DEVELOPMENT AND EVALUATION OF A SORGHUM TISANE

by

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Abstract

Known for its antioxidant activity and other health benefits, tea is the second most consumed beverage in the world, following water. With up to 6% (w/w) of phenolic compounds, sorghum has the highest content compared to other cereals. The objective of this research was to develop and analyze a sorghum tisane using two different red sorghum hybrids. Tisanes are herbal infusions composed of anything other than the leaves from the *Camellia sinensis* plant. The sorghum kernel was cracked using an Allis experimental roll stand equipped with a Le Page cut mill. Samples were sifted at 180 RMP- 4" diameter throw for 2 min. The two hybrids were roasted in a Whirlpool convection oven at 212°C for 13 or 15 min. Three fruit and herbal combinations were tested to increase consumer acceptability. Samples was brewed for 4 min in 8fl.oz at 100°C. Oxygen Radical Absorbance Capacity(ORAC) and Total Phenolic Content (TPC) were used to analyze the beverage along with chemical, physical and sensory tests. TPC results showed sorghum tisane to have 38.5±6.91 mg gallic acid equivalence/ 8fl oz. and 433.7 ±7.11 μM Trolox equivalence/ 236.6 mL (8 fl.oz, 1 cup) for an ORAC value. Fruit and herbal combinations were also added to the sorghum to increase overall consumer acceptability. These combinations included strawberry mixed with lemon, blood orange mixed with pear, and pineapple mixed with orange. A consumer acceptance test was performed on the three different sorghum tisanes using a 9 point hedonic scale. Results showed an overall acceptability at 6.63±1.54 for the sorghum tisane infused with a strawberry and lemon combination while the sorghum tisane with pineapple orange scored 6.72 . These results demonstrate the potential for introducing a consumer acceptable sorghum tisane into the market.

Table of Contents

List of Figures

List of Tables

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CHAPTER 1 - LITERATURE REVIEW

Introduction to Tea

Tea as a word formally refers to the agricultural products of the leaves, leaf buds, and internodes of the *Camellia sinensis* plant, prepared and cured by various methods. The plant is an evergreen shrub native to the hillsides of China, India, Ceylon, Malaysia, and Indonesia. Leading countries in tea production today consist of India, China and Sri Lanka with 60% of the worlds production (Klasra and others 2007). The remaining 40% of tea, however, is cultivated in over 30 countries. With an estimated 18 to 20 billion cups consumed daily, tea is the second most consumed beverage in the world. Originating in China, tea was traditionally consumed as a medicinal beverage by Chinese monks. With time, the beverage diffused westward to Great Britain and eventually to other parts of the world (Cabrera and others 2003). The actual history of tea varies with each story, but the role it has played over thousands of years while traveling from east to west is un-deniably significant. "Influencing medicine, politics, the arts, culture, and religion, tea has become a beverage idolized by poets and revered in spiritual practices. Behind this beverage lies stories of treachery, violence, smuggling, drug trading, international espionage, slavery, and revolution" (Hohenegger 2006). It is known, however, that tea was used to barter trade by the Turkish traders for Chinese produce in the 5th century which allowed it to be carried westward. In the 100 years that followed, tea became a beverage instead of just a medicinal drink. By 1610, it had been carried through Dutch and Holland to reach Europe (Klasra and others 2007). Each region around the world perceived tea in their own way and even though it was categorized under many different names and varieties, true tea only comes from a single species of plant, *Camellia sinensis*, which is in the family Theaceae. "*Camellia sinensis* is an

evergreen shrub that produces small aromatic flowers with white petals and numerous golden stamens" (Martin 2007). The plant, if left alone will grow 9 to 12 meters tall, however the shrubs during cultivation are normally trimmed and maintained at ~ one meter. The tea shrub grows in warm climates where rainfall is evenly distributed throughout the season. The plant can be found at high and low elevation and on terraces or open fields. While lower elevations produce a greater quantity of tea, the high elevation will produce a finer quality of teas. The plant is harvested only after it has matured two to five years. This process begins by an experienced plucker picking the bud and the two terminal leaves from the shoot. An experienced plucker can pick between thirty and thirty-five kilograms of tea each day.

Teas from the *Camellia sinensis* plant are classified in three major groups: non-fermented green tea; semi-fermented oolong tea; and fermented black and red teas (Cabrera and others 2003). However, these classes are recognized more commonly by four different names; black, green, white, and oolong. The only difference between each of these types of tea is the way that they are processed. Tea, in a general sense, refers to an array of different types of beverages. For example; mate, rooibos, and herbal teas are all types of teas but are not from the *Camellia sinensis* plant. Rooibos tea, also known as red tea, is an infusion made from the South African rooibos plant, while herbal tea refers to an infusion or tisane of flowers, fruit, herbs, and leaves from other plant material. Mate tea is an infusion of toasted leaves and twigs from the yerba mate plant. An herbal infusion of anything other than the leaves from the *Camellia sinensis* plant is known by three different names; tisane, herbal tea or ptisan.

Each region of the world is favorable to a particular tea; however, black tea with 76-78% worldwide consumption is the most popular, followed by green tea at 20-22% consumption (Cabrera and others 2003).

Processing of Black Tea

The processing of *Camellia sinensis* is the underlying characteristic that makes each type of tea different. Since black tea is the most widely consumed tea in the world, it is important to understand how the processing of this type of tea is unique to the flavor and overall characteristics developed during the brewing process (Wang and Ho 2009).

The processing of the *Camellia sinensis* leaves to make black tea can best be described as an oxidation (fermentation) process used on the raw green leaves before they are dried out. Due to this extensive fermentation process that black tea must go through, it has the highest caffeine content of the four types of tea (black, oolong, green and white) but the lowest level of antioxidants (Cabrera and others 2003).

Once the tea leaves are harvested from the *Camellia sinensis* plant by plucking the bud and the second and third leaves, they go through five different processing steps; withering, rolling, oxidation, and drying or firing in order to create the final product. Each plant produces around three thousand tea leaves in one year, however after being fully processed this will only yield about one pound of tea (Griffiths 2007).

Once harvested the leaves enter the withering stage, where newly plucked leaves are thinly spread in a cool breezy room or under direct sunlight to accelerate the drying. If the climate is not suitable, heated air is forced over the leaves in order to reduce the water content. This process can take between 8 to 24 hours, with the final goal being that the tea leaves are pliable enough to roll (Griffiths 2007).

From the withering racks, the leaves are twisted and rolled either by machinery or by hand so that the leaf cells are broken up. This is done in order to expose the enzymes that are housed inside the veins of the leaf. As these enzymes are exposed to the outside of the leaf

oxidation begins. The amount of oxidation depends upon how much of the enzymes are exposed and for how long.

Once the oxidation process is started the leaf begins to turn bright copper in color. This process is the main deciding factor for which type of tea the leaf will become (Griffiths 2007). Black tea requires a full oxidation of the leaves, which allows the deep black color to be formed (Cabrera and others 2003). This extensive oxidation is also responsible for the taste and body that is characteristic to black tea (Griffiths 2007).

After the leaves are fully oxidized they are placed in an oven with temperatures reaching up to 93°C. When the leaves have 20% moisture content remaining they are transferred to a wood fire to complete their drying. The resulting product is then sorted according to size and packaged. Figure 1.1 illustrates how each type of the four teas goes through similar manufacturing steps but at different specifications in order to create the variations in taste, flavor and body.

