Phenotypic diversity of colored phytochemicals in sorghum accessions with various pericarp pigments

by

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B.S., Northwest Missouri State University, 2016

A REPORT

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Food Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2018

Approved by:

Major Professor
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Abstract

Sorghum is a versatile grain that is generally consumed in Asian and African countries but is gaining interest in the United States due to its gluten-free and bioactive compound-enriched health benefits. There are many varieties of sorghum that come in a wide range of colors. These genetic factor-depended phenotypic colors are contributed by various phytochemical pigments that reside within different components of the sorghum kernel, especially in the pericarp and endosperm. Various pericarp pigments are reflective of the certain phytochemical levels which may include anthocyanins, carotenoids, and condensed tannins. This article reviews recent studies on the association of pericarp pigments in various sorghum accessions with anthocyanins and carotenoids, respectively. It covers aspects of the potential health benefits of these colored dietary constituents. However, further investigations are warranted to clarify the diversity of these bioactive constituent interactions with genetic and environmental factors. How these phytochemicals correlate to the sorghum pericarp pigments could be important in future use of sorghum as a functional food with potential health benefits.

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Acknowledgements

This paper could not have been completed without the encouragement and unwavering support of my academic advising team. Thank you to my major professor, Dr. Weiqun Wang, the committee members for helping me complete my goals here at Kansas State University. Also, I especially wanted to thank my friends and family for their support and dedication.

Chapter 1 - Introduction

The philosophy that most Americans share is that eating fruits and vegetables of all different colors is important because colorful food is healthy for you. But what is not known is that there are many other colorful foods, like sorghum, that could be beneficial to the diet as well. Sorghum is the fifth leading whole grain cereal crop in the world after wheat, maize, rice, and barley; primarily grown in tropical, subtropical, and arid regions such as Asia and Africa (1). More than 35% of sorghum is grown directly for human consumption and used in a variety of foods. For instance, white sorghums are processed into flours and expanded snacks like cookies (11). In countries like Africa, sorghum, preferably tannin sorghum, is processed into porridge where the slow digestibility of the sorghum helps them feel fuller for longer (11). Whereas, in countries like the United States, Australia, and Brazil, this cereal is mainly used as animal feed and alcoholic products. However, using sorghum in food is gaining interest in the United States due to its gluten-free and bioactive compound-rich health properties. Sorghum could potentially be used as a substitute for conventional cereals due to its high phenolics, fiber, and carotenoids. (1). These bioactive compounds have been linked to multiple health benefits, including cholesterol-lowering, antioxidants, slow digestibility, anti-inflammatory, and anticarcinogenic properties (2).

Being a gluten-free product, sorghum has the potential to be a useful grain alternative for individuals with gluten sensitivity or celiac disease. It also contains high fiber content with a lower glycemic index compared to other grains, which could help reduce obesity and Type-2 diabetes. On the other hand, compared to wheat, corn, and rice, sorghum has a lower sensory quality and reduced protein digestion (3). One of the big possibilities for sorghum is cereal products, as potential expanded and extruded sorghum products. Grain based snack foods like breakfast cereal are common and widely consumed in many parts of the world. These products are typically made with corn, rice, or wheat in countries such as the United States and Brazil (11). Even though sorghum has a lower cost and is easier to produce, only recently has it been used for this purpose.

But sorghum-based products are showing good acceptability and have the potential to replace traditional cereals. With their agronomic advantages outweighing any negatives such as astringency. The antioxidant activity of sorghum flours is also high because of high levels of phenolic compounds such as luteolinidin, apeigninidin, and 3-deoxyanthocyanidins (3). This is especially true of brown tannin-sorghum which demonstrates excellent flavor, appearance, and texture (1). For instance, bran from high tannin sorghums were incorporated in bread and cookies at up to 15% and 30%, respectively, without significant differences in texture or flavor profiles compared to whole-wheat products. Thus, high tannin sorghum grains and their bran could find use as parts of ingredients in such foods (11).

Sorghum contains the highest phytochemical contents among cereals, including anthocyanins, carotenoids, phenolic acids, and condensed tannins. As you can see in Figure 1, the sorghum kernel is divided into three components: seed coat, endosperm, and embryo or germ. The seed coat is divided even further into the pericarp and testa (4). These phytochemicals are usually contained in the pericarp, endosperm and testa. Sorghum also typically contains provitamin A carotenoids, but the association of sorghum intake and vitamin A deficiency is shown to be contradictory. Studies conducted in Africa indicated various types of malnutrition including vitamin A deficiency when sorghum was the principle grains in the diet (5). However, other studies have suggested that the sorghum consumed in Africa and Asia are critical sources of dietary carotenoids that might provide the needed provitamin A (6). These inconsistencies might be due to the different varieties of sorghum consumed (3). Various sorghum varieties differ in the color of the pericarp and/or endosperm at a wide range from red to white. These colors are contributed by the pigmented phytochemicals such as anthocyanins and carotenoids, which are considered to be a dependable indicator of sorghum varieties (3, 7).

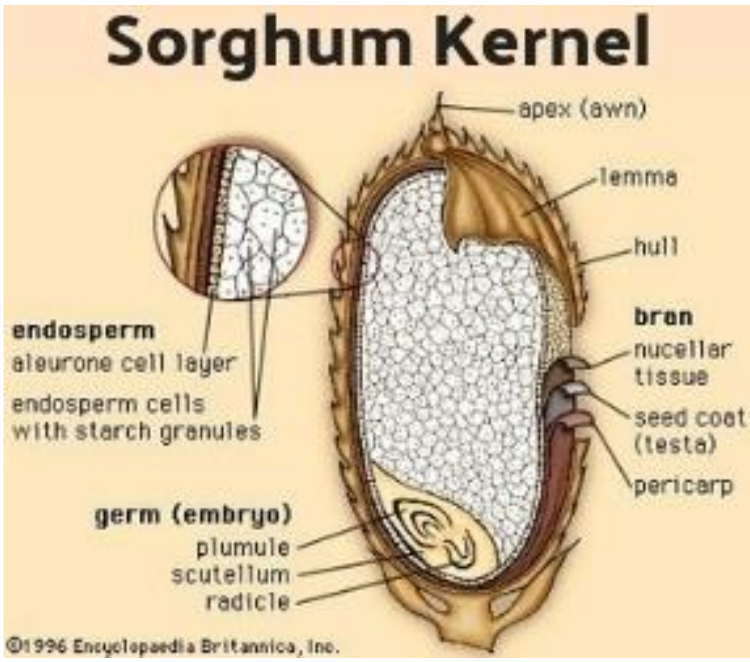


Figure 1. Detailed structure of the sorghum kernel. Modified from reference (27).

Chapter 2 - Pericarp Pigments

Sorghum grains come in a wide variety of colors such as red, yellow, white, and black. These colors typically come from the pericarp, endosperm, and testa. The pericarp is the outer layers of the seed coat of the sorghum kernel. The phenotypic pigments of sorghum pericarp and endosperm are determined by genotypic factor (3-4). Genetic factors control pericarp color, pericarp thickness, and secondary plant color (8-9). The pericarp colors are controlled by the R and Y genes. The white color occurs when Y is homozygous recessive, yellow occurs when R and Y are homozygous recessive and homozygous dominant, and red occurs when both R and Y are dominant. Black sorghum is a special red sorghum that turns black in the presence of sunlight (8). Normally the endosperm is yellow or white and the pericarp is usually red or black. The Z gene controls pericarp thickness which does not affect the flavonoids levels (9).

The wide array of phenolic compounds is concentrated in the pericarp. Phenolic compounds are present in all sorghums, while their composition, levels, and types are affected by the genotype. The phenolic compounds include phenolic acids, flavonoids, and condensed tannins. Sorghum flavonoids are 3-deoxyanthocyanins, orange luteolinidin, and yellow apigeninidin. The 3-deoxyanthocyanins are more stable than common anthocyanins, making them a potential natural food colorant (8). Figure 2 shows the chemical structures of the common anthocyanins presented in sorghum. Sorghums with red/purple secondary plant colors and sorghum with black pericarp have the highest levels of 3-deoxyanthocyanins. Red sorghum with tan secondary plant color has higher apigeninidin and luteolinidin levels than sorghum with red/purple secondary plant color (8).

Another important genotypic factor affecting pericarp color is other colored phytochemicals such as carotenoids. Carotenoids are one of the colored phytochemicals that may account for phenotypic color of sorghum pericarp (7). These carotenoids include lutein, zeaxanthin, α-carotene, and β-carotene as shown in Figure 2. Yellow pericarp/yellow endosperm sorghum accessions show the highest contents of carotenoids, followed by brown pericarp/yellow endosperm and white pericarp/yellow endosperm. The lowest carotenoids were found in the accessions with white pericarp/white endosperm (7). It appears that the phenotypic diversity of sorghum pericarp colors is associated with the content of carotenoids and pro-vitamin A, indicating a different impact of sorghum varieties on vitamin A deficiency and suggesting a possible prevention of vitamin A deficiency by breeding sorghum varieties with specific pericarp pigments (7). This potential health benefit, as well as many others, are due in part to the various phytochemicals found in sorghum.

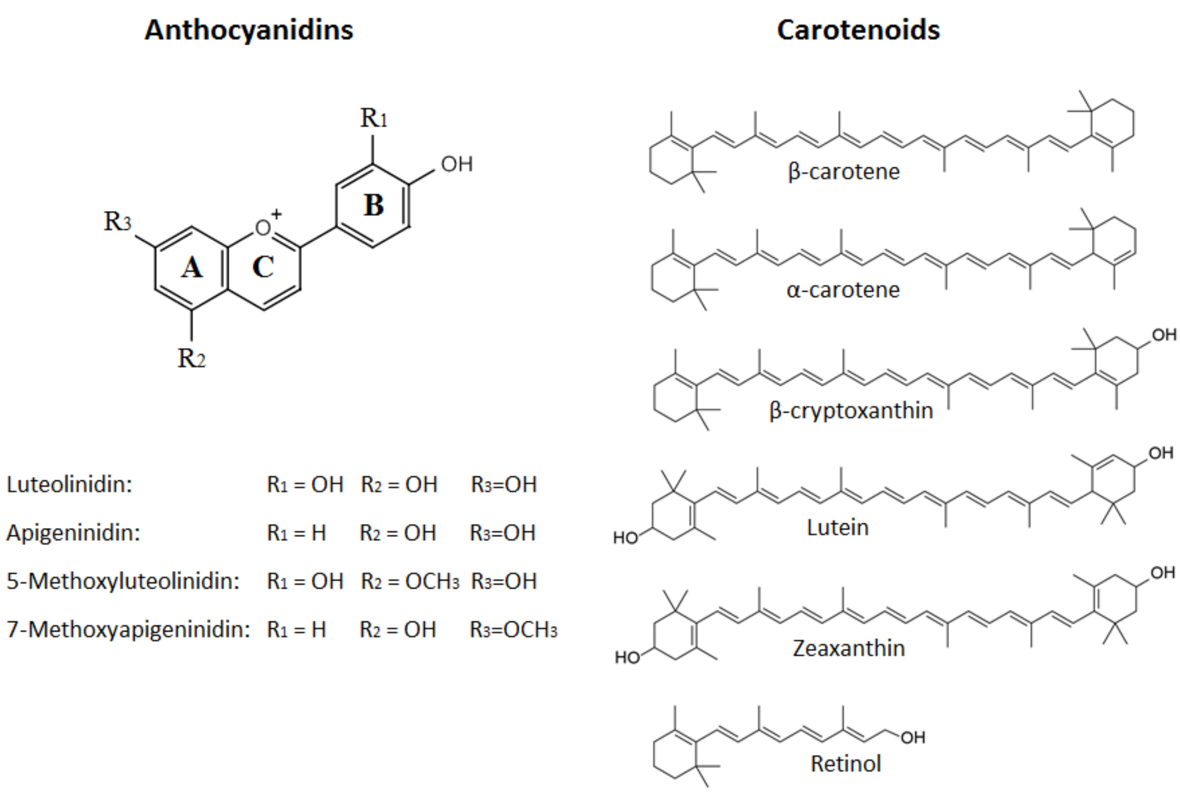


Figure 2. Chemical structures of the common anthocyanins (left) or carotenoids (right) found in sorghum

Chapter 3 - Phytochemicals

Phytochemicals or "plant chemicals" are non-nutritive, biologically active compounds naturally found in plants. As shown in Figure 3, sorghum contains various phytochemicals including phenolics, carotenoids, sterols, and policosanols. Plant sterols and policosanols are mostly components of wax and oils, but phenolics are used as natural defense of plants against pests and diseases (2). Phenols, also known as polyphenols, in sorghum are classified into two categories: phenolic acids and flavonoids. Anthocyanins and condensed tannins fall under the flavonoid category. Only varieties with a pigmented testa have condensed tannins (10). Carotenoids are also categorized as phytochemicals. Studies have indicated potential health benefits of using sorghum for food consumption due to its bioactive compounds. Sorghum has the highest content of phenolic compounds among cereals (1). Evidence suggests that sorghum consumption, for example, may reduce the risk of certain types of cancer in humans compared to other cereals which could be due the high concentration of phytochemicals in the sorghum (11).

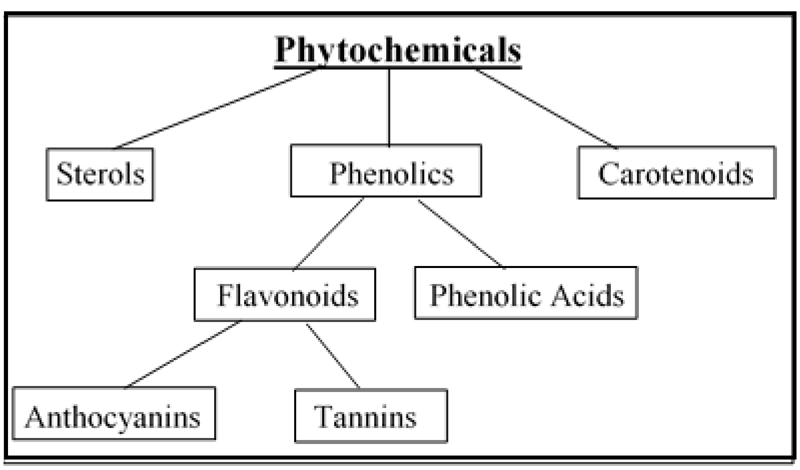
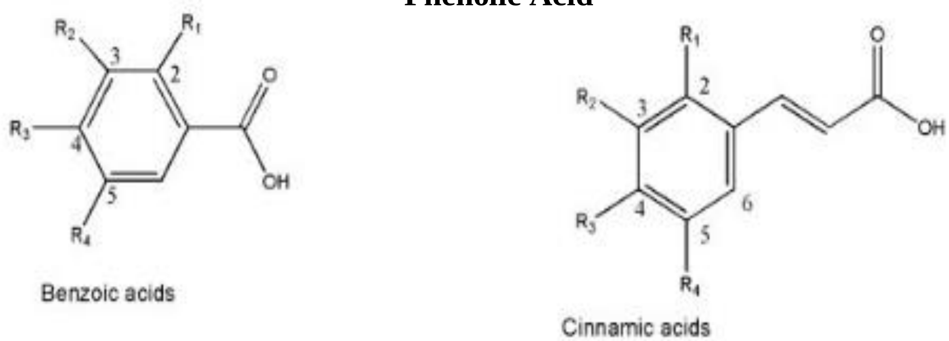


Figure 3. Phytochemical tree

Phenolic acids are located in the pericarp, testa, and endosperm. The phenolic acids of sorghum largely occur as either benzoic or cinnamic acid and their derivatives (Figure 4) (11).

They are generally classified into two types: free and bound phenolic acids. Free phenolic acids are found in the outer layers of the kernel, whereas the bound phenolic acids are associated within the cell walls. Free phenolic acids are soluble in nature and bound acids are insoluble, covalently bound to structural components of cells like cellulose, hemicellulose, lignin, and pectin (12). Phenolic compounds are created from the secondary metabolism of plants when they are subjected to stressful conditions such as infections and mechanical injuries. These compounds act as a natural defense mechanism in the plants by protecting them from pests and disease (13).

Phenolic Acid



Gallic acid: R_1 = H, R_2 = R_3 = R_4 = OH
Gentisic acid: R_1 = R_4 = OH, R_2 = R_3 = H
Salicylic acid: R_1 = OH, R_2 = R_3 = R_4 = H
p-hydroxybenzoic acid: R_1 = R_2 = R_4 = H, R_3 = OH
Syringic: R_1 = H, R_2 = R_4 = OCH_3 R_3 = OH
Protocatechuic: R_1 = R_4 = H, R_2 = R_3 = OH

Caffeic acid: R_1 = R_4 = H, R_2 = R_3 = OH
Ferulic acid: R_1 = R_4 = H, R_2 = OCH_3 R_3 = OH
o-coumaric acid: R_1 = OH, R_2 = R_3 = R_4 = OH
p-coumaric acid: R_1 = R_2 = R_4 = H, R_3 = OH
Sinapic: R_1 = H, R_2 = R_4 = OCH_3, R_3 = OH

Figure 4. Chemical structures of common phenolic acid monomers identified in sorghum. Modified from the reference (11).

Tannins, also known as proanthocyanidins, are large molecular weight polyphenols which are present in sorghum testa (Figure 5). The testa is located between the pericarp and endosperm. Sorghum accessions with pigmented testa are classified as type II and III sorghums. These sorghums have dominant B1 and B2 genes. The B1 and B2 genes control the presence or absence of the pigmented testa layer and both genes must be dominant for a pigmented testa to develop. When the spreader S gene is dominant concurrently with the dominant B1 and B2 genes, pericarp color becomes phenotypically brown (11).

Tannins bind to and reduce digestibility of various food/feed nutrients, such as proteins, carbohydrates, and calories. Thus, limiting nutritional value, decreasing digestibility and negatively affecting productivity of livestock. This aspect of tannins has resulted in decades of breeding efforts to eliminate tannins from sorghum in the US. Whereas, in other parts of the world where pest and diseases are common, tannin sorghums are still grown due to the ability to tolerate these conditions compared to non-tannin varieties (11). But this property of tannins could potentially be useful in reducing obesity in humans due to its reduced caloric availability. There have been numerous studies done on the weight loss of animals when consuming tannin sorghums with varying degrees of success. But there is a lack of epidemiological data regarding tannin sorghums potential use in lowering caloric intake in overweight humans (11). Also, due to tannins ability to bind to free radicals, sorghums containing tannins have higher antioxidant capacity than sorghums that do not contain tannins (13). This along with the tannin sorghums slow digestibility and nutrient release properties should be investigated further.

Finally, there are the most well-known phytochemicals found in sorghum: anthocyanins and carotenoids. These phytochemicals provide many of the colors found in sorghum's several varieties, but the most important aspect is the potential health benefits they can offer. To understand how these two phytochemicals contribute to numerous health benefits, it is imperative to know their makeup and overall function in sorghum. Therefore, the following sections go further into the specifics of these important phytochemicals and the roles they play in prevention of certain diseases and cancers.

Proanthocyanidins

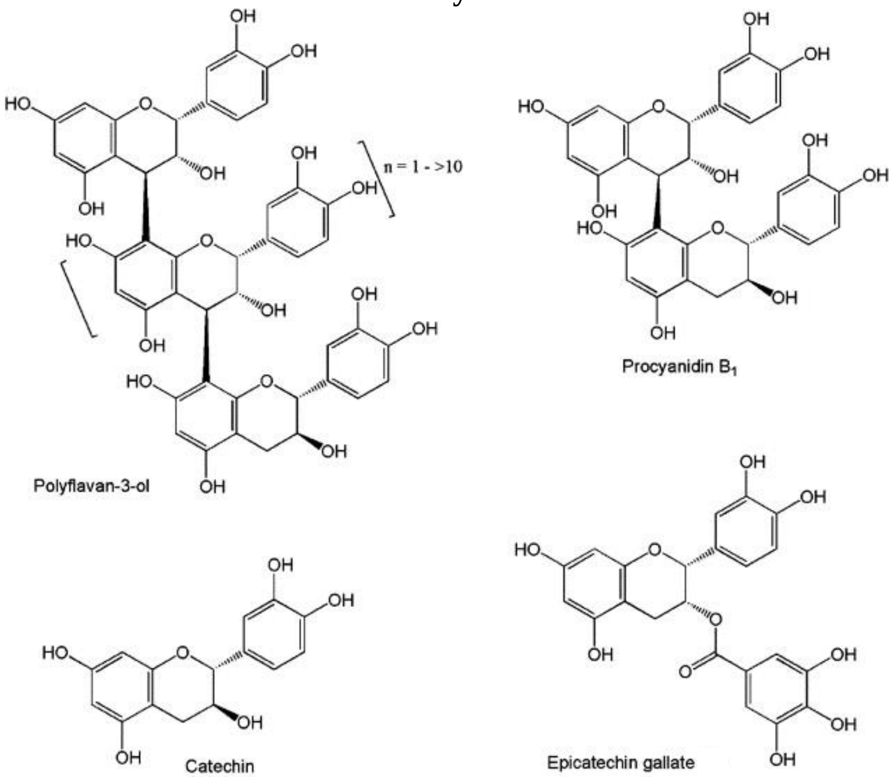


Figure 5. Proanthocyanidins commonly found in sorghum. Modified from the reference (11).

Chapter 4 - Anthocyanins

Anthocyanins are members of the flavonoid group of phytochemicals and are the major class of flavonoids studied in sorghum. There are over 5,000 flavonoids which include the groups anthocyanins, flavonols, flavones, flavanones, flavan-3-ols, and isoflavones (2). These phytochemicals are also referred to as bioflavonoids due to their many roles in human health maintenance. Anthocyanins are one of the most important water-soluble plant pigments and are predominantly found in grapes, wines, fruits, vegetables, nuts, and purple-fleshed sweet potatoes (14). In general, this class of compounds contributes the blues, purples, and reds in plants. Anthocyanins are typically ingested as part of a complex mixture of flavonoids and their intake has been projected to be nine times higher than any other dietary flavonoids (15-16). They are synthesized by the flavonoid branch of the phenylpropanoid pathway through secondary metabolism in the plants (3).

Plants typically produce anthocyanins as a protective mechanism against environmental stress such as cold temperatures, drought, and UV light (16). Among the over 600 types of anthocyanins, the majority of anthocyanins founds in food items usually consists of six anthocyanidins, including cyanidin, delphinidin, malvidin, pelargonidin, petunidin, and peonidin (17). These exist in nature as mostly glycoside anthocyanins. Most studies that are related to anthocyanins are based on the compounds from fruit and vegetables. However, due to a rising demand for cost-effective sources of natural, stable pigments, there is an increased interest in alternative sources of anthocyanins. Sources like cereal, corn, rice, and wheat. Pigmented sorghums are also potentially rich sources of unique anthocyanins (18).

Sorghum anthocyanins are called 3-deoxyanthocyanins which are comprised of luteolinidin and apigeninidin, including their methoxylated derivatives of 5-methoxyluteolinidin and 7-methoxyapigeninidin (14). The anthocyanins in sorghum are unique because they do not contain the hydroxyl group in the 3-position of the C-ring (Figure 2). This feature increases their stability at high pH compared to other anthocyanins, indicating these compounds can be used as potential natural food colorants. These anthocyanins are also called phytoalexins since they are produced as a response to mold or bacterial infection (10). These compounds are more prevalent in purple-pigmented plants than in tan-pigmented plant sorghums. The two most common sorghum 3-deoxyanthocyanins are the yellow apigeninidin and the orange luteolinidin. These 3-deoxyanthocyanidins represent 36-50% of the total anthocyanin content in black and brown sorghum brans (3). Sorghum with a black pericarp, which are concentrated in the bran, have the highest levels of 3-deoxyanthocyanins. The black sorghum bran has twice the level of anthocyanins as red and brown sorghum brans (10).

The profile of sorghum anthocyanins was recently identified and quantified in the selected twenty-five sorghum accessions with various phenotypic pericarp pigments (3). As shown in Figure 6, the high levels of total anthocyanins were found in sorghum accessions with red pericarp, followed by brown pericarp and yellow pericarp sorghums. Sorghum accessions with white pericarp generally contained the least to undetectable amount. Although anthocyanins appeared to be associated with the pericarp color, a distinguishable diversity of anthocyanin contents was present among and between the phenotypic pericarp colors. This suggests that other colorful phytochemicals such as carotenoids might have also contributed (3).

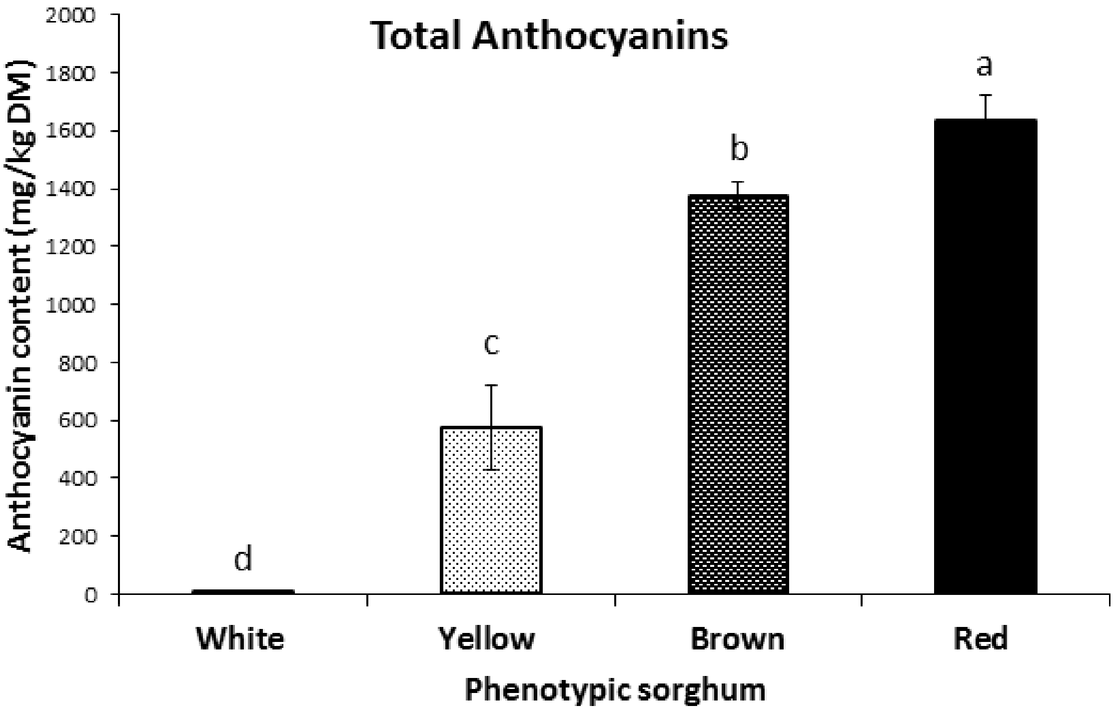


Figure 6. The contents of total anthocyanins in four sorghum accessions with various pigments of pericarp: White (PI656079), Yellow (PI229838), Brown (PI221723), and Red (PI297139). Data represent mean values ± SD. Bars marked with different letters are statistically significant at $p < 0.05$, $n = 2$ **(3)**.

Chapter 5 - Carotenoids

Carotenoids are a family of compounds of over 600 fat-soluble pigments. They function in plants and photosynthetic bacteria as accessory pigments in photosynthesis. Carotenoids are responsible for many different colors in nature such as the red, yellow, and orange color. They play an important role in photosynthesis as light-harvesting pigments and protecting molecules from the formation of singlet oxygen. The most prevalent carotenoids are α-carotene, β-carotene, lycopene, lutein, zeaxanthin, and β-cryptoxanthin. The known carotenoid function in humans is vitamin A activity, but only provitamin A carotenoids have this function (7).

Carotenoids can be classified into two groups based on their functional groups: xanthophylls and carotene. Xanthophylls has oxygen as the functional group, this includes lutein, zeaxanthin, and β-cryptoxanthin. Carotene which contains only a hydrocarbon chain without any oxygenated functional group, includes α-carotene, β-carotene, and lycopene (19). Carotenoids typically contain forty carbon atoms with an extensive system of conjugated double bonds. They have one or two cyclic structures at the end of the conjugated chains. One of the exceptions to this is lycopene, an acyclic carotenoid with eleven conjugated double bonds with no retinol structure or no vitamin A activity (20).

Naturally occurring carotenoids are in the all-trans-configuration. The configuration of double bonds is either E or Z, which corresponds to trans or cis. Cis isomers are usually less thermodynamically stable than trans isomers. Thus, trans forms of carotenoids are more prevalent in nature. Although cis isomers are more soluble than trans and therefore are more readily absorbed and transported within cellular compartments (21). Out of the six common carotenoids, three of them, α-carotene, β-carotene, and β-cryptoxanthin, are precursors of vitamin A, meaning they can be converted into retinol, and thus referred to as provitamin A carotenoids. The other three, lycopene, lutein, and zeaxanthin have no vitamin A activity and are thus referred to as non-provitamin A carotenoids (7).

Vitamin A is a family of molecules that contain a 20-carbon structure with β-ionone ring and tetraene side chain with either a hydroxyl group (retinol), aldehyde group (retinal), carboxylic acid group (retinoic acid) or ester group (retinyl ester) at carbon-15 (15). To convert provitamin A carotenoids to vitamin A, the process involves cleaving of the central double bond. An example would be the cleaving of the central double bond of β-carotene molecule, yielding two 20-carbon molecules of vitamin A (7).

Intestinal absorption of dietary carotenoids is enabled by the formation of bile acid micelles. Like other nonpolar lipids, the hydrocarbon backbone of carotenoids makes them insoluble in water and thus must be solubilized within micelles in the gastrointestinal tract to allow for absorption. Absorption requires that the carotenoids first be freed from the food matrix and solubilized in oil droplets. This transfer of carotenoids and other fat-soluble compounds from the food matrix into micelles during digestion is referred to as bioaccessibility (22). It is a necessary preliminary step, so the compounds become accessible for uptake by the intestinal mucosa.

Carotenoids are then transferred to bile salt micelles which are generated during the digestion of fat-soluble compounds including triglyceride, phospholipids and cholesterol ester. Once inside mucosal cells, carotenoids must also be incorporated into chylomicrons and released into the lymphatics. The carotenoids and other fat-soluble compounds interact with brush border proteins for transfer to the cell interior. The bile salts in the micelles dissociate and are reabsorbed in the ileum (22-23). Absorption of carotenoids from plant foods are relatively inefficient. This is due to numerous factors such as the physicochemical characteristics of carotenoids, physical and chemical barriers within the plant tissue, and processing and preparation of foods. These impede the solubilization of carotenoids emulsified in lipid droplets (22).

The most widely studied carotenoid is β-carotene, which is also the major carotenoid in our diet and in human blood and tissues. The major sources of dietary β-carotene include green leafy vegetables as well as orange and yellow fruits and vegetables. However, there are factors besides just the type of food that are important in the bioavailability of β-carotene. These factors include cooking, chopping, and the presence of dietary fat, all of which improve bioavailability. Thus, the bioavailability of β-carotene from green leafy vegetables such as spinach is thought to be low (23).

Another important carotene is lycopene, which is derived predominately from tomatoes and tomato products. Lycopene bioavailability is greater from tomato paste compared to fresh tomatoes. Other dietary sources include dried apricots, guava, watermelon, papaya and pink grapefruit. Dietary sources of lutein and zeaxanthin include spinach, kale, broccoli, peas, and brussels sprouts. There are no adverse effects of consuming large amounts β-carotene or other carotenoids from foods except for carotenodermia. Carotenodermia is a harmless, biological effect of high carotenoid intake. Characterized by a yellowish discoloration of the skin due to an elevation of carotene concentrations (23).

When it comes to sorghum, the level of carotenoids is related to the pericarp pigment. The highest content of total carotenoids is found to be in sorghum accessions with yellow pericarp. The highest β-carotene content is found in accessions with brown or yellow pericarp. Five carotenoids (α-carotene, β-carotene, lutein, zeaxanthin, and β-cryptoxanthin) were recently identified and quantitated, in the selected sorghum accessions with various pericarp pigments (7).

As shown in Figure 7, the highest content of total carotenoids was found in the sorghum accessions with yellow pericarp/yellow endosperm, followed by brown pericarp/yellow endosperm. The lowest carotenoids were found in the accessions with white pericarp/white endosperm and/or white pericarp/yellow endosperm. The results suggested a possible prevention of vitamin A deficiency by consuming selected sorghum varieties with yellow pericarp pigments (7).

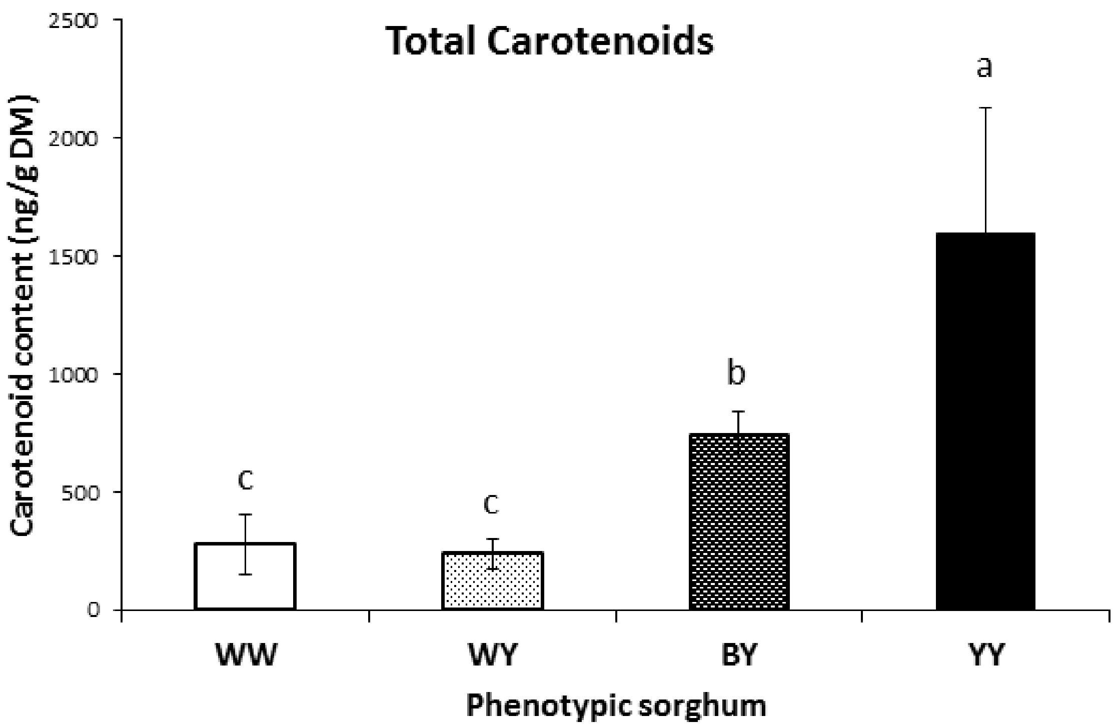


Figure 7. The contents of total carotenoids in selected sorghum accessions with various pigments of pericarp and endosperm: WW (white pericarp/white endosperm), WY (white pericarp/yellow endosperm), BY (brown pericarp/yellow endosperm), and YY (yellow pericarp/yellow endosperm). Data represent mean values ± SD. Bars marked with different letters are statistically significant at $p < 0.05$, $n = 3$ **(7)**.

Chapter 6 - Health Benefits of Colored Phytochemicals

One of the most studied aspects of phytochemicals are their antioxidant properties. Antioxidants come in many forms and are found in numerous food products. They are the primary phytochemical contributors to the potential prevention of certain diseases and cancers, like prostate cancer or Cardiovascular Disease. The definition of antioxidants is based on several criteria. First, the substance needs to be found in the human diet, second the content of the substances must have been measured in foods, and third the substances, in humans, must decrease the adverse effects of reactive species, such as reactive oxygen and nitrogen species *in vivo* (24).

Free radicals can be defined as molecules containing one or more unpaired electrons. They are mostly derived from oxygen, known as reactive oxygen species (ROS) or from nitrogen, known as reactive nitrogen species (RNS). Both ROS and RNS play a role as beneficial and harmful species. Overproduction of these species, either by external or internal factors, can result in a state called oxidative stress. This can cause damage to cell structures, including DNA, proteins, and membrane lipids. Antioxidants act on biological systems to balance the toxic action of free radicals (25). ROS include a group of oxidants which consist of free radical and non-free radical oxygen intermediates. These include hydrogen peroxide (H2O2), superoxide (O2•), singlet oxygen (1O2), and the hydroxyl radical (•OH) (26).

Another type of reactive radical that is derived from oxygen and can be formed in living systems, is peroxyl radicals (ROO•). The simplest form of this radical is the perhydroxyl radical (HOO•). Hydroxyl radicals have a very high reactivity and a very short lifetime. This makes these radicals responsible for the damage to DNA, protein, and membranes and due to their short lifetime, these radicals react close to the site of formation. Nitric oxide (NO) is a radical that derived from nitrogen that also plays a role in living systems. It is a small molecule that contains an unpaired electron and is generated by nitric oxide synthase. NO acts as an important oxidative biological signaling molecule in a variety of physiological processes, such as neurotransmission, blood pressure regulation, smooth muscle relaxation, defense mechanisms, and immune regulation (21).

Anthocyanins have been extensively studied in fruits and vegetables due to their antioxidant properties. However, there is limited data on the types and levels of anthocyanins in cereals due to the belief that cereals are not a commercially significant source. Sorghum fractions have a higher anthocyanin activity *in vitro* than other cereals and fruits and may have similar health benefits usually associated with fruits (11). Various beneficial properties reported for anthocyanins are largely attributed to their antioxidant properties. Benefits include vasoprotective and anti-inflammatory properties, anticancer, and chemoprotective properties. However, antioxidant activity alone is not a good predictor of potential biological benefits because the chemical structure such as the position, number, and types of substitutions, of anthocyanins play an important role in the biological activity exerted. For instance, it has been reported that substitution on the B-ring of the anthocyanidins affected their ability to suppress carcinogenesis (16, 18).

In both *in vitro* and *in vivo* research trials, anthocyanins have shown the ability to reduce cancer cell proliferation and inhibit tumor formation (14-15). Anthocyanins ability to interfere with the process of carcinogenesis is possibly linked to multiple mechanisms of action which include inhibition of cyclooxygenase enzymes and antioxidant potential. Research has shown that significant anthocyanin concentration is effective against various stages of carcinogenesis but the individual role of anthocyanins is not yet determined. This is due in part to the fact that anthocyanins were easily degraded during bioassays if separated from the stabilizing cofactors, such as other phenolic compounds (15). Typically, anthocyanins interact with other flavonoid components or anthocyanins to provide full potency. Anthocyanins almost never act independently when it comes to biological activity in the human body. Within a plant, phytochemical interaction is key and thus any plant with anthocyanins includes a complex phytochemical mixture. Anthocyanins and other flavonoids are secondary products that are produced as defensive and protective agents which are used together to combat diseases and pests. This multiplicity of bioactive phytochemicals is also useful in humans and animals who can benefit from this interaction of flavonoids (15).

One of the larger roles of anthocyanin, as well as other dietary flavonoids, is the protection against cardiovascular disease (CVD), which is the most common cause of mortality among men and women. Studies have shown beneficial effects of flavonoids, including anthocyanins, on the biomarkers of CVD risk, which are Nitric Oxide (NO), inflammation, and endothelial dysfunction. Inflammation is defined by heat, redness, and swelling which plays a large role in the development of CVD. For anthocyanins, this protection is strongly linked to the oxidative stress protection that anthocyanins provide to endothelial cells. Also, there are several mechanisms that have been proposed to explain the *in vivo* anti-inflammatory action of flavonoids such as anthocyanins (11). Unfortunately, there is limited epidemiological data on sorghum consumption and cardiovascular health in humans. Which may be due to in the regions were sorghum consumption as food is high, CVD is not regarded as a major problem since there are other diseases that are of bigger concern. These studies would be important in helping to validate some of the evidence reported for in vitro and controlled animal studies and help determine the specific role sorghum may play in fighting CVD (11).

Carotenoids are known to have vitamin A activity but may possibly have antioxidant activity as well. They are thought to be associated with various health effects such as decreased risk of macular degeneration and cataracts, particularly lutein and zeaxanthin which may protect from eye disease due to their ability to absorb damaging blue light that enters the eye (23). But carotenoids may also decrease the risk of certain cancers and cardiovascular events as well. These beneficial effects are thought to be due to their role as antioxidants which are a result of the conjugated double bond structures ability to delocalize unpaired electrons. This is the feature that is responsible for carotenoids exceptional ability to quench singlet oxygen and terminate free radicals that have formed in tissues (21). The presence of these numerous conjugated double bonds and functional end groups are crucial for the function of carotenoids. This is essential for light absorption in photosynthetic organisms. Carotenoids absorb radiant energy to chlorophyll molecules in a light harvesting function during photosynthesis. This dissipates excess energy through the xanthophyll cycle and quench excited-state chlorophylls directly (26).

One of the factors responsible for oxidative damage to plant leaves is singlet oxygen and carotenoids are the first line of defense by quenching them. This can be done physically, by energy transfer or chemically, by direct reaction with the radical. In plants, organelles such as chloroplast and peroxisome have an intense rate of electron flow, which generate ROS as byproducts during respiration and photosynthesis. They also host antioxidants to harmlessly dissipate ROS like carotenoids (26). The function of carotenoids is determined by their molecular properties, such as size, structure, presence of functional group, and potential interaction with other carotenoids. Carotenoids most important aspect is the double bond conjugation which is linked to its antioxidant properties (21, 26).

Since carotenoids are a part of a hydrophobic group of antioxidants, the major mechanism of this action is located within the lipid membranes. They are also associated with proteins or lipoprotein structures. This means that the local environment of carotenoids affects its properties and vis versa. The structural differences of carotenoids mean different types of interaction with the membrane lipids, affecting membrane fluidity and thermo-stability in different ways (21). One of the main potential health benefits of the antioxidant function of carotenoids is cancer prevention. One way is carotenoids have shown to inhibit the growth of tumor cells by interfering at different phases of the cell cycle. Deregulated cell cycles are one of the major hallmarks of cancer cells. This means the cells lose their ability to regulate the cell cycle and controls the rate of proliferation.

Carotenoids modulate the cell cycle arrest by multiple mechanisms in cancer cells. One such carotenoid is lycopene, which has been reported to significantly suppress the proliferation of prostate cancer cells. Lycopene has also shown to produce an anti-proliferative effect in human colon cancer and breast cancer cells (26). Another hallmark of cancer cells is a defect in the apoptosis mechanism. Apoptosis is a form of programmed cell death. This defect is characterized by morphological changes including membrane blabbing and formation of apoptotic bodies. Cancer cells resist apoptosis by the expression of anti-apoptotic proteins. Carotenoids possess chemo-preventive activity by reducing cancer incidence through apoptosis.

Another way cancer occurs, in addition to atherosclerosis and diabetic retinopathy, is through the formation of tumors. Angiogenesis is fundamental for the growth and metastasis of tumors. Carotenoids, such as β-carotene and lycopene, has shown to have excellent anti-angiogenic properties (26). Specifically, reports have shown the higher the intake of carotenoids, the lower the risk of lung cancer, the leading cause of death worldwide. High levels of carotenoids, such as α-carotene, trans-β-carotene, lutein, and zeaxanthin, have been reported to reduce the risk of prostate cancer, the second common cancer in men (26). For sorghum, available data on cancer are too limited to draw reasonable conclusions. Additional in vitro data as well as controlled animal studies are necessary to understand how the levels and compositions of phytochemicals in sorghum, like carotenoids, affect cancer and which specific components are responsible (11).

Chapter 7 - Discussion

Anthocyanins and carotenoids were the two major phytochemicals addressed in this paper because they are major components in the color and health benefits of sorghum. However, there are a few other phytochemicals mentioned that can also be linked to the color and possible health benefits. For instance, condensed tannins, as previously stated, are polyphenols present in sorghum with pigment testas. Sorghum varieties with pigmented testas are classified as type II and type III sorghum. The difference between the two types is the S gene. The S gene or spreader gene spreads the pigment of the testa to the pericarp. This means that if the gene is regressive, like in the type II sorghum, the pigment will not spread to the pericarp and thus the sorghum has a white appearance while still containing polyphenols like tannins. If the gene is dominate, like in type III, the pigment will spread, which gives a white or red pericarp color, but an overall brownish red/white sorghum (4). Like other polyphenols, tannins also show antioxidant properties, as well as anti-inflammatory, antihypertensive, anti-cancer, and anti-carcinogenic properties. There has been a wide range of research conducted regarding the antioxidant properties within tannins, but little has been done with condensed tannins in sorghum accessions with various testa pigments.

Chapter 8 - Conclusion

Sorghum is the fifth leading whole grain cereal crop in the world and is found primarily in tropical, subtropical, and arid regions such as Asia and Africa. In countries such as the United States and Brazil, sorghum is mainly used for animal feed. But sorghum use in food is on rise due to its functional and health properties. Most research on sorghum has been performed on its high phytochemical contents, including phenolic anthocyanins, polyphenolic tannins, and carotenoids, that are mostly presented in the pericarp, endosperm, and testa. The major colored phytochemicals in sorghum are anthocyanins and carotenoids. The contents of anthocyanins appear to be associated with the sorghum pericarp color, but a diversity of anthocyanin contents was present among and between the phenotypic pericarp colors. The content of total carotenoids was correlated with sorghum pericarp and endosperm pigments. Both anthocyanins and carotenoids are believed to be bioactive compounds and have potential health benefits including antioxidant, anticancer, vasoprotective, and anti-inflammatory properties. There is also potential for more research to be performed on the other phytochemicals, such as condensed tannins, that contribute to sorghum color and provide potential health benefits. Along with many other functional and healthful properties, sorghum shows promise to be potentially functional in health benefit-promoted food products.

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