghum quercetin content ranged from 6.7 ug/g (Sumac & SC 84) to 7.2 ug/g (SC991). Rutin was not detected in rice or sorghum. In corn, rutin was detected in Maize Morado (50.5 ug/g) and Heirloom Purple (13.2 ug/g).

Taxifolin was the only detectable flavanonol, where it was only detected in the Sumac (429.6 ug/g), SC84 (297.3 ug/g), and the SC991 (402.5 ug/g) sorghum samples.

Apigenidin is a 3-deoxyanthocyanidin (3-DA), which is reported to be unique to sorghum grains. Apigenidin was not detected in the rice or corn samples. In sorghum, the apigenidin content ranged from 6.7 ug/g (Sumac) to 21.3 ug/g (SC84). Luteolinidin, also a 3-DA, was not detected in rice or corn samples. The luteolinidin content of sorghum ranged from 27.0 ug/g (SC84) to 179.5 ug/g (Black). 7-methoxyapigenidn, also a 3-DA was only detectable in Black sorghum (55.1 ug/g).

2.3.7 NP-HPLC

The normal phase high pressure liquid chromatography (RP-HPLC) results of rice, corn and sorghum flours are represented in **Table 2.5**

Table 2.5 Degree of polymerization of rice, corn, and sorghum samples

Grain	Variety	Total PA (µg/g)			DPi			%Polymer		
Rice	***242***	0.360 ± 0.0	DE		5.847 ± 0.2	AC		25.823 ± 2.0	BD	
	MNSN	0.348 ± 0.0	DE		6.592 ± 0.0	AC		34.835 ± 0.3	AD	
	Tiara	0.027 ± 0.0	E		3.108 ± 0.5	C		19.897 ± 5.2	CD	
	Scarlet	0.190 ± 0.0	E		6.332 ± 0.1	AC		29.650 ± 3.6	BD	
	IAC600	0.041 ± 0.0	E		7.128 ± 0.1	AB		66.083 ± 1.3	AB	
	China	0.028 ± 0.0	E		4.602 ± 0.7	BC		34.096 ± 6.6	AD	
Corn	***Maize Morado***	0.196 ± 0.0	E		6.913 ± 4.0	AC		54.106 ± 44.9	AD	
	Heirloom Purple	0.135 ± 0.0	E		8.721 ± 0.1	A		76.170 ± 1.6	A	
	Red	0.024 ± 0.0	E		6.283 ± 1.4	AC		52.825 ± 13.7	AD	
	Yellow	0.046 ± 0.0	E		6.373 ± 2.5	AC		53.726 ± 25.2	AD	
Sorghum	***Black***	1.082 ± 0.1	C		7.845 ± 0.2	AB		50.088 ± 2.2	AD	
	Sumac	3.148 ± 0.4	B		6.014 ± 0.3	AC		34.130 ± 3.5	AD	
	SC84	6.887 ± 0.3	A		4.858 ± 0.2	AC		24.946 ± 1.4	BD	
	SC991	3.170 ± 0.5	B		3.919 ± 0.8	BC		14.541 ± 11.2	D	
	PI570481	0.836 ± 0.1	CD		8.741 ± 0.0	A		61.438 ± 2.2	ABC	

Total PA = proanthocyanidin content expressed as µg/g sample. Sample means that do not share a letter within the same column are significantly different ($p<0.05$).

The total proanthocyanidin content of rice samples ranged from 0.028 (China) to 0.360 (242). In corn, the total proanthocyanidin amount ranged from 0.024 (Red) to 0.196 (Maize Morado). The total proanthocyanidin content in sorghum ranged from 0.836 (PI570481) to 6.887 (SC84). The DPI in rice ranged from 3.108 (Tiara) to 7.128 (IAC600). In corn, DPI ranged from 6.283 (Red) to 8.721(Heirloom Purple). Lastly, DPI in sorghum ranged from 3.919 (SC991) to 8.741 (PI570481). The %P is the percentage of polymers with DP>10 present in the samples. In rice, the sample with the lowest %P value was Tiara and the sample with the highest %P was IAC 600 (19.9% and 66.03%, respectively). For corn samples, the %P ranged from 52.83%

(Red) to 76.17% (Heirloom Purple). The %P in sorghum samples ranged from 14.45% (SC991) to 61.44% (PI570481).

2.3.8 Glycation inhibition

The samples highest in TPC and AA were chosen for glycation inhibition analysis. The results from the glycation inhibition analysis are represented in **Figure 2.6.**

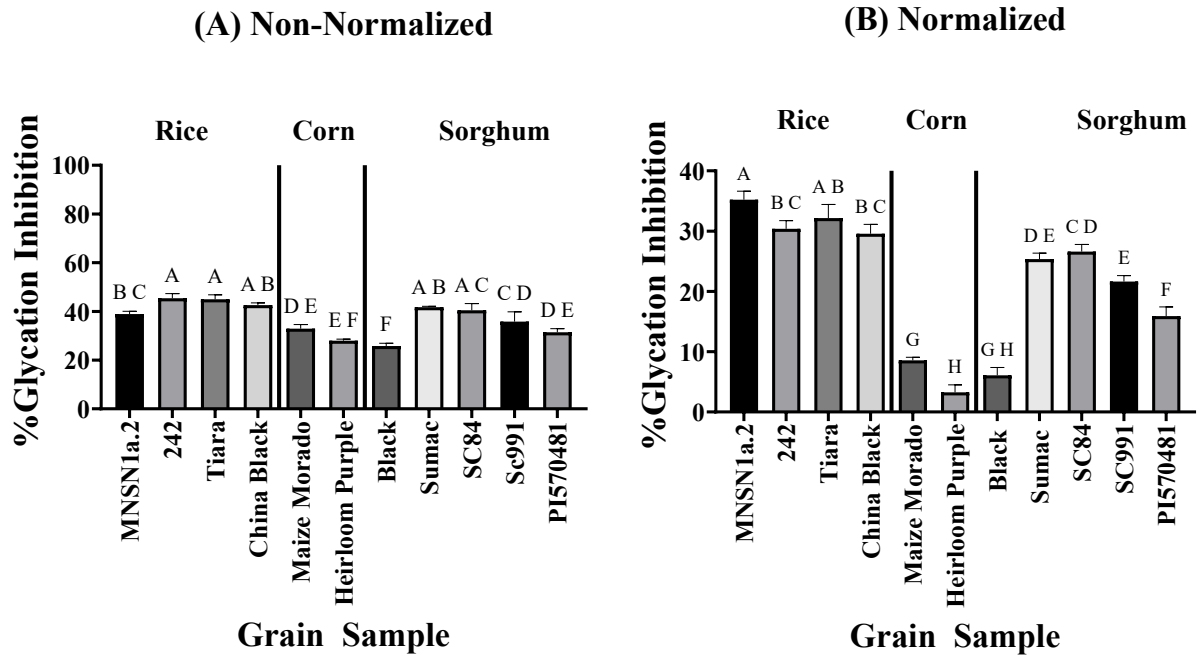


Figure 2.6 Glycation inhibition of (A) Non-normalized and (B) normalized extracts of rice, corn, and sorghum samples.

Samples were extracted uniformly and analyzed as is. However, since some inhibition assays are concentration dependent, samples were then "normalized" to the same phenolic concentration (0.5 mg GAE/g) to examine the effect of polyphenol blends rather than polyphenol concentration. The as is or "non-normalized" samples are presented in **Figure 2.6A.** In rice samples, the average glycation inhibition ranged from 38.95% (MNSN1a.2) to 45.43% (242). The 242, Tiara, and China Black rice varieties were not significantly different than Sumac, and SC84 sorghum. Corn, which had the lowest glycation inhibition in comparison to rice and

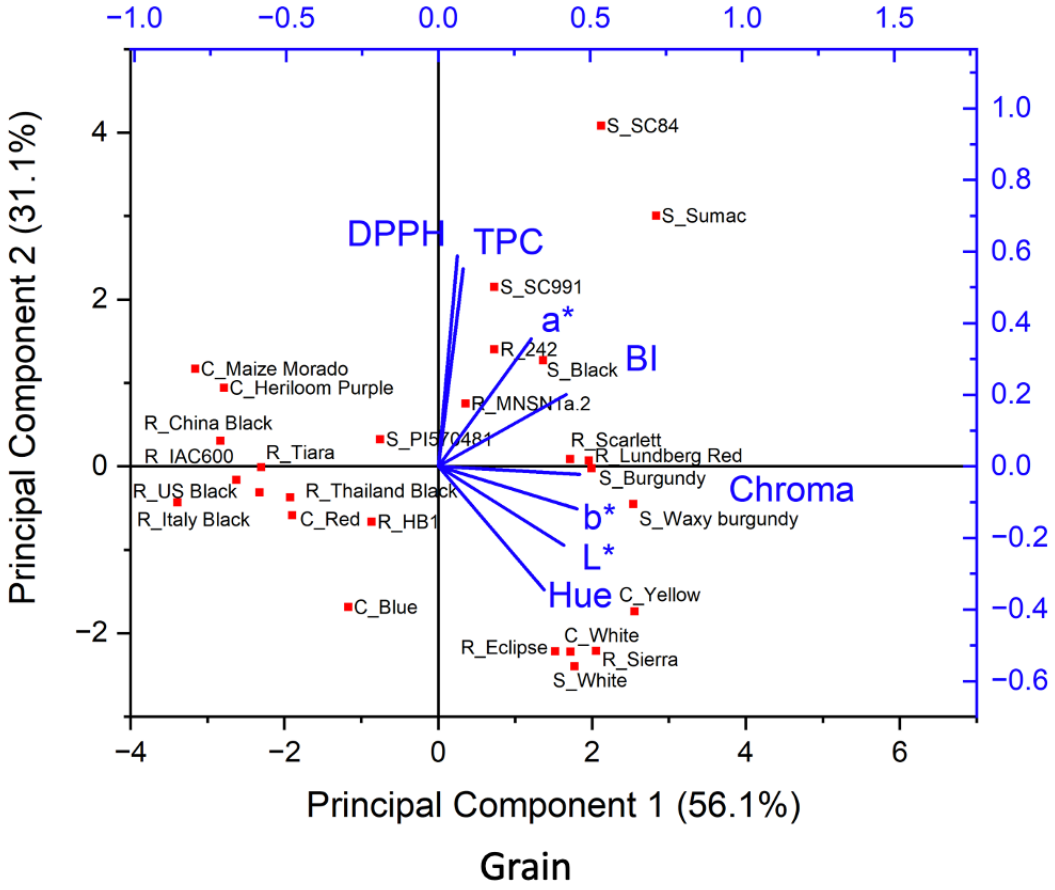
sorghum. The average glycation inhibition of sorghum ranged from 25.82% (black) to 41.74 (Sumac).

The glycation inhibition of normalized rice samples (**Figure 2.6.B**) ranged from 29.58% (China Black) to 35.21% (MNSN1a.2). The glycation inhibition of normalized corn samples ranged from 3.27% (Heirloom Purple) to 8.57% (Maize Morado). The glycation inhibition of normalized sorghum samples ranged from 6.08% (Black) to 26.61% (SC84). The average glycation inhibition of rice (31.84%) was significantly ($p<0.05$) higher than the glycation inhibition of corn (5.92%) and sorghum (19.12%).

2.3.9 Principal component analysis and Pearson's correlations

The quality attributes of grain and flour sample are influenced by their interrelationships to total phenolic content, antioxidant capacity, browning index, and color attributes. **Figure 2.7** represents the principal component analysis (PCA) results for both the grain and flour samples in relationship to each of the 8 other attributes examined in this study

A)


−1.0
−0.5
0.0
0.5
1.0
1.5
4
2
0
−2
−4
−2
0
2
4
6
1.0
0.8
0.6
0.4
0.2
0.0
−0.2
−0.4
−0.6
Principal Component 2 (31.1%)
Principal Component 1 (56.1%)
DPPH
TPC
a*
BI
Chroma
b*
L*
Hue
S_SC84
S_Sumac
S_SC991
R_242
S_Black
R_MNSN1a.2
S_PI570481
R_Scarlett
R_Lundberg Red
S_Burgundy
S_Waxy burgundy
C_Yellow
C_White
R_Sierra
S_White
R_Eclipse
C_Blue
C_Maize Morado
C_Heriloom Purple
R_China Black
R_IAC600
R_Tiara
R_US Black
R_Thailand Black
R_Italy Black
C_Red
R_HB1

Grain

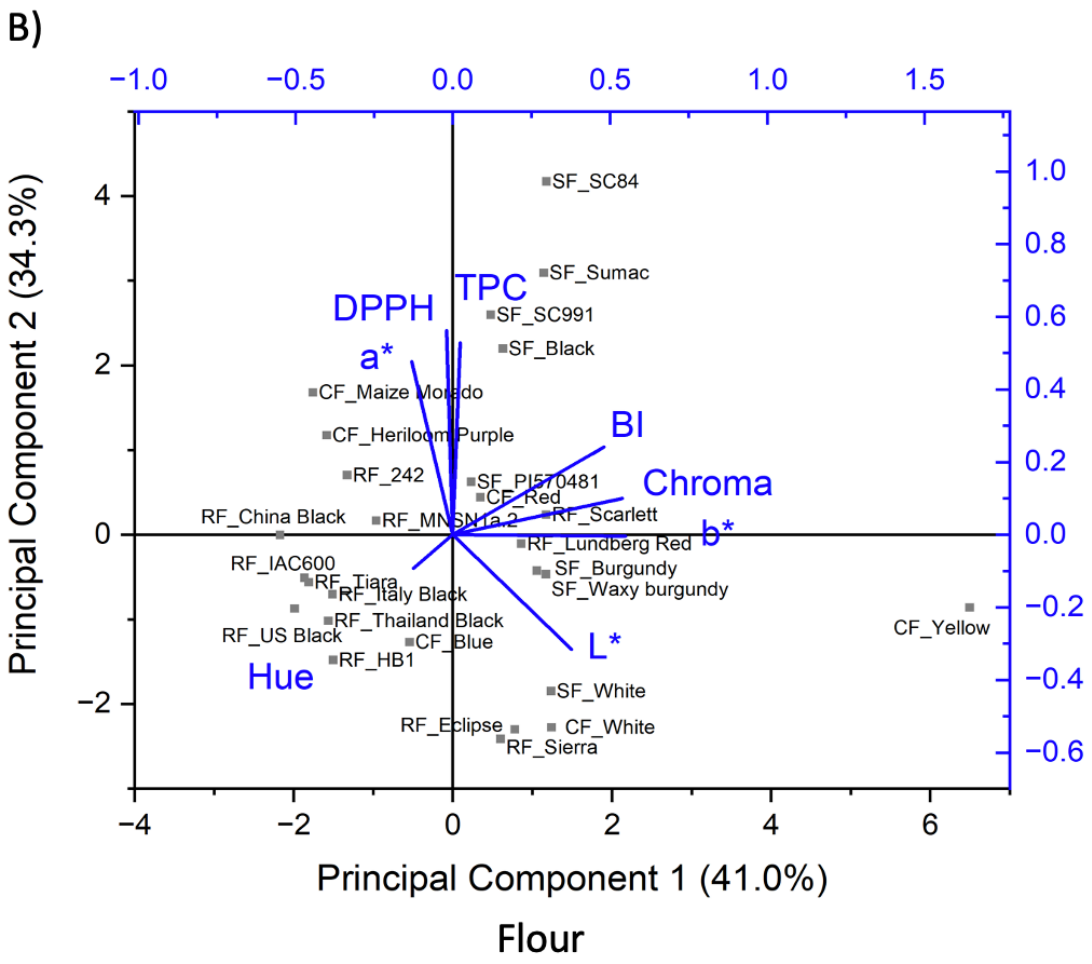


Figure 2.7 PCA plots of rice, corn, and sorghum samples.

In **Figure 2.7.A**, two principal components from the 8 dependent variables were identified through multivariate analysis in whole grain samples. The new variable was principal component 1 (PC1) and principal component 2 (PC2). For the grains, around 87% of the data can be accounted for in PC1 and PC2 where PC1 was 56.1% while PC 2 was 31.1%.

In **Figure 2.7.B** The new PC1 and PC 2 are determined with the 8 dependent variables and the flour samples. In a bi-rotated space orthogonal to PC1 and PC2, more than 75% of the dataset's variability can be accounted for. Specifically, PC1 explains 41.0% of the variability, while PC2 accounts for 34.3%.

Pearson's coefficient correlation results of rice, corn, and sorghum grain and flour are represented in **Figure 2.8.**

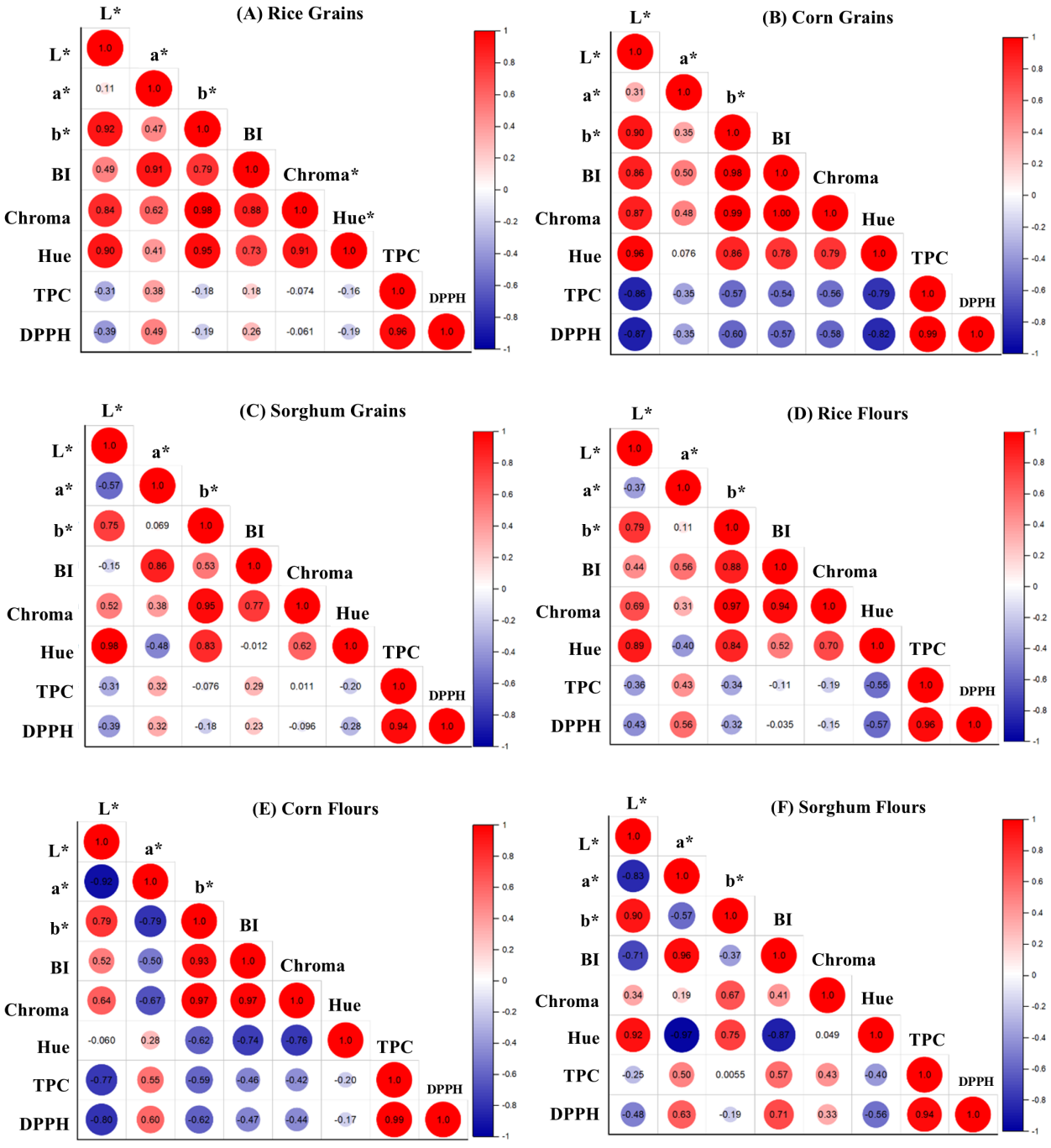


Figure 2.8 Pearson's correlation of rice, corn, and sorghum samples.

Rice, corn, and sorghum sample results were combined for each attribute to evaluate the correlation of attribute to another. The rice grain (**Figure 2.8A**) neither *L*, a*, b*,* hue, or

chroma were strongly correlated to TPC or DPPH in rice grains. The *L** value of corn grain (**Figure 2.8B**) had strong negative correlations to both TPC and DPPH. The *a** value of corn grains did not have strong positive or negative correlations with any one attribute. The hue of corn grains had strong negative correlations to both TPC and DPPH. In sorghum grains (**Figure 2.8C**) neither *L*, a*, b*,* hue, or chroma had strong positive or negative correlations to TPC or DPPH.

In rice flour (**Figure 2.8D**) none of the attributes related to color (*L*, a*, b*,* hue, or chroma) were strongly correlated to TPC or DPPH in rice flours. The *L** values of corn flour (**Figure 2.8E**) had strong negative correlations to TPC and DPPH while *a** values showed moderately positive correlations to TPC and DPPH. The other attributes (BI*,* hue, and chroma) did not have strong positive or negative correlations to TPC or DPPH. In sorghum flour (**Figure 2.8F**) *L** values had neither strong positive nor negative correlations to TPC and DPPH. However, the *a** value and BI had strong positive correlations to TPC and DPPH. TPC and DPPH were highly correlated to one another (Pearson's r value greater than 0.94) for all rice, corn, and sorghum grains and flours.

2.4 Discussion

2.4.1 Proximate analysis

The proximate analysis of rice, corn, and sorghum where similar to previously reported proximate analysis profiles of other pigmented rice (Petroni et al., 2017), corn (Libron et al., 2021), and sorghum grains (Ape David et al., 2016). The moisture content of all grains were close to the recommended 12% moisture level (wet basis) in order to achieve safe storage conditions.

2.4.2 Color analysis

There were detectable differences in color among the grain samples, as was to be expected. There is currently a misconception that pigmentation correlates well to TPC and AA. Based on the result of our study, no color indicators (*L*, a*, b*, hue or chroma)* had a clear direct correlation to TPC or AA. This was not in agreement with (Choi et al., 2019) who reported that *a** was highly correlated to TPC in sorghum. It is therefore important to be mindful when selecting a grain for future phenolic studies to select a grain high in polyphenols, rather than selecting a grain based on color alone.

2.4.3 Total phenolic content

Sorghum was the highest in TPC which is line with other literature reviews comparing the TPC of sorghum to other cereal grains (Van Hung, 2016). To our knowledge this is the first time in literature that whole grain rice, corn and sorghum samples have been evaluated in one study for their total phenolic content, condensed tannin content, and glycation inhibition.

2.4.4 Condensed tannin content

Using the vanillin-HCl method was not effective to detect condensed tannin contents in rice or corn. However, condensed tannins, also called proanthocyanidins, have been reported in rice grains (Goufo and Trindade, 2014) and corn (Lu et al., 2023). The most likely reason that no condensed tannins (proanthocyanidins) were detected in rice or corn samples via the modified vanillin-HCl method is because this method was developed specifically for sorghum condensed tannins. In addition, the spectrophotometric based assay may not be sensitive enough to detect the amounts of CT in rice and corn samples. For this reason, HPLC was utilized since it has greater sensitivity and may be able to detect condensed tannins in smaller quantities.

2.4.7 Antioxidant activity

Sorghum had the highest antioxidant activity, which is line with other review articles (Awika and Rooney, 2004). However, it should also be noted that TPC and DPPH are both spectrophotometric techniques based on a redox reaction. They will therefore have high correlations with one another.

2.4.5 RP-HPLC

It is reported that 3-deoxyanthcyanidins are present in purple corn (Sweeny and Iacobucci, 1983). However, there seems to be limited recent literature suggesting corn contains 3-deoxyanthocyanidins. In our study, the detectable 3-DA were unique to sorghum. Additionally, rice and corn contained cyanic compounds that sorghum did not. In order to positively say if 3-DAs are unique to sorghum in comparison to other grains, more research is needed to see if other varieties of purple corn contain 3-DAs.

2.4.6 NP-HPLC

Normal phase HPLC was implemented to detect the presence of condensed tannins in rice, corn, and sorghum grains. If condensed tannins are present, they will show fluorescence at an excitation peak of 230 nm and emission peak 320 nm (Langer et al., 2011). Both rice and corn showed fluorescence, indicating small quantities of condensed tannins were present in comparison to sorghum. This result could be due to the preparation method (70% v/v acetone) and may need to be altered in order to better detect rice and corn condensed tannins. The calculated DPI of sorghum was in line with other previously published works. A previous publication (Kaufman et al., 2009) mentioned that the actual DP of sorghum polyphenols ranges from DP=1 to around DP=22.

2.4.8 Glycation inhibition

From our studies, it is important to first select a grain high in polyphenol content to observe proper glycation inhibition effects. Corn was consistently low in glycation inhibition, most likely due to the decreased amount of TPC. However, this hypothesis is not supported when reviewing the rice data. Rice had fewer TPC than sorghum yet showed similar glycation inhibition amounts. This suggests that glycation inhibition, and potentially other in vitro bioactivity assay, could be dependent on the blend of phenolics rather than the concentration. This is further supported by the normalization of the samples. Once the phenolic content was equalized (0.5mg GAE/ sample) rice showed the greatest glycation inhibition. If the glycation inhibition were dependent on concentration, all samples would have shown the same glycation inhibition levels.

2.4.9 Principal component analysis and Pearson's correlations

Overall, the principal component analysis showed that *L*, a*, b**, hue, and chroma were highly correlated with one another. The red value (*a**) was highly correlated with browning index. In performing these analyses, no definitive trend between color, TPC, and DPPH appeared. This further justifies the hypothesis that color alone is not always an accurate indicator for strong AA or high TPC levels in pigmented grains.

2.5 Conclusion

In conclusion, sorghum had higher TPC, CTC, and AA of the grains sampled in this study. In spite of sorghum having higher TPC and CTC, rice had comparable glycation inhibition to sorghum before normalization. After normalization, rice was significantly higher in glycation inhibition in comparison to the other grains. The anthocyanin compounds were unique to rice and corn and not detectable in sorghum. Furthermore, sorghum was the only detectable source

for 3-DAs. Additionally, it is important to select a grain based on its measurable total phenolic content or other quantitative parameter instead of color alone, as color is not a strong indicator for TPC or AA.

Chapter 3 - Impact of heat and high-moisture pH treatments on starch digestibility, phenolic composition, and cell bioactivity in sorghum (*Sorghum bicolor* L. Moench) flour

3.1 Introduction

Sorghum (*Sorghum bicolor* L. Moench) is a commonly grown dryland cereal crop originating from Africa (Teferra, 2019). Sorghum is considered an agronomically advantageous crop for its drought tolerance and adaptability to grow in a varied of soil conditions and altitudes (Rashwan et al., 2021). According to a recent report from the Foreign Agricultural Service (FAS), the United States is the top producer of sorghum grain in the world. In 2023, the US produced 8,175 metric tons, ranking sorghum the 3rd most produced cereal grain in the country. In the US, Kansas and Texas produced 71% of the country's entire sorghum production (USDA-FAS, 2023). Sorghum is considered a food staple in many Asian and African countries, where it is used to make breads, porridges, and even fermented alcoholic beverages (O. Aluge et al., 2016). Sorghum contains many major nutrients, including starch, protein, lipids, minerals, and vitamins. Additionally, sorghum is high in fiber, gluten free, and is a non-genetically modified organism (Hassani et al., 2014). Despite its nutritional benefits, little sorghum grown in the US is used for human consumption.

Despite the underutilization of sorghum, sorghum grain has gained recent attention for containing health-promoting compounds called polyphenols. Polyphenols are secondary plant metabolites mostly found in fruits, vegetables, and grains. Polyphenols consist of multiple phenol units and contain many different families of phenolic compounds defined by differences in their structure. Detected families of phenolic compounds reported in sorghum include phenolic acids, flavonoids, and condensed tannins. Polyphenols found in sorghum are reported to have many health benefits. One health benefit of importance is its anticancer property. For example, ethanolic sorghum bran extracts have shown antiproliferative properties in human adenocarcinoma and liver cancer cells (Cox et al., 2019), human colon cancer cells (Lee et al., 2020, Lee et al., 2021), and in colon cancer-induced mice models (Lee et al., 2021). Moreover, ethanolic extracts from whole sorghum flour have also shown effective cell growth inhibition in human hepatocarcinoma and colorectal cancer cells (Chen et al., 2021). In addition to the anticancer properties that have been demonstrated in sorghum derived polyphenols, they may also serve as natural alternatives for some synthetic compounds used in food manufacturing.

Other uses of sorghum derived polyphenols include serving as a natural source of antioxidants, food colorants, and dyes. For example, 3-deoxyanthocyanidins (3-DAs) have gained attention in the food industry as natural colorants and antioxidants, especially given their improved stability during processing, when compared to anthocyanins (Xiong et al., 2019b). They are water-soluble pigments responsible for either orange-red or blue-violet pigmentation in plants. During food processing, however, there are many factors that might affect the amount, structure, stability, and bioactivity of polyphenols found in sorghum. For example, defatting and deproteinating sorghum flour has been shown to reduce antioxidant activity (Irondi et al., 2022).

If sorghum is to be fully utilized for its health benefits and agronomical advantages, it is imperative to understand the effects of processing on polyphenols and other nutritional components of high polyphenol-containing sorghum. Understanding the effect of processing on sorghum polyphenol composition may increase its potential for value-added or functional food formulations. The objective of this study was to determine the effects of heating time and pH on high polyphenol sorghum lines for starch digestibility, phenolic profile, and cell bioactivity in a high-moisture environment.

3.2 Materials and Methods

3.2.1 Materials

Grain samples from high phenolic, high tannin brown sorghum grain (SC84) (Rhodes et al., 2017) were used in this study. Grain samples were grown in the winter nursery at Puerto Vallarta, Mexico, in 2018–2019 by the sorghum breeding program, Kansas State University, Agricultural Research Center (Hays, Kansas, United States). The reagents, vanillin and Folin–Ciocalteu (FC), were purchased from Sigma-Aldrich (Milwaukee Wisconsin, United States). Absolute ethanol (grade USP), hydrochloric acid (HCl), Trolox, potassium phosphate monobasic and dibasic, gallic acid, and anhydrous sodium bicarbonate were purchased from Thermo Fischer Scientific (Waltham, Massachusetts, United States). ACS reagent grade absolute methanol was purchased from Ricca Chemical Company (Arlington, Texas, United States).

3.2.2 Sample Preparation

Whole sorghum kernels were ground through a 0.5mm screen using a UDY Mill (UDY Corporation Cyclone Sample Mill, Fort Collins, CO, United States). Sorghum flour (1g) was combined with 0.02M sodium phosphate buffer solutions (20 mL) at pH 3, 4, 5, 7, and 8. Samples were placed in a water bath at 100°C and heated for 0, 10, 30, 60, or 120min. Unheated

samples (0min) were used as a control. After the heating process, the entire samples (liquid and solid portions) were freeze-dried for 48h and stored at −80°C until further use.

3.2.3 Starch Digestibility

Digestible and resistant starch was measured using the K-DSTRS kit from Megazyme (Megazyme LTD, Ireland). Flour samples were incubated with continuous shaking in a sodium maleate buffer (50 mM, pH 6) with a mixture of pancreatic α-amylase and amyloglucosidase for 4 h at 37°C. Aliquots were removed at 240 min for digestible starch (DS) and resistant starch (RS). To measure DS, a 1 mL aliquot was added to 20 mL of 50 mM acetic acid in order to terminate the reaction. Then, 0.1 mL aliquots were incubated with 0.1 mL of amyloglucosidase (100U/mL) at 50°C for 30 min. A final incubation with GOPOD reagent was conducted at 50°C for 20 min before absorbance was measured at 510 nm. To measure RS, a 4 mL aliquot was removed and added to 4 mL of ethanol (95% v/v). The samples were centrifuged at 4,000 rpm for 10 min. and the supernatant was discarded. The pellet was washed twice with aqueous ethanol (50% v/v) and then suspended in 2 mL sodium hydroxide (1.7M) to dissolve the resistant starch. Then, 8 mL of sodium acetate buffer (1M) was added to neutralize the solution. A portion (0.1 mL) of amyloglucosidase (3,300U/mL) was added before incubating for 30 min at 50°C. Aliquots (0.1 mL) were incubated with GOPOD reagent at 50°C for 20 min before absorbance was read at 510 nm. Total starch (TS) content was calculated according to Equation 1 and reported in “as is” basis.

Digestible Starch + Resistant Starch = Total Starch Content (5)

3.2.4 Total Phenolic Content

One gram of flour samples was combined with 9mL ethanol (70% v/v) and placed on a shaker for 2h. After stirring, samples sat overnight (18h) at −20°C and were centrifuged for 10

min at 4°C at 2970×g the next day. Supernatants were collected and analyzed for total phenolic content (TPC) using the Folin–Ciocalteu (FC) method as described by Herald et al. (2012). Briefly, a standard curve of gallic acid ranging from 0 to 800 μg/L was used to quantify gallic acid equivalents (GAE) in mg per milliliter of sample. FC reagent was diluted in a 1:1 working ratio with Deionized (DI) water. Samples were diluted with 70% ethanol solvent to achieve acceptable absorbance values. The diluted sample (25 uL) was combined with 75μL of DI water and 25μL of FC reagent in a 96 well plate. After a brief 6 min incubation period, 100μL of 7.5% (w/v) Na2CO3 was added to the each well simultaneously using a 96 channel semi-automated pipettor (Sorenson Bioscience Inc., Radnor PA, United States). The samples were sealed with heat seal film and incubated in the dark for 90min. After the final incubation period, sample absorbance values were measured at 765nm using a Biotek synergy 2 multi-detection plate reader (Winooski, VT, United States). TPC was calculated based on the gallic acid standard curve and expressed as mg gallic acid equivalents (GAE) per g sample.

3.2.5 Condensed Tannin Content

The condensed tannin content (CTC) of the samples were determined with slight modification using a previously published method (Herald et al., 2014). Briefly, 0.1 g of sample was extracted for 20 min in 1mL 1%HCl-methanol at 20°C. Samples were centrifuged at 805×g for 6min and decanted. Aliquots were used for analysis and diluted (if needed) in 1%HCl-Methanol. Catechin standard was prepared by dissolving catechin in 100% (v/v) methanol at a concentration of 10mg/mL. The calibration curve was diluted in range of 0–5.0mg/ mL catechin in 1%HCl-methanol. Both standards and samples were added (30μL) to their corresponding wells in a 96-well plate. Vanillin reagent (1% vanillin w/v and 8% HCl v/v) (150μL) and 4% HCl-methanol (150μL) were added to the well plate using a 96 channel semi-automated pipettor

(Sorenson Bioscience Inc., Radnor PA, United States). After incubating for 20min at 30°C, absorbance values were measured at 500nm using a Biotek synergy 2 multidetector plate reader (Winooski VT, United States). The absorbance of the blank (no vanillin reagent) was subtracted from the corresponding standard or sample with vanillin and plotted against the standard curve to calculate catechin equivalents (CE) in mg per g flour.

3.2.6 HPLC Analysis

Phenolic profiles of samples were determined using HPLC methods previously described by (Lee et al., 2022) adapted from Irakli et al. (2012) using a 1290 Infinity II (Agilent, Santa Clara, United States) HPLC coupled with a diode array detector.

A C18 column (Kinetex®, 2.6 μm, 100Å, 150 mm × 4.6 mm size, Phenomenex, Torrance, CA, United States) attached to a guard column (SecurityGuard™, Phenomenex, Torrance, CA, United States) was used for sample analysis. The mobile phases contained acetonitrile (A), methanol (B), and water-glacial acetic acid, 0.5% v/v (C). The elution gradient flowed at 1 mL/min at a temperature of 30°C and consisted of: initial composition of 5:5:90 (A:B:C), followed by 0–5 min of 8:8:84, 5–15 min of 10:10:80, 15–25 min of 25:0:75, 25–35 min of 30:0:70, 35–45 min of 60:0:40. To stabilize the base line for future runs, a post-time of 5 min with the initial gradient was used.

Selected samples heated for 0, 10, 30, and 60 min at pH 3, 5, and 8 were analyzed in triplicate. Samples were combined with ethanol (70% v/v) at a ratio of 1:10 (w:v) and centrifuged for 10 min at 4°C at 2,970×g. Supernatants were collected and dried at 60°C (Rocket Synergy 2 Evaporation System, Thermo Fisher, Pittsburgh, United States). The dried extracts were mixed with 10 mL methanol: ethanol (90,10, v/v) in an ultrasonic bath (ULTRAsonik, Simi

Valley, CA, United States) for 40 min. Samples were then passed through a 0.45 μm polyvinylidene difluoride (PVDF) syringe filter before injection.

Working solutions of phenolic standards were prepared in methanol: ethanol (90,10, v/v) for the calibration curves (9.00× 10−5 –0.09 mg/mL, R2≥0.99). The chromatograms at 280, 320, and 510 nm were selected. The standards eriodyctiol, protocatechuic acid, catechin, epicatechin, quercetin, luteolin, and naringenin were quantified at 280 nm. Apigenin, chlorogenic-, caffeic-, p-coumaric- and ferulic acids were quantified at 320 nm. Luteolinidin, apigenidin, and the 7-methoxyapigenidin were quantified at 510 nm. Target compounds were calculated based on standard curves and expressed as μg of target compound per g sample.

3.2.7 Cell bioactivity

To determine the cell bioactivity of sorghum phenolics, a cancer cell viability assay (MTS) was performed. Human colorectal carcinoma cells, lines HCT 116 and SW480, were grown in McCoy's media and RPMI 1640 media, containing 10% v/v fetal bovine serum (FBS) and 1X antibiotic antimycotic were added to both media. Cells were maintained at 37°C and 5% CO2.

HCT116 and SW480 Cells were plated at 5,000 and 10,000 cells/ well, respectively, in 96 well plate, and allowed to grow overnight before treatment. Cells were treated with 3 different concentrations of aqueous ethanolic extracts (20, 10, 5, mg/mL) with 70% ethanol serving as vehicle control. Cells were incubated for 48 h, and cell viability was measured using the MTS assay kit (Promega Cell Titer 96tm Aqueous One Solution Cell Proliferation Assay) from Fisher scientific (Pittsburg, PA, United States). In short, the media was removed from the wells and the plates were washed with PBS. Media (100 μL) containing MTS reagent at 20 μL/mL was added to each well and incubated at 37°C and 5% CO2 for 1 h (HCT116) and 1.5 h (SW480). The

plates were then read at 490 nm on a Biotek H4 Plate Reader (Winooski, VT, United States). Results are expressed as percent cell inhibition and were calculated according to Equations 2, 3.

$$\text{Cell viability (CV)} = (\text{Abs sample} - \text{Abs blank})/(\text{Abs vehicle control} - \text{Abs blank}) \quad (6)$$

$$\% \text{ Cell inhibition} = (1 - \text{CV}) \times 100 \quad (7)$$

3.2.8 Statistical Analysis

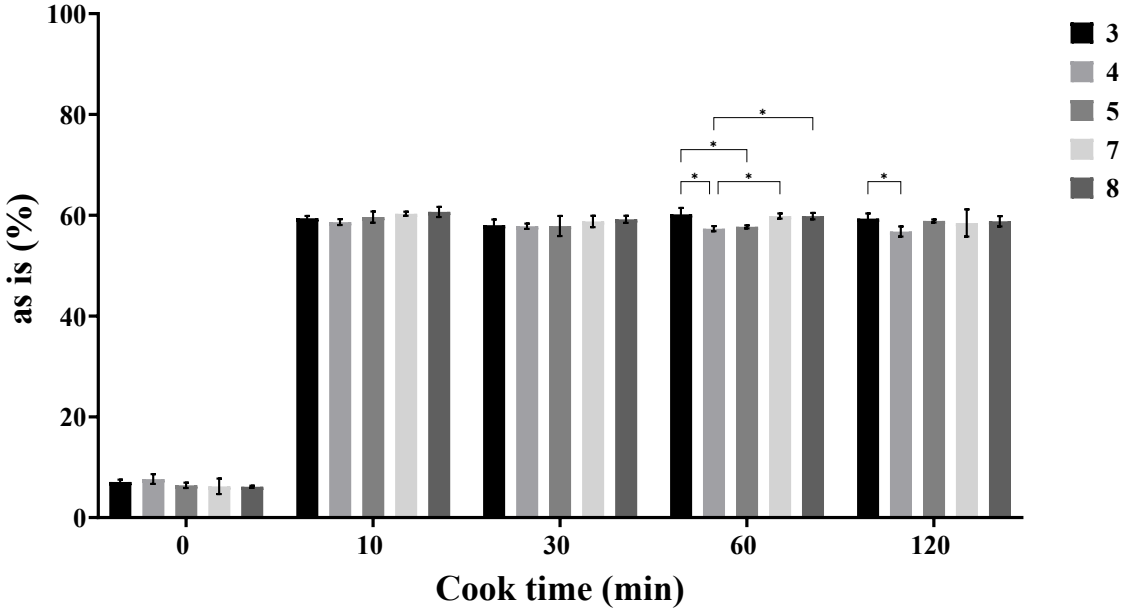
Data represents the averages of at least three independent experiments. Results were analyzed using 2-way analysis of variance (ANOVA) with Tukey's post hoc test using GraphPad prism version 9.4.0 (GraphPad, San Diego, CA). Mean values and standard deviations were reported with a level of significance at p-value less than 0.05. Statistical significance is represented by: *p≤0.5, **p≤0.1, ***p≤0.01, and ****p≤0.0.001. Pearson's correlation coefficient, or Pearson's r, was performed to examine the relationship of TPC and CTC on %Cell inhibition.

3.3 Results

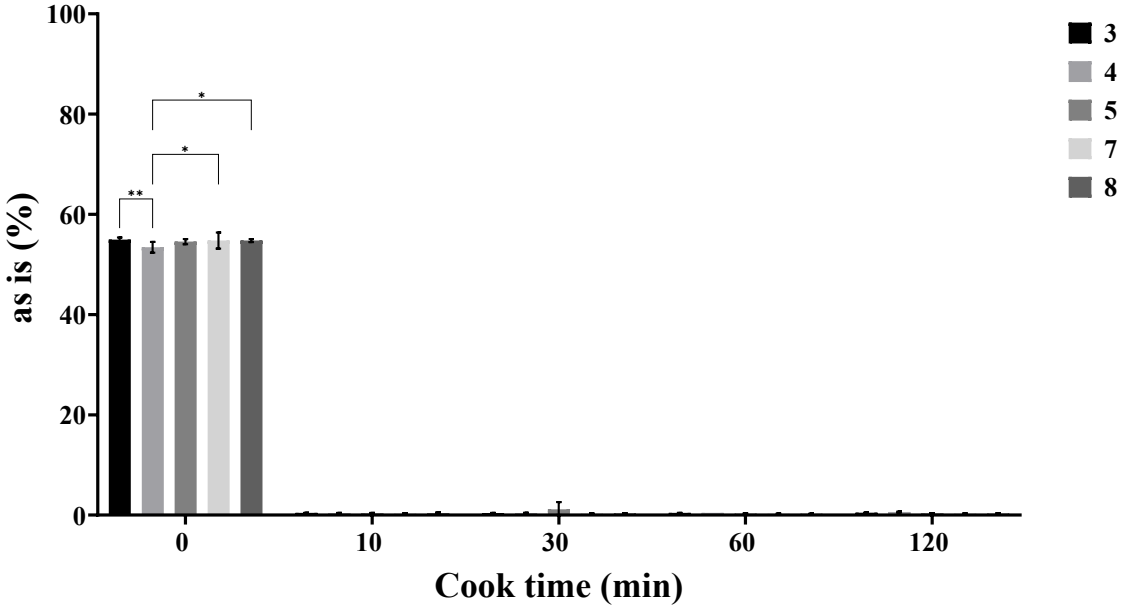
3.3.1 Starch Digestibility

The digestible starch significantly increased ($p < 0.05$) from approximately 7 g/100 g flour in unheated samples to 60 g/100 g flour after 10 min of heating for all pH treatments. Heating the samples for longer than 10 min did not significantly affect total starch digestibility, regardless of pH treatments. The total starch digestibility was only affected by pH after heating for 30min (Figure 1A).

(A) Digestible Starch



(B) Resistant Starch



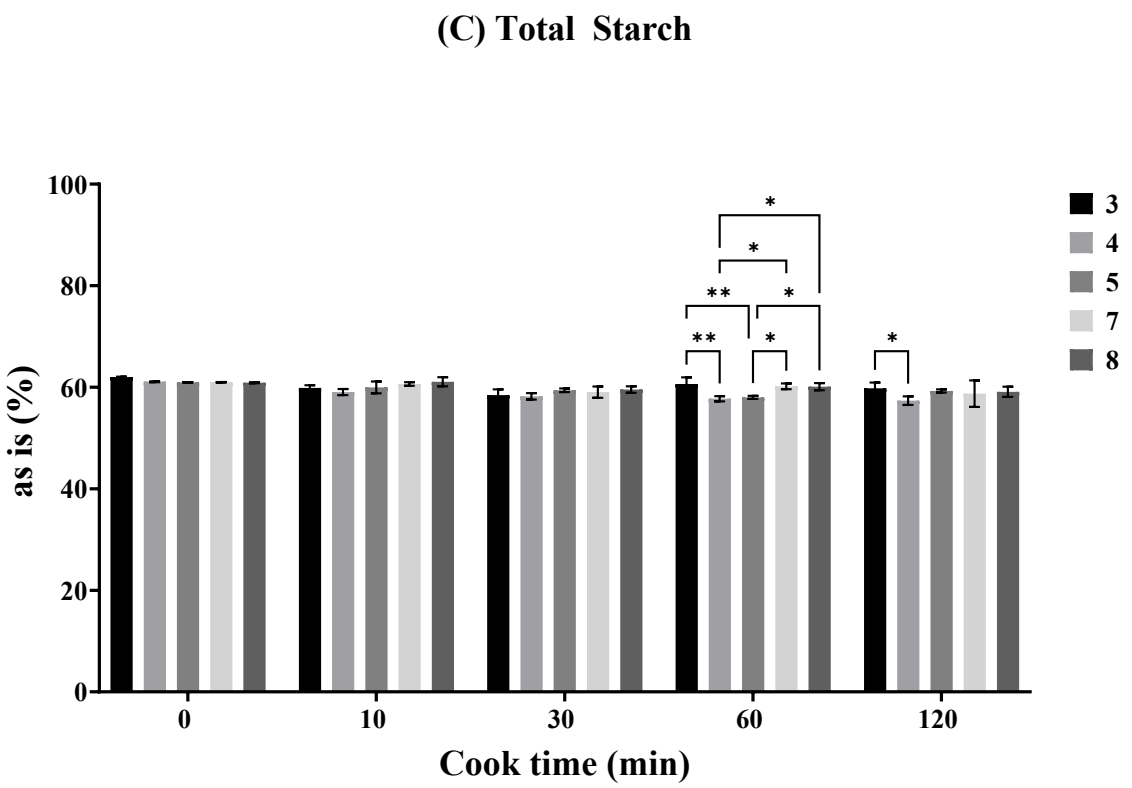


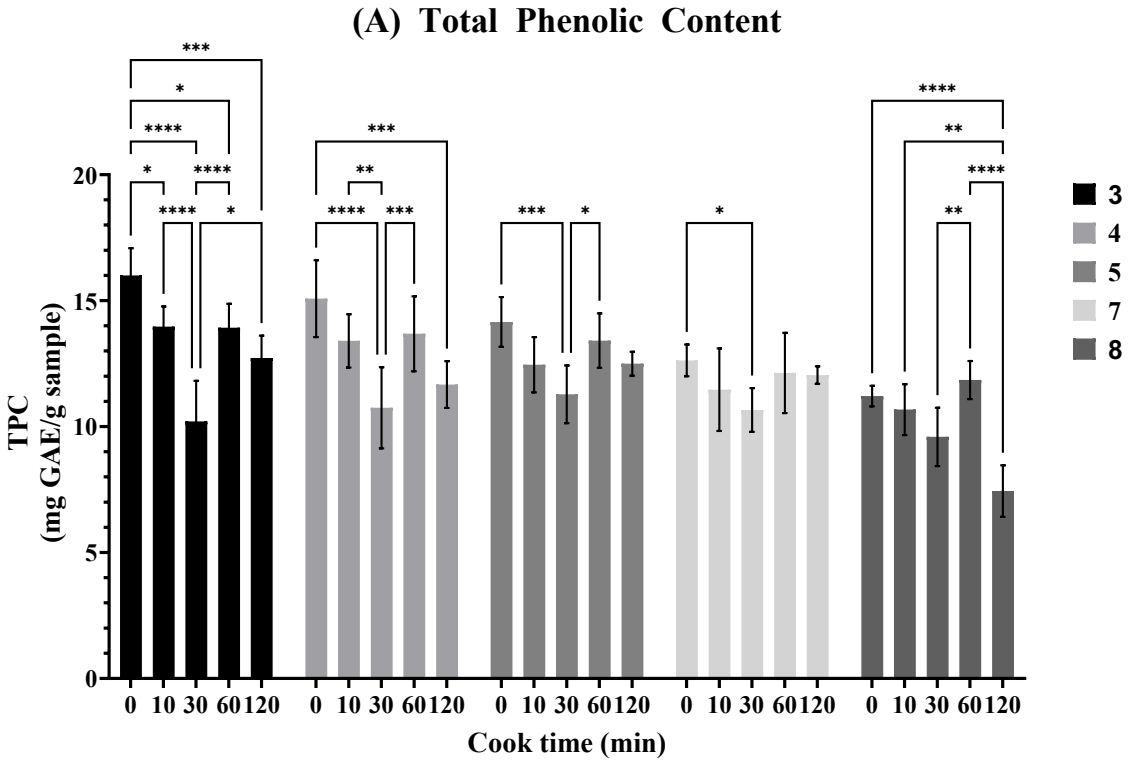
Figure 3.1 Overall Changes in starch. Digestible starch (A), resistant starch (B), and total starch (C) in sorghum flour calculated on a % as is basis (g/100g sample) after heat-moisture and pH treatments.

Resistant starch significantly decreased after heating for 10 min. Heating for longer than 10 min did not significantly affect resistant starch. Smaller, significant changes in resistant starch from the pH treatments were only detected in unheated samples (Figure 1B). The total starch (TS) was calculated from the digestible and resistant starch fractions. The TS was not significantly affected by heating, regardless of time. There were small, but significant, differences in total starch across pH groups for the 60 and 120 min time points (Figure 1C).

3.3.2 Total Phenolic Profile

The TPC of unheated samples ranged from 11.21 to 16.0mg GAE/g across pH treatments. Heating the samples for 10min did not significantly decrease TPC, except for pH 3. TPC decreased significantly after heating samples for 30min, with the exception of pH 8.

Surprisingly, after heating for 60 min, there was a significant increase in TPC when compared to 30 min for samples buffered at pH 3, 4, 5, and 8. Throughout the 120 min heating time, total phenolic content was most stable at pH 5 and 7 (Figure 2A).



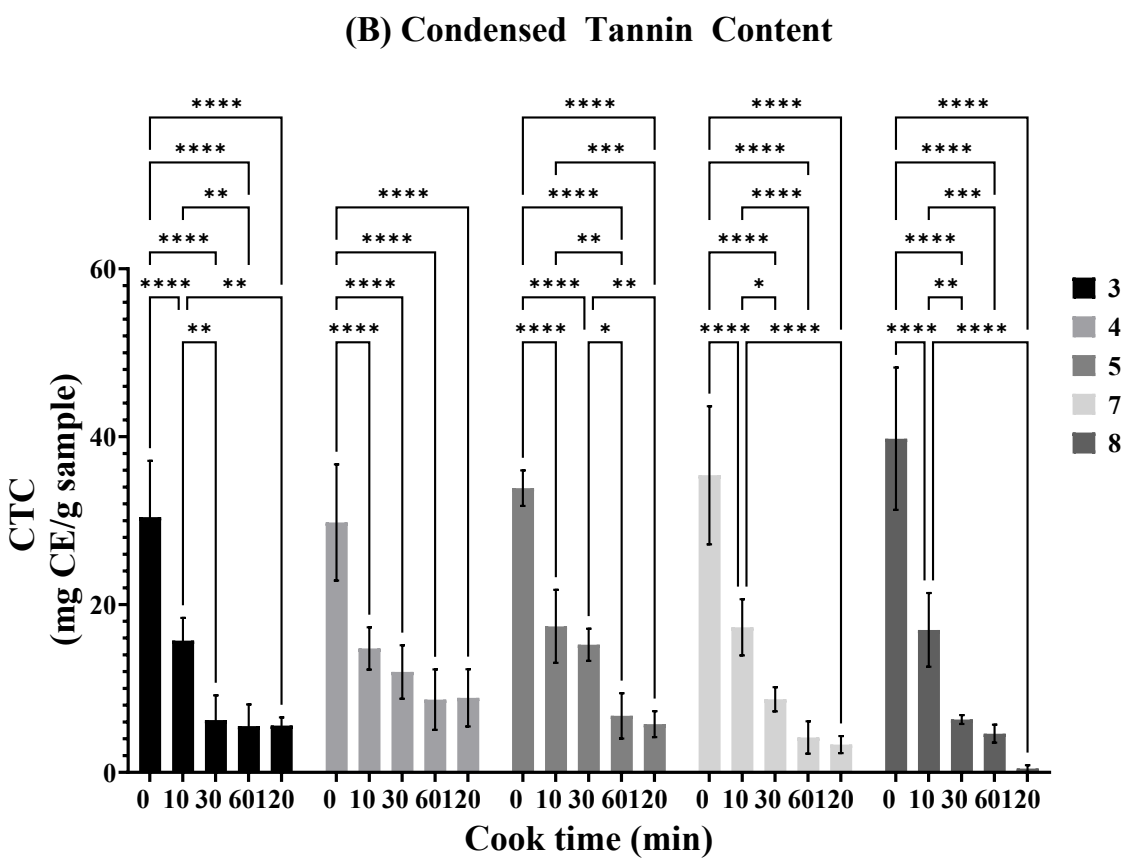


Figure 3.2 Phenolic profile. Total phenolic content (A) and condensed tannin content (B) of sorghum.

Total phenolic content expressed as milligram gallic acid equivalents per gram (mg GAE/g). Condensed tannin content expressed as milligram catechin equivalents per gram (mg CE/g).

3.3.3 Condensed tannin content

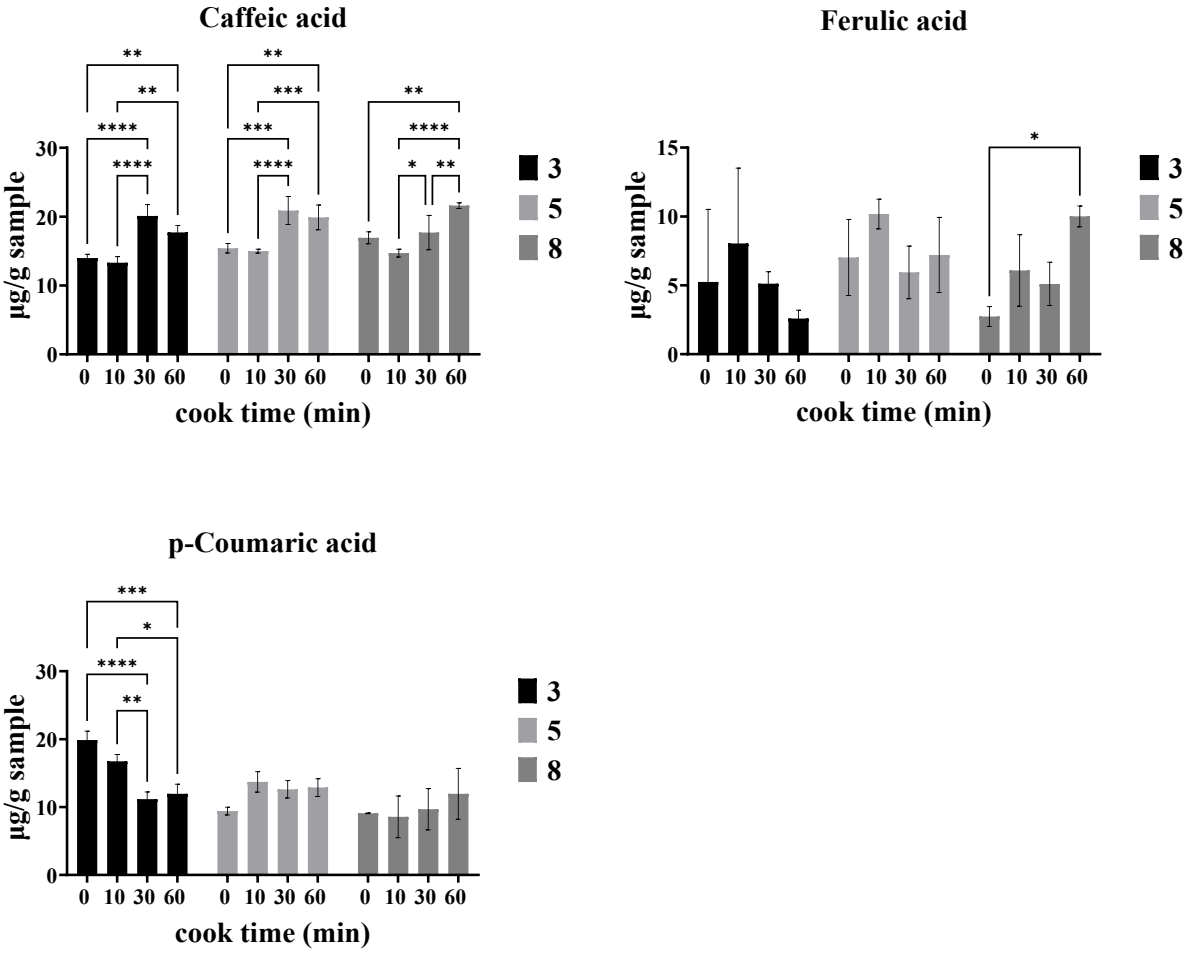
The CTC of unheated samples ranged from 29.77 to 39.76CE (mg/g) across all pH treatments. Heating the samples for 10 min significantly decreased CTC across all pH groups by an average of 51.46% Heating the samples for longer than 10 min continued to decrease CTC across all pH groups (Figure 2B). Unheated samples buffered at pH 8 were significantly higher in CTC than samples at pH 3 and 4. Although unheated samples buffered at pH 8 started with a higher CTC as compared to pH 3 and 4, they were the lowest in CTC (0.46mgCE/g) after heating for 120 min (Supplementary Table S1).

3.3.4 HPLC Analysis

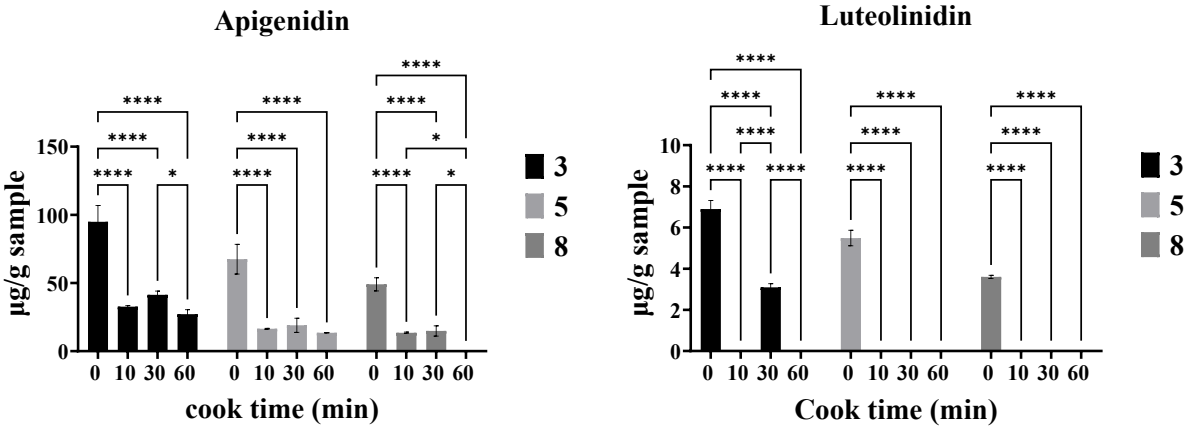
Selected samples heated at pH 3, 5, and 8 were selected for further in-depth analysis using HPLC. The following phenolic compounds were detected: caffeic acid, ferulic acid, p-coumaric acid, luteolin, apigenin, catechin-, epicatechin, naringenin, eriodyctiol, taxifolin, quercetin, luteolinidin, apigenidin, and 7-methoxyapigdenidin.

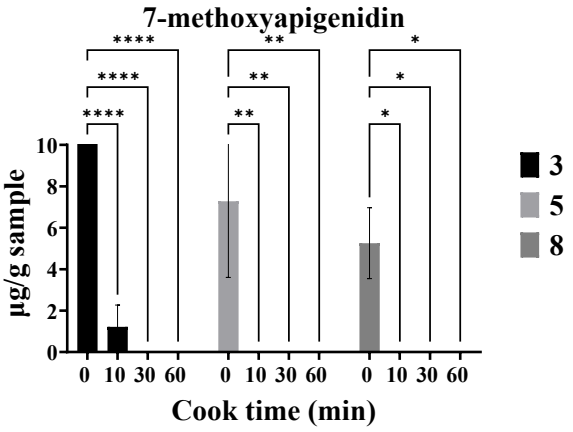
Caffeic acid concentrations significantly increased after 30 min of heating for all pH groups. Ferulic acid significantly increased at 60 min, when compared to unheated control at pH 8. The compound p-coumaric acid significantly decreased over time in pH 3. The results indicated that cinnamic acids are more stable and/or increased by heating at higher pH (5 and 8), except for caffeic acid, which increased at all three pH levels (**Figure 3.3A**).

(A)

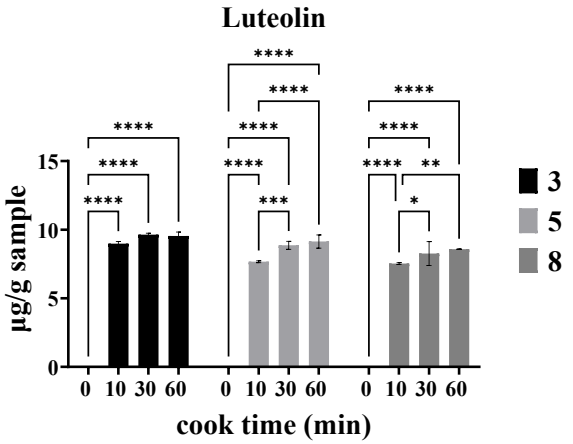

Caffeic acid
µg/g sample
cook time (min)
0 10 30 60
3
5
8
Ferulic acid
µg/g sample
cook time (min)
p-Coumaric acid
µg/g sample
cook time (min)

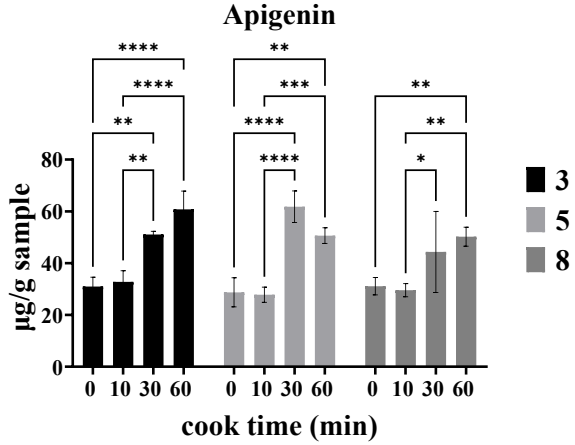
(B)


Apigenidin
µg/g sample
cook time (min)
0 10 30 60
3
5
8
Luteolinidin
µg/g sample
Cook time (min)

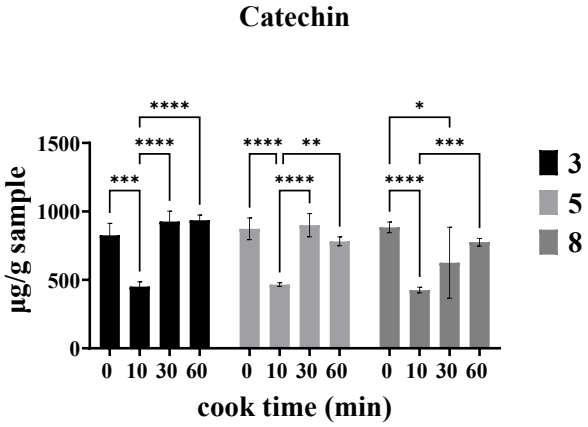

7-methoxyapigenidin
µg/g sample
Cook time (min)
0 10 30 60 0 10 30 60 0 10 30 60
3
5
8

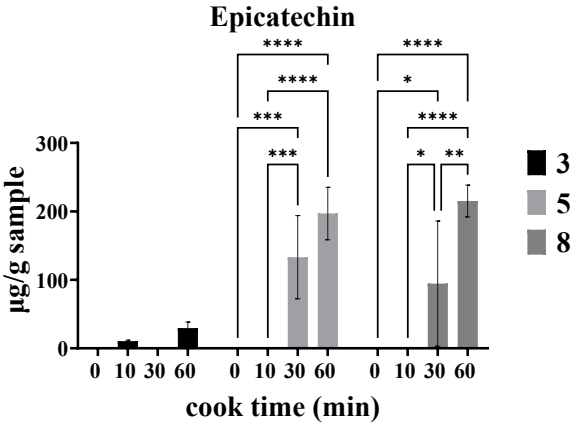
(C)


Luteolin
µg/g sample
cook time (min)
0 10 30 60 0 10 30 60 0 10 30 60
3
5
8


Apigenin
µg/g sample
cook time (min)
0 10 30 60 0 10 30 60 0 10 30 60
3
5
8

(D)


Catechin
µg/g sample
cook time (min)
0 10 30 60 0 10 30 60 0 10 30 60
3
5
8


Epicatechin
µg/g sample
cook time (min)
0 10 30 60 0 10 30 60 0 10 30 60
3
5
8

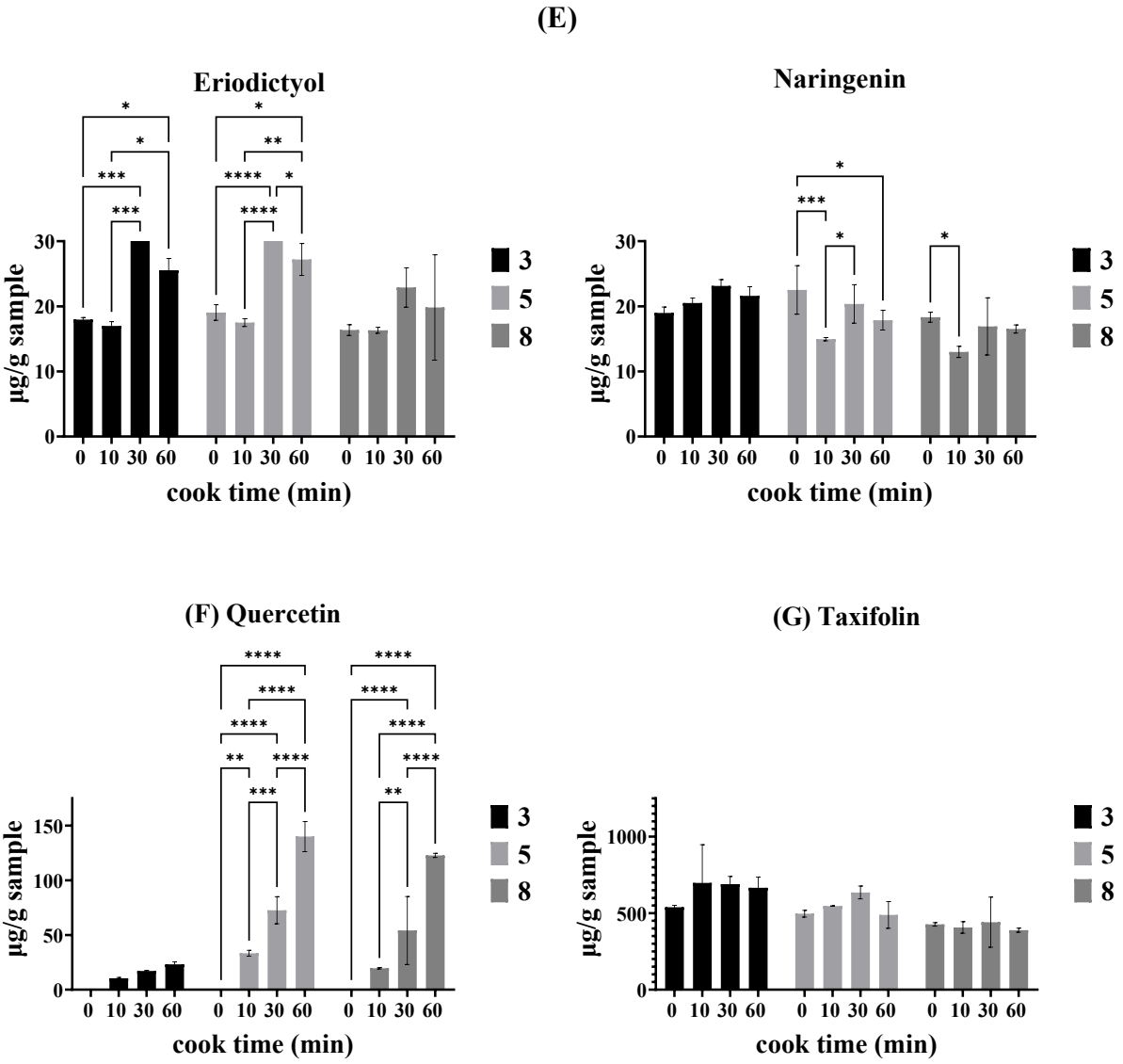


Figure 3.3 RP-HPLC results for cinnamic acids (A), 3-deoxyanthocyanidins (B), flavones (C), flavanols (D), flavanones (E), flavonols (F), and flavononol (G) detected in sorghum before and after processing.

Samples expressed as microgram per gram sample.

The detectable 3-deoxyanthocyanidins (3-DAs), apigenidin, luteolinidin, and 7-methoxyapigenidin all decreased after 10 min of heating, regardless of pH level. Heating for longer than 10 min continued to significantly decrease 3-DA content. Apigenidin was the most abundant 3-DA, with concentrations around 10 times higher than the other 3-DAs detected.

Luteolinidin was detected in such small quantities that the signal to noise ratio is likely too high for results to be evaluated accurately (Figure 3B).

Both flavone compounds, luteolin and apigenin, increased after heating, regardless of pH. Luteolin was not detected in unheated samples and was detectable after 10 min of heating. Apigenin content significantly increased after 30min of heating across all three pH groups (Figure 3C).

The flavanol compound, catechin, significantly decreased after 10min of heating, but increased to unheated levels by 60min. Epicatechin was not detectable in unheated samples but increased significantly with heating time in samples buffered at pH 5 and pH 8, but not at pH 3, where the increase was not significant (Figure 3D).

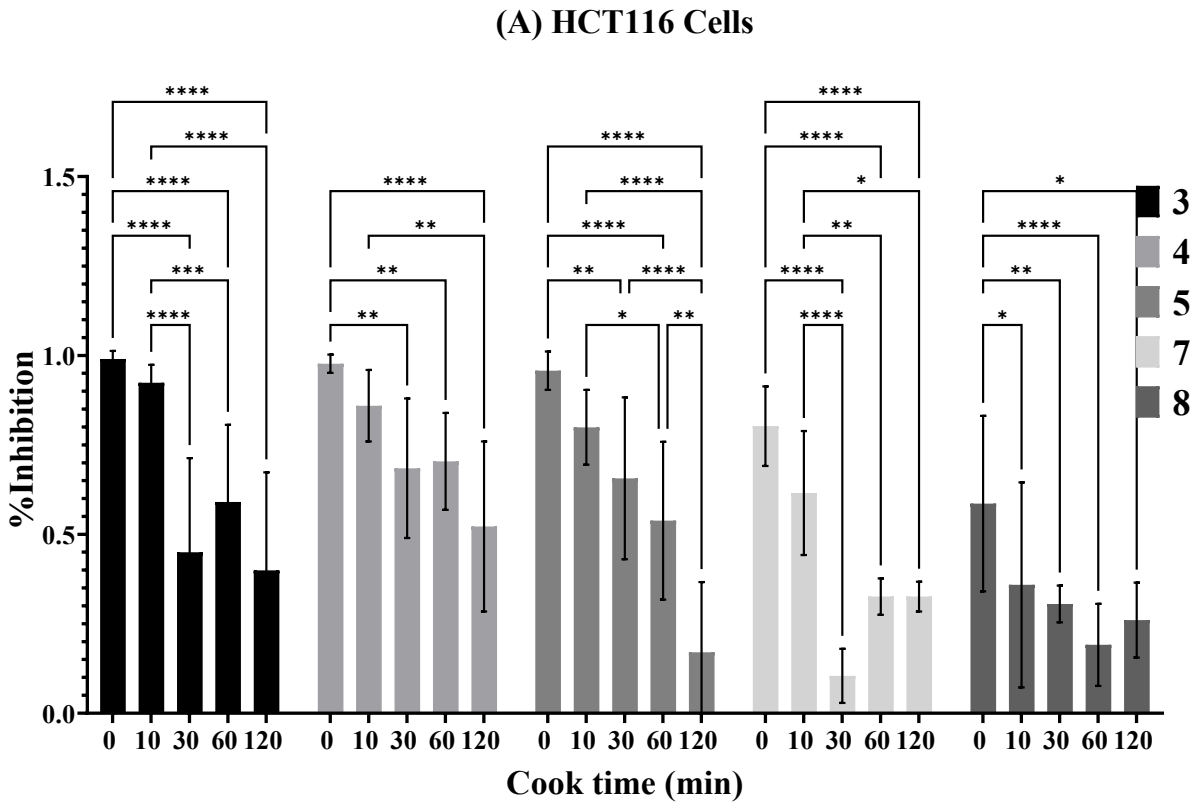
The flavanone, naringenin, significantly decreased in concentration after 10min of heating in samples buffered at pH 5 and pH 8. (Figure 3E). After 30 min, eriodyctiol, also a flavanone, significantly increased in concentration from 17.96µg/g in unheated samples to 30.24µg/g at pH 3, and from 19.05µg/g to 35.53µg/g at pH 5.

Quercetin content increased over heating time at pH 5 and 8, while at pH 3 the increase was not significant. Heating for 60 min significantly raised quercetin content from 0µg/g in unheated samples to 139.95µg/g, and 122.73µg/g in samples buffered at pH 5 and pH 8, respectively (Figure 3F). Quercetin was the only detected flavonols.

The only detectable flavanonol was taxifolin. Taxifolin content did not significantly change with heat time, regardless of pH level (Figure 3G). Taxifolin content was significantly higher in samples buffered at pH 3, when compared to samples at pH 8 across all heat times (Supplementary Table S2).

3.3.5 Cell bioactivity

Cell inhibition properties of extracts did not significantly decrease after heating for 10 min, except for pH 8 in HCT116 cells. Heating for 30 min significantly lowered cell inhibition of all samples regardless of pH level (Figures 4A, B).



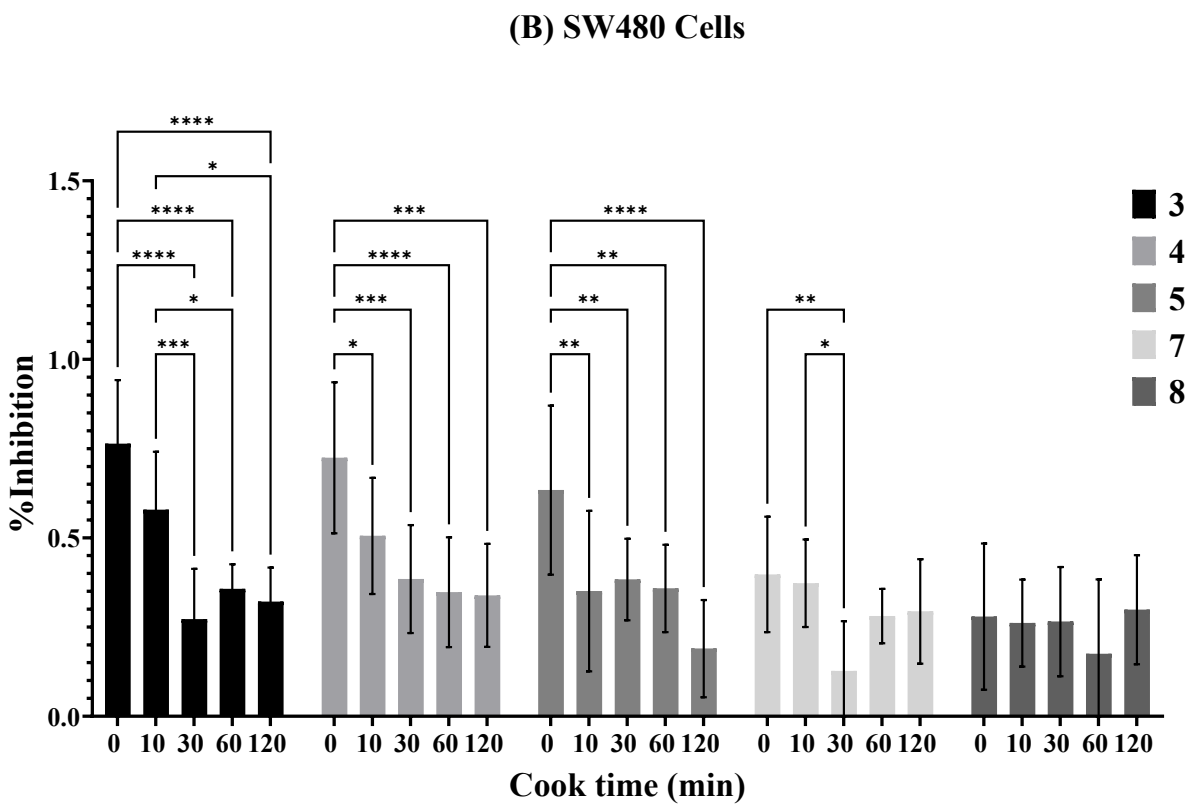


Figure 3.4 Cell bioactivity of phenolics on cell inhibition in HCT 116 (A) and SW480 (B) human colon cancer cells.

Samples buffered at pH 3, 4, and 5 were significantly higher in cell inhibition than pH 8 across all heat times, except for 120 min (Supplementary Table S3). Samples buffered at pH 8 had a significantly lower cell inhibition, when compared to pH 3 and pH 4 before and after heating samples for 10 min. After heating for 60 min, pH had no effect on cell inhibition of samples (Supplementary Table S3).

A correlation analysis to evaluate whether total phenolic content and/ or condensed tannin content was correlated to cell inhibition for both cell lines was conducted. Both analyses were positively correlated overall with percent cell inhibition for both cell lines. For HCT116, the correlation for TPC was r=0.67, while CTC was r=0.69. For SW480 cells, TPC was r=0.70 and CTC was r=0.59 (Figure 5). Based on these results, both condensed tannin and polyphenols

are correlated with cancer cell inhibition.

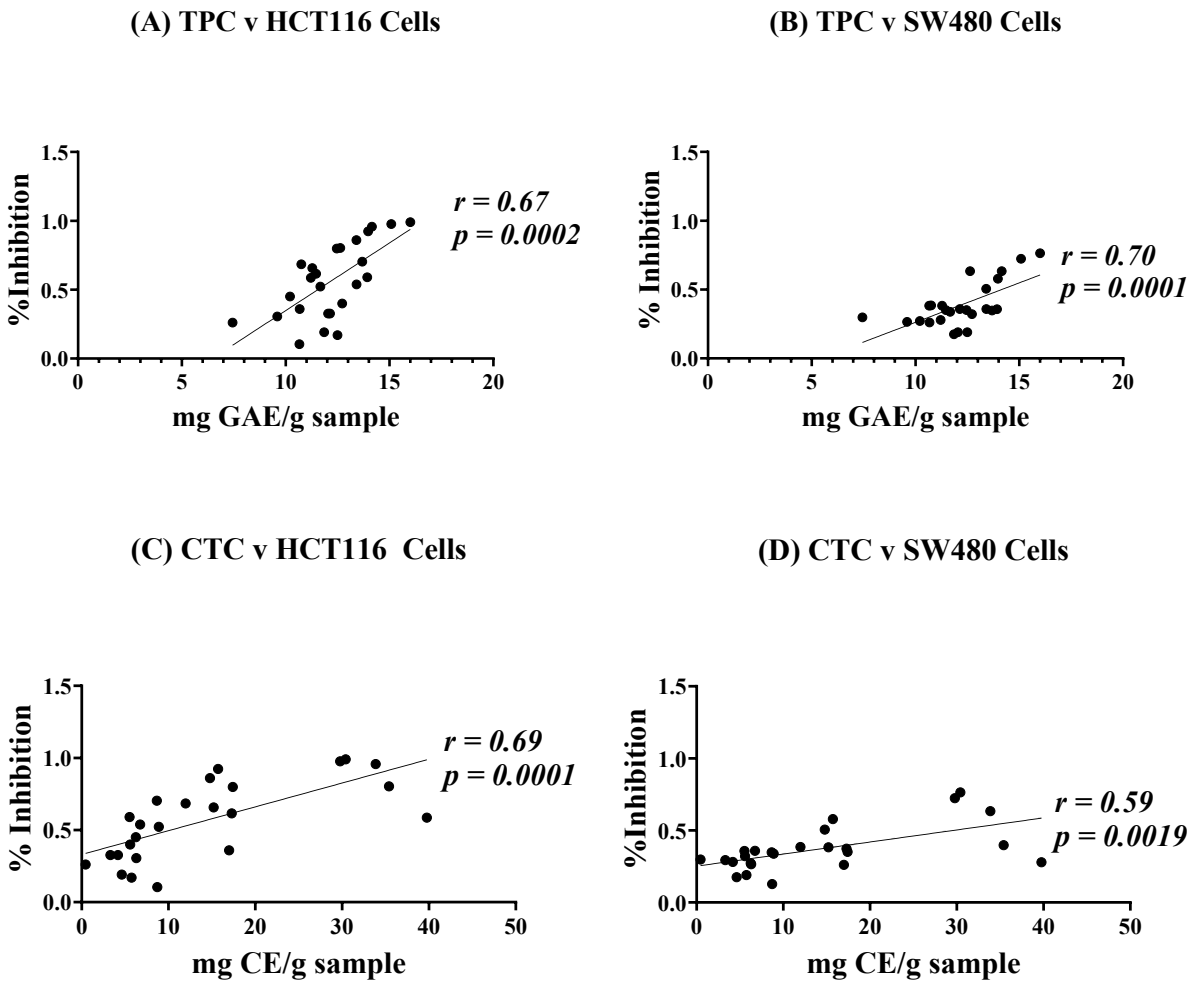


Figure 3.5 Pearson's correlation of total phenolic content of (A) HCT116 and (B) SW480 and the condensed tannin content of (C) HCT116 and (D) SW480 cells compared to percent growth inhibition.

3.4 Discussion

3.4.1 Starch digestibility

In the present study, digestible starch significantly increased after heating, when compared to the non-heated samples in an excess moisture environment. The digestible starch of samples was around 60g/100g flour after 10 min of heating. This was comparable to Ezeogu et

al. (2005), who reported 85% digestible starch in a decorticated red sorghum variety after heating for 10 min. Differences in starch digestibility could be due to the presence (or lack of) specific polyphenolic compounds. Specific types of polyphenols impact starch digestibility differently depending on the type (ex; proanthocyanidins vs. monomeric phenolics) and polyphenolic concentration (Barros et al., 2012). The increase in digestible starch is inversely related to the resistant starch content in sorghum after heat and high-moisture pH treatments.

The amount of resistant starch in raw samples was around 55g/100g. The heat and high-moisture pH treatments presented in our study significantly reduced the resistant starch of our samples from 55g/100g to less than 1g/100g for all samples. This trend was also observed by Teixeira Nde et al. (2016), where in RS of samples after wet-heating decreased by 94%. The same decrease in RS from heat and high moisture treatments were not reported by Vu et al. (2017) who examined heat and moisture treatments in white sorghum flours and reported a 53–140% increase in resistant starch content after heat moisture treatments. Differences in final resistant starch levels can be attributed to differences in methodology. Vu et al. (2017)evaluated high heat treatments with low-moisture levels for longer time periods, when compared to the current study. Other differences may be accounted for by differences in starting material composition and water content (excess water vs. low moisture). For example, Vu et al. (2017) who examined decorticated white sorghum flour commercially purchased from Archer Daniels, while the sorghum studied in our present study was high-polyphenol whole grain sorghum. The presence of pericarp present in whole grain flours will influence resistant starch content. In addition, pigmented sorghum grains are reported to have a higher initial RS content than white lines (Rocchetti et al., 2020). Another factor that influences RS in grains is the proportion of water to grain during heat treatment. The greater the proportion of water present during the

heating process, the greater degree of gelatinization, thus increasing digestibility of starch (Teixeira Nde et al., 2016). To our knowledge, the direct effect of high-moisture pH heat treatments on starch digestibility has not been extensively studied in sorghum.

Total starch content for sorghum was around 60g/100g, which is comparable to the 70–75g/100g reported in Italian pigmented sorghum grain by Rocchetti et al. (2020). Total starch content for sorghum may vary depending on genotype and cultivation conditions.

3.4.2 Total phenolic profiled

The TPC of samples decreased, but not significantly after 10 min of heating for pH 4, 5, 7, and 8. It is commonly reported that wet-heating decreases TPC in sorghum samples (Li et al., 2022b). An additional study examining the effect of wet-heating (boiling) on sorghum phenolic content also reported a reduction in TPC by 18% in an unreported sorghum variety after 10 min of heating (Hithamani and Srinivasan, 2014). A similar study performed by Sorour et al. (2017) reported a 32 and 41% loss in TPC after boiling for 2h in low-tannin and high-tannin sorghum lines, respectively. Differences in TPC reduction could be due to differences in variety, water ratio, and presence of buffer. For example, both studies by Li et al. (2022b) and Sorour et al. (2017) mixed sorghum flour or grain with water at ratio of 1:5 (w/v). The methods described in our study examined wet heating of sorghum flour to pH buffers at 1:20 (w/v) ratio and were performed with different sorghum lines than previously reported. After heating for 30 min, samples decreased by 24.0% on average. TPC continued to decrease with longer heat times. Samples buffered at pH 8 were significantly lower in TPC than samples at pH 3, except for samples in the 30 min heating group. This is in line with the findings from Friedman and Jürgens (2000) who reported a 67% reduction in TPC from high pH levels in plant derived phenolics.

3.4.3 Condensed tannin content

Unlike the TPC results, the CTC significantly decreased after 10min of heating, regardless of pH level. Overall, the CTC reduced by 51.46% (10 min), 71.38% (30 min), 82.46% (60 min) and 85.81% (120 min) on average, across all pH groups. The reduction in CTC is similar to the findings of Sorour et al. (2017), who reported a 91.9% reduction in CTC after wet-heating a high tannin sorghum grain for 2h. Other studies using wet-heating also saw a 54% reduction in CTC after 10 min of heating (Hithamani and Srinivasan, 2014). Additional high-moisture heat methods were examined during tea processing, where the CTC of two sorghum grains decreased by 22 and 56% (Xiong et al., 2020). The effect of pH and heat treatments on the condensed tannic content in sorghum is not commonly reported in literature.

3.4.4 HPLC analysis

As previously mentioned, there is limited literature investigating wet-heating and pH effects on sorghum phenolics. Therefore, comparisons to studies examining other plant derived phenolics have been evaluated for the purpose of this discussion. Caffeic acid increased in concentration after 30 min of heating. Caffeic acid was the only cinnamic acid to consistently increase with heating. Ferulic acid only increased in content after 60min of heating for pH 8. The increase in both phenolic compounds from wet-heating is unexpected but could be attributed to increased extractability (Ghafoor et al., 2019). Hong et al. (2023) reported decreases in caffeic and ferulic acids after wet-heating Qingke (barley) for 40 min. The last cinnamic acid, p-coumaric acid, decreased by 39% in unheated samples when compared to samples heated for 60 min at pH 3. This result was in line with Hong et al. (2023) who reported a 26% loss in p-coumaric acid after wet-heating.

All three detectable 3-DAs significantly decreased after heating for 10 min, regardless of pH. This agrees with other research groups that used wet-heating methods on whole grain sorghum and saw significant decreases in apigenidin, luteolinidin, and 7-methoxyapigeninidin. The authors hypothesized that the decrease in 3-DA content was likely due to the 3-DAs leaching out during heating (Cardoso et al., 2015, Cardoso et al., 2014). In our study, however, whole flour is heated with pH buffers in a closed environment, and the whole sample was lyophilized. This prevents potential 3-DAs from leaching out during the heat process and indicates the change in 3-DA content was likely caused by the thermal processing and not leaching out. Although initial apigenidin content decreased after 10 min, apigenidin content did not significantly decrease with heat time in lower pH levels (pH 3 and 5). While an initial decrease is observed, the lack of change in content with increased heat time suggests that some 3-DAs present in sorghum are more thermally stable at lower pH levels. This is in agreement with Yang et al. (2014), who reported 3-DAs from sorghum maintained thermal stability over a range of pH levels. It was further stated by the authors that the type of acid or base used also plays a significant role in determining 3-DA heat stability.

To date, studies on stability of flavones in sorghum during wet heating are unavailable. Therefore, other heating methods were evaluated. The flavone, luteolin, was not detected in unheated samples, until the samples were heated for 30 min or more. This disagrees with the findings from (Cardoso et al., 2015). who saw a 53% decrease in luteolin after using a conventional oven. Differences in luteolin content may be due to differences in sorghum variety, as well as heating methods and moisture content.

Another detected flavone, apigenin, also increased concentration with heat time across all pH levels. Interestingly, apigenin was already detectable in raw sorghum samples prior to

heating, and only significantly increased after 30 min of heating. This disagrees with Cardoso et al. (2015), who reported a loss of 50% after using dry heat. The reported differences in luteolin and apigenin content may be explained by the use of different processing types (dry vs. wet heating) and need to be examined more closely.

Catechin was detected in unheated samples and decreased in content after heating for 10 min. Interestingly, catechin did not continue to decrease with heating time. Rather, catechin increased in content from the 10 to 30 and 60 min time groups. After 30 min of heating, the catechin content was similar to the unheated samples. This trend is in alignment with other research groups who studied eight of the most common catechin standards found in teas under similar heating conditions (60–90°C). After heating solutions at 90°C for 8h, it was reported that non-epistructured catechin compounds increased in concentration due to epimerization of other compounds into catechin, which was found to take place between 60 and 90°C (Fan et al., 2016). This process likely explains the increase in catechin content after the initial decrease.

Naringenin decreased in content after 10 and 60 min of heating at pH 5. However, both the higher and lower levels pH values remained unchanged in final concentration after 60 min of heating. Approximately 20μg/g of naringenin was detected in sorghum, while 37μg/g was reported in wet-heated barley Hong et al. (2023). Naringenin, like eriodyctiol, indicates some heat stability, given the lack of significant change over time at specific pH levels. This is further supported by (Chaaban et al., 2017), who studied the heat stability of naringenin standards against various temperatures and times and saw little reduction in concentration.

Initial quercetin concentration of raw samples was not detectable. After wet-heating, however, quercetin content increased across all pH levels. The increase in quercetin content after heating may be attributed to the cleavage of quercetin from the cell walls or other macronutrients

present during the heating process. Thermal processing can separate bound phenolics from plant cell walls or other macronutrients present in the food matrix (Ribas-Agusti et al., 2018) This may explain why quercetin was detected in the heated samples and not the unheated samples.

Taxifolin was the only detected flavononol and was not affected by heat or pH treatments. This is similar to other flavonoids in our study. In fact, except for 3-DAs, all flavonoid groups either increased in content or were relatively unaffected by heating at various pH levels. Our findings suggest that sorghum flavonoids may be pH- and heat-stable in excess moisture environments; however, further research is needed to understand this relationship.

Literature examining the effects of thermal processing on specific sorghum phenolic compounds is limited and should therefore be studied further. It was previously reported that roasting red sorghum decreased phenolic content (Irondi et al., 2019), which is a different process than cooking in excess moisture. Nonetheless, similar reductions were observed for the majority of the polyphenols studied. It is important to note that all changes in phenolic compounds reported in this study are from one sorghum genotype. Additionally, the phenolics were extracted in 70% (v/v) ethanol. While changes in phenolic content may be from the pH or high-moisture heat treatments, it may also be affected by the individual phenolic compound's extractability in 70% ethanol.

3.4.5 Cell bioactivity

The effect of heating and high-moisture pH treatments on sorghum polyphenol content and its anticancer activity is not currently well reported. In our study, anticancer activity was more effective at lower pH levels. This is in line with the findings from (Cox et al., 2019), who studied the effects of different ethanol-based extraction conditions and their effect on human cancer cell inhibition. It was observed that solvents with lower pH values had greater cell

inhibition rates (Cox et al., 2019), The increased cell inhibition at lower pH values may be explained by changes in plant phenolic structure and stability at different pH levels (Hithamani and Srinivasan, 2014). Altering the structure of phenolic compounds may affect their anticancer activity. However, more research is needed to understand the effect of structural changes and their relationship to anticancer activities.

Pearson's correlation factor of 1 are indicators of high positive correlations among trends, while a value of −1 indicates relationships that are strongly inversely related. The findings from our study suggest that TPC and CTC are both positively correlated with SW480 and HCT 116 cells. More studies of isolated condensed tannin and isolated non-condensed tannin polyphenols are needed to understand specific correlation among cell lines.

3.5 Conclusion

The objective of this study was to determine the effects of heating time and pH on high polyphenol sorghum for starch digestibility, phenolic profile, and cell bioactivity in a high moisture environment. Heating consistently enhanced the starch digestibility of the sorghum samples, irrespective of pH treatment and time. Notably, total phenolic content and bioactivity exhibited minimal alterations following a 10 min heating duration. These results hold promising implications for the development of high-phenolic sorghum products and provide valuable insights to guide forthcoming animal and clinical studies. The demonstrated impact of wet-heating on starch digestibility, coupled with the preservation of phenolic content and bioactivity, underscores the potential of incorporating high-phenolic sorghum lines in future functional food formulations.

Chapter 4 – Examining various processing effects on phenolic profile and bioactivity in high and low polyphenol sorghum (*Sorghum bicolor* L. Moench) grains and flours

4.1 Introduction

Advanced glycation end products (AGE) are formed when protein or lipids become glycated in the presence of sugars. They are often used as biomarkers for the onset of many diseases like diabetes, kidney disease and Alzheimer's. Phenolics, which have antioxidative properties, can therefore be used to inhibit the formation of AGE by blocking the receptor of AGEs (Yeh et al., 2017). A less reported on health benefit of interest is the potential ability of sorghum polyphenols to be used as a novel ingredient against the formation of advanced glycation end (AGE) products (Farrar et al., 2008).

As previously discussed in **Chapter 2** and **Chapter 3**, sorghum phenolics may offer potential health benefits, however, current research has focused on the sorghum phenolics extracted from raw grains. To deliver potential health benefits of sorghum to consumers, there is an urgent need to study the effect of processing (thermal, physical, chemical, enzymatic, etc.) on sorghum phenolic bioactivity in food models. In addition, it is imperative that functional foods formulated with sorghum aimed at delivering additional health benefits will need to be palatable and attractive to consumers. It is therefore imperative that the effects of processing on sorghum phenolics be examined, especially on high polyphenol sorghum samples. A majority of sorghum phenolic processing research currently available in literature use white, red, or an undisclosed sorghum variety for their phenolic model. In doing so, it becomes difficult to gauge how polyphenols are affected by processing. For example, Stefoska-Needham et al. (2016), used a red

and brown sorghum as their high polyphenol model, which contained 2.27 and 2.99 mg GAE/g, respectively. Presently, there are commercially available sumac sorghum samples that contain 22.4 mg GAE/g **(Figure 2.2).** These varieties are low in polyphenol content prior to processing, therefore making it difficult to examine the effect of processing and due to high signal to noise ratio of the assays commonly used to measure total phenolic content and antioxidant activities.

The objective of this study was to examine the effects of various heat processing methods on sorghum phenolics in Sumac (high polyphenol), White (no/low polyphenol) and Black (moderate polyphenol amounts) sorghum samples.

4.2 Materials and Methods

4.2.1 Chemicals

The chemicals used for this chapter are the same chemicals and suppliers used in **Chapter 2.** Please reference section 2.2.1 for further information on chemicals and manufacturers used for this chapter.

4.2.3 Sample preparation

Three sorghum varieties were purchased from Nu Life Market (Scott City, KS). All sorghum grain was purchased from the same grow out year, thus reducing differences in phenolic profile due differences in growing conditions by year. In this study 8 processing methods were examined to determine the effects of processing on sorghum phenolics. The process names and corresponding methods are recorded in Table 4.1. Briefly, boiled flour (200g) was prepared by boiling flour at 100°C for 10 minutes in 400g water until a thick paste formed similar to porridge, oatmeal, or cream of wheat products. Boiled whole grain (200g) was boiled in 400 g water for 30 minutes (T1) or 50 minutes (T2). Toasted grain was produced and purchased by Nu Life Market. High pressure grain (200 g) was cooked in an Instapot® on high

pressure for 30 minutes while low pressure grain was cooked on low pressure for 30 minutes. Popped and extruded samples were also produced and purchased from Nu Life Market. For sumac, no toasted grain or popped grain could be produced due to constraints in kernel size and machinery capabilities. A summarized list of processing methods and their intended model food applications can be found in **Table 4.1.**

Table 4.1 Processing method and model food application

Processing Name	**Method**	**Model Food Application**
Raw	No heat processing applied	Harvested Grain
Boiling Flour	Flour boiled for 10 minutes	Porridges, Cream of Wheat
Boiling Grain T1	Grain boiled for 30 minutes	Ready Rice, Rice
Boiling Grain T2	Grain boiled for 50 minutes	Ready Rice, Rice
Toasted Grain	NA, purchased	Snack Foods
High Pressure	Grain cooked under high pressure for 30 min	Soups, Rice, Ready Rice
Low Pressure	Grain cooked under low pressure for 30 min	Soups, Rice, Ready Rice
Popped	NA, purchased	Popcorn, Snack Foods
Extruded	NA, purchased	Snacks, Breakfast Cereals, Pet Foods

4.2.4 Total phenolic content

Phenolic extractions were produced following the methodology in section 2.2.6 Total Phenolic Content and section 3.2.4.1 Total Phenolic Content. The TPC of samples are expressed as milligram gallic acid equivalents per gram of sample (mg GAE/g).

4.2.5 Condensed tannin content

The condensed tannin content was determined using similar methods to section 2.2.7 and 3.2.4.2. Condensed tannin content is expressed as milligram Catechin equivalent per grom of sample (mg CE/g sample).

4.2.6 Antioxidant activity

Antioxidant activity was determined using the 2,2-diphenyl- 1-picrylhydrazyl (DPPH*) method from section 2.2.10.

4.2.7 RP – H LC

The detection and quantification of phenolic compounds present in corn, rice, and sorghum samples were determined using reversed phase high pressure liquid chromatography (RP-HPLC). Phenolic profile determination was determined using the methods in section 2.29 and 3.2.4.3. Results were expressed in terms of microgram of phenolic compound per gram sample.

4.2.8 NP – HPLC

Normal-phase HPLC was used to detect monomers, oligomers and polymeric proanthocyanidins (condensed tannins) present in samples. For methodology see section 2.4.6 NP-HPLC.

4.2.9 Glycation inhibition

The glycation inhibition potential of samples was analyzed using the methods from section 2.2.11.

4.2.10 Statistical Analysis

The data reported is representative of at least three independent experiments. Statistical analysis was conducted using one-way analysis of variance (ANOVA) with Tukey's post hoc

testing using GraphPad Prism, version 10.1.0 (GraphPad, San Diego, CA). Significant difference in mean values was determined at p-value < 0.05.

4.3 Results

4.3.1 Total phenolic content

The total phenolic content of sumac, white, and black sorghum after various processing methods are represented in **Figure 4.1**. In sumac sorghum (**Figure 4.1A**), the extruded samples were significantly higher in TPC after processing (17.89 mg GAE/g) in comparison to the raw sample (14.58 mg GAE/g). The boiling flour, boiling grain T1, high pressure and low-pressure samples were not significantly different from one another. Sumac grain after the boiling grain T2 treatment resulted in the lowest TPC content (5.98 mg GAE/g). In white sorghum (**Figure 4.1B**), toasted grain, high pressure, low pressure, popped and extruded were all significantly higher in TPC in comparison to the raw grain (1.41 mg GAE/g) after processing. Boiling grain T2 had the lowest TPC (0.95 mg GAE/g). In Black sorghum (**Figure 4.1C**), extrusion (11.4 mg GAE/g) and toasting (12.0 mg GAE/g) did not significantly decrease the TPC content of raw grains (10.23 mg GAE/g). Toasted grain and extruded grain were the highest in TPC after processing. Boiling flour, high pressure and popped grain did not significantly reduce TPC content in comparison to the raw grain. Both boiling grain methods and low pressure processing significantly decrease TPC content in comparison to raw grain

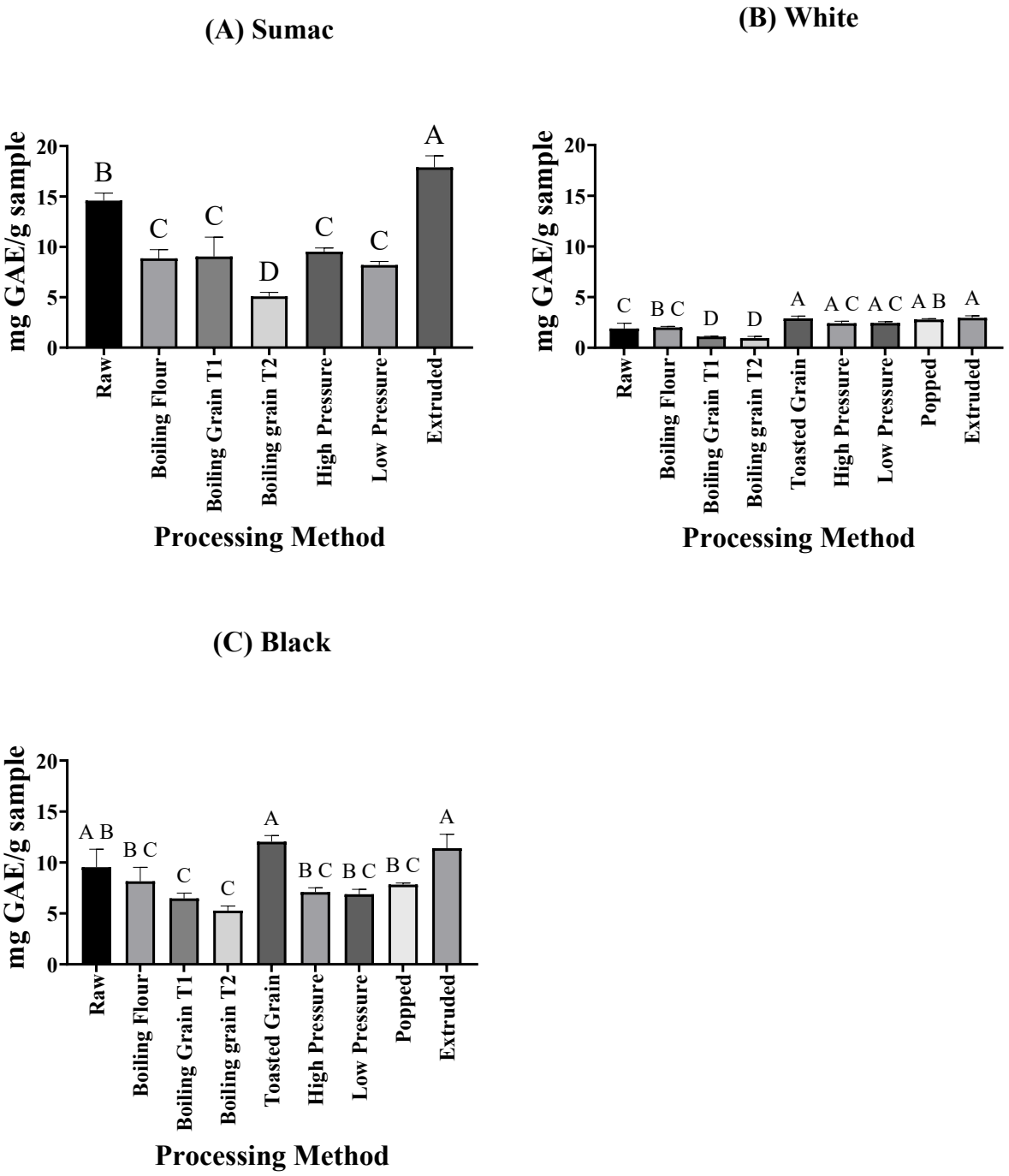
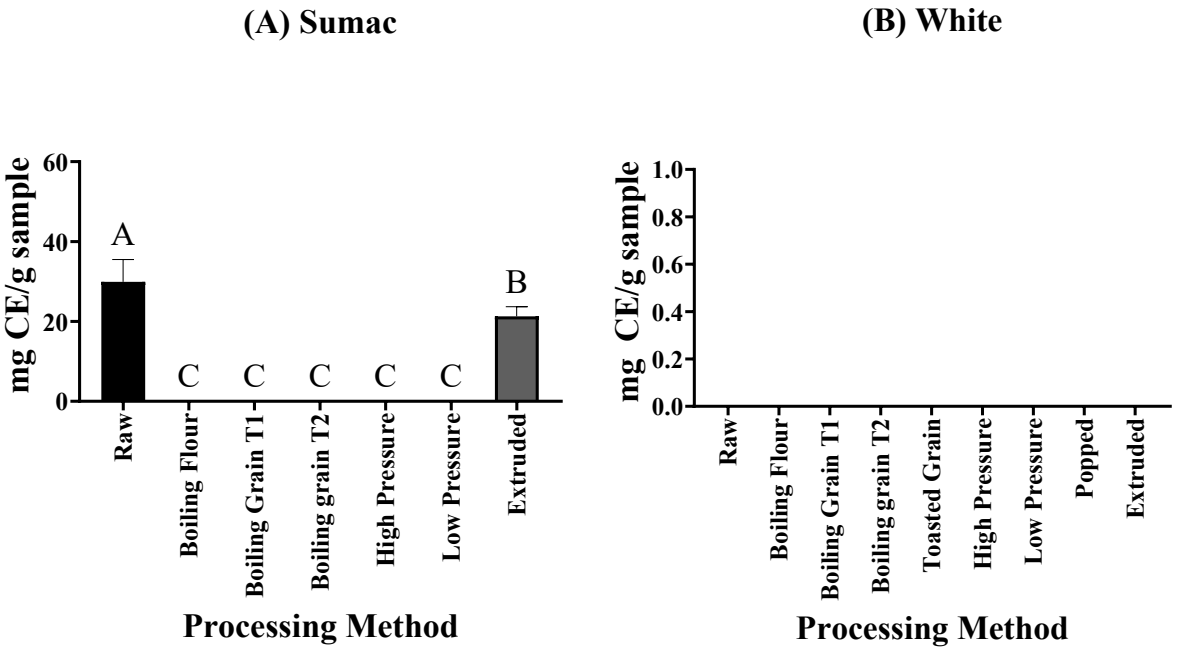


Figure 4.1 Total phenolic content of (A) Sumac, (B) White, and (C) Black sorghum after various cooking methods.

4.3.2 Condensed Tannin Content

The results of the condensed tannin content of sumac, white, and black sorghum before and after processing are represented in **Figure 4.2**. In sumac sorghum (**Figure 4.2A)**, the condensed tannin content was significantly reduced in all processing methods except for extrusion. All methods significantly reduced condensed tannin content to undetectable levels with the exception of extrusion. Extruded samples contained 28% less CTC than raw samples. There were no detectable condensed tannins present in the white sorghum using the modified vanillin HCl method previously discussed **(Figure 4.2B)**. For black sorghum, the condensed tannin content of raw grain was 36 mg GAE/g sample. After processing, CTC was significantly reduced in all samples except for toasted grains, which did not significantly decrease after processing **(Figure 4.2C).**



(C) Black

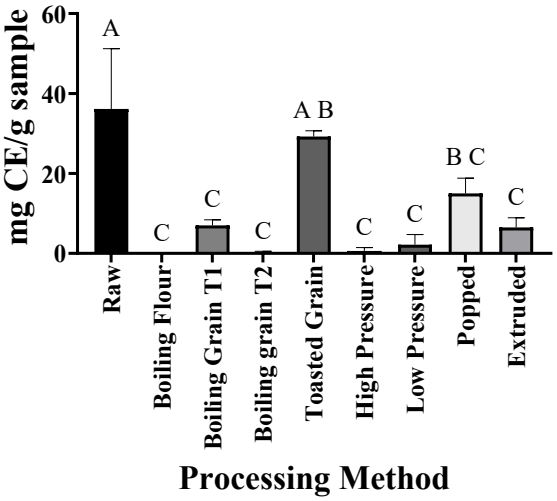


Figure 4.2 Condensed tannin content of (A) Sumac, (B) White, and (C) Black sorghum after various cooking methods.

4.3.3 Antioxidant activity

The results of the antioxidant activity (AA) of sumac, white, and black sorghum after various processing methods are reported in **Figure 4.3.** In sumac (**Figure 4.3A**), the extruded sample was significantly higher in AA than the raw grain. In sumac, Boiled flour, boiled grain, high pressure, and low pressure samples all significantly decreased the AA in comparison to raw sumac but were not significantly different from one another. Boiling grain T2 was the lowest in AA of all the sumac samples. White sorghum is generally lower in polyphenol and condensed tannin content than sumac and black sorghum. For example, in white sorghum (**Figure 4.3B**) the antioxidant activity of raw grain was 33.49 mg TE/g. After boiling (T2) and low pressure processing, there was a significant reductio in AA. In black sorghum (**Figure 4.3C**) there was a significant increase in AA after toasting grains in comparison to raw sorghum. All other processing methods, with exception of extrusion, significantly decreased AA of black sorghum.

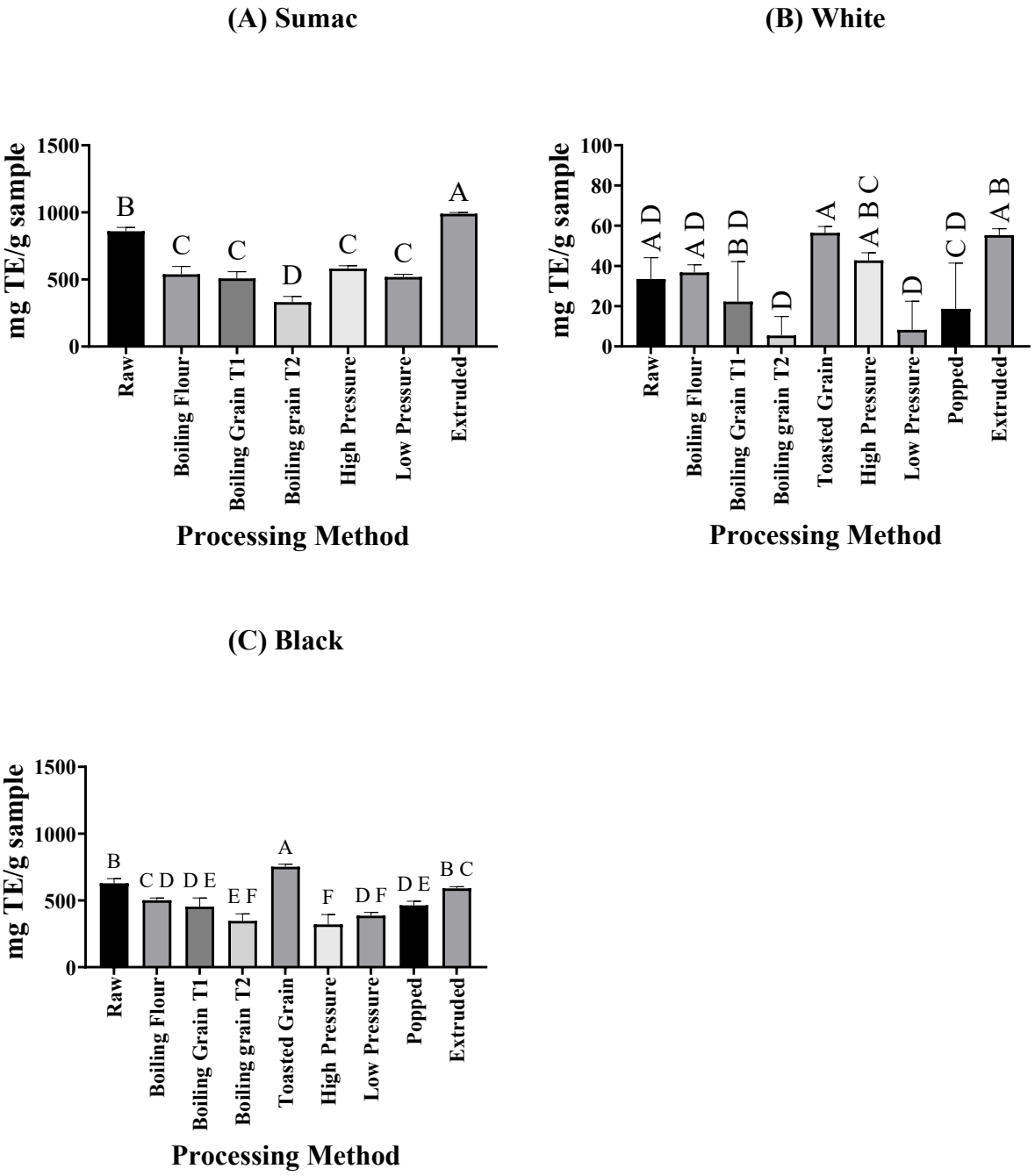


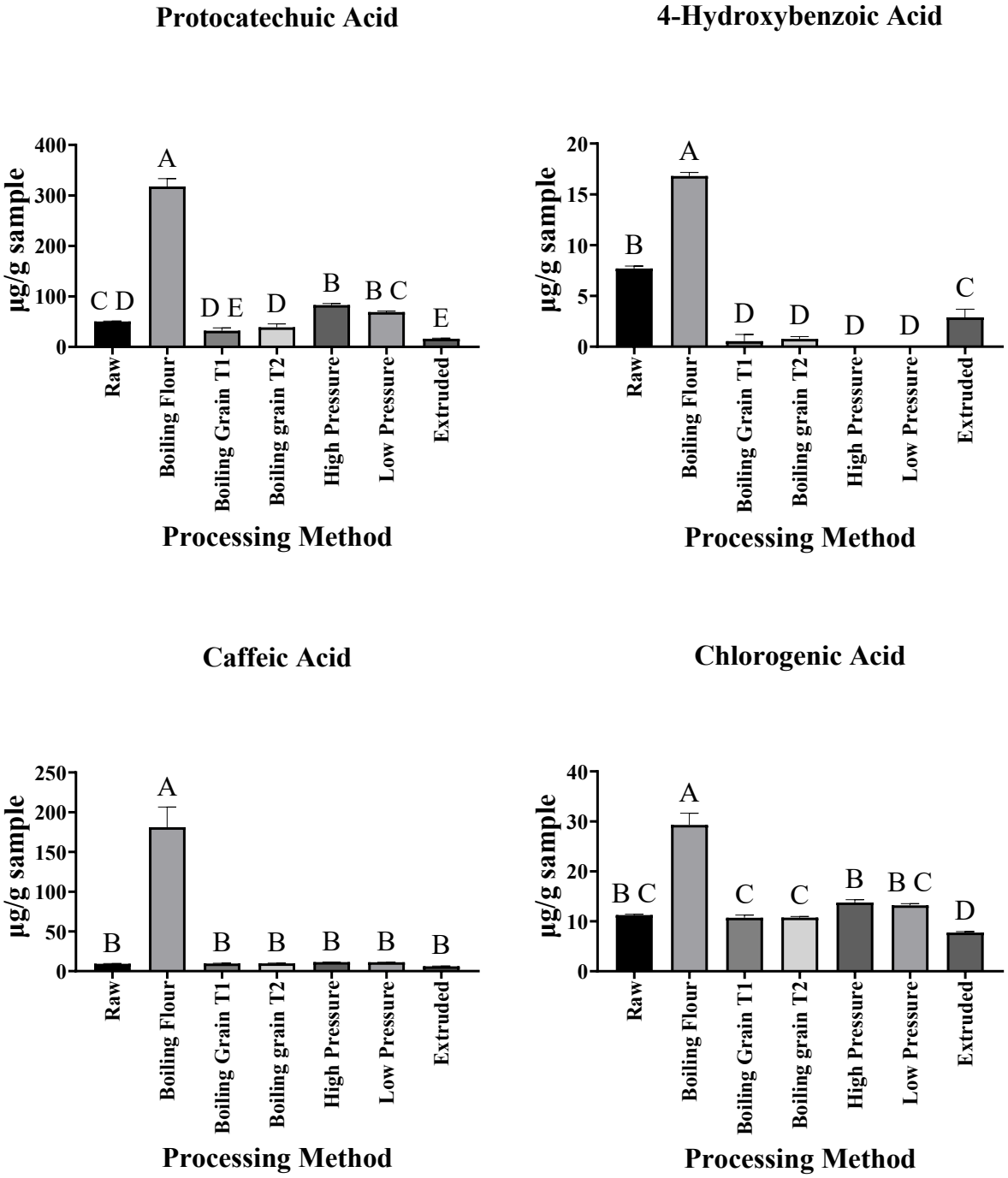
Figure 4.3 Antioxidant activity of (A) sumac, (B) white, and (C) black sorghum grain after different processing methods.

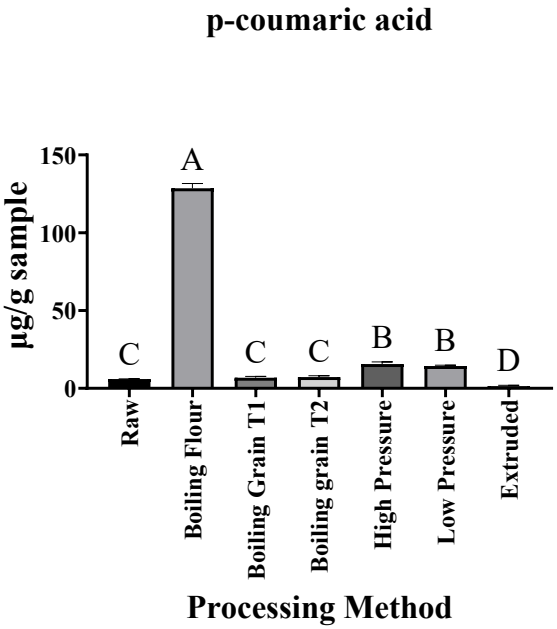
4.3.4 RP – HPLC

Figure 4.4 contains the phenolic profile of sumac sorghum samples after various processing methods as detected by RP-HPLC. In **Figure 4.4A** The detectable phenolic acids of sumac sorghum are shown after various processing methods. In total, five phenolic acids were detected. The protocatechuic acid content in sumac significantly increased after boiling (317 µg/g) and high pressure processing (82.9 µg/g) in comparison to raw sumac (50.43 µg/g). Extrusion processing significantly decreased protocatechuic acid content of sumac sorghum.

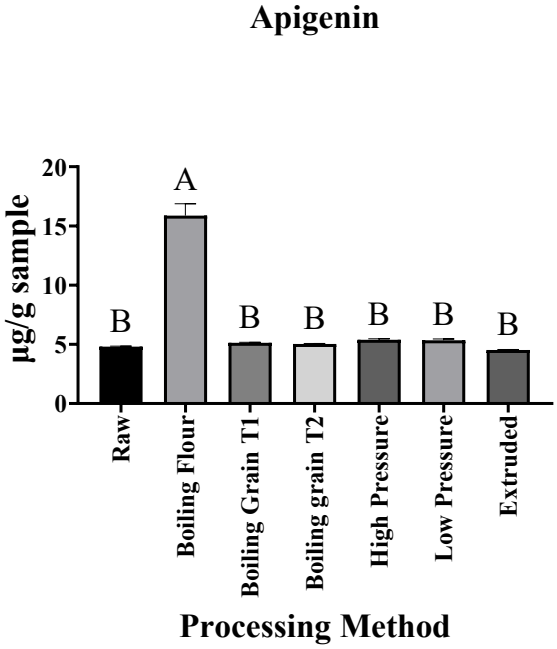
All detectable flavonoid compounds in sumac sorghum are reported in **Figure 4.4B.** Boiled flour significantly increased apigenin content, while no other processing effect significantly affected the apigenin content of sumac. Boiling flour, high pressure processing, and low pressure processing significantly increased luteolin content of sumac sorghum. Neither boiling grain processes (T1 or T2) affected luteolin content while extrusion significantly decreased luteolin content. High pressure and low pressure processing significantly increased catechin concentration while boiling flour significantly decreased catechin concentration. Boiling flour, high pressure, and low pressure significantly increased eriodyctiol content while both boiling methods and extrusion significantly decreased eriodyctiol content. All processing methods significantly increased quercetin content except for extrusion, which did not have a significant effect. Boiling flour significantly increased taxifolin content, while extrusion significantly decreased taxifolin content. All other processing methods did not have a significant impact on taxifolin content in sumac samples. Apigenidin, luteolinidin, and 5-methoxyluteolinidin (**Figure 4.4C)** content were significantly decreased by all processing methods.

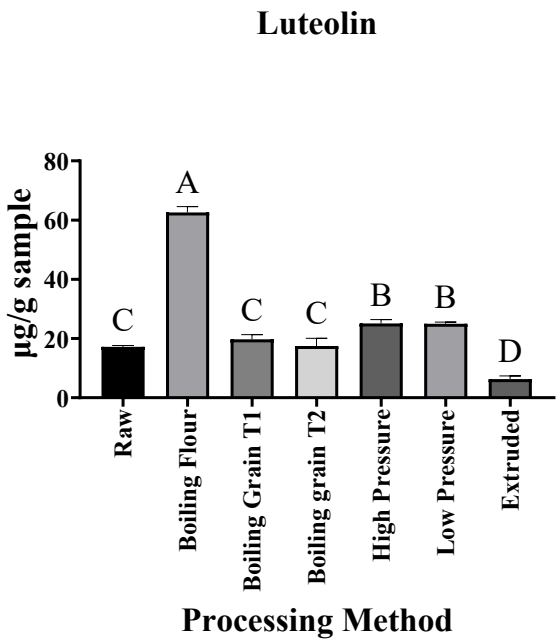
(A)

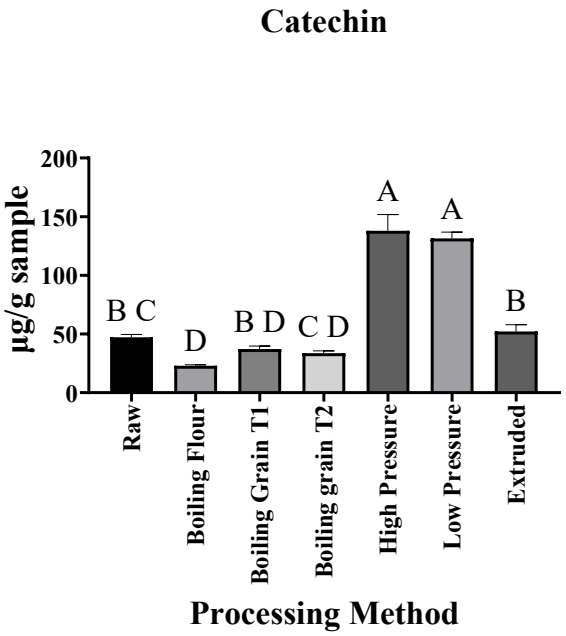

Protocatechuic Acid
µg/g sample
400
300
200
100
0
C D
A
D E
D
B
B C
E
Raw
Boiling Flour
Boiling Grain T1
Boiling grain T2
High Pressure
Low Pressure
Extruded
Processing Method
4-Hydroxybenzoic Acid
µg/g sample
20
15
10
5
0
B
A
D
D
D
D
C
Raw
Boiling Flour
Boiling Grain T1
Boiling grain T2
High Pressure
Low Pressure
Extruded
Processing Method
Caffeic Acid
µg/g sample
250
200
150
100
50
0
B
A
B
B
B
B
B
Raw
Boiling Flour
Boiling Grain T1
Boiling grain T2
High Pressure
Low Pressure
Extruded
Processing Method
Chlorogenic Acid
µg/g sample
40
30
20
10
0
B C
A
C
C
B
B C
D
Raw
Boiling Flour
Boiling Grain T1
Boiling grain T2
High Pressure
Low Pressure
Extruded
Processing Method

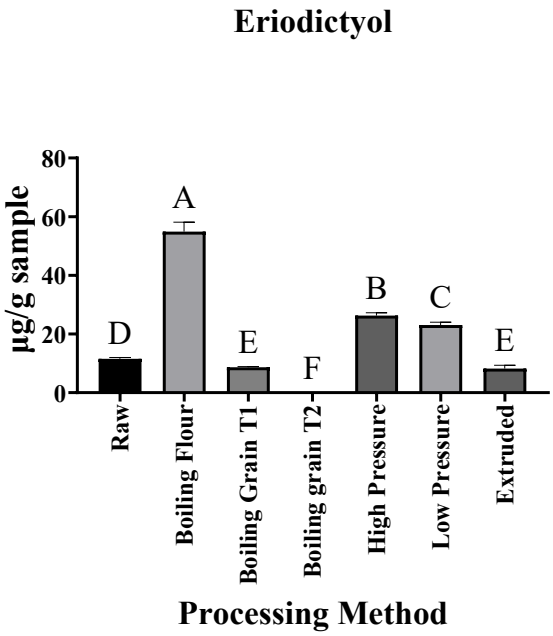

p-coumaric acid
µg/g sample
150
100
50
0
A
C
C
C
B
B
D
Raw
Boiling Flour
Boiling Grain T1
Boiling grain T2
High Pressure
Low Pressure
Extruded
Processing Method

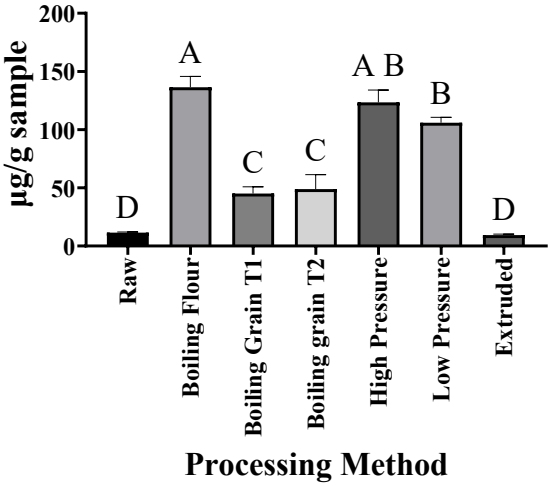
(B)

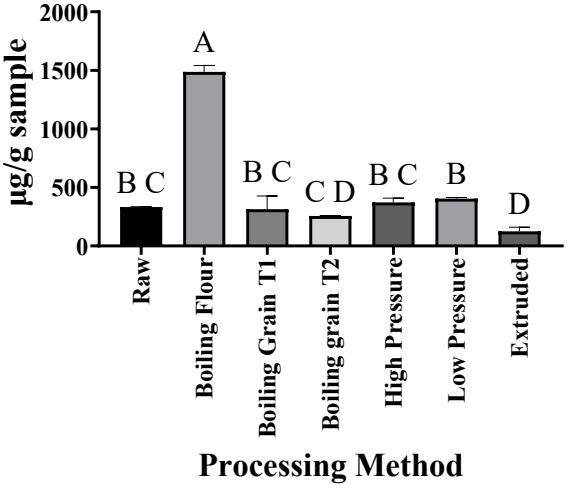

Apigenin
µg/g sample
20
15
10
5
0
B
A
B
B
B
B
B
Raw
Boiling Flour
Boiling Grain T1
Boiling grain T2
High Pressure
Low Pressure
Extruded
Processing Method


Luteolin
µg/g sample
80
60
40
20
0
C
A
C
C
B
B
D
Raw
Boiling Flour
Boiling Grain T1
Boiling grain T2
High Pressure
Low Pressure
Extruded
Processing Method


Catechin
µg/g sample
200
150
100
50
0
B C
D
B D
C D
A
A
B
Raw
Boiling Flour
Boiling Grain T1
Boiling grain T2
High Pressure
Low Pressure
Extruded
Processing Method


Eriodictyol
µg/g sample
80
60
40
20
0
D
A
E
F
B
C
E
Raw
Boiling Flour
Boiling Grain T1
Boiling grain T2
High Pressure
Low Pressure
Extruded
Processing Method


Quercetin
µg/g sample
200
150
100
50
0
D
A
C
C
A B
B
D
Raw
Boiling Flour
Boiling Grain T1
Boiling grain T2
High Pressure
Low Pressure
Extruded
Processing Method


Taxifolin
µg/g sample
2000
1500
1000
500
0
B C
A
B C
C D
B C
B
D
Raw
Boiling Flour
Boiling Grain T1
Boiling grain T2
High Pressure
Low Pressure
Extruded
Processing Method

(C)

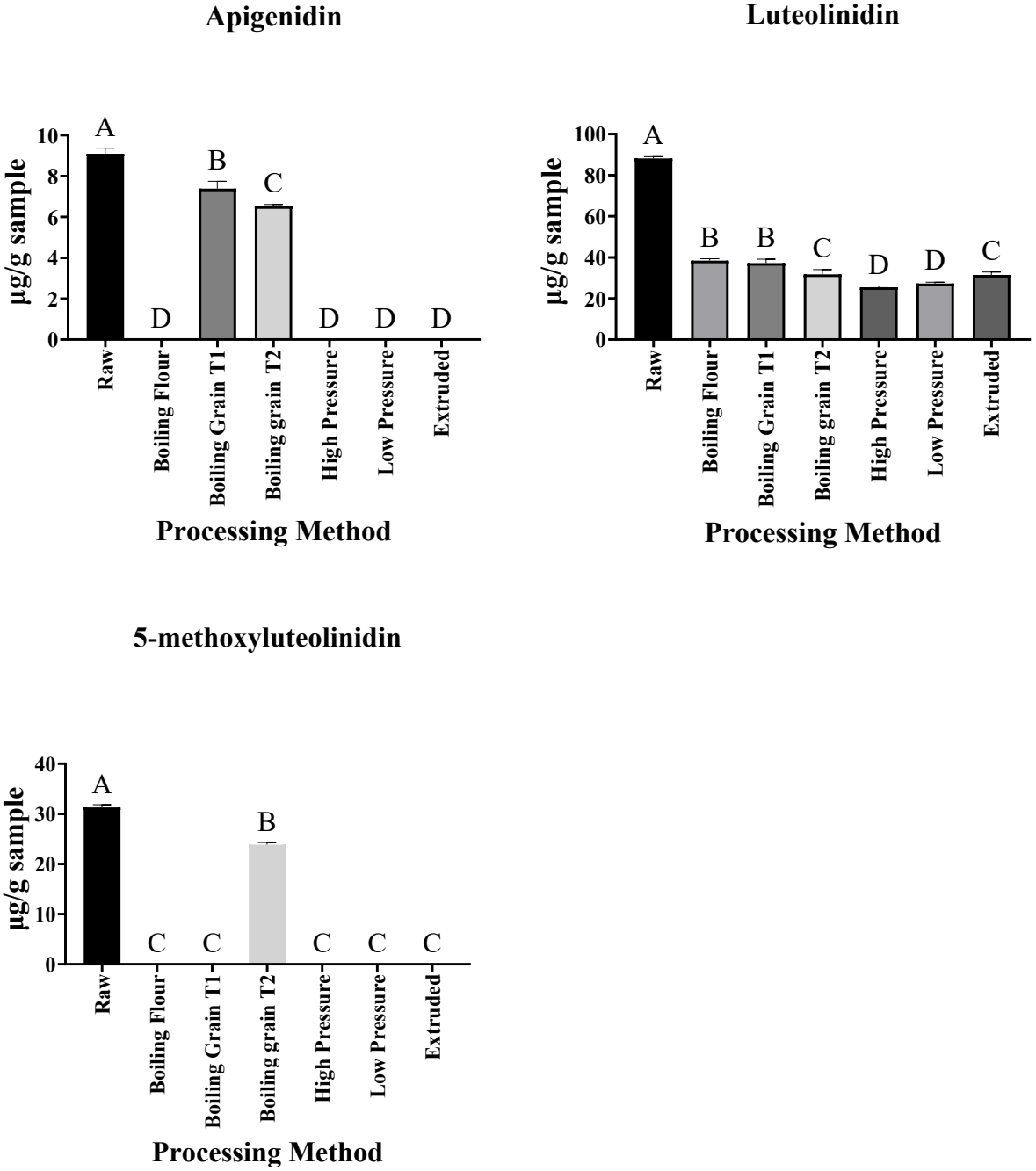
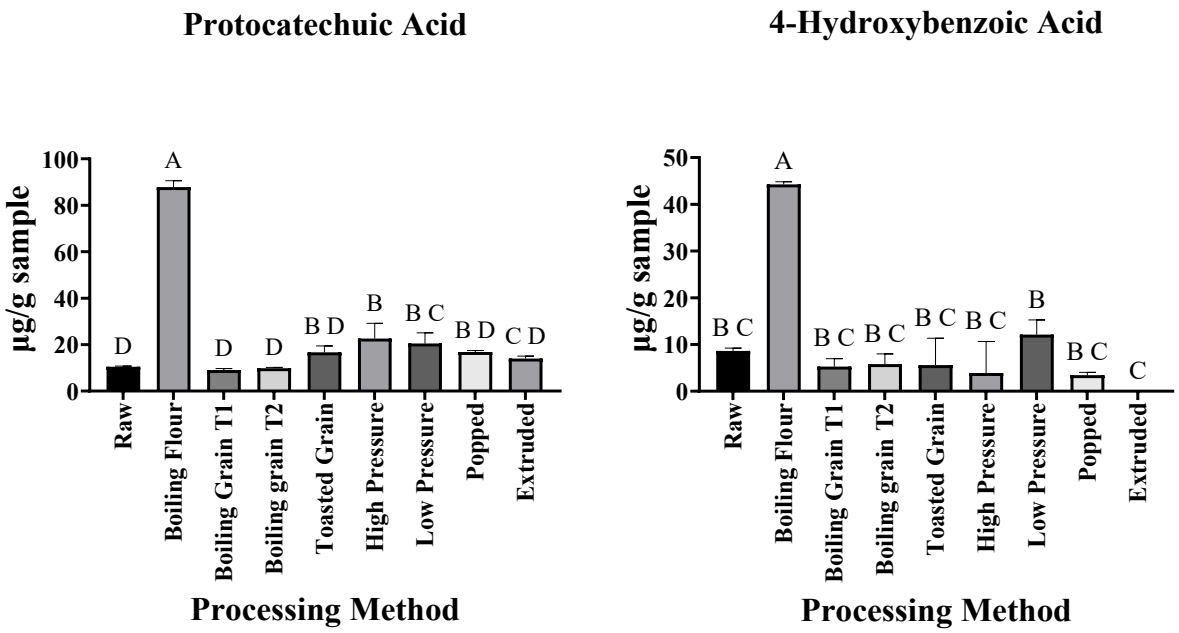


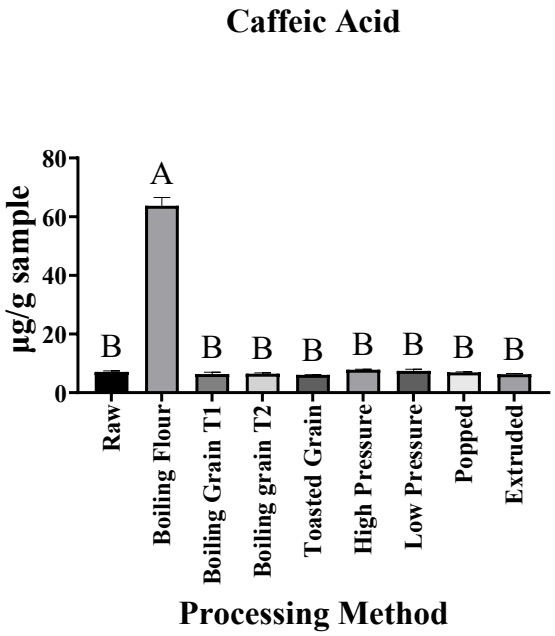
Figure 4.4 Detected phenolic acids (A), flavonoids (B), and 3-deoxyanthocyanidins (C) in sumac sorghum before and after processing using RP-HPLC.

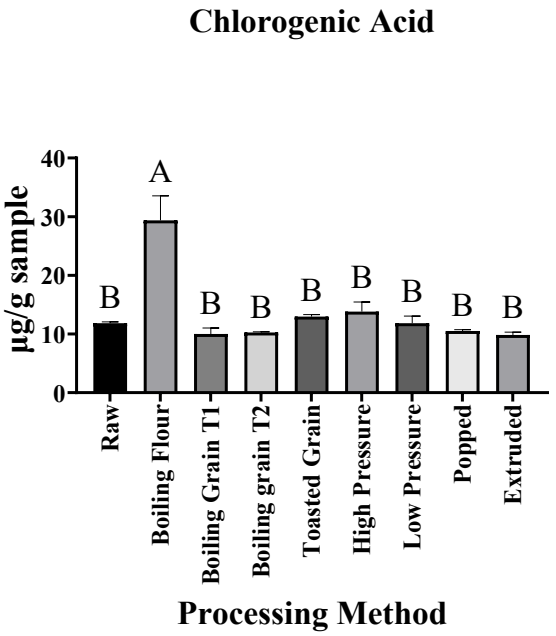
In **Figure 4.5A**, the phenolic profile of black sorghum via RP-HPLC analysis is represented. Like sumac, boiling flour increased protocatechuic acid, 4-hydroxy benzoic acid, caffeic acid, chlorogenic acid, and p-coumaric acid. Both boiling methods did not affect any of the phenolic acids. Toasted grain, high pressure, low pressure, and popping, significantly increased protocatechuic acid content. Extrusion did not significantly decrease phenolic acid content with exception of 4-hydroxy benzoic acid and ferulic acid.

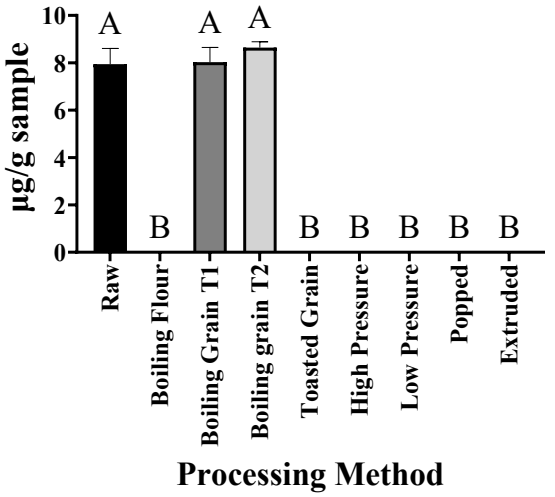
All detected flavonoid compounds in black sorghum are reported in **Figure 4.5B**. All 3-DA content decreased after processing with the exception of boiling flour, in which 5-methoxyluteolinidin was not affected and extrusion where in 7-methoxyapigenidin was significantly increased after processing (**Figure 4.5C).**

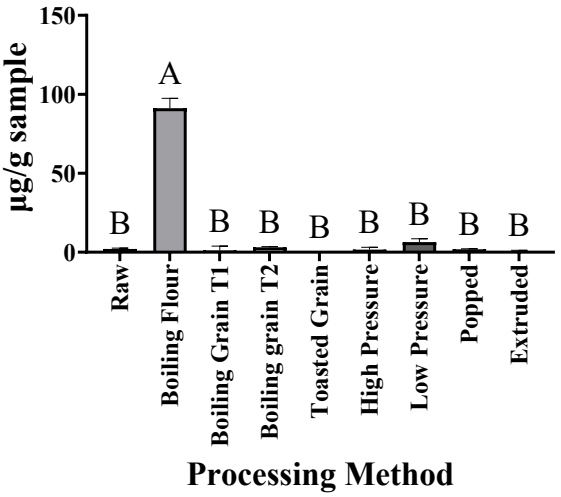
(A)



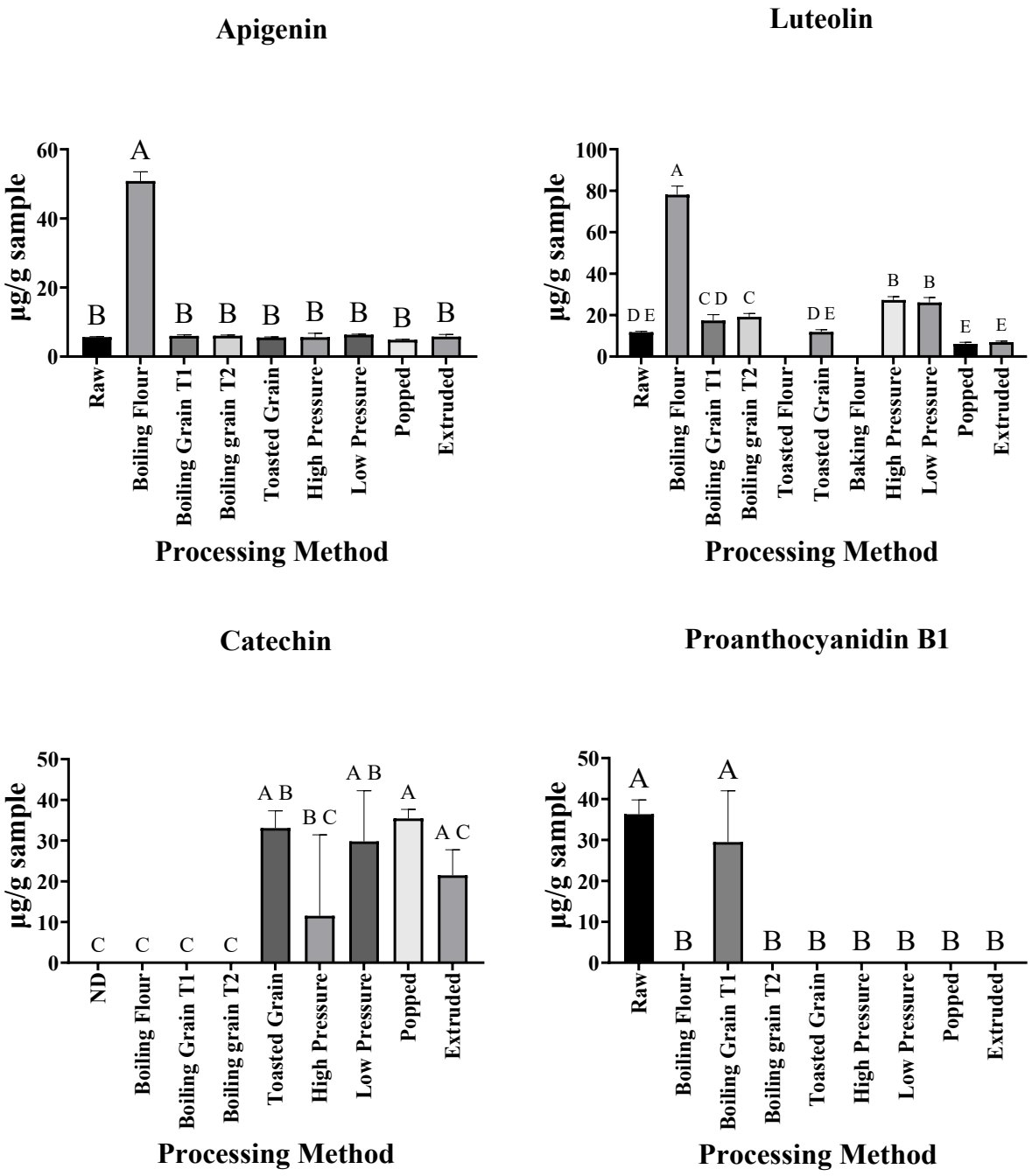

Caffeic Acid
µg/g sample
80
60
40
20
0
A
B
B
B
B
B
B
B
B
Raw
Boiling Flour
Boiling Grain T1
Boiling grain T2
Toasted Grain
High Pressure
Low Pressure
Popped
Extruded
Processing Method

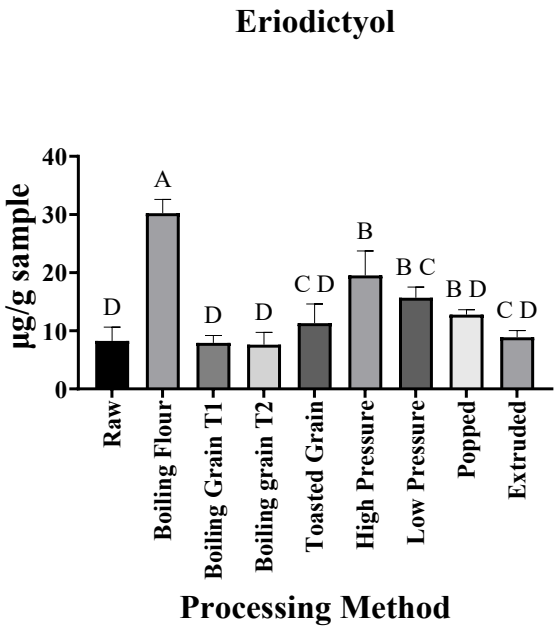

Chlorogenic Acid
µg/g sample
40
30
20
10
0
B
A
B
B
B
B
B
B
B
Raw
Boiling Flour
Boiling Grain T1
Boiling grain T2
Toasted Grain
High Pressure
Low Pressure
Popped
Extruded
Processing Method

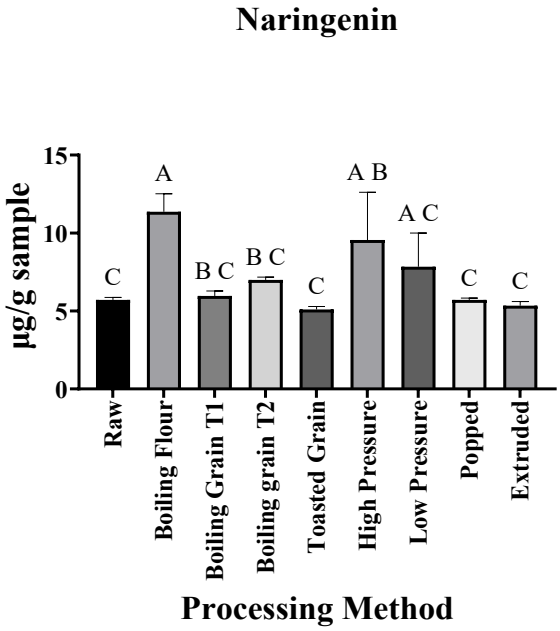

Ferulic acid
µg/g sample
10
8
6
4
2
0
A
B
A
A
B
B
B
B
B
Raw
Boiling Flour
Boiling Grain T1
Boiling grain T2
Toasted Grain
High Pressure
Low Pressure
Popped
Extruded
Processing Method


p-coumaric acid
µg/g sample
150
100
50
0
B
A
B
B
B
B
B
B
B
Raw
Boiling Flour
Boiling Grain T1
Boiling grain T2
Toasted Grain
High Pressure
Low Pressure
Popped
Extruded
Processing Method

(B)


Apigenin
µg/g sample
60
40
20
0
A
B B B B B B B B
Raw
Boiling Flour
Boiling Grain T1
Boiling grain T2
Toasted Grain
High Pressure
Low Pressure
Popped
Extruded
Processing Method
Luteolin
µg/g sample
100
80
60
40
20
0
A
D E
C D
C
D E
B
B
E
E
Raw
Boiling Flour
Boiling Grain T1
Boiling grain T2
Toasted Flour
Toasted Grain
Baking Flour
High Pressure
Low Pressure
Popped
Extruded
Processing Method
Catechin
µg/g sample
50
40
30
20
10
0
C C C C
A B
B C
A B
A
A C
ND
Boiling Flour
Boiling Grain T1
Boiling grain T2
Toasted Grain
High Pressure
Low Pressure
Popped
Extruded
Processing Method
Proanthocyanidin B1
µg/g sample
50
40
30
20
10
0
A
A
B B B B B B B
Raw
Boiling Flour
Boiling Grain T1
Boiling grain T2
Toasted Grain
High Pressure
Low Pressure
Popped
Extruded
Processing Method


Eriodictyol
µg/g sample
40
30
20
10
0
D
A
D
D
C D
B
B C
B D
C D
Raw
Boiling Flour
Boiling Grain T1
Boiling grain T2
Toasted Grain
High Pressure
Low Pressure
Popped
Extruded
Processing Method


Naringenin
µg/g sample
15
10
5
0
C
A
B C
B C
C
A B
A C
C
C
Raw
Boiling Flour
Boiling Grain T1
Boiling grain T2
Toasted Grain
High Pressure
Low Pressure
Popped
Extruded
Processing Method

(C)

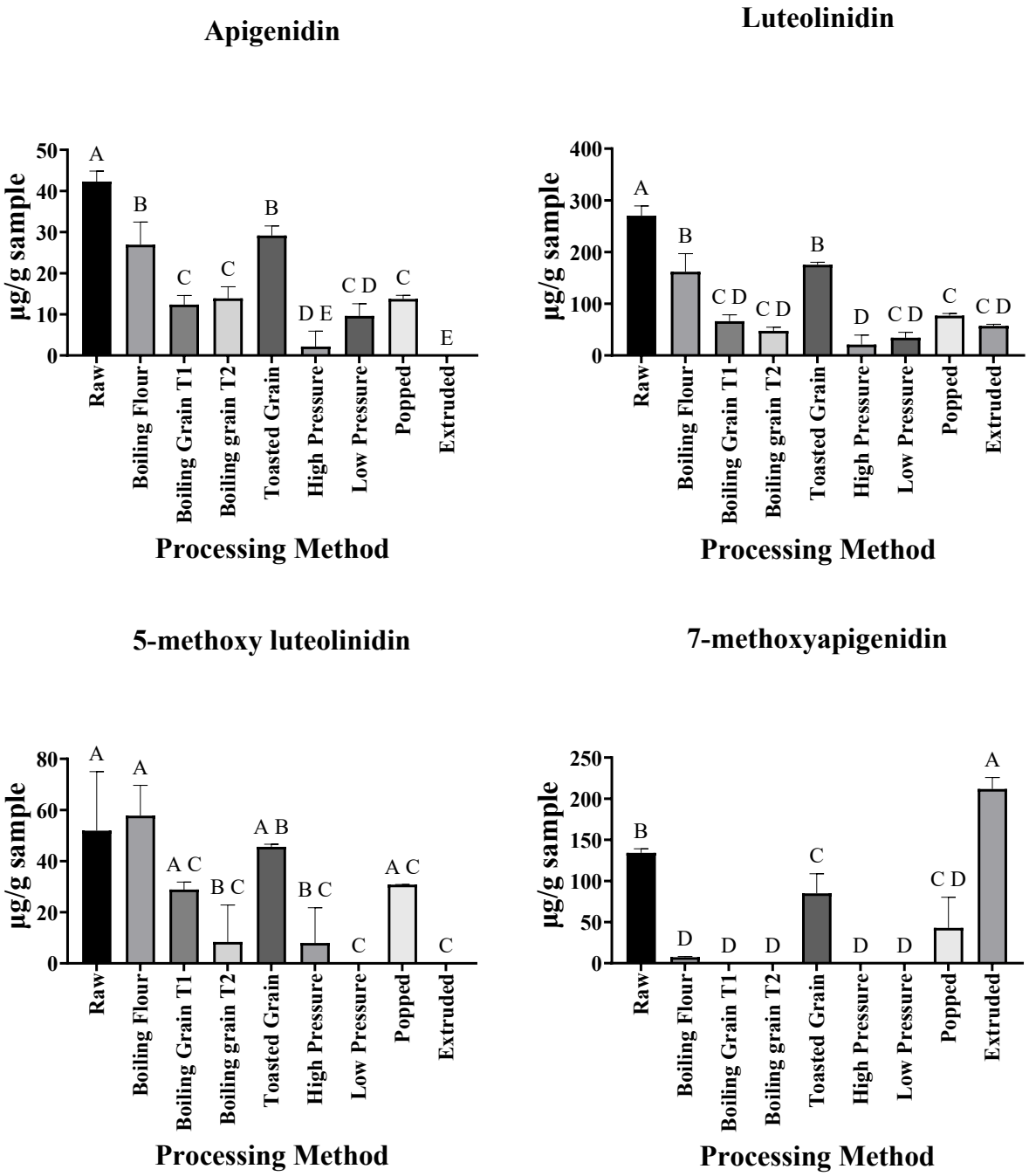


Figure 4.5 Detected phenolic acids (A), flavonoids (B,) and 3-deoxyanthocyanidins (C) in black sorghum using RP-HPLC.

4.3.5 NP – HPLC

Table 4.2 contains the degree of polymerization results for sumac sorghum via NP-HPLC. The total proanthocyanidin content (condensed tannin content) of sumac (**Table 4.2**) significantly decreased after boiling grain T1, high pressure, and low pressure processing. Boiled flour, boiled grain T2 and extrusion processing did not significantly decrease total proanthocyanidin content of sumac samples.

Table 4.2 Total proanthocyanidin content, degree of polymerization index and %polymer results of sumac sorghum after processing via NP-HPLC

Grain	Process	Total PA (µg/g)			DPi			%Polymer		
Sumac	***Raw***	1.29 ± 0.19	AB		6.35 ± 0.45	A		38.52 ± 2.61	AB	
	Boiled Flour	0.93 ± 0.05	BC		2.10 ± 0.06	D		19.63 ± 0.94	C	
	Boiled grain T1	0.69 ± 0.13	C		6.36 ± 0.91	A		42.72 ± 9.44	A	
	Boiled Grain T2	0.95 ± 0.95	BC		5.01 ± 0.32	B		27.55 ± 3.13	AC	
	High Pressure	0.74 ± 0.23	C		2.97 ± 0.24	D		18.19 ± 10.27	C	
	Low Pressure	0.83 ± 0.05	C		3.27 ± 0.31	CD		15.82 ± 3.41	C	
	Extruded	1.35 ± 0.04	A		4.34 ± 0.07	BC		24.01 ± 1.01	BC	

Toal proanthocyanidin (PA) content expressed as µg/g per sample. Means with different letters within a column are statistically significant ($p<0.05$).

The total proanthocyanidin content of black sorghum (**Table 4.3**) significantly decreased in boiled flour, high pressure, and low pressure samples. All other processing methods did not significantly impact total proanthocyanidin content.

Table 4.3 Total proanthocyanidin content, degree of polymerization index and %polymer results of black sorghum after processing via NP-HPLC

Grain	Process	Total PA (µg/g)			DPi			%Polymer		
Black	***Raw***	1.30 ± 0.14	A		7.00 ± 0.12	A		44.23 ± 0.91	AB	
	Boiled Flour	0.87 ± 0.03	C		2.85 ± 0.13	C		32.79 ± 1.01	AC	
	Boiled grain T1	0.99 ± 0.08	BC		6.58 ± 0.08	A		40.39 ± 1.51	AC	
	Boiled Grain T2	0.93 ± 0.04	BC		6.14 ± 0.05	AB		36.36 ± 0.81	AC	
	Toasted Grain	1.08 ± 0.07	B		7.17 ± 0.06	A		47.80 ± 0.45	A	
	High Pressure	0.58 ± 0.03	D		4.50 ± 1.68	BC		25.59 ± 14.37	C	

Low Pressure	0.58	±	0.04	D	5.13	±	1.36	AB	30.31	±	11.10	BC
Popped	0.83	±	0.03	C	6.36	±	0.12	AB	41.03	±	1.14	AC
Extruded	0.88	±	0.05	C	5.30	±	0.16	AB	29.15	±	0.45	BC

Toal proanthocyanidin (PA) content expressed as µg/g per sample. Means with different letters within a column are statistically significant ($p<0.05$).

4.3.6 Glycation inhibition

In comparison to white and black sorghum, sumac sorghum had the highest glycation inhibition in raw grain (data not shown). As a result, the effects of thermal processing on glycation inhibition in sumac sorghum was evaluated. The glycation inhibition results of sumac are reported in **Figure 4.6** The glycation inhibition was significantly decreased by all processing methods. In addition, boiling sumac flour significantly decreased the glycation inhibition more than other processing methods.

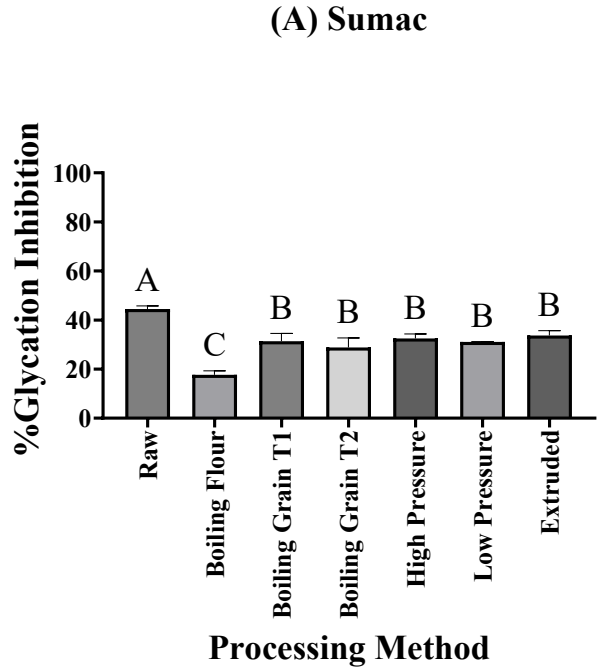


Figure 4.6 Glycation inhibition of sumac sorghum before and after processing

4.4 Discussion

4.4.1 Total phenolic content

4.4.1.1 Boiling Flour

In sumac sorghum, boiling flour significantly decreased TPC in comparison to raw grains. This is similar to Collins et al. (2024) who made a sorghum porridge with eight different varieties of sorghum. Four red varieties were used, of which two samples significantly decreased in TPC after a 10 min cook time at 95°C. The decrease in total phenolic content could be due to the formation of starch-lipid complexes. The formation of these complexes during cooking can then interfere with the detection or bioavailability of bioactive compounds (Collins et al., 2024). The decrease in TPC in sumac, is also in line with Kayodé et al. (2007) who studied changes in phenolic content of red sorghum after cleaning and porridge processing. For all three cleaning methods and subsequent heating methods, the TPC of their red sorghum saw a 38-65% decrease in TPC in comparison to their raw grain counterparts. It is hypothesized by the authors that the decrease in TPC is likely due to the formation of insoluble complexes between the phenolics and other food components (protein, lipids, starch, etc.). In comparison, the white and black sorghum samples did not significantly decrease after boiling the flours. This is in line with Kayitesi et al. (2012) who studied sorghum and sorghum marama bean (legume native to southern Africa) composite flours and porridges. The authors examined sorghum flour and sorghum porridge alone after processing and did not see a significant decrease in TPC. It should be noted however, that the type of sorghum used is not clearly mentioned. The lack of change could be explained by structural changes that occur during the cooking process. During the cooking process structural change of some phenolic compounds may occur which could increase their accessibility during analysis or detection. Differences in TPC may also be due to differences in processing method

and starting material. It is therefore important to clearly describe both the starting material of sorghum and the processing method to help guide future studies

4.4.1.2 Boiling Grain (T1 &T2)

In contrast to boiling whole flour, boiling whole grain at both time points significantly decreased the TPC in all sorghum varieties. To date, there is limited information on the effect of boiling on whole grain sorghum. The effect of boiling sorghum flour is more often reported wherein boiling sorghum flour decreases total phenolic content (Hamad et al., 2019, Luzardo-Ocampo et al., 2020). The decrease in boiled grain is likely also explained by changes in phenolic structure during processing or the formation of phenolic complexes with food components. However, to evaluate the effect of boiling on other whole grain sorghum varieties, more research is needed.

4.4.1.3 Toasted Grains

All toasted grains were purchased from Nu Life Marker. As previously mentioned, due to the size of the kernel, sumac was not able to be toasted for analysis in this study. In white and black sorghum grains, phenolic content increased after toasting. This is in agreement with Li et al. (2022b) who saw a similar increase in total phenolic content after roasting. It is hypothesized that since roasting happens on the surface of the kernel, there is a breakdown of the cell wall, which released bound phenolics, thus increasing their detectability during analysis.

4.4.1.4 Pressure Cooking

In sumac, both high pressure and low pressure cooking significantly decreased total phenolic content. This is in agreement with Bianco-Gomes et al. (2022) who performed a similar pressure cooking method as described in this study. After 15 minutes of high-pressure cooking, TPC decreased by 93%. It is notable that the type of sorghum is not specified by the authors in

this study. In white and black sorghum, both high pressure and low-pressure cooking did not significantly impact TPC. This disagrees with Luo et al. (2020) who reported an increase in total phenolic content after high pressure processing red sorghum bran for 30 minutes in comparison to 10 minutes. It is notable that the authors did not examine differences in processed grain in comparison to raw grain and may therefore not be an appropriate assessment. Lastly, the differences in results between grain samples may be due to differences in grain characteristics. For example, raw sumac sorghum is higher in TPC than both black and white sorghum samples.

4.4.1.5 Popped

All popped, toasted, and extruded grains were purchased from Nu Life Market (Scott City, KS) from the same grow out as the raw grain samples. As previously mentioned, popped and toasted sumac was not available for purchase due to incompatibility with equipment and kernel size. White sorghum saw a significant increase in total phenolic content after popping. Although the use of popping with low polyphenolic sorghum has been reported, many of these studies examine other nutritional parameters like protein content, starch digestibility, protein digestibility and phytic content (Parker et al., 1999, Duodu et al., 2001, Castro-Campos et al., 2021). There is little research available regarding the effect of popping on sorghum polyphenols and even fewer published studies on the effect of popping on high polyphenol sorghum varieties. Black sorghum TPC was not significantly impacted by popping which is in agreement with Cardoso et al. (2014) who examined the effect of popping on red sorghum varieties. To fully understand the effects of popping on sorghum phenolics, more studies are needed examine the effect of this processing method on high polyphenol sorghum varieties. To our knowledge, this is the first time the effects of popping on high polyphenol sorghum samples have been evaluated.

Additionally, given that TPC was not significantly affected by popping, this processing method shows promising applications for sorghum in functional food applications.

4.4.1.6 Extruded

Extruding sorghum increased TPC for all sorghum varieties. This is in line Salazar Lopez et al. (2016) who examined the effect of various extrusion parameters on unpigmented sorghum bran. It is hypothesized that the extrusion process releases phenolics from plant cell walls, thereby making them more available for detection. This hypothesis is supported by the findings of Ortiz-Cruz et al. (2020) who examined the effect of bound and free phenolics on sorghum after extrusion. The findings indicate that the extrusion process increased availability of phenolics for detection due to the increase in free phenolics and significant decrease in bound phenolics after processing. The increase in TPC is also in agreement with other extrusion studies examining different cereal grains like rice, barley and oats (Zielinski et al., 2001).

4.4.2 Condensed Tannin Content

4.4.2.1 Boiling Flour

In white sorghum samples, condensed tannins were not detected, regardless of processing method. Boiling sorghum flour significantly reduced CTC of sorghum regardless of variety. The decrease in CTC agrees with our previous study in which a 51% reduction in condensed tannin content after boiling for 10 minutes was observed (Peterson et al., 2024). The decrease in CTC after boiling disagrees with Collins et al. (2024) who reported that boiling flour did not significantly impact the CTC of 5 of their 6 pigmented sorghum samples. Difference in results is likely due to starting material. For example, the CTC of Black raw grain present in our study was 38mg CE/g while the raw CTC of sorghum studied by Collins et al. (2024) ranged from 0.78 mg

to 8.32 mg CE/g. In addition, the authors also used an alternate method of detection (total proanthocyanidin content assay or TPAC) that may influence results.

4.4.2.2 Boiling Grain (T1 and T2)

The effect of boiling whole grain sorghum on condensed tannin content is not well reported in current literature using spectrophotometric assays. Other studies have been published examining the effect of boiled whole grain on sorghum condensed tannin content via NP-HPLC analysis (Dlamini et al., 2009) and will be discussed in section 4.4.5 of this study.

4.4.2.3 Toasted Grain

As previously mentioned, sumac sorghum was not toasted for this study due to processing constraints. In addition, condensed tannins were not detected in white sorghum. In black sorghum grain, however, toasting grain did not significantly reduce the CTC. This disagrees with (Wu et al., 2013) who examined the effect of soaking, steaming and roasting on tannin containing sorghum. In comparison to the raw grain, the authors reported a 48% decrease in CTC after roasting. Differences in CTC may be due to differences in processing method and detection method (Vanillin-HCl vs Butanolysis). The effect of roasting on whole grains is less commonly reported than their whole flour counterparts. Although the CTC of toasted flours have been studied and reported in literature, the lack of appropriate controls specifically comparing the raw flours to the toasted flours, are not available for comparison (Silva et al., 2020, Paes et al., 2024). More studies are needed to examine the effect of toasting on whole grain and whole flour, with appropriate controls, to understand how toasting influences condensed tannin content in sorghum.

4.4.2.4 Pressure Cooking

High pressure and low pressure cooking significantly reduced the CTC in sumac and black sorghum. The effect of pressure cooking is not well reported in literature. To our knowledge this is the first time in literature that the effects of high and low pressure processing on whole sorghum grain condensed tannins have been reported.

4.4.2.5 Popping

Popping sorghum decreased the condensed tannin content in black sorghum. Although there was a significant decrease in CTC in comparison to raw grain, the CTC of popped black sorghum was comparable to toasted black sorghum grain. The decrease in CTCT in black sorghum is likely due to structural changes within the condensed tannin. This could be loosely supported by the findings that popping black sorghum did not significantly reduce the TPC of black grains. It is possible that the popping method either releases bound phenolics or creates structural changes in condensed tannins from large polymers into smaller molecular weight phenolics. However, to investigate this hypothesis more research is needed. To date, there is limited research available on the effect of popping on sorghum condensed tannins.

4.4.2.6 Extruded

Extruding sorghum decreased the CTC in both sumac and black varieties. This is in agreement with (Adarkwah-Yiadom and Duodu, 2017) who reported a decrease in CTC after various extrusion methods on a red sorghum variety. Other studies have been published examining the effect of extrusion on sorghum condensed tannin content via NP-HPLC analysis (Dlamini et al., 2009) and will be discussed in section 4.4.5 of this study.

4.4.3 Antioxidant activity

Boiling sumac sorghum flour decreased AA. This disagrees with Collins et al. (2024) who reported a significant increase in AA in 3 of 4 red sorghum varieties after boiling. In the

fourth red sorghum sample, the AA was not significantly impacted. These results align more closely with our white sorghum flour which was not significantly impacted by boiling. Like sumac, boiling black sorghum flour significantly reduced the AA. This partially aligns with Collins et al. (2024) who reported a decrease in AA in one of the two black sorghums analyzed in their study.

Like boiled flour, boiling the whole grains of sumac and black sorghum decreased the AA at both time points. In white grain, however, boiling grain did not significantly impact the AA. Differences in AA may be due to differences in initial AA in the raw grains. For example, the AA in sumac and black sorghum is at least 25 times higher than that of white sorghum. To date, there is limited information on the effect of boiling on AA in whole grain sorghum. To understand the mechanism that influences AA changes due to boiling in sorghum, more research is needed.

For black grain, toasting increased AA. This was not in agreement with (Xiong et al., 2019a) who found a significant reduction in sorghum AA after roasting during processing. However, differences in AA could be due to starting material, as Xiong et al., (2019a) used a white non-tannin grain. The white sorghum in our study did not significantly change after toasting, which does agree with Li et al. (2019a). This supports the previously acknowledged problem in literature that current research focusing on processing effects on sorghum polyphenols may not be using appropriate models that contain high levels of polyphenols.

High pressure and low pressure processing significantly decreased the AA of sumac and black sorghum. This agrees with Bianco-Gomes et al. (2022) who reported a decrease in AA after high pressure cooking sorghum grains for 15 minutes. White sorghum was not significantly

decreased by high pressure processing, but AA was significantly reduced after low pressure cooking.

Popping white sorghum did not significantly impact AA. This agrees with the findings of Cardoso et al. (2014) who investigated the effect of popping on red, non-tannin containing sorghum. The authors reported no significant changes in AA after popping. However, popping black sorghum significantly decreased AA in this current study. Although c the mechanism that influences changes to AA during popping is not well reported, it is notable that the concentration of AA in white and black sorghum is significantly different. This further supports the previous claim that studying processing effects on appropriate sorghum models is imperative if sorghum polyphenols are to be used in functional food applications.

Extruding sumac sorghum increased AA which supports the results of Rudra et al. (2015) who extruded sorghum-barely composite snacks. After extrusion at high temperature and low moisture conditions, antioxidant activity was significantly increased. In white and black sorghum extrusion did not significantly increase or decrease AA. This is also still in agreement with Rudra et al. (2015) who examined three other extrusion parameters on sorghum AA. The effect of extrusion on sorghum AA is therefore beneficial. As extrusion released bound phenolics, thus making them more available for detection. It is also reported that during extrusion, the formation of melanoidins from the Maillard browning reaction also contribute to increased AA in extruded cereal grains (Zieliński and Troszyńska, 2000).

4.4.4 RP – HPLC

Boiling the sumac and black sorghum flour did not significantly affect the 3-Da content. This is in agreement with the findings in **Chapter 3**, wherein all detectable 3-Da in SC84 sorghum significantly decreased after 30 minutes of boiling at 100°C. Due to the fact that

samples were processed in a closed system and freeze dried as a whole, changes in 3-Da content are likely due to structural changes in polyphenols or the formation of insoluble complexes with food matrices and not due to leaching out (Peterson et al., 2024).

Boiling sumac and black sorghum grains at both time points did not significantly affect the chlorogenic acid and quercetin content. This disagrees with Luzardo-Ocampo et al. (2020) who reported a decrease in both compounds in white and red sorghum grains after boiling.

Popping black sorghum grain decreased all 3-Da content except for 5-methoxy luteolindin, which did not significantly decrease. This somewhat disagrees with Cardoso et al. (2014) who examined the effect of popping grains on red sorghum. The authors did not report a significant decreased in any of the detectable 3-Das after popping. The reason for the apparent lack of change observed by the authors is not well understood. Likewise, further studies are needed to understand the effect of popping on 3-Da content in sorghum.

After extrusion, the flavones (luteolin and apigenin) of sumac and black sorghum significantly decreased. These results agree with Cardo et al., (2015) who reported that extrusion processing decreased apigenin and luteolin to an undetectable (not detectable) level. In addition to the flavones, the 3-DA content decreased in sumac and black sorghum after extrusion. This is line with Cardoso et al. (2015) who reported extrusion processing decreasing apigenidin, luteolindin, and 5-methoxyapigenidin in three sorghum cultivars.

4.4.5 NP – HPLC

Examining the effect of processing on the condensed tannin content via NP-HPLC is not well reported in literature. For boiling flour, toasted grain, or popped grain. To the best knowledge of the authors this is the first time the effects of those processing methods on sorghum condensed tannin content analyzed via NP-HPLC have been reported.

Boiling sumac grain for 30 minutes and boiling black sorghum grain for both 30 minutes and 50 minutes did not significantly reduce the DPi or %P of samples. This agrees with Dlamini et al. (2009) who examined two separate porridge processing methods on sorghum. In the traditionally prepared samples (boiled grains), the oligomer and monomer content of traditional porridges were not significantly different from grains.

The effect of high and low pressure cooking on sorghum polyphenols is not well reported. In the current study, both sumac and black sorghum varieties decreased in total proanthocyanidin content and %Polymer after pressure cooking. This agrees with Barros et al. (2012) who examined the effect of polyphenols on starch pasting properties and amylose and amylopectin interactions. The authors noted their result indicate proanthocyanidins depolymerize during heat processing and form insoluble complexes with gelatinized starch. Although the results from the current study are similar to those of Barros et al. (2012), it should be noted that the authors used a rapid visco analyzer (RVA) and an autoclave to process their samples. In addition, their paper utilized freeze dried phenolics and isolated starch, amylose, and amylopectin, whereas the present study observed whole food models. Therefore, to further understand changes in proanthocyanidin content due to high and low pressure processing, more studies are needed.

Extruding sumac and black sorghum did not significantly decrease their Dpi or %Polymer content in comparison to raw grains. This disagrees with Dlamini et al. (2009) who reported a significant decrease in extractable proanthocyanidin content after extrusions processing. Differences in results could be due to differences in extrusion processing parameters as it has been reported that different moisture, pressure, and temperature parameters will affect the amount of extractable CT in samples (Adarkwah-Yiadom and Duodu, 2017).

4.4.6 Glycation Inhibition

The glycation of proteins is a main factor in the contribution of aging and the onset of many diseases. AGEs are precursors for many chronic diseases, therefore inhibiting there formation may prevent the onset of some diseases. In literature, extracted sorghum phenolics have been used for AGE inhibition (Farrar et al., 2008, Senevirathne et al., 2021, Ben El Mahdi et al., 2024, Ofosu et al., 2020). There is a significant gap in knowledge for processed sorghum phenolics and their ability to inhibit AGE formation. To the best of knowledge, using processed sorghum phenolics for glycation inhibition has not been reported in literature on boiled flours, boiled grains, toasted grain, high pressure cooking, low pressure cooking, popped sorghum or extruded sorghum samples.

Boiling sumac sorghum flour significantly decreased the glycation inhibition. Although glycation inhibition decreased by 60% after boiling the flour, the samples still retained some of their glycation inhibition, measuring at 17%. Given the relatively low TPC and AA levels in white sorghum, it was not selected for the glycation analysis portion of this dissertation. Black grain likewise had low levels of initial glycation inhibition and was not studied further for this dissertation.

Boiling sumac sorghum grain for 30 and 50 minutes decreased glycation inhibition by 31% and 35%, respectively. Although glycation inhibition decreased, the glycation inhibition of boiled sumac grain was significantly higher than the glycation inhibition of boiled sumac flour. Boiling whole grains may protect bound phenolics from thermal degradation during heat processing, thus making them more available for detection.

Using high and low pressure cooking for sumac sorghum significantly decreased the glycation inhibition of samples. Although a notable decrease in glycation occurred after pressure

cooking sumac, the glycation inhibition of pressure cooked sumac was significantly higher than boiled flours in sumac samples.

In sumac sorghum, extrusion significantly decreased the glycation inhibition of sample extracts. This is interesting as extrusion increased the TPC and AA in suma. Given that processing greatly affected the level of AGE inhibition in every processing method, it is vital that more research be conducted on processed sorghum phenolics to determine their potential use for functional food systems.

4.5 Conclusion

In conclusion, each processing method had various effects on TPC, CTC, phenolic profile, AA, and glycation inhibition. Specific trends of note were boiling flour significantly increased phenolic acids and most flavonoids in both sumac and black sorghum. Boiling grain (T1 & T2), high pressure, and low-pressure processing significantly decreased TPC and AA in sumac and black sorghum. All 3-DAs significantly decreased after processing with exception of extrusion where in 7-methoxyapigenidin increased in black sorghum only. Additionally, glycation inhibition was negatively impacted by processing in all sumac samples. Future work looking at the effect of processing on other potential health benefits, for example, cancer cell inhibition or starch digestibility, should be examined to better understand the effect of processing on sorghum for health food applications.

Chapter 5 – Conclusions and future needs

Sorghum polyphenols show great potential for human health food applications. In this current work, the health benefits of sorghum and their current health food applications have been discussed. In **Chapter 2** rice, corn, and sorghum grain were compared for their phenolic profile

and bioactivity. Sorghum had the overall highest TPC, CTC, and AA content in comparison to rice and corn grains. Rice had comparable glycation inhibition to sorghum after extract normalization. The phenolic profiled of samples were also compared in which rice and corn had detectable anthocyanins while sorghum did not. The detectable 3-DAs were not detectable in rice or corn samples and were only detected in sorghum samples. This supports the current ongoing hypothesis that 3-DAs are unique to sorghum in comparison to other cereal grains. Additionally, some pigmented sorghum varieties are known to be sources for condensed tannins. When analyzing the degree of polymerization in rice, corn, and sorghum samples, sorghum had more total proanthocyanidins (condensed tannins) than rice and corn. The findings of **Chapter 2** suggested that sorghum was a suitable model to study the effect of processing conditions on sorghum polyphenols.

In **Chapter 3** the effect of high moisture pH treatments on SC84 sorghum flour was examined. In this study, heating sorghum for 10 minutes greatly increase digestible starch content. Additionally, heating sorghum for 10 minutes did not significantly reduce the TPC content or decrease the cancer cell inhibition capacity of the sorghum phenolic extracts. The CTC and 3-DA content of sorghum did significantly decrease. Other flavonoid groups of interest either remain unchanged or increase in concentration after heating, regardless of pH. The results of this study showed further promise for sorghum phenolics to be applied in health food or functional food formulations.

In **Chapter 4** the effect of processing on sorghum was expanded by including 8 processing methods and three sorghum varieties. A model food and processing method was applied to each variety of sorghum (Sumac, White, and Black). In all sorghum varieties, extruding sorghum increased TPC, AA, and Glycation inhibition. In White and Black varieties,

toasted grain also had increased TPC, AA, and glycation inhibition in comparison to raw grain. Overall, the processing method that consistently decreased TPC, AA, and glycation inhibition was the boiled grain T2 treatment.

The work presented in this dissertation continues to demonstrate the potential for high polyphenol sorghum as a cost-effective functional food ingredient. To date there are numerous studies examining the changes in phenolic content and consumer acceptance of foods formulated with sorghum. Many of these studies, however, were performed with white, red, or undefined sorghum varieties. Based on the finding from Chapter 2, using the proper starting material greatly affects the TPC, CTC, AA, and glycation inhibition of pigmented grains. Further proven in **Chapter 3** and **Chapter 4**, having a high polyphenol sorghum variety can increase the chances of polyphenols and their potential health benefits being retained or increased post processing. There is therefore a gap in knowledge in current literature surrounding the use of "high polyphenol" sorghum lines and their ability to withstand processing methods and their potential health benefits.

Regardless of the numerous health benefits that sorghum is reported to offer, consumers are unlikely to consume sorghum if it is not a palatable or attractive food product. To further validate high polyphenol sorghum as a functional food ingredient, more work is needed to examine 1) the potential health benefits of sorghum after processing and 2) the consumer acceptability of high polyphenol sorghum formulated foods. In future studies, it is paramount that high polyphenol sorghum lines are examined for their potential health benefits in vitro post processing. In conjunction with this work, studies using high polyphenol sorghum in food formulations should include consumer acceptability, shelf life, and physiochemical studies to understand changes in foods after inclusion of high polyphenol sorghum.

Appendix A Definitions

3-Da – 3-deoxy anthocyanidin(s)

AA – antioxidant activity

ABTS – 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

ACS – American Chemical Society

AGE – advanced glycation end products

ANOVA – analysis of variance

AOAC – Association of Official Analytical Chemists

BSA – bovine serum albumin

BSA-FRU – bovine serum albumin fructose

CE – catechin equivalents

CFR – code of federal regulations

CTC – condensed tannin content

DI – deionized (water)

DP – degree of polymerization

Dpi – degree of polymerization index

DPPH – 2,2-Diphenyl-1-picrylhydrazyl

DS – digestible starch

FBS – fetal bovine serum

FC – Folin Ciocalteu

GAE – gallic acid equivalents

GF – gluten free

HCl – hydrochloric acid

HORAC – hydroxyl radical antioxidant capacity

HPLC – high pressure liquid chromatography

MTS – 3-(4,5-dimethylthizaol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium

NP-HPLC – normal phase high pressure liquid chromatography

RP-HPLC– reverse phase high pressure liquid chromatography

RS – resistant starch

RVA – rapid visco analyzer

SOD – super oxide radical scavenging activity

TE – Trolox equivalents

TPAC – total proanthocyanidin content

TPC – total phenolic content

TS – total starch

US – United States

USDA – United States Department of Agriculture

USDA-ARS – United States Department of Agriculture, Agricultural Research Service

USDA-FAS – United States Department of Agriculture, Foreign Agriculture Service

WHO – World Health Organization

References

ADARKWAH-YIADOM, M. & DUODU, K. G. 2017. Effect of extrusion cooking and simulated in vitro gastrointestinal digestion on condensed tannins and radical scavenging activity of type II and type III whole grain sorghum. *International Journal of Food Science & Technology,* 52**,** 2282-2294.

AFIFY, A. E.-M. M., EL-BELTAGI, H. S., ABD EL-SALAM, S. M. & OMRAN, A. A. 2011. Bioavailability of iron, zinc, phytate and phytase activity during soaking and germination of white sorghum varieties. *Plos one,* 6**,** e25512.

AFIFY, A. E.-M. M., EL-BELTAGI, H. S., ABD EL-SALAM, S. M. & OMRAN, A. A. 2012a. Biochemical changes in phenols, flavonoids, tannins, vitamin E, β–carotene and antioxidant activity during soaking of three white sorghum varieties. *Asian Pacific Journal of Tropical Biomedicine,* 2**,** 203-209.

AFIFY, A. E.-M. M., EL-BELTAGI, H. S., ABD EL-SALAM, S. M. & OMRAN, A. A. 2012b. Effect of soaking, cooking, germination and fermentation processing on proximate analysis and mineral content of three white sorghum varieties (Sorghum bicolor L. Moench). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca,* 40**,** 92-98.

AFIFY, A. E.-M. M., EL-BELTAGI, H. S., ABD EL-SALAM, S. M. & OMRAN, A. A. 2015. Effect of soaking, cooking, germination and fermentation processing on physical properties and sensory evaluation of sorghum biscuits. *Notulae Scientia Biologicae,* 7**,** 129-135.

AL-ZOREKY, N. S. 2009. Antimicrobial activity of pomegranate (Punica granatum L.) fruit peels. *Int J Food Microbiol,* 134**,** 244-8.

ALAM, M. N., BRISTI, N. J. & RAFIQUZZAMAN, M. 2013. Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharm J,* 21**,** 143-52.

ALMAIMAN, S. A., ALBADR, N. A., ALSULAIM, S., ALHUTHAYLI, H. F., OSMAN, M. A. & HASSAN, A. B. 2021. Effects of microwave heat treatment on fungal growth, functional properties, total phenolic content, and antioxidant activity of sorghum (Sorghum bicolor L.) grain. *Food Chemistry,* 348**,** 128979.

ANDRESSA RODRIGUES DE SOUSAA, M. E. D. C. M., RENATA CELI LOPES TOLEDOA, LAÉRCIO DOS ANJOS BENJAMINB, VALÉRIA APARECIDA VIEIRA QUEIROZC, MARCIA PARANHO VELOSOD, KASSIUS DE SOUZA REISD, HÉRCIA STAMPINI DUARTE MARTINOA, 2018. Extruded sorghum (Sorghum bicolor L.) reduces metabolic risk of hepatic T steatosis in obese rats consuming a high fat diet. *Food Research International,* 112**,** 7.

ANITA STEFOSKA-NEEDHAM, E. J. B., STUART K. JOHNSON, JIAYUE CHU & TAPSELL1, A. L. C. 2016. Flaked sorghum biscuits increase postprandial GLP-1 and GIP levels and extend subjective satiety in healthy subjects. *Mol. Nutr. Food Res,* 60**,** 10.

ANN E. HAGERMAN, A. L. G. B. 1989. Choosing appropriate methods and standards for assaying tannin. *Journal of Chemical Ecology,* 15**,** 16.

ANN E. HAGERMAN, K. M. R., G. ALEXANDER JONES, KARA N. SOVIK, NICOLE T. RITCHARD, PAUL W. HARTZFELD, AND THOMAS L. RIECHEL 1998. High Molecular Weight Plant Polyphenolics (Tannins) as Biological Antioxidants. *Journal of Agriculture and Food Chemistry,* 46.

ANUNCIAÇÃO, P. C., CARDOSO, L. D. M., QUEIROZ, V. A. V., DE MENEZES, C. B., DE CARVALHO, C. W. P., PINHEIRO-SANT'ANA, H. M. & ALFENAS, R. D. C. G.

2018. Consumption of a drink containing extruded sorghum reduces glycaemic response of the subsequent meal. *European journal of nutrition,* 57**,** 251-257.
ANUNCIACAO, P. C., CARDOSO, L. M., GOMES, J. V. P., DELLA LUCIA, C. M., CARVALHO, C. W. P., GALDEANO, M. C., QUEIROZ, V. A. V., ALFENAS, R. C. G., MARTINO, H. S. D. & PINHEIRO-SANT'ANA, H. M. 2017. Comparing sorghum and wheat whole grain breakfast cereals: Sensorial acceptance and bioactive compound content. *Food Chem,* 221**,** 984-989.
APE DAVID, I., NWONGU NWANYINNAYA, A., UWAKWE EZEKIEL, I. & IKEDINOBI CHINELO, S. 2016. Comparative proximate analysis of maize and sorghum bought from Ogbete main market of Enugu State, Nigeria. *Greener Journal of Agricultural Sciences,* 6**,** 272-375.
ARDOIN, R., SMITH, B., LEA, J., BOUE, S., SMOLENSKY, D., SANTANA, A. L. & PETERSON, J. 2023. Consumer perceptions and antioxidant profiling of acidified cold-brewed sorghum bran beverages. *Journal of Food Science,* 88**,** 2301-2312.
ARI AKIN, P., DEMIRKESEN, I., BEAN, S. R., ARAMOUNI, F. & BOYACI, I. H. 2022. Sorghum Flour Application in Bread: Technological Challenges and Opportunities. *Foods,* 11**,** 2466.
ASHLEY, D., MARASINI, D., BROWNMILLER, C., LEE, J. A., CARBONERO, F. & LEE, S. O. 2019. Impact of Grain Sorghum Polyphenols on Microbiota of Normal Weight and Overweight/Obese Subjects during In Vitro Fecal Fermentation. *Nutrients,* 11.
AWIKA, J. M., MCDONOUGH, C. M. & ROONEY, L. W. 2005. Decorticating Sorghum To Concentrate Healthy Phytochemicals. *Journal of Agricultural and Food Chemistry,* 53**,** 6230-6234.
AWIKA, J. M. & ROONEY, L. W. 2004. Sorghum phytochemicals and their potential impact on human health. *Phytochemistry,* 65**,** 1199-221.
AWIKA, J. M., ROONEY, L. W. & WANISKA, R. D. 2004. Properties of 3-Deoxyanthocyanins from Sorghum. *Journal of Agriculture and Food Chemistry,* 52**,** 7.
BARROS, F., AWIKA, J. M. & ROONEY, L. W. 2012. Interaction of tannins and other sorghum phenolic compounds with starch and effects on in vitro starch digestibility. *J Agric Food Chem,* 60**,** 11609-17.
BARROS, F., DYKES, L., AWIKA, J. & ROONEY, L. 2013. Accelerated solvent extraction of phenolic compounds from sorghum brans. *Journal of Cereal Science,* 58**,** 305-312.
BEN EL MAHDI, N., LEMÉE, L., REMAURY, Q. B., ELOY, L., NHIRI, N., LAKHSSASSI, N., CACCIOLA, F. & NHIRI, M. 2024. Potential of Sorghum Seeds in Alleviating Hyperglycemia, Oxidative Stress, and Glycation Damage. *Molecules,* 29**,** 3445.
BIANCO-GOMES, A. C., NOGUEIRA, L. D. S., BONO-LOPES, N. V. M., GOUVÊA-SOUZA, C. P., BOLDRINI-FRANÇA, J., GOMES, V. M., CHERENE, M. B., ALVES, N. E. G. & VASCONCELOS, C. M. 2022. Dry heat and pressure favor bioactive compounds preservation and peptides formation in sorghum [Sorghum bicolor (L.) Moench]. *Current Research in Food Science,* 5**,** 117-124.
BLACKWELL, D. L., HERALD, T., BEAN, S. R. & GADGIL, P. 2012. Alkaline extraction of phenolic compounds from intact sorghum kernels.
BLANCH, G. P. & RUIZ DEL CASTILLO, M. L. 2021. Effect of Baking Temperature on the Phenolic Content and Antioxidant Activity of Black Corn (Zea mays L.) Bread. *Foods,* 10.

BRANTSEN, J. F., HERRMAN, D. A., RAVISANKAR, S. & AWIKA, J. M. 2021. Effect of tannins on microwave-assisted extractability and color properties of sorghum 3-deoxyanthocyanins. *Food Research International,* 148**,** 110612.

BRÖHAN, M., JERKOVIC, V. & COLLIN, S. 2011. Potentiality of Red Sorghum for Producing Stilbenoid-Enriched Beers with High Antioxidant Activity. *Journal of Agricultural and Food Chemistry,* 59**,** 4088-4094.

BVOCHORA, J., REED, J., READ, J. & ZVAUYA, R. 1999. Effect of fermentation processes on proanthocyanidins in sorghum during preparation of Mahewu, a non-alcoholic beverage. *Process Biochemistry,* 35**,** 21-25.

CARDOSO, L. D. M., MONTINI, T. A., PINHEIRO, S. S., PINHEIRO-SANT'ANA, H. M., MARTINO, H. S. D. & MOREIRA, A. V. B. 2014. Effects of processing with dry heat and wet heat on the antioxidant profile of sorghum. *Food Chemistry,* 152**,** 210-217.

CARDOSO, L. D. M., PINHEIRO, S. S., DE CARVALHO, C. W. P., QUEIROZ, V. A. V., DE MENEZES, C. B., MOREIRA, A. V. B., DE BARROS, F. A. R., AWIKA, J. M., MARTINO, H. S. D. & PINHEIRO-SANT'ANA, H. M. 2015. Phenolic compounds profile in sorghum processed by extrusion cooking and dry heat in a conventional oven. *Journal of Cereal Science,* 65**,** 220-226.

CASTRO-CAMPOS, F. G., CABRERA-RAMÍREZ, A. H., MORALES-SÁNCHEZ, E., RODRÍGUEZ-GARCÍA, M. E., VILLAMIEL, M., RAMOS-LÓPEZ, M. & GAYTÁN-MARTÍNEZ, M. 2021. Impact of the popping process on the structural and thermal properties of sorghum grains (Sorghum bicolor L. Moench). *Food Chemistry,* 348**,** 129092.

CDC 2022. Center for Disease Control.

CHAABAN, H., IOANNOU, I., CHEBIL, L., SLIMANE, M., GÉRARDIN, C., PARIS, C., CHARBONNEL, C., CHEKIR, L. & GHOUL, M. 2017. Effect of heat processing on thermal stability and antioxidant activity of six flavonoids. *Journal of Food Processing and Preservation,* 41.

CHEN, X., SHEN, J., XU, J., HERALD, T., SMOLENSKY, D., PERUMAL, R. & WANG, W. 2021. Sorghum Phenolic Compounds Are Associated with Cell Growth Inhibition through Cell Cycle Arrest and Apoptosis in Human Hepatocarcinoma and Colorectal Adenocarcinoma Cells. *Foods,* 10.

CHIREMBA, C., TAYLOR, J. R. N. & DUODU, K. G. 2009a. Phenolic Content, Antioxidant Activity, and Consumer Acceptability of Sorghum Cookies. *Cereal Chemistry,* 86**,** 590-594.

CHIREMBA, C., TAYLOR, J. R. N. & DUODU, K. G. 2009b. Phenolic Content, Antioxidant Activity, and Consumer Acceptability of Sorghum Cookies. *Cereal Chemistry,* 86.

CHOBOT, V., HADACEK, F., BACHMANN, G., WECKWERTH, W. & KUBICOVA, L. 2016. Pro- and Antioxidant Activity of Three Selected Flavan Type Flavonoids: Catechin, Eriodictyol and Taxifolin. *International Journal of Molecular Sciences,* 17**,** 1986.

CHOI, S., KIM, J., LEE, Y. & KIM, C. 2019. Antioxidant activity and contents of total phenolic compounds and anthocyanins according to grain colour in several varieties of Sorghum bicolor (L.) Moench. *Cereal research communications,* 47**,** 228-238.

CHUNG, I.-M., KIM, E.-H., YEO, M.-A., KIM, S.-J., SEO, M. C. & MOON, H.-I. 2011. Antidiabetic effects of three Korean sorghum phenolic extracts in normal and streptozotocin-induced diabetic rats. *Food Research International,* 44**,** 127-132.

CLOSE, G. L., ASHTON, T., CABLE, T., DORAN, D., HOLLOWAY, C., MCARDLE, F. & MACLAREN, D. P. 2006. Ascorbic acid supplementation does not attenuate post-exercise muscle soreness following muscle-damaging exercise but may delay the recovery process. *British Journal of Nutrition,* 95**,** 976-981.

COLLINS, A., SANTHAKUMAR, A., LATIF, S., CHINKWO, K., FRANCIS, N. & BLANCHARD, C. 2024. Impact of Processing on the Phenolic Content and Antioxidant Activity of Sorghum bicolor L. Moench. *Molecules,* 29**,** 3626.

COX, S., NORONHA, L., HERALD, T., BEAN, S., LEE, S.-H., PERUMAL, R., WANG, W. & SMOLENSKY, D. 2019. Evaluation of ethanol-based extraction conditions of sorghum bran bioactive compounds with downstream anti-proliferative properties in human cancer cells. *Heliyon,* 5.

DAG, D. & OZTOP, M. H. 2017. Formation and Characterization of Green Tea Extract Loaded Liposomes. *J Food Sci,* 82**,** 463-470.

DE CAMARGO, A. C., VIDAL, C. M., CANNIATTI-BRAZACA, S. G. & SHAHIDI, F. 2014. Fortification of cookies with peanut skins: effects on the composition, polyphenols, antioxidant properties, and sensory quality. *J Agric Food Chem,* 62**,** 11228-35.

DE MESA-STONESTREET, N. J., ALAVI, S. & BEAN, S. R. 2010. Sorghum proteins: the concentration, isolation, modification, and food applications of kafirins. *J Food Sci,* 75**,** R90-R104.

DE MORAIS CARDOSO, L., PINHEIRO, S. S., DE CARVALHO, C. W. P., QUEIROZ, V. A. V., DE MENEZES, C. B., MOREIRA, A. V. B., DE BARROS, F. A. R., AWIKA, J. M., MARTINO, H. S. D. & PINHEIRO-SANT'ANA, H. M. 2015. Phenolic compounds profile in sorghum processed by extrusion cooking and dry heat in a conventional oven. *Journal of Cereal Science,* 65**,** 220-226.

DE OLIVEIRA, L. D. L., DE ORLANDIN, L. C., DE AGUIAR, L. A., QUEIROZ, V. A. V., ZANDONADI, R. P., BOTELHO, R. B. A. & DE ALENCAR FIGUEIREDO, L. F. 2022. Gluten-Free Sorghum Pasta: Composition and Sensory Evaluation with Different Sorghum Hybrids. *Foods,* 11**,** 3124.

DIMIDI, E., COX, S. R., ROSSI, M. & WHELAN, K. 2019. Fermented Foods: Definitions and Characteristics, Impact on the Gut Microbiota and Effects on Gastrointestinal Health and Disease. *Nutrients,* 11.

DLAMINI, N. R., DYKES, L., ROONEY, L. W., WANISKA, R. D. & TAYLOR, J. R. N. 2009. Condensed Tannins in Traditional Wet-Cooked and Modern Extrusion-Cooked Sorghum Porridges. *Cereal Chemistry,* 86**,** 191-196.

DUKE, K. M. 2021. *EFFECT OF MICROWAVE ASSISTED EXTRACTION ON THE PHENOLIC CONTENT AND PROFILE OF DIFFERENT SORGHUM PHENOTYPES.*

DUODU, K., TANG, H., GRANT, A., WELLNER, N., BELTON, P. & TAYLOR, J. 2001. FTIR and solid state13C NMR spectroscopy of proteins of wet cooked and popped sorghum and maize. *Journal of Cereal Science,* 33**,** 261-269.

DUODU, K. G. & AWIKA, J. M. 2019. Phytochemical-Related Health-Promoting Attributes of Sorghum and Millets. *Sorghum and Millets.*

DUODU, K. G., NUNES, A., DELGADILLO, I., PARKER, M. L., MILLS, E. N. C., BELTON, P. S. & TAYLOR, J. R. N. 2002. Effect of Grain Structure and Cooking on Sorghum and Maize in vitro Protein Digestibility. *Journal of Cereal Science,* 35**,** 161-174.

DUODU, K. G., TAYLOR, J. R. N., BELTON, P. S. & HAMAKER, B. R. 2003. Factors affecting sorghum protein digestibility. *Journal of Cereal Science,* 38**,** 117-131.

DYKES, L. 2019. Tannin Analysis in Sorghum Grains. *Methods Mol Biol,* 1931**,** 109-120.
DYKES, L., PETERSON, G. C., ROONEY, W. L. & ROONEY, L. W. 2011. Flavonoid composition of lemon-yellow sorghum genotypes. *Food Chem,* 128**,** 173-9.
DYKES, L. & ROONEY, L. W. 2006. Sorghum and millet phenols and antioxidants. *Journal of Cereal Science,* 44**,** 236-251.
DYKES, L. & ROONEY, L. W. 2007. Phenolic compounds in cereal grains and their health benefits. *cereal foods world,* 52**,** 7.
DYKES, L., ROONEY, L. W., WANISKA, R. D. & ROONEY, W. L. 2005. Phenolic Compounds and Antioxidant Activity of Sorghum Grains of Varying Genotypes. *Journal of Agricultural and Food Chemistry,* 53**,** 6813-6818.
ELMAKI, H. B., BABIKER, E. E. & EL TINAY, A. H. 1999. Changes in chemical composition, grain malting, starch and tannin contents and protein digestibility during germination of sorghum cultivars. *Food Chemistry,* 64**,** 331-336.
EMBASHU, W. & NANTANGA, K. K. M. 2019. Malts: Quality and phenolic content of pearl millet and sorghum varieties for brewing nonalcoholic beverages and opaque beers. *Cereal Chemistry,* 96**,** 765-774.
EMMAMBUX, N. M. & TAYLOR, J. R. N. 2003. Sorghum kafirin interaction with various phenolic compounds. *Journal of the Science of Food and Agriculture,* 83**,** 402-407.
EZEOGU, L. I., DUODU, K. G. & TAYLOR, J. R. N. 2005. Effects of endosperm texture and cooking conditions on the in vitro starch digestibility of sorghum and maize flours. *Journal of Cereal Science,* 42**,** 33-44.
FAN, F. Y., SHI, M., NIE, Y., ZHAO, Y., YE, J. H. & LIANG, Y. R. 2016. Differential behaviors of tea catechins under thermal processing: Formation of non-enzymatic oligomers. *Food Chem,* 196**,** 347-54.
FARRAR, J. L., HARTLE, D. K., HARGROVE, J. L. & GREENSPAN, P. 2008. A novel nutraceutical property of select sorghum (Sorghum bicolor) brans: inhibition of protein glycation. *Phytotherapy Research,* 22**,** 1052-1056.
FERREIRA, D., NEL, R. J. J. & BEKKER, R. 1999. 3.19 - Condensed Tannins. *In:* BARTON, S. D., NAKANISHI, K. & METH-COHN, O. (eds.) *Comprehensive Natural Products Chemistry.* Oxford: Pergamon.
FOREIGN AGRICULTURAL SERVICE, U. S. D. O. A. 2021. Foreign Demand Fuels U.S. Corn and Sorghum Exports. *International Agricultural Trade Report.* June 16th, 2021 ed.
FRANCIS, N., RAO, S., BLANCHARD, C. & SANTHAKUMAR, A. 2019. Black Sorghum Phenolic Extract Regulates Expression of Genes Associated with Oxidative Stress and Inflammation in Human Endothelial Cells. *Molecules,* 24.
FRIEDMAN, M. & JÜRGENS, H. S. 2000. Effect of pH on the Stability of Plant Phenolic Compounds. *Journal of Agricultural and Food Chemistry,* 48**,** 2101-2110.
GALLE, S., SCHWAB, C., DAL BELLO, F., COFFEY, A., GÄNZLE, M. G. & ARENDT, E. K. 2012. Influence of in-situ synthesized exopolysaccharides on the quality of gluten-free sorghum sourdough bread. *Int J Food Microbiol,* 155**,** 105-12.
GAYTÁN-MARTÍNEZ, M., CABRERA-RAMÍREZ, Á. H., MORALES-SÁNCHEZ, E., RAMÍREZ-JIMÉNEZ, A. K., CRUZ-RAMÍREZ, J., CAMPOS-VEGA, R., VELAZQUEZ, G., LOARCA-PIÑA, G. & MENDOZA, S. 2017. Effect of nixtamalization process on the content and composition of phenolic compounds and antioxidant activity of two sorghums varieties. *Journal of Cereal Science,* 77**,** 1-8.

GHAFOOR, K., AHMED, I. A. M., DOĞU, S., USLU, N., FADIMU, G. J., JUHAIMI, F. A., BABIKER, E. E. & ÖZCAN, M. M. 2019. The Effect of Heating Temperature on Total Phenolic Content, Antioxidant Activity, and Phenolic Compounds of Plum and Mahaleb Fruits. *International Journal of Food Engineering,* 15.

GIERGIELEWICZ-MOZAJSKA, H., DĄBROWSKI, Ł. & NAMIEŚNIK, J. 2001. Accelerated Solvent Extraction (ASE) in the analysis of environmental solid samples - Some aspects of theory and practice. *Critical Reviews in Analytical Chemistry,* 31**,** 149-165.

GIRARD, A. L. & AWIKA, J. M. 2018. Sorghum polyphenols and other bioactive components as functional and health promoting food ingredients. *Journal of Cereal Science,* 84**,** 112-124.

GITERU, S. G., COOREY, R., BERTOLATTI, D., WATKIN, E., JOHNSON, S. & FANG, Z. 2015. Physicochemical and antimicrobial properties of citral and quercetin incorporated kafirin-based bioactive films. *Food Chem,* 168**,** 341-7.

GOUFO, P. & TRINDADE, H. 2014. Rice antioxidants: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid. *Food science & nutrition,* 2**,** 75-104.

HAGERMAN, A. E. & BUTLER, L. G. 1978. Protein precipitation method for the quantitative determination of tannins. *Journal of Agricultural and Food Chemistry,* 26**,** 809-812.

HAMAD, S. A., MUSTAFA, A. I., MAGBOUL, B. I., QASEM, A. A. & AHMED, I. A. M. 2019. Nutritional quality of raw and cooked flours of a high β-glucan sorghum inbred line. *Journal of Cereal Science,* 90**,** 102857.

HASSANI, A., ZARNKOW, M. & BECKER, T. 2014. Influence of malting conditions on sorghum (Sorghum bicolor (L.) Moench) as a raw material for fermented beverages. *Food Sci Technol Int,* 20**,** 453-63.

HERALD, T. J., GADGIL, P., PERUMAL, R., BEAN, S. R. & WILSON, J. D. 2014. High-throughput micro-plate HCI-vanillin assay for screening tannin content in sorghum grain. *J Sci Food Agric,* 94**,** 2133-6.

HERALD, T. J., GADGIL, P. & TILLEY, M. 2012. High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour. *J Sci Food Agric,* 92**,** 2326-31.

HERRMAN, D. A., BRANTSEN, J. F., RAVISANKAR, S., LEE, K.-M. & AWIKA, J. M. 2020. Stability of 3-deoxyanthocyanin pigment structure relative to anthocyanins from grains under microwave assisted extraction. *Food Chemistry,* 333**,** 127494.

HITHAMANI, G. & SRINIVASAN, K. 2014. Bioaccessibility of polyphenols from wheat (Triticum aestivum), sorghum (Sorghum bicolor), green gram (Vigna radiata), and chickpea (Cicer arietinum) as influenced by domestic food processing. *Journal of agricultural and food chemistry,* 62**,** 11170-11179.

HO, C.-T. 1992. Phenolic Compounds in Food An Overview. *American Chemical Society*.

HONG, Q., CHEN, G., WANG, Z., CHEN, X. & KAN, J. 2023. Effects of different thermal processing methods on bioactive components, phenolic compounds, and antioxidant activities of Qingke (highland hull-less barley). *Food Science and Human Wellness,* 12**,** 119-129.

INDRIANINGSIH, A. W., KHASANAH, Y., NOVIANA, E., RAHAYU, E., HASTUTI, H. P., NI'MATURROHMAH, D., SURYANI, A. E., DARSIH, C., WINDARSIH, A. & HANDAYANI, S. 2023. The effect of soaking of white and red varieties of Sorghum

bicolor flour on its antioxidant, antidiabetic and physicochemical properties. *Food and Humanity,* 1**,** 1531-1538.
IRAKLI, M. N., SAMANIDOU, V. F., BILIADERIS, C. G. & PAPADOYANNIS, I. N. 2012. Simultaneous determination of phenolic acids and flavonoids in rice using solid-phase extraction and RP-HPLC with photodiode array detection. *J Sep Sci,* 35**,** 1603-11.
IRONDI, E. A., ADEGOKE, B. M., EFFION, E. S., OYEWO, S. O., ALAMU, E. O. & BOLIGON, A. A. 2019. Enzymes inhibitory property, antioxidant activity and phenolics profile of raw and roasted red sorghum grains in vitro. *Food Science and Human Wellness,* 8**,** 142-148.
IRONDI, E. A., ADEWUYI, A. E. & AROYEHUN, T. M. 2022. Effect of Endogenous Lipids and Proteins on the Antioxidant, in vitro Starch Digestibility, and Pasting Properties of Sorghum Flour. *Frontiers in Nutrition,* 8.
IZZA, H. F., SUSANTI, D. Y., MARIYAM, S. & SAPUTRO, A. D. 2023. Performance of microwave-assisted extraction of proanthocyanidins from red sorghum grain in various power and citric acid concentration. *Journal of the Saudi Society of Agricultural Sciences*.
JIANG, H., HETTIARARCHCHY, N. S. & HORAX, R. 2018. Physical properties and estimated glycemic index of protein-enriched sorghum based chips. *Journal of Food Science and Technology,* 55**,** 891-898.
KANG, J., PRICE, W. E., ASHTON, J., TAPSELL, L. C. & JOHNSON, S. 2016. Identification and characterization of phenolic compounds in hydromethanolic extracts of sorghum wholegrains by LC-ESI-MSn. *Food Chemistry,* 211**,** 215-226.
KAYITESI, E., DE KOCK, H. L., MINNAAR, A. & DUODU, K. G. 2012. Nutritional quality and antioxidant activity of marama–sorghum composite flours and porridges. *Food Chemistry,* 131**,** 837-842.
KAYODÉ, A. P., LINNEMANN, A. R., NOUT, M. J. & VAN BOEKEL, M. A. 2007. Impact of sorghum processing on phytate, phenolic compounds and in vitro solubility of iron and zinc in thick porridges. *Journal of the Science of Food and Agriculture,* 87**,** 832-838.
KHAN, I., YOUSIF, A., JOHNSON, S. K. & GAMLATH, S. 2013. Effect of sorghum flour addition on resistant starch content, phenolic profile and antioxidant capacity of durum wheat pasta. *Food Research International,* 54**,** 578-586.
KHAN, I., YOUSIF, A. M., JOHNSON, S. K. & GAMLATH, S. 2014. Effect of Sorghum Flour Addition on In Vitro Starch Digestibility, Cooking Quality, and Consumer Acceptability of Durum Wheat Pasta. *Journal of Food Science,* 79**,** S1560-S1567.
KHAN, I., YOUSIF, A. M., JOHNSON, S. K. & GAMLATH, S. 2015. Acute effect of sorghum flour-containing pasta on plasma total polyphenols, antioxidant capacity and oxidative stress markers in healthy subjects: A randomised controlled trial. *Clinical Nutrition,* 34**,** 415-421.
KHODDAMI, A., MOHAMMADREZAEI, M. & ROBERTS, T. H. 2017. Effects of Sorghum Malting on Colour, Major Classes of Phenolics and Individual Anthocyanins. *Molecules,* 22**,** 1713.
KHODDAMI, A., WILKES, M. A. & ROBERTS, T. H. 2013. Techniques for Analysis of Plant Phenolic Compounds. *Molecules,* 18**,** 2328-2375.
KIRTIL, E., DAG, D., GUNER, S., UNAL, K. & OZTOP, M. H. 2017. Dynamics of unloaded and green tea extract loaded lecithin based liposomal dispersions investigated by nuclear magnetic resonance T2 relaxation. *Food Res Int,* 99**,** 807-814.

KONFO, C. T. R., CHABI, N. W., DAHOUENON-AHOUSSI, E., CAKPO-CHICHI, M., SOUMANOU, M. M. & SOHOUNHLOUE, D. C. K. 2015. Improvement of African traditional sorghum beers quality and potential applications of plants extracts for their stabilization: a review. *The Journal of Microbiology, Biotechnology and Food Sciences,* 5**,** 190.

KUDITA, S., SCHOUSTRA, S., MUBAIWA, J., SMID, E. J. & LINNEMANN, A. R. 2023. Substitution of maize with sorghum and millets in traditional processing of Mahewu, a non-alcoholic fermented cereal beverage. *International Journal of Food Science & Technology*.

KULAMARVA, A. G., SOSLE, V. R. & RAGHAVAN, G. S. V. 2009. Nutritional and Rheological Properties of Sorghum. *International Journal of Food Properties,* 12**,** 55-69.

KYSELOVA, Z. 2011. Toxicological aspects of the use of phenolic compounds in disease prevention. *Interdisciplinary toxicology,* 4**,** 173-83.

LANGER, S., MARSHALL, L. J., DAY, A. J. & MORGAN, M. R. 2011. Flavanols and methylxanthines in commercially available dark chocolate: a study of the correlation with nonfat cocoa solids. *J Agric Food Chem,* 59**,** 8435-41.

LEE, H. S., SANTANA Á, L., PETERSON, J., YUCEL, U., PERUMAL, R., DE LEON, J., LEE, S. H. & SMOLENSKY, D. 2022. Anti-Adipogenic Activity of High-Phenolic Sorghum Brans in Pre-Adipocytes. *Nutrients,* 14.

LEE, S. H., LEE, H. S., LEE, J., AMARAKOON, D., LOU, Z., NORONHA, L. E., HERALD, T. J., PERUMAL, R. & SMOLENSKY, D. 2021. Polyphenol Containing Sorghum Brans Exhibit an Anti-Cancer Effect in Apc Min/+ Mice Treated with Dextran Sodium Sulfate. *Int J Mol Sci,* 22.

LEE, S. H., LEE, J., HERALD, T., COX, S., NORONHA, L., PERUMAL, R., LEE, H. S. & SMOLENSKY, D. 2020. Anticancer Activity of a Novel High Phenolic Sorghum Bran in Human Colon Cancer Cells. *Oxid Med Cell Longev,* 2020**,** 2890536.

LI, R., WANG, Q., PENG, H., ZHAO, G., ZHANG, D. & LI, Z. 2022a. Exploring the effect of microwave treatment on phenolic flavonoids, antioxidant capacity, and phenolic in vitro bioaccessibility of sorghum. *International Journal of Food Science & Technology,* 57**,** 2510-2522.

LI, Z., ZHAO, X., ZHANG, X. & LIU, H. 2022b. The Effects of Processing on Bioactive Compounds and Biological Activities of Sorghum Grains. *Molecules,* 27**,** 3246.

LIBRON, J. A. M. A., CARDONA, D. E. M., MATEO, J. M. C., BELTRAN, A. K. M., TUAÑO, A. P. P. & LAUDE, T. P. 2021. Nutritional properties and phenolic acid profile of selected Philippine pigmented maize with high antioxidant activity. *Journal of Food Composition and Analysis,* 101**,** 103954.

LIU, L., HERALD, T. J., WANG, D., WILSON, J. D., BEAN, S. R. & ARAMOUNI, F. M. 2012. Characterization of sorghum grain and evaluation of sorghum flour in a Chinese egg noodle system. *Journal of Cereal Science,* 55**,** 31-36.

LOPES, R. D. C. S. O., DE LIMA, S. L. S., DA SILVA, B. P., TOLEDO, R. C. L., DE CASTRO MOREIRA, M. E., ANUNCIAÇÃO, P. C., WALTER, E. H. M., CARVALHO, C. W. P., QUEIROZ, V. A. V. & RIBEIRO, A. Q. 2018. Evaluation of the health benefits of consumption of extruded tannin sorghum with unfermented probiotic milk in individuals with chronic kidney disease. *Food Research International,* 107**,** 629-638.

LU, N., JUN, J. H., LI, Y. & DIXON, R. A. 2023. An unconventional proanthocyanidin pathway in maize. *Nature Communications,* 14**,** 4349.
LUCKEMEYER, T., LUCKEMEYER, T., HESTEANDE, B., MILLER, R., KERTH, C., AWIKA, J. & ROONEY, L. 2015. Sorghum bran addition in bratwurst, pre-cooked pork patties and pre-cooked turkey patties. *Meat Science***,** 118.
LUO, M., HOU, F., DONG, L., HUANG, F., ZHANG, R. & SU, D. 2020. Comparison of microwave and high-pressure processing on bound phenolic composition and antioxidant activities of sorghum hull. *International Journal of Food Science & Technology,* 55**,** 3190-3202.
LUO, X., CUI, J., ZHANG, H., DUAN, Y., ZHANG, D., CAI, M. & CHEN, G. 2018. Ultrasound assisted extraction of polyphenolic compounds from red sorghum (Sorghum bicolor L.) bran and their biological activities and polyphenolic compositions. *Industrial Crops and Products,* 112**,** 296-304.
LUZARDO-OCAMPO, I., RAMÍREZ-JIMÉNEZ, A. K., CABRERA-RAMÍREZ, Á. H., RODRÍGUEZ-CASTILLO, N., CAMPOS-VEGA, R., LOARCA-PIÑA, G. & GAYTÁN-MARTÍNEZ, M. 2020. Impact of cooking and nixtamalization on the bioaccessibility and antioxidant capacity of phenolic compounds from two sorghum varieties. *Food chemistry,* 309**,** 125684.
MARSTON, K., KHOURYIEH, H. & ARAMOUNI, F. 2016. Effect of heat treatment of sorghum flour on the functional properties of gluten-free bread and cake. *LWT - Food Science and Technology,* 65**,** 637-644.
MCCLEMENTS, D. J. 2015. Encapsulation, protection, and release of hydrophilic active components: potential and limitations of colloidal delivery systems. *Adv Colloid Interface Sci,* 219**,** 27-53.
MCGRATH, R. M., KALUZA, W. Z., DAIBER, K. H., VAN DER RIET, W. B. & GLENNIE, C. W. 1982. Polyphenols of sorghum grain, their changes during malting and their inhibitory nature. *Journal of Agricultural and Food Chemistry,* 30**,** 450-456.
MOHAMED, L. K., SULIEMAN, M. A., YAGOUB, A. E. A., MOHAMMED, M. A., ALHUTHAYLI, H. F., MOHAMED AHMED, I. A., ALMAIMAN, S. A., ALFAWAZ, M. A., OSMAN, M. A. & HASSAN, A. B. 2022. Changes in Phytochemical Compounds and Antioxidant Activity of Two Irradiated Sorghum (Sorghum bicolor (L.) Monech) Cultivars during the Fermentation and Cooking of Traditional Sudanese Asida. *Fermentation,* 8**,** 60.
MORAES, É. A., MARINELI, R. D. S., LENQUISTE, S. A., QUEIROZ, V. A. V., CAMARGO, R. L., BORCK, P. C., CARNEIRO, E. M. & MARÓSTICA JÚNIOR, M. R. 2018. Whole sorghum flour improves glucose tolerance, insulin resistance and preserved pancreatic islets function in obesity diet-induced rats. *Journal of Functional Foods,* 45**,** 530-540.
N'DRI, D., MAZZEO, T., ZAUPA, M., FERRACANE, R., FOGLIANO, V. & PELLEGRINI, N. 2013. Effect of cooking on the total antioxidant capacity and phenolic profile of some whole-meal African cereals. *Journal of the Science of Food and Agriculture,* 93**,** 29-36.
NCUBE, T., SIBANDA, T. & NGOROMANI, N. 2015. Rheological Properties and Bread Making Quality of White Grain Sorghum-Wheat Flour Composites. *International Journal of Food Science and Nutrition Engineering,* 5**,** 176-182.

NGUYEN, P. H., DUNG, V. V., ZHAO, B. T., KIM, Y. H., MIN, B. S. & WOO, M. H. 2014. Antithrombotic and antidiabetic flavonoid glycosides from the grains of Sorghum bicolor (L.) Moench var. hwanggeumchal. *Arch Pharm Res,* 37**,** 1394-402.

NWANGUMA, B. C. & EZE, M. O. 1996. Changes in the Concentrations of the Polyphenolic Constituents of Sorghum during Malting and Mashing. *Journal of the Science of Food and Agriculture,* 70**,** 162-166.

O. ALUGE, O., A. AKINOLA, S. & F. OSUNDAHUNSI, O. 2016. Effect of Malted Sorghum on Quality Characteristics of Wheat-Sorghum-Soybean Flour for Potential Use in Confectionaries. *Food and Nutrition Sciences,* 07**,** 1241-1252.

OFOSU, F. K., ELAHI, F., DALIRI, E. B.-M., YEON, S.-J., HAM, H. J., KIM, J.-H., HAN, S.-I. & OH, D.-H. 2020. Flavonoids in decorticated sorghum grains exert antioxidant, antidiabetic and antiobesity activities. *Molecules,* 25**,** 2854.

OLOJEDE, A. O., SANNI, A. I., BANWO, K. & MICHAEL, T. 2022. Improvement of Texture, Nutritional Qualities, and Consumers’ Perceptions of Sorghum-Based Sourdough Bread Made with Pediococcus pentosaceus and Weissella confusa Strains. *Fermentation,* 8**,** 32.

ORTIZ-CRUZ, R. A., RAMÍREZ-WONG, B., LEDESMA-OSUNA, A. I., TORRES-CHÁVEZ, P. I., SÁNCHEZ-MACHADO, D. I., MONTAÑO-LEYVA, B., LÓPEZ-CERVANTES, J. & GUTIÉRREZ-DORADO, R. 2020. Effect of extrusion processing conditions on the phenolic compound content and antioxidant capacity of sorghum (Sorghum bicolor (L.) Moench) bran. *Plant Foods for Human Nutrition,* 75**,** 252-257.

PAES, L. T., DOS SANTOS D'ALMEIDA, C. T., DO CARMO, M. A. V., DA SILVA CRUZ, L., DE SOUZA, A. B., VIANA, L. M., MALTAROLLO, V. G., MARTINO, H. S. D., DE ALMEIDA LIMA, G. D. & FERREIRA, M. S. L. 2024. Phenolic-rich extracts from toasted white and tannin sorghum flours have distinct profiles influencing their antioxidant, antiproliferative, anti-adhesive, anti-invasive, and antimalarial activities. *Food Research International,* 176**,** 113739.

PANCHE, A. N., DIWAN, A. D. & CHANDRA, S. R. 2016. Flavonoids: an overview. *J Nutr Sci,* 5**,** e47.

PARKER, M., GRANT, A., RIGBY, N., BELTON, P. & TAYLOR, J. 1999. Effects of popping on the endosperm cell walls of sorghum and maize. *Journal of Cereal Science,* 30**,** 209-216.

PERIERA, A. P., FERREIRA, I. C. F. R., MARCELINO, F., VALENTÃO, P., ANDRADE, P. B., SEABRA, R., ESTEVINHO, L., BENTO, A. & PERIERA, J. A. 2007. Phenolic Compounds and Antimicrobial Activity of Olive (Olea europaea L. Cv. Cobrançosa) Leaves *Molecules***,** 9.

PERIERA, J. A., PERIERA, A. P. G., FERREIRA, I. C. F. R., VELENTÃO, P., ANDRADE, P. B., SEABRA, R., ESTEVINHO, L. & BENTO, A. 2006. Table Olives from Portugal-Phenolic Compounds, Antioxidant Potential, and Antimicrobial Activity. *Journal of Agriculture and Food Chemistry,* 54**,** 6.

PESTA, D. & RODEN, M. 2017. The Janus head of oxidative stress in metabolic diseases and during physical exercise. *Current diabetes reports,* 17**,** 1-13.

PETERSON, J., SANTANA, A. L., COX, S., PEREZ-FAJARDO, M., COVARRUBIAS, J., PERUMAL, R., BEAN, S., WU, X., WANG, W. & SMOLENSKY, D. 2024. Impact of heat and high-moisture pH treatments on starch digestibility, phenolic composition, and cell bioactivity in sorghum (Sorghum bicolor L. Moench) flour. *Front Nutr,* 11**,** 1428542.

PETRONI, K., LANDONI, M., TOMAY, F., CALVENZANI, V., SIMONELLI, C. & CORMEGNA, M. 2017. Proximate Composition, Polyphenol Content and Anti-inflammatory Properties of White and Pigmented Italian Rice Varieties. *Universal Journal of Agricultural Research,* 5**,** 312-321.

PISOSCHI, A. M. & NEGULESCU, G. P. 2012. Methods for Total Antioxidant Activity Determination: A Review. *Biochemistry & Analytical Biochemistry,* 01.

PLANK, D. W., SZPYLKA, J., SAPIRSTEIN, H., WOOLLARD, D., ZAPF, C. M., LEE, V., CHEN, C. O., LIU, R. H., TSAO, R. & DÜSTERLOH, A. 2012. Determination of antioxidant activity in foods and beverages by reaction with 2, 2′-Diphenyl-1-picrylhydrazyl (DPPH): Collaborative study first action 2012.04. *Journal of AOAC International,* 95**,** 1562-1569.

PONTIERI, P. & DEL GIUDICE, L. 2016. Sorghum: A Novel and Healthy Food. *In:* CABALLERO, B., FINGLAS, P. M. & TOLDRÁ, F. (eds.) *Encyclopedia of Food and Health.* Oxford: Academic Press.

POQUETTE, N. M., GU, X. & LEE, S.-O. 2014. Grain sorghum muffin reduces glucose and insulin responses in men. *Food & function,* 5**,** 894-899.

PRIOR, R. L. & GU, L. 2005. Occurrence and biological significance of proanthocyanidins in the American diet. *Phytochemistry,* 66**,** 2264-80.

PROESTOS, C., N.CHORIANOPOULOS, NYCHAS, G.-J. E. & KOMAITIS, M. 2005. RP-HPLC Analysis of the Phenolic Compounds of Plant Extracts. Investigation of Their Antioxidant Capacity and Antimicrobial Activity. *Journal of Agriculture and Food Chemistry,* 54**,** 5.

QUEIROZ, V. A. V., CORREIA, V. T. D. V., MENEZES, C. B. D., MIGUEL, R. D. A., CONCEIÇÃO, R. R. P. D., PAIVA, C. L. & FIGUEIREDO, J. E. F. 2021. Retention of phenolic compounds and acceptability of gluten-free churros made with tannin or tannin-free sorghum flour. *Pesquisa Agropecuaria Brasileira,* 55.

RANDHIR, R., KWON, Y.-I. & SHETTY, K. 2008. Effect of thermal processing on phenolics, antioxidant activity and health-relevant functionality of select grain sprouts and seedlings. *Innovative Food Science & Emerging Technologies,* 9**,** 355-364.

RAO, S., SANTHAKUMAR, A. B., CHINKWO, K. A., WU, G., JOHNSON, S. K. & BLANCHARD, C. L. 2018. Characterization of phenolic compounds and antioxidant activity in sorghum grains. *Journal of Cereal Science,* 84**,** 103-111.

RASHWAN, A. K., YONES, H. A., KARIM, N., TAHA, E. M. & CHEN, W. 2021. Potential processing technologies for developing sorghum-based food products: An update and comprehensive review. *Trends in Food Science & Technology,* 110**,** 168-182.

RAVISANKAR, S., DIZLEK, H. & AWIKA, J. M. 2021. Changes in extractable phenolic profile during natural fermentation of wheat, sorghum and teff. *Food Research International,* 145**,** 110426.

RHODES, D., GADGIL, P., PERUMAL, R., TESSO, T. & HERALD, T. J. 2017. Natural Variation and Genome-Wide Association Study of Antioxidants in a Diverse Sorghum Collection. *Cereal Chemistry,* 94**,** 190-198.

RIBAS-AGUSTI, A., MARTIN-BELLOSO, O., SOLIVA-FORTUNY, R. & ELEZ-MARTINEZ, P. 2018. Food processing strategies to enhance phenolic compounds bioaccessibility and bioavailability in plant-based foods. *Crit Rev Food Sci Nutr,* 58**,** 2531-2548.

ROCCHETTI, G., GIUBERTI, G., BUSCONI, M., MAROCCO, A., TREVISAN, M. & LUCINI, L. 2020. Pigmented sorghum polyphenols as potential inhibitors of starch digestibility: An in vitro study combining starch digestion and untargeted metabolomics. *Food Chem,* 312**,** 126077.

ROONEY, L. W. & AWIKA, J. M. 2004. Specialty sorghums for healthful foods. *Specialty grains for food and feed***,** 283-312.

RUDRA, S. G., JAKHAR, N., NISHAD, J., SAINI, N., SEN, S., BHARDHWAJ, R., JAISWAL, S., SUNEJA, P., SINGH, S. & KAUR, C. 2015. Extrusion conditions and antioxidant properties of sorghum, barley and horse gram based snack. *Vegetos-An International Journal of Plant Research,* 28**,** 171-182.

RUIZ-HERNÁNDEZ, A. A., ROUZAUD-SÁNDEZ, O., FRIAS, J., AYALA-ZAVALA, F., ASTIAZARÁN-GARCÍA, H., SALAZAR–LÓPEZ, N. J., LÓPEZ-SAIZ, C. M., SANDRA, C. & SÁNCHEZ, M. R. 2023. Antioxidant and anti-inflammatory potential of a food produced from irradiated (UV-A LED) sorghum sprouts subjected to in vitro gastrointestinal simulation. *Journal of Functional Foods,* 110**,** 105857.

RYMBAI, H., SHARMA, R. R. & SRIVASTAV, M. 2011. Bio-colorants and its implications in health and food industry–a review. *International Journal of Pharmacological Research,* 3**,** 2228-2244.

SALAZAR LOPEZ, N. J., LOARCA-PIÑA, G., CAMPOS-VEGA, R., GAYTÁN MARTÍNEZ, M., MORALES SÁNCHEZ, E., ESQUERRA-BRAUER, J. M., GONZALEZ-AGUILAR, G. A. & ROBLES SÁNCHEZ, M. 2016. The extrusion process as an alternative for improving the biological potential of sorghum bran: phenolic compounds and antiradical and anti-inflammatory capacity. *Evidence-Based Complementary and Alternative Medicine,* 2016.

SANAEI NASAB, S., ZARE, L., TAHMOUZI, S., NEMATOLLAHI, A., MOLLAKHALILI-MEYBODI, N., ABEDI, A.-S. & DELSHADIAN, Z. 2023. Effect of irradiation treatment on microbial, nutritional and technological characteristics of cereals: A comprehensive review. *Radiation Physics and Chemistry,* 212**,** 111124.

SÁNCHEZ-RANGEL, J. C., BENAVIDES, J., HEREDIA, J. B., CISNEROS-ZEVALLOS, L. & JACOBO-VELÁZQUEZ, D. A. 2013. The Folin–Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination. *Analytical methods,* 5**,** 5990-5999.

SANG, Y., BEAN, S., SEIB, P. A., PEDERSEN, J. & SHI, Y.-C. 2008. Structure and Functional Properties of Sorghum Starches Differing in Amylose Content. *Journal of Agricultural and Food Chemistry,* 56**,** 6680-6685.

SANTANA, Á. L., PETERSON, J., PERUMAL, R., HU, C., SANG, S., SILIVERU, K. & SMOLENSKY, D. 2023. Post Acid Treatment on Pressurized Liquid Extracts of Sorghum (Sorghum bicolor L. Moench) Grain and Plant Material Improves Quantification and Identification of 3-Deoxyanthocyanidins. *Processes,* 11**,** 2079.

SCHOBER, T. J., MESSERSCHMIDT, M., BEAN, S. R., PARK, S.-H. & ARENDT, E. K. 2005. Gluten-Free Bread from Sorghum: Quality Differences Among Hybrids. *Cereal Chemistry,* 82**,** 394-404.

SCHOFIELD, P., MBUGUA, D. & PELL, A. 2001. Analysis of condensed tannins: a review. *Animal feed science and technology,* 91**,** 21-40.

SEN, S. & CHAKRABORTY, R. 2011. The Role of Antioxidants in Human Health. *Oxidative Stress: Diagnostics, Prevention, and Therapy.* American Chemical Society.

SENEVIRATHNE, I. G. N. H., ABEYSEKERA, W. K. S. M., ABEYSEKERA, W. P. K. M., JAYANATH, N. Y., GALBADA ARACHCHIGE, S. P. & WIJEWARDANA, D. C. M. S. I. 2021. Antiamylase, antiglucosidase, and antiglycation properties of millets and sorghum from Sri Lanka. *Evidence-Based Complementary and Alternative Medicine,* 2021**,** 5834915.

SHELEMBE, J. S., CROMARTY, D., BESTER, M., MINNAAR, A. & DUODU, K. G. 2014. Effect of acidic condition on phenolic composition and antioxidant potential of aqueous extracts from sorghum (Sorghum bicolor) bran. *Journal of Food Biochemistry,* 38**,** 110-118.

SHUKLA, S., LOHANI, U. C., SHAHI, N. C. & DUBEY, A. 2022. Extraction of natural pigments from red sorghum (Sorghum bicolor) husk by ultrasound and microwave assisted extraction: A comparative study through response surface analysis. *Journal of Food Process Engineering,* 45**,** e14130.

SILVA, T. L., LACERDA, U. V., DA MATTA, S. L. P., QUEIROZ, V. A. V., STRINGHETA, P. C., MARTINO, H. S. D. & DE BARROS, F. A. R. 2020. Evaluation of the efficacy of toasted white and tannin sorghum flours to improve oxidative stress and lipid profile in vivo. *Journal of food science,* 85**,** 2236-2244.

SINGLA, R. K., DUBEY, A. K., GARG, A., SHARMA, R. K., FIORINO, M., AMEEN, S. M., HADDAD, M. A. & AL-HIARY, M. 2019. Natural Polyphenols: Chemical Classification, Definition of Classes, Subcategories, and Structures. *J AOAC Int,* 102**,** 1397-1400.

SOOBRATTEE, M. A., NEERGHEEN, V. S., LUXIMON-RAMMA, A., ARUOMA, O. I. & BAHORUN, T. 2005. Phenolics as potential antioxidant therapeutic agents: mechanism and actions. *Mutat Res,* 579**,** 200-13.

SOROUR, M. A., MEHANNI, A. E., TAHA, E. M. & RASHWAN, A. K. 2017. Changes of Total Phenolics, Tannins, Phytate and Antioxidant Activity of Two Sorghum Cultivars as Affected by Processing. *Journal of Food and Dairy Sciences,* 8**,** 267-274.

SPERANZA, S., KNECHTL, R., WITLACZIL, R. & SCHÖNLECHNER, R. 2021. Reversed-phase HPLC characterization and quantification and antioxidant capacity of the phenolic acids and flavonoids extracted from eight varieties of sorghum grown in Austria. *Frontiers in Plant Science,* 12**,** 769151.

STALIKAS, C. D. 2007. Extraction, separation, and detection methods for phenolic acids and flavonoids. *Journal of separation science,* 30**,** 3268-3295.

STEFOSKA-NEEDHAM, A., BECK, E. J., JOHNSON, S. K., BATTERHAM, M. J., GRANT, R., ASHTON, J. & TAPSELL, L. C. 2017. A diet enriched with red sorghum flaked biscuits, compared to a diet containing white wheat flaked biscuits, does not enhance the effectiveness of an energy-restricted meal plan in overweight and mildly obese adults. *Journal of the American College of Nutrition,* 36**,** 184-192.

STEFOSKA-NEEDHAM, A., BECK, E. J., JOHNSON, S. K., CHU, J. & TAPSELL, L. C. 2016. Flaked sorghum biscuits increase postprandial GLP-1 and GIP levels and extend subjective satiety in healthy subjects. *Mol Nutr Food Res,* 60**,** 1118-28.

SVENSSON, L., SEKWATI-MONANG, B., LUTZ, D. L., SCHIEBER, A. & GANZLE, M. G. 2010. Phenolic acids and flavonoids in nonfermented and fermented red sorghum (Sorghum bicolor (L.) Moench). *Journal of Agricultural and Food Chemistry,* 58**,** 9214-9220.

SWEENY, J. G. & IACOBUCCI, G. A. 1983. Effect of substitution on the stability of 3-deoxyanthocyanidins in aqueous solutions. *Journal of Agricultural and Food Chemistry,* 31**,** 531-533.
SYTAR, O. 2012. Plant phenolic compounds for food, pharmaceutical and cosmetics production. *Journal of Medicinal Plants Research,* 6.
TALEON, V., DYKES, L., ROONEY, W. L. & ROONEY, L. W. 2012. Effect of genotype and environment on flavonoid concentration and profile of black sorghum grains. *Journal of Cereal Science,* 56**,** 470-475.
TAN, S. H., BLANCHARD, C. L., ROBERTS, T. H., WATERS, D. L. E. & MAWSON, A. J. 2023. Effects of Sorghum Grain and Wort Composition on Dry Grind Fermentation Performance: A Model for Baijiu Production. *Beverages,* 9**,** 29.
TAYLOR, J. Overview: Importance of sorghum in Africa. Afripro: workshop on the proteins of sorghum and millets: enhancing nutritional and functional properties for Africa, Pretoria, 2003. 1-21.
TAYLOR, J., BEAN, S. R., IOERGER, B. P. & TAYLOR, J. R. N. 2007. Preferential binding of sorghum tannins with γ-kafirin and the influence of tannin binding on kafirin digestibility and biodegradation. *Journal of Cereal Science,* 46**,** 22-31.
TAYLOR, J. R., BELTON, P. S., BETA, T. & DUODU, K. G. 2014. Increasing the utilisation of sorghum, millets and pseudocereals: Developments in the science of their phenolic phytochemicals, biofortification and protein functionality. *Journal of Cereal Science,* 59**,** 257-275.
TAYLOR, J. R. N., SCHOBER, T. J. & BEAN, S. R. 2006. Novel food and non-food uses for sorghum and millets. *Journal of Cereal Science,* 44**,** 252-271.
TEFERRA, T. F. 2019. Quinoa and Other Andean Ancient Grains: Super Grains for the Future. *Cereal Foods World,* 64.
TEIXEIRA NDE, C., QUEIROZ, V. A., ROCHA, M. C., AMORIM, A. C., SOARES, T. O., MONTEIRO, M. A., DE MENEZES, C. B., SCHAFFERT, R. E., GARCIA, M. A. & JUNQUEIRA, R. G. 2016. Resistant starch content among several sorghum (Sorghum bicolor) genotypes and the effect of heat treatment on resistant starch retention in two genotypes. *Food Chem,* 197**,** 291-6.
TOWO, E., MATUSCHEK, E. & SVANBERG, U. 2006. Fermentation and enzyme treatment of tannin sorghum gruels: effects on phenolic compounds, phytate and in vitro accessible iron. *Food Chemistry,* 94**,** 369-376.
TRAPPEY, E. F., KHOURYIEH, H., ARAMOUNI, F. & HERALD, T. 2014. Effect of sorghum flour composition and particle size on quality properties of gluten-free bread. *Food Science and Technology International,* 21**,** 188-202.
USDA-FAS. 2023. *Sorghum 2023World Production: 59,920 (1000 MT)* [Online]. United States Department of Agriculutre, Foreign Agricultural Service. Available: https://ipad.fas.usda.gov/cropexplorer/cropview/commodityView.aspx?cropid=0459200 [Accessed January 4th 2024].
VAN BUITEN, C. B., LAMBERT, J. D. & ELIAS, R. J. 2018. Green Tea Polyphenols Mitigate Gliadin-Mediated Inflammation and Permeability in Vitro. *Mol Nutr Food Res,* 62**,** e1700879.
VAN HUNG, P. 2016. Phenolic compounds of cereals and their antioxidant capacity. *Critical reviews in food science and nutrition,* 56**,** 25-35.

VINGRYS, K., MATHAI, M., ASHTON, J. F., STOJANOVSKA, L., VASILJEVIC, T., MCAINCH, A. J. & DONKOR, O. N. 2022. The effect of malting on phenolic compounds and radical scavenging activity in grains and breakfast cereals. *Journal of Food Science,* 87**,** 4188-4202.
VIRUPAKSHA, T. & SASTRY, L. 1968. Protein content and amino acid composition of some varieties of grain sorghum. *Journal of Agricultural and Food Chemistry,* 16**,** 199-203.
VU, T. H., BEAN, S., HSIEH, C. F. & SHI, Y. C. 2017. Changes in protein and starch digestibility in sorghum flour during heat-moisture treatments. *J Sci Food Agric,* 97**,** 4770-4779.
WANISKA, R. D. & ROONEY, L. K. 2003. Sorghum Grain Quality for Increased Utilization. *Sorghum and Millets Diseases.*
WHO. 2021. *Cardiovascular Diseases (CVDs)* [Online]. World Health Organization. Available: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds) [Accessed 10/08/2024 2024].
WHO. 2024. *Obesity and overweight* [Online]. World Health Organization. Available: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight [Accessed 10/8/2024].
WINGER, M., KHOURYIEH, H., ARAMOUNI, F. & HERALD, T. 2014. Sorghum Flour Characterization and Evaluation in Gluten-Free Flour Tortilla. *Journal of Food Quality,* 37**,** 95-106.
WU, L., HUANG, Z., QIN, P. & REN, G. 2013. Effects of processing on phytochemical profiles and biological activities for production of sorghum tea. *Food Research International,* 53**,** 678-685.
XIANLI WU, G. R. B., JOANNE M HOLDEN, DAVID B HAYTOWITZ, SUSAN E GEBHARDT, RONALD L PRIOR 2004. Lipophilic and Hydrophili Antioxidant Capacities of Common *Journal of Agriculture and Food Chemistry,* 52**,** 12.
XIAO, J., CHEN, Y. & HUANG, Q. 2017. Physicochemical properties of kafirin protein and its applications as building blocks of functional delivery systems. *Food Funct,* 8**,** 1402-1413.
XIONG, Y., ZHANG, P., JOHNSON, S., LUO, J. & FANG, Z. 2020. Comparison of the phenolic contents, antioxidant activity and volatile compounds of different sorghum varieties during tea processing. *Journal of the Science of Food and Agriculture,* 100**,** 978-985.
XIONG, Y., ZHANG, P., LUO, J., JOHNSON, S. & FANG, Z. 2019a. Effect of processing on the phenolic contents, antioxidant activity and volatile compounds of sorghum grain tea. *Journal of Cereal Science,* 85**,** 6-14.
XIONG, Y., ZHANG, P., WARNER, R. D. & FANG, Z. 2019b. 3-Deoxyanthocyanidin Colorant: Nature, Health, Synthesis, and Food Applications. *Compr Rev Food Sci Food Saf,* 18**,** 1533-1549.
XIONG, Y., ZHANG, P., WARNER, R. D. & FANG, Z. 2019c. Sorghum Grain: From Genotype, Nutrition, and Phenolic Profile to Its Health Benefits and Food Applications. *Comprehensive Reviews in Food Science and Food Safety,* 18**,** 2025-2046.
YAN, Z., LI, J., LI, S., CUI, T., JIANG, Y., CONG, G. & YU, M. 2014. Impact of Lignin Content on the Sweet Sorghum Bagasse Enzymatic Hydrolysis. *Energy Procedia,* 61**,** 1957-1960.
YANG, L., DYKES, L. & AWIKA, J. M. 2014. Thermal stability of 3-deoxyanthocyanidin pigments. *Food Chem,* 160**,** 246-54.

YEH, W.-J., HSIA, S.-M., LEE, W.-H. & WU, C.-H. 2017. Polyphenols with antiglycation activity and mechanisms of action: A review of recent findings. *Journal of Food and Drug Analysis,* 25**,** 84-92.

YOUSIF, A., NHEPERA, D. & JOHNSON, S. 2012. Influence of sorghum flour addition on flat bread in vitro starch digestibility, antioxidant capacity and consumer acceptability. *Food Chem,* 134**,** 880-7.

ZHU, X., ZHANG, H. & LO, R. 2004. Phenolic Compounds from the Leaf Extract of Artichoke (Cynara scolymus L.) and Their Antimicrobial Activities. *Journal of Agriculture and Food Chemistry,* 52**,** 6.

ZIELINSKI, H., KOZLOWSKA, H. & LEWCZUK, B. 2001. Bioactive compounds in the cereal grains before and after hydrothermal processing. *Innovative Food Science & Emerging Technologies,* 2**,** 159-169.

ZIELIŃSKI, H. & TROSZYŃSKA, A. 2000. Antioxidant capacity of raw and hydrothermal processed cereal grains. *Pol. J. Food Nutr. Sci,* 9**,** 79-83.

ZONG, X., WEN, L., MOU, T., WANG, Y. & LI, L. 2022. Effects of multiple cycles of sorghum starch gelatinization and fermentation on production of Chinese strong flavor Baijiu. *Journal of Cereal Science,* 108**,** 103561.