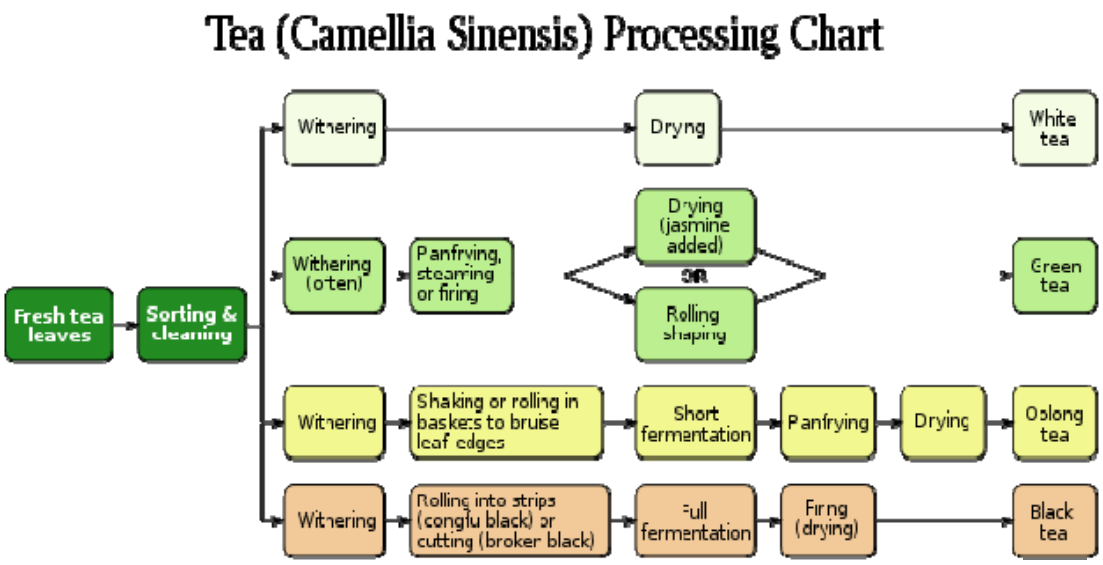


Figure 1.1-Flow Diagram of Tea Production (Taken from http://teavortal.com/?page_id=10, last accessed on April 3, 2010)

Antioxidants

The processing of tea leaves not only affects the flavor and color of the tea but it also contributes to the levels of caffeine and antioxidant activity. Tea has been used for its medicinal purposes since ancient times, but recently has been highlighted for its polyphenols, which act as strong antioxidants. Tea is a complex beverage made of a combination of polyphenols, amino acids, alkaloids, proteins, and carbohydrates. (Cabrera and others 2003). Polyphenols are recognized for their strong antioxidant activity *in vitro* and *in vivo.*

Polyphenols are chemical compounds found in the leaves and seeds of plants. Their function is to protect the plant from oxidative damage through their natural antioxidant activity (Fukushima and others 2009). They are characterized by the presence of more than one phenol unit or building block per molecule and are divided into hydrolyzable tannins (gallic acid esters of glucose and other sugars) and phenylpropanoids, such as lignins, flavonoids, and condensed

tannins. Condensed tannins are the most abundant type of polyphenol found in virtually all families of plants and comprising up to 50% of the dry weight of leaves.

Since polyphenols act as antioxidants, and oxidative stress is a key factor for human disease risk, they protect cells and body chemicals against damage caused by free radicals (reactive atoms that contribute to tissue damage in the body) (Fukushima 2009). For this reason polyphenols have been reported to reduce the risk of several diseases such as cancer, Alzheimer's, Parkinson's, type II diabetes and cardiovascular disease.

Foods and beverages that have a high polyphenol content including: coffee, green tea, cocoa, wine, and some fruits and vegetables have been shown to reduce the risk of these diseases along with many others. Each of these types of food contain particular molecules that provide the antioxidant activity associated with disease prevention. There are over 5,000 species of polyphenol molecules. One species in particle is flavonoids, which includes catechins found in tea, isoflavons in beans and lutein in vegetables (Fukushima and others 2009)."

Depending on the food source and the type of polyphenol present the method for measurement will vary depending on the type of molecule being extracted and examined. Since tea has a phenolic composition primarily of catechin, it was shown that by using a modified Folin-Ciocalteu method total polyphenols (TP) could be measured by reverse-phase column chromatography (Fukushima and others 2009). This study also examined three different types of antioxidant activity by measuring beverages, fruits, and vegetables. These activities, Copper reducing power, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging power, and superoxide scavenging activity were positively correlated to the TP contents. Figures 1.2 and 1.3

illustrate the different TP contents in various beverages, fruits and vegetables along with the correlation graphs between antioxidant activity and TP.

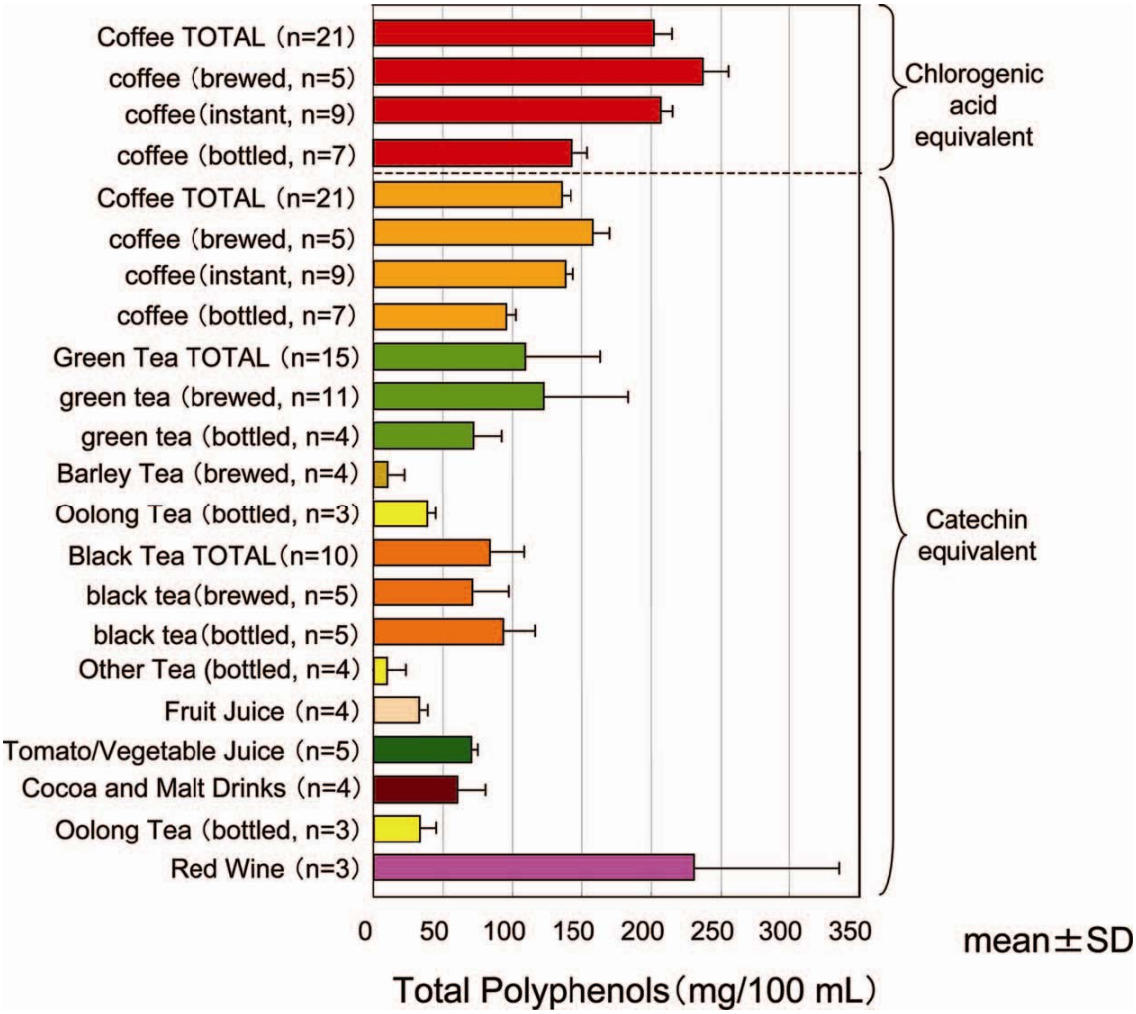


Figure 1.2-Total polyphenols in beverage. (Adapted from Fukushima and others 2009)

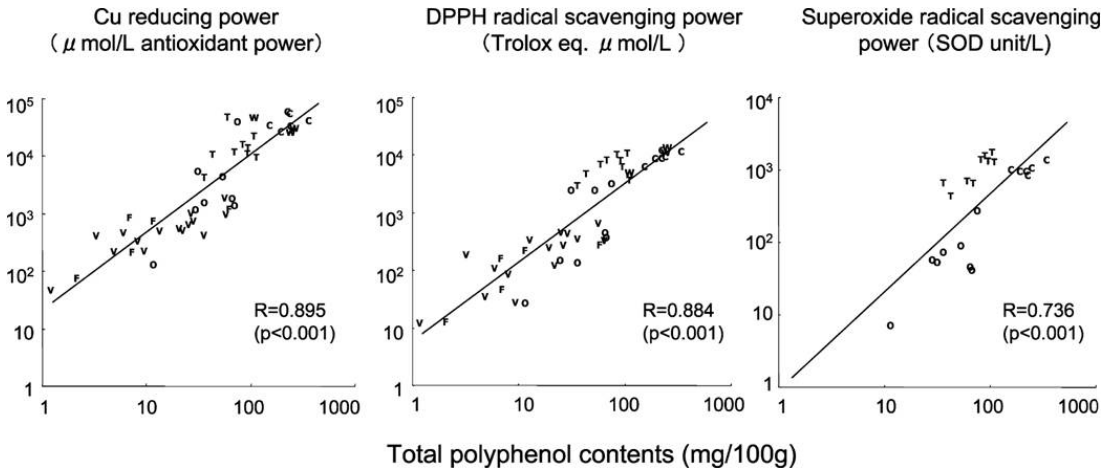


Figure 1.3-Correlation between total polyphenol contents and antioxidant activities in beverages and extracts from fruits and vegetables. C: Coffee; T: Green Tea, Black Tea, and Oolong tea; O: Other beverages including juices and cocoa; W: Red Wine; V:vegetables; F: Fruits. (Adapted from Fukushima and others 2009)

Other ways of analyzing antioxidants is by looking at the antioxidant capacity (AC), the condensed tannin content (CT), high performance liquid column chromatography (HPLC) phenolic profile, and the tocopherol content. The AC values can be assessed with the Oxygen Radical Absorbance Capacity (ORAC) assay while the DPPH assay can be used to measure the TP, CT, and AC. Phenolic compounds can also be detected using the HPLC phenolic profile. Measuring polyphenols is not limited to just these methods, but each experiment must be looked at individually in order to assess which type of method is appropriate (Schlesier and others 2001).

The measurement of polyphenols is also affected by other limiting conditions that may be present in the environment. For each food material, chemical and physical factors must be considered in order to understand how the measurement may be effected. In one study conducted by Bach and Munch (1998) studies how the effect of dietary polyphenols influenced the digestibility of dietary fiber and protein by reducing the digestibility of the protein and amino

acids from being exposed to acidification and cooking temperatures. The treatments were thought to free up the polyphenols from the matrix, thus allowing them to react with the amino acids found in various foods containing protein. The study therefore concluded that diets high in acidified and cooked foods will have lower polyphenol and tannin contents compared to diets that are raw and lower in acid (Bach and Munck 1988). Even though each study of polyphenols and tannins needs to be considered independently to understand chemical and physical effects, it can be assumed that heat and acid have a degrading effect in most situations.

Tea and fruits have been recognized the most in recent years as great sources of antioxidants. However, grains such as sorghum also play a significant role in contributing phenolic acids and tannins to the diet.

Sorghum

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is the second most important crop in Africa after maize with production levels of 44.7 million metric tons in 2005 (Kobue-Lekalake and others 2007). A few common names of sorghum include milo, jowar, kafir corn, guinea corn, and cholam. Gaining recent recognition in the United States and Europe for its gluten-free status, it has shown to be an acceptable alternative for wheat in bakery and snack items for those who have a wheat allergy. Additionally, sorghum varieties also offer the benefit of phenolic acid compounds, condensed tannins, and flavonoids which exist in free form mainly in the bran and bound forms when esterfied to cell wall polymers (Dykes and Rooney 2006). These phenolic acids are particularly of interest in food and beverage products due to their astringency, bitterness, color, browning reactions, and health benefits (Lekalake and other 2007). Many

studies have been conducted to measure the phenolic values of sorghum and explore ways to retain the phenolic contents throughout processing in order to be used in food products. Minimal research has been conducted in order to understand how sorghum performs when used in a beverage matrix (Dykes and Rooney 2006). Therefore, it is important to understand the composition, origins, genetics, and world production of sorghum so that utilization and further applications (i.e. beverages using sorghum) can be achieved.

Production

Sorghum is considered the fifth most important cereal crop in the world and ranked third in the U.S. for cereal crop production (U.S. Grains Council 2010). Its uses range from broom straw to syrup with applications in food, tools, shelter, and sugar. Thirty-nine countries produced at least 100,000 milli-tons of sorghum grain during 1998 (Smith 2000). The U.S. produced 13.2 million metric tons of this yield on 3.1 million hectares. Total world production of sorghum amounted to approximately 65 million metric tons according to one report with the majority of that coming from Africa and Asia at ~55%. The United States follows in production at ~29% with about 90% of that grown in Kansas, Texas, Nebraska, Oklahoma, and Missouri (Smith 2000) (Table 1.1).

Table 1.1-Sorghum production by state in 2008

State	Bushels of Sorghum harvested
Kansas	**214,500,000**
Texas	**158,600,000**
Nebraska	**19,110,000**
Oklahoma	**13,950,000**
Arkansas	**10,120,000**
Louisiana	**9,570,000**
Illinois	**7,828,000**
Missouri	**7,760,000**
South Dakota	**7,360,000**
Mississippi	**5,822,000**

Source: U.S. Department of Agriculture 2008

Sorghum is grown throughout the world because of its ability to grow in an array of conditions. Low temperatures, not the length of the growing season, is the limiting factor for this grain. The average minimum temperature needed for this crop to grow is ~27°C with daytime temperature needing to reach 32°C for proper photosynthesis. The seeds germinate well in temperatures between 10-35°C, however a frost will kill the plant. For this reason, this crop is typically planted during the early summer months in the Midwest. Sorghum will tolerate a wide range of soil conditions including wet and semiarid to heavy clay and sandy soils. It will also grow in a wide range of soil acidity (5.0 to 8.5) and will tolerate altitudes from sea level to 3000 meters (Kimber 2000). Therefore, sorghum can be grown throughout the world as long as these conditions are present (Figure 1.4).

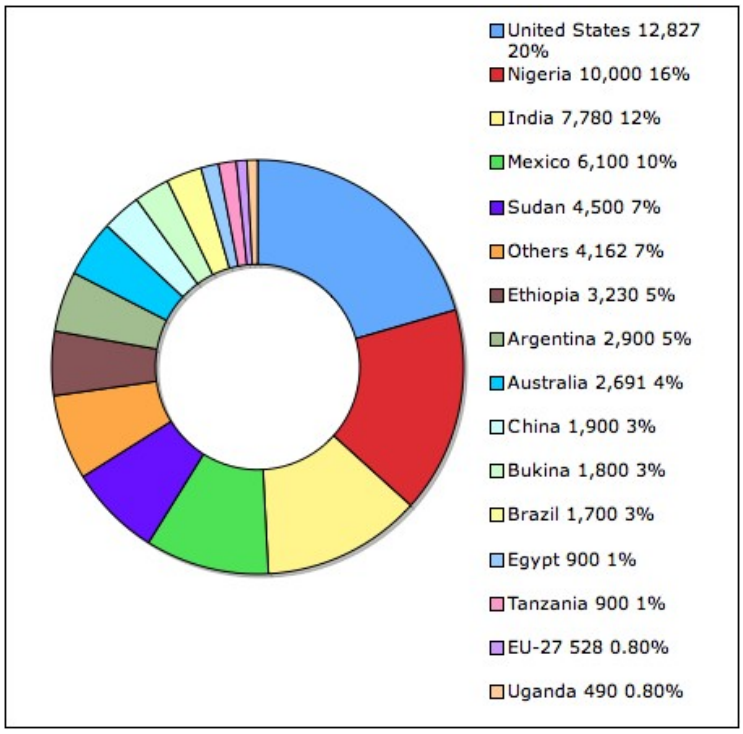


Figure 1.4-World sorghum production (Adapted from U.S. Grains Council 2010).

Sorghum Description and Composition

Botanical Description:

Sorghum can be described as a hearty grass similar to corn in vegetative appearance, but distinguished by having more tillers and finely branched roots than corn. It can be recognized by its cane-like grass appearance with tall slender stocks, which average 14-18 leaves that grow upward on alternate sides of the plant. Sorghum has a fibrous root system that may penetrate into the ground about 152 to 243 cm and waxy leaves that vary from 1.5-13 cm wide and 15-35 cm long depending on the variety (Dahlberg 2000). The sorghum seed is a flattened sphere approximately 4.0 mm long by 2 mm wide and 2.5 mm thick (Rooney and Serna-Sadivar 2000). The variety of sorghum determines many of its characteristics including the size and shape of the seed head (panicle), which typically measures 25-35 cm in length but varies based on the density

of the seed (Rooney and Serna-Sadivar 2000). Furthermore, the variety of the sorghum plant will also determine the seeds chemical and physical composition along with its color (Figure 1.5).

Figure 1.5-Sorghum anatomical features. A. Grain Head B. Leaves C. Stalk D. Flower E. Roots F. Seed. (Image and labels courtesy of Nebraska Foundation for Agriculture Awareness 2010).

Species and Race Description:

Sorghum is classified under the family *Poaceae*, tribe *Andropogoneae*, subtribe *Sorghinae* and genus *Sorghum* Moench (Dahlberg 2000). The genera is further divided into five subgenera: *Sorghum, Chaetosorghum, Heterosorghum, Parasorghum and Stiposorghum*. However, cultivated sorghum is classified under *Sorghum bicolor* L. *Moenchis* (Dahlberg 2000). *Sorghum bicolor* (L.) *Moenchis* is further divided into three subspecies; *S. bicolor* subsp. *bicolor, S. bicolor* subsp. *drummondii,* and *S. bicolor* subsp. *verticilliflorum* (formally subsp. *arundinaceum*) (Dahlberg 2000). Great millet, kafir corn, jowar, milo and shatter-cane are among the several common names used to classify these subspecies. Therefore, for the purpose

of this report, sorghum will be used in reference to *Sorghum bicolor* L. *Moenchis.* Five major races were used to partition *Sorghum bicolor* (L.) *Moenchis*; bicolor, guinea, caudatum, kafir, and durra (figure 1.6) . All combinations of these races are possible and would make up the intermediate races (Dahlberg 2000).

Bicolor:

Thought to be the most primitive grain sorghum, it can be characterized by open medium size panicles with long branches terminating with glumes that clasp to the caryopsis. The caryopsis can be brown, red or purple and is recognized by being small, elongated, and symmetrical in shape. The plants are fairly low yielding, medium in height, and are typically found in Africa, Asia, India, and Indonesia (Dahlberg 2000).

Guinea:

Proposed to have originated 3,000 years ago in West Africa, guinea is unlike the bicolor race in that it is characterized by having long, loose, and pendulous panicles. The glumes are involuted, thus covering most of the caryopsis. The grain tends to be light in color or slightly pigmented with a oval kernel shape that is flattened but can appear twisted (Figure 1.6). Plants are low yielding, medium to tall in height, and resistant to animal and insect predation (Dahlberg 2000).

Caudatum:

Associated with the Chari-Nile languages in Africa and found widely distributed throughout northeastern Nigeria, Chad, Sudan, Ethiopia, and Uganda, caudatums are one of the most important races ergonomically since they provide a high yield and excellent seed quality. The panicles are medium to large in size, oblong, and dense to slightly open. The kernel is flat on one side while round or bulging on the other (Figure 1.6). They are chalky white and do not

contain a testa layer (Dahlberg 2000).

Kafir:

Dominating the southern regions of Africa and widely distributed in northern Nigeria, kafir is thought to be derived from early bicolor of northern Africa and carried south. It is distinguished by its elongated, erect and cylindrical panicles and small glumes which cover symmetrical, spherical grains (Figure 1.6). The kernels are elliptical and flattened while the plant is medium in height and typically high yielding (Dahlberg 2000).

Durra:

Tolerant to dry arid environments from suggested early crossings between bicolor and wild forms, durra is characterized by dense and compact panicles that are oblong in shape (Figure 1.6). The caryopsis is red, yellow or white and is large, broad on top and a wedge shape at the base. Plants tend to be medium to tall and of good quality (Dahlberg 2000).

While any of these races can be crossed to make other variations of sorghum, the overall grain composition is fairly comparative throughout the races.

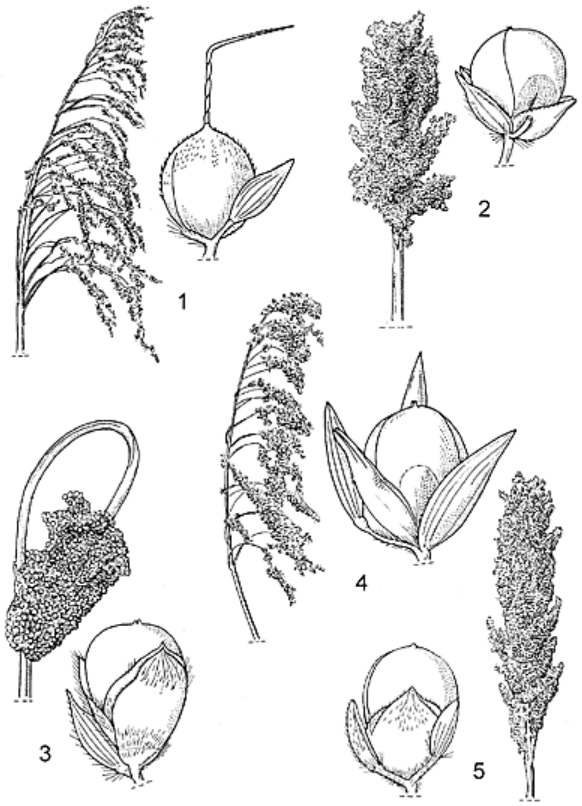


Figure 1.6-Panicles and spikelets of bicolor (1), caudatum (2), durra (3), guinea (4), and kafir (5) (Taken from Balole and Legwaila 2006).

Grain Composition:

Very similar to maize, sorghum is composed of three main anatomical parts: pericarp (outer layer), endosperm (storage tissue), and germ (embryo) (Rooney and Serna-Sadivar 2000). The relative proportions of these components vary but they generally make up 6, 84 and 10% of the kernel, respectively. The pericarp is further divided into three parts: epicarp, mesocarp, and endocarp.

Starch Components:

Making up the majority of the kernel, starch is found throughout the floury and corneous parts of the endosperm. In most varieties, starch is composed of 70-80% branched amylopectin and 20-30% amylose. However, there are genetic variations, such as waxy or glutinous sorghums which contain 100% amylopectin (Rooney and Serna-Sadivar 2000). While sorghum is strongly related to corn in composition and shape, the primary differences are present in the size of the starch component and gelatinization temperatures. Sorghum starch granules are ~ 25 μm while corn granules (waxy and non waxy varieties) are ~15 μm. Furthermore, sorghum gelatinizes at 68-78°C which differs from corns 62-72°C gelatinization temperature range (Rooney and Serna-Sadivar 2000).

Protein:

Protein content also differentiates the two types of grains with sorghum being more variable and ~1-2% points higher in protein compared to corn. The protein in sorghum is found throughout the caryopsis with a ratio of 80% in the endosperm, 16% in the germ, and 3% in the pericarp (Rooney and Serna-Sadivar 2000). Of these proteins, kafirins make up about 50% of the protein fraction. Alcohol soluble, kafirins are hydrophobic, rich in proline, aspartic acid, and glutamic acid, and are found in tightly packed protein bodies in both hard and soft endosperms. (Rooney and Serna-Sadivar 2000). Kafirins are composed of three fractions, α-kafirin, β-karfirin, and γ- kafirin. The major fraction α is located in the inner most region of the protein body while the β and γ fractions are located in the periphery (Rooney and Serna-Sadivar 2000). With high levels of cysteine, the β and γ fractions undergo disulfide linkages which creates a protective cap around the α-kafirin and retards their digestion (Rooney and Serna-Sadivar 2000).

Glutelins, albumins, and globulins are the other proteins in sorghum, which make up the enzymes, cell material, and structure needed by the plant for development.

Fiber:

Sorghum is primarily composed of high levels of insoluble fiber with low levels of β-glucans and soluble fiber. The majority of the crude fiber is in the pericarp and endosperm cell walls and is composed of cellulose, hemicellulose, and small amount of lignin (Rooney and Serna-Sadivar 2000). Ferulic and caffeic acids, phenolic compounds in sorghum, can cause higher amounts of dietary fiber in high tannin sorghums due to the complexes between the tannins and proteins (Rooney and Serna-Sadivar 2000).

Lipids:

Sorghum generally contains about 1% less fat than corn but has considerably more wax, which is located on the outer part of the pericarp (Table 1.2). The lipid fraction is primarily composed of linoleic, oleic, and palmitic acids. Other fatty acids present in smaller quantities include: palmitoleic, steric, linolenic, and arachic. The germ and aleurone layer are the main contributors of the lipid fraction with ~80% of the oil coming from the germ and the remainder from the aleurone layer (Rooney and Serna-Sadivar 2000).

Table 1.2-Typical fatty acid composition of sorghum lipids

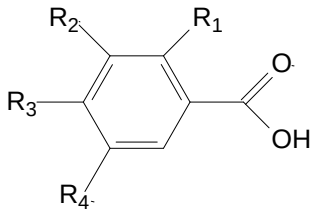
Component	Sorghum (%)
Palmitic (16:0)	14.3
Palmitoleic (16:1)	1.0
Stearic (18:0)	2.1
Oleic (18:1)	31.0
Linoleic (18:2)	49.0
Linolenic (18:3)	2.7
Arachidic (20:0)	0.2

Fatty acid composition is expressed as percent of total
Ether extract equals 3.4%
Source: Taken from Rooney and Serna-Saldivar 2000

Phenolics:

Phenols, which can be found in all varieties of sorghum, have the ability to affect color, appearance, and nutritional quality of the grain and product the grain is used in (Rooney and Serna-Sadivar 2000). Among cereals, sorghum has the highest content of phenolic compounds reaching up to 6% (w/w) in some varieties (Deshpande and others 1986, Beta and others 1999, Doka and others 2004, Awika and Rooney 2004, Dicko and others 2005). Phenols can be divided into three sub-categories: phenolic acids, flavonoids, and tannins. All varieties of sorghum contain phenols and most contain flavonoids, however, only sorghums with a pigmented testa layer contain tannins. Phenolic acids are derived from benzoic or cinnamic acid, which are linked together to form larger units (Figure 1.7). Like other cereals, sorghum phenolic acids (PA) are concentrated in the bran and exist mostly in bound forms (esterfied to cell wall polymers), with ferulic acid being the most abundant bound PA in sorghum (Hahn and others 1983). Other PA identified in sorghum include: syringic, protpcatechuic, caffeic, *p*-coumaric, and sinapic. Flavanoids consist of two units: a C6-C3 fragment from cinnamic and a C6 fragment from

malonyl-CoA. The major groups of flavanoids found in sorghum are the flavans (flavan-3-en-3-ols with a double bond between C3 and C4 and hydroxylated at C3 are anthocyanidins. Tannins protect the kernel from attacks from microorganisms, insects, and birds. They are oligomers composed of five to seven flavan-3-ol subunits (catechin) linked through acid labile carbon-carbon bonds and are only present in the condensed form.



Benzoic acids (11-16)

Gallic acid (11): R_1=H, R_2 = R_3 =R_4 = OH

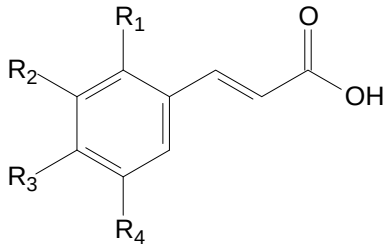
Gentisic acid (12): R_1 = R_4 = OH, R_2 = R_3= H

Salicylic acid (13): R_1=OH, R_2 = R_3 = R_4 = H

p-hydrobenzoic acid (14): R_1 = R_2 = R_4 = H, R_3 = OH

Syringic (15): R_1 = H, R_2 = R_4 = OCH_3, R_3 = OH

Protocatechuic (16): R_1 = R_4 =H, R_2 = R_3 = OH



Cinnamic acids (17-21)

Caffeic acid (17): R_1 = R_4 = H, R_2 = R_3= OH

Ferulic acid (18): R_1 = R_4 = H, R_2 = OCH_3, R_3 = OH

o-coumaric (19): R_1= OH, R_2 = R_3 =R_4 = H

p-coumaric acid (20): R_1 = R_2 =R_4 = H, R_3= OH

Sinapic (21): R_1= H, R_2 =R_4 = OCH_3, R_3= OH

Figure 1.7-Some phenolic acid monomers identified in sorghum (Adapted from Awika and Rooney 2004)

Even though tannins are commonly associated with sorghums, 99% of cultivated sorghum in the U.S. does not contain tannins even though non-tannin phenolic compounds are occasionally reported as tannins. Tannins bind to and reduce digestibility of various food/feed nutrients, thus negatively impacting livestock productivity. The resulting low feed value of tannin sorghums therefore motivated the elimination of tannins from sorghum by crop breeding over the past decades.

Sorghums are classified as type I (without tannins), type II (tannins present in pigmented testa), or type III (tannins present in pigmented testa or pericarp). However, this classification system does not account for varying levels of other major phenolic compounds such as anthocyanins. Therefore, another broad way to classify this grain is by appearance and total extractable phenols (TEP); thus, there are white sorghums (no detectable tannins or anthocyanins and very low TEP levels); red sorghums (no tannins but a red pericarp with significant levels of TEP); black sorghums (a black pericarp and very high levels of anthocyanins); and brown sorghums (pigmented testa and contain significant levels of tannins with varying degrees of pericarp pigmentation) (Awika and Rooney 2004).

Sorghum's phenolic compounds, like all others, have a very strong antioxidant effect. Currently, antioxidant activity is the most common *in vitro* parameter used to assess or predict potential health benefits of plant phytochemical compounds. However, correlations between actual health benefits and *in vitro* antioxidant activity are unknown. Studies that show *in vitro* antioxidant activity often ignore other potentially beneficial or harmful effects of phytochemicals, such as modification of enzyme activity and/or cell signaling pathways (Awika and Rooney 2004). Furthermore, antioxidant activity data tells us nothing about release or uptake of the compounds, as well as their distribution and metabolism within the body. It does provide

information to help identify the plants that have these desirable compounds. Phenol content in sorghum has been shown to be strongly correlated to antioxidant activity when measured by various methods, indicating that phenols are a major source for the activity (Awika 2003). Sorghums high antioxidant activity is comparable to those activities seen in fruits and vegetables (Table 1.3). Tannin sorghums rich in antioxidants have been shown to slow hydrolysis in foods, produce naturally dark-colored products, and increase dietary fiber levels of food products. Epidemiological evidence suggests that sorghum consumption reduces the risk of certain types of cancer in humans compared to other cereals (Awika and Rooney 2004).

Table 1.3-Antioxidant activity of different types of grain sorghum compared to some common fruits (Modified from Awika and Rooney 2004).

Sample	*Oxygen Radical Absorbance Capacity (dry wt)*[a]
White Sorghum	22
Red Sorghum	140
Black Sorghum	220
Sumac Sorghum	870
Sumac Bran	3100
Blueberries	87-870
Plums	452-600

[a] μmol TE/g using fluorescein as a probe

Grain Teas

Due to its relatively low cost and availability, coffee is one of the top beverages consumed around the world today. For centuries though, this was not the case and substitutes were made to replace coffee. These substitutes were commonly made out of plants, seeds, and roots, which like coffee beans contained large amounts of carbohydrates, protein, and sometimes other compounds with physiological activity, but rarely caffeine (Clarke and Macrae 1987). Many of these beverages were made out of cereal grains and eventually took on the name grain teas since they were roasted and then steeped in hot water. This type of beverage contains no parts of the *Camellia sinensis* plant but is rather a type of herbal infusion. One of the most popular forms of an infused grain beverage is barley tea, which is a common beverage found in Japan and Korea. Known for its dark amber color and roasted coffee flavor, this naturally caffeine free beverage has been used for centuries not only as a coffee substitute but also as a medicinal beverage. Limited published data is available on production conditions and flavor characteristics of barley tea but a few reports have been made about the physiological functions this beverage may have. Therefore, understanding this beverage and how it is made will be important for future development of grain infused beverages.

Grain Beverages on the Market

Currently, grain beverages are on the market primarily to serve as coffee substitutes or health beverages (Clarke and Macrae 1987). These products include Caro (a blend of instant cereal and chicory), Pero (a blend of malted barley, chicory and rye) and Inka (a blend of roasted barley, rye, chicory and beet roots). Asian tisanes (grain teas) are primarily found in Asian markets but are slowly moving west and can be found on the internet for purchase and in specialty stores within the U.S. (Ames and others 2006). They include barley tea (mugicha),

brown rice tea with green tea (genmaicha), and roasted brown rice tea (Hyeonmi cha). These products are either instant beverages that dissolve into water and can be served hot or cold (i.e. Pero, Caro and Inka) or in tea bags that are to be steeped in hot or cold water and then consumed. Besides being naturally caffeine free, grain beverages have been recognized throughout history and through a few physiological studies as having positive health affects (Suganuma and others 2001).

Health Effects of Grain Beverages

Known throughout history as being medicinal beverages, grain teas are believed to help the seasonal cold or flu, help inflammatory arthritis and break up congestion and phlegm. However, only a few physiological studies have been done to indicate actual medicinal benefit. These studies have shown barley tea, in particular, to have a protective effect against gastric stress ulcers and reduce blood pressure, which may reduce the risk of circulatory diseases related to lifestyle (Suganuma and others 2002). Barley tea also has antioxidative activity since it contains catechol and is high in soluble fiber, which has been shown to reduce blood cholesterol in humans. Cereal fibers in general have beneficial effects on glucose metabolism (Jenkins and others 1978; Yokoyama and others 1997; Hallfrisch and Behall, 2000), blood lipids (Akerberg and others 1998), and risk of colon cancer (McIntosh 1993) but very little published information is available in how this correlates to their benefits in a steeped beverage. Therefore, there is a strong need to further understand the processing of grain teas and their potential health benefits.

Handling and Processing of Grain Teas

Grain teas, in particular barley tea is an herbal tea that starts with the raw grain and is then typically treated, roasted, milled, and then packaged. Limited published data exists on actual conditions for producing barley tea but Wang and others (1968) and Suh and others (1981) reported that the brownness of barley during roasting was a good indicator for the flavor produced, and the roasting time and temperature had a great effect on the color and yield of barley tea. Roasting temperature is also known as an important factor for coffee roasting, along with grain size and extraction conditions (Kim and Others 1998). Treatments are also used on the grain to obtain optimum flavor from roasting.

Japanese Patent No. 3396/73 claims a process in which raw barley is roasted and impregnated with caramel obtained by treating the grain with glucose or crude sugar prior to roasting. Once optimum roasting is obtained the grain goes through a milling step, which allows the kernel to be cracked or ground. This procedure allows for a greater surface area which results in better results during the steeping of the tea. Once the grain is cracked or ground, it is then put through sieves to obtain the ideal particle size. Lastly, the grain tea goes into packaging, which typically entails individual tea bags being filled, sealed, and then packaged in a bulk package. This method allows for the consumer to prepare individual cups of tea by steeping the tea bag in hot water. However, some varieties of grain teas are also bulk packaged and sold in a loose form, which enables the consumer to brew it in similar ways a loose leaf tea would be brewed. Due to the lack of processing information available and the unique nature individual grains present there is a need for further investigation of processing parameters needed to make grain teas.

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CHAPTER 2 - PRELIMINARY RESEARCH

Introduction

Preliminary experimental work was done with a variety of sorghum hybrids including white, red, and black varieties to determine how milling, roasting and steeping affected final sensory attributes of the sorghum tisane. Prototypes of the sorghum tisane were informally evaluated by a panel of faculty and students at Kansas State University as well as the personnel from the Center for Grain and Animal Health Research (CGAHR) in Manhattan, KS.

A series of milling procedures were tested to determine optimal kernel particle size. Additionally, characterization of different granular screens supplemented the milling procedures to evaluate how particle size affected the end product. Roasting time and temperatures were also evaluated to determine how they affected the color, flavor, and aroma characteristics of the beverage. Lastly, best steeping treatment was determined by testing different steeping times and water temperatures typically used for herbal teas.

Lastly, various herbal flavor combinations were tested to identify flavor combinations that would enhance the overall acceptability of the grain beverage. Six different combinations of fruits and herbs were evaluated. Three were eliminated due to unfavorable flavor characteristics and three were further tested in a consumer study.

Materials and Methods

Minimal published information on processing sorghum for use as a tisane is available. Therefore, preliminary processing was conducted to determine the most reproducible, consistent and efficient methods of milling, roasting, and brewing sorghum. Initial methods of milling the sorghum consisted of cracking the whole kernel using a mortal and pestle to produce an external cracking of the caryopsis but with minimal endosperm release. This method was eliminated due to inconsistency and efficiency reasons. Therefore, further testing was done at the Grain Science and Industry Department at Kansas State University using a Ross Roller Mill (Ferrell-Ross Roll Manufacturing Inc, Hereford, TX) but was also eventually eliminated due to an excessive endosperm release. An Allis Roller Mill (Utah Machine and Mill Supply Inc., Salt Lake City Utah) was then tested, which resulted in optimal caryopsis cracking. The mill was set with 22.9 cm x 15.2 cm rolls, 16 corrugations/inch with a 1.0:1.0 differential and the gap set at 0.1 cm. Different varieties of sorghum were used during the primary experiments, however, due to availability a white sorghum variety was primarily used to develop the milling and roasting procedures.

Particle size showed to be of particular importance for final beverage characteristics. Therefore, preliminary research consisted of passing the sorghum through the Allis Roller Mill for one, three and five, times in order to understand how granulation size effected final beverage characteristics. Granular screen characterization was also conducted to determine particle size distribution. Multiple screen combinations were tested to determine proper particle size for the grain. First characterization consisted of screen sized (in microns) 2540, 2030, 1659, 1358, 1190 and 750. The second test for screen characterization consisted of screen sizes (in microns) 3530, 2920, 2540, 2030, 1358, and 750. Based on distribution of particles a series of four screens were determined to be optimum for sifting (Table 2.1).

Limited published data exists for the actual conditions for producing grain teas, in particular barley tea, but Wang and others (1968) and Suh and others (1981) reported that the brownness of barley during roasting was a good indicator for the flavor produced, and the roasting time and temperature had a great effect on the color and yield of barley tea. Roasting temperature is also known as an important factor for coffee roasting, along with grain size and extraction conditions (Kim and others 1998). Therefore, preliminary roasting procedures followed parameters typically seen in coffee roasting. Initial roasting took place on a hot plate using a stainless steel pan and roasting till a dark brown/black color was formed and a strong pungent roasted aroma developed. Further testing showed convection roasting to be more consistent than roasting over a hotplate, therefore multiple time and temperature combinations were tested. These included, 212°C for 14 min, 232°C for 12 min and 260°C for 10 min. Characteristics of the final brewed beverage were then evaluated.

Steeping times were tested and evaluated using an informal panel of Kansas State University's faculty and students. Sorghum was combined with 236.6 mL (8 fl.oz, 1 cup) of water and steeping times were tested at three, four and five minutes, which are typical steeping times for herbal teas. Optimal steeping time was determined based on the intensity of the flavor, color, and aroma of the beverage.

Informal sensory evaluations, consisting of 10 faculty and students from the Food Science Institute at Kansas State University, were also done on the herbal combinations that were added to the grain to enhance acceptability. All herbal and fruit blends were from Cooks Corner (3 N. New York Rd. #12. Galloway, NJ 08205 USA). Samples were prepared using 12 grams of sorghum and 6 grams of herbal mixture followed by steeping for 4 min in 8 fl. oz of

100°C water. Three mixtures were determined to be favorable over the others and were used in a consumer study for further analysis.

Herbal and fruit combinations included: angel falls mist (strawberry and lemon combined with hibiscus petals, rosehip chips, calendula petals, dried apple pieces, dried sweet orange peel, and natural flavors), blood orange pear (pear pieces, blood orange, dried apple pieces, hibiscus petals, roships and natural flavors), pineapple orange (dried pineapple, natural orange flavor, dried apple pieces, rosehip chips, hibiscus petals, dried orange peel and natural flavor), berry berry (black currants, red currants, raisins, hibiscus, rosehip chips and natural flavors), cranberry apple (hibiscus petals, rosehip chips, dried apple pieces, dried sweet orange peel and natural flavors), and apricot supreme (hibiscus petals, rosehip chips, calendula petals, dried apple pieces, dried sweet orange peel, and natural flavors).

Results and Discussion

Preliminary work proved that cracking the sorghum using the Allis experimental roll stand equipped with a Le Page cut mill was effective. Five passes through the mill set at 0.034 inches provided the most favorable finished product characteristics. Preliminary results showed one and three passes through the mill did not result in optimum final product characteristics. Five passes provided a finished product with the highest intensity of color and flavor when compared to the samples that had one or three passes through the mill. Final granular screen characterization was determined and used for both grain types during primary experiments (Table 2.1). Removing endosperm components that had been released from the milling was necessary to minimize the amount of starch gelatinized during the steeping process. The sieving process also proved beneficial for removing other contaminants not wanted in the grain sample.

Table 2.1-Screen characterization for granulation size of sorghum

Screen[A]	Inches[B]	Micron[C]	% Open[D]
7 SSMW	.028	2920	64.8
8 SSMW	.025	2540	64.0
10 SSMW	.020	2030	64.0
12 SSMW	.018	1659	60.8

[A]Screen type= Stainless Steel Mill Wire
[B] Size of screen openings in inches
[C]Size of screen openings in microns
[D] Percent of open area on the screen

Preliminary roasting procedures showed 212°C for 14 min to be the optimum temperature and time for roasting when compared to 260°C and 232°C due to the color, aroma and flavor produced in the final product, as determined by the sensory panel.

Time and temperature requirements for steeping herbal teas varies depending on the type of infusion but in most cases 212°C water is used with an average of three to five min steep time. Preliminary experiments for the sorghum grain beverage showed a three minute steep time to be insufficient in order to produce the desired flavor, color, and aroma. However, there was not a significant difference seen between the fourth min and fifth minute for color, flavor, and aroma characteristics. Therefore, a four minute steep time was determined to be sufficient for optimum sensory characteristics.

Adding other herbal mixtures to the sorghum beverage proved to enhance all sensory characteristics in the preliminary experiments. Three fruit and herb mixtures were chosen to be acceptable additives for the grain tea. These mixtures were pineapple orange herbal tea blend, angel falls mist tea blend and blood orange pear tea blend. The three combinations that were not selected were either too tart or had undesirable off flavors.

Conclusion and Implications

Preliminary research provided direction for further testing of sorghum tisane blends with fruit and herbal infusions. First, it was found that different milling and sieving procedures affect the sensory characteristics of the tisane. Second, roasting and steeping procedures also showed an effect on the final product along with the addition of herbal mixtures.

Preliminary studies showed potential for developing a tisane that could be acceptable to consumers, however, further consumer testing and development of processing parameters is needed.

CHAPTER 3 - PRIMARY RESEARCH

Introduction

Tea is the second most consumed beverage in the world (Cabrera 2003). Consumers are now looking for new health beverages that offer convenience, good flavor, and positive health benefits. Grain teas (tisanes), established health beverages in the Asian market have been around for years, but have only recently emerged in the North American beverage industry due to the changing demographics. Known for their positive health benefits, grain teas are caffeine-free and have been claimed to offer a range of health benefits such as: fiber, antioxidant activity, protective effects against gastric stress ulcers, and reduction of blood pressure and cholesterol. Due to the lack of published data on manufacturing parameters and flavor characteristics, the objective of this study was to determine appropriate milling, roasting, and steeping procedures and evaluate the chemical and sensory properties of three sorghum tisanes with fruit and herbal combinations.

Materials and Methods

Two different red sorghum hybrids and three different types of fruit and herbal combinations were used for this research. The experiments were either done in duplicate or triplicate.

The two sorghum varieties were sorghum variety SP 217-X 2009 red sorghum (Lane County, KS) provided by the Center for Grain and Animal Health Research (CGAHR) (Manhattan, KS) and MMR Genetics 381/73 2009 red sorghum (Vega, TX), (CGAHR, Manhattan, KS). The three fruit and herb blends were pineapple orange, angel falls mist, and blood orange pear (Cooks Corner, 3 N. New York Rd. #12. Galloway, NJ 08205 USA). German rock cane sugar (Teavana, Kansas City, KS) was also used. For simplicity, abbreviations were used to label the beverages while experiments and evaluations were conducted. The same abbreviations will be used throughout this document. The beverage varieties and abbreviations are as follows:

18 g SP 217- X 2009	**18-217**
18 g MMR Genetics 381/73 2009	**18-381**
50/50 mix of the two sorghums (9 g SP 217-X 2009 and 9 g MMR Genetics 381/73 2009)	**50/50**
Pineapple Orange with 50/50 sorghum mix (6 g pineapple orange with 6 g SP 217-X 2009 and 6 g MMR Genetics 381/73 2009)	**PO**
Angel Falls Mist with 50/50 sorghum mix (6 g Angel Falls Mist with 6 g SP 217-X 2009 and 6 g MMR 381/73 2009)	**AFM**
Blood Orange Pear with 50/50 sorghum mix (6 g Blood Orange Pear with 6 g SP 217-X 2009 and 6 g MMR Genetics 381/73 2009)	**BOP**

Upon delivery of the grain, the CGAHR reported SP 217-X 2009 as containing 10.79 mg/g gallic acid equivalence (GAE) total phenolics (d/w basis) and 268.48 ± 42.78 μM equivalence Trolox/1g for an Oxygen Absorbance Radical Capacity (ORAC) value. MMR Genetics 381/73 2009 was reported to have an ORAC value of 47.6 ± 8.6 μM equivalence Trolox/1g.

Milling

Both red sorghum hybrids (SP 217-X 2009, MMR Genetics 381/73 2009) were milled on the same day at the Department of Grain Science and Industry, Kansas State University using an Allis experimental roll stand equipped with a Le Page cut mill (Utah Machine and Mill Supply Inc, Salt Lake City, UT) (Figure 3.1). The mill was set with 22.9 cm x 15.2 cm rolls, 16 corrugations/inch with a 1.0:1.0 differential and the gap set at 0.1 cm. Each hybrid was stored at 22°C prior to milling in a clear plastic Ziploc® bag. Individually, each hybrid was passed through the Le Page cut Mill five times with the rolls set at 0.1 cm. Samples were collected and immediately sifted through granular screens. Preliminary testing determined optimum screen size characterization. Screens were arranged in an automatic shaker in descending order and samples were individually sifted at 180 RPM-10.2 cm diameter throw for 2 min in the automatic sifter (Table 3.1). Sifted grain from each screen was collected, weighed and then combined and stored in Ziploc® bags. Particles in the pan were thrown away. The two sorghum hybrids (SP 217-X 2009 and MMR Genetics 381/73 2009) showed different granulation sizes when same screen sizes were used for sifting. The differences in the particle size resulted in SP217-X 2009 as being coarser, which may affect phenolic and antioxidant properties of the grain (Figure 3.1).

Table 3.1-Sorghum granulation distribution for two different red sorghum hybrids.

Screen[A]	Micron[B]	Grams[C]	Percent[D]	Cumulative Percent[E]
Sample SP 217-X				
7 SSMW	2920	1.6	0.11	0.11
8 SSMW	2540	101.3	7.5	7.61
10 SSMW	2030	1085.7	80	87.61
12 SSMW	1659	4.5	0.33	87.94
Pan	0	167.4	12.06	100
Sample MMR-381				
7 SSMW	2920	12.6	0.96	0.96
8 SSMW	2540	91.5	7	7.96
10 SSMW	2030	847.1	65.1	73.06
12 SSMW	1659	170	13	86.06
Pan	0	180	13.9	99.96

[A]Screen type: number of openings/inch using stainless steel mill wire.
[B]Screen opening size in microns.
[C]Grams of sorghum that were held over.
[D]Percent of sorghum that was held over.
[E]Cumulative percent of sorghum that was held over.

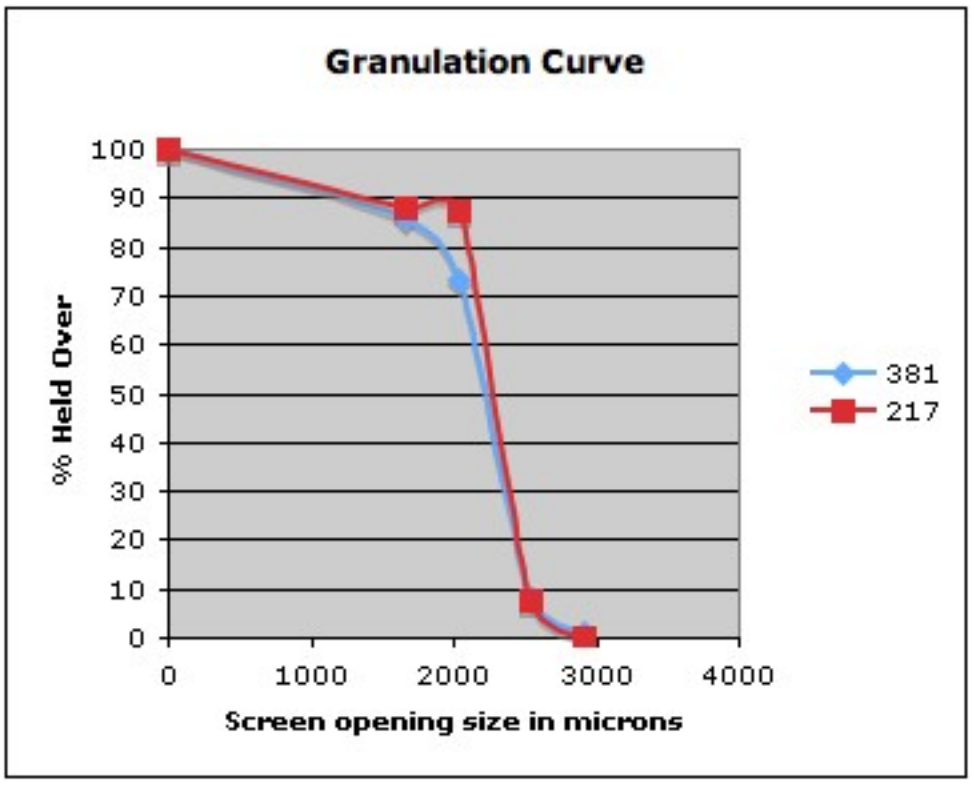

Figure 3.1-Granulation curve for two different red sorghum hybrids.

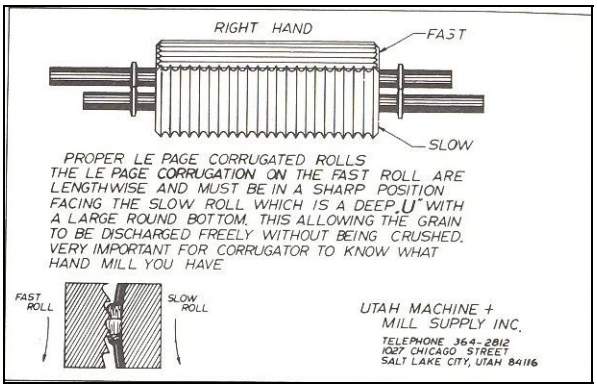

Figure 3.2-Picture of Le Page Corrugated Rolls. (Taken from Associated of Operative Millers Technical Bulletins 1969

Roasting

Both hybrids of sorghum were roasted using two separate Whirlpool convection ovens (Model WFE381LVS, Benton Harbor, MI). Roasting was done simultaneously using 300 gram batches that were evenly distributed on 11' x 16' metal pans. Hybrid SP 217- X 2009 was roasted in the convection oven for 13 minutes at 212° C while the MMR 381/73 2009 hybrid was roasted for 15 min at 212° C in the convection oven. The longer roast time was necessary for MMR 381/73 2009 in order for optimum color and aroma to be developed. Under or over roasting the grain samples will significantly affect the final product characteristics. Both samples were removed promptly and allowed to cool completely on the pans at 21°C. Samples were stored for approximately five days before testing.

Brewing

Samples were prepared by weighing 6 grams of the SP 217- X 2009 hybrid and 6 grams of the MMR 381/73 2009 and combining with 6 grams of the appropriate fruit and herbal mixture. The 18 gram sample was then placed in a Teavana® perfect tea maker (Figure 3.3) (Atlanta, GA). Next, 236.6mL (8 fl.oz., 1 cup) of 100° C distilled water was added, the lid was closed and the mixture was allowed to steep for four min. The infused product was then promptly sieved using the Teavana® perfect teamaker into a glass beaker for analysis and covered with aluminum foil.

Figure 3.3-Illustration of Teavana® Perfect Tea Maker (http://www.tofslie.com/hey/wp-content/uploads/2008/04/teavanatmaker.jpg, last accessed on March 16, 2010).

Physical and Chemical Measurements

pH Measurements

The pH measurements were taken on the beverages using a Fisher Scientific Accumet 25-pH meter (Denver Instrument Company, Denver, CO) with automatic temperature compensation (ATC). A Fisher Universal glass pH electrode was attached for measurements. The pH meter was calibrated before each use with two buffer solutions, pH 4.0 and pH 7.0. 50 mL of each prepared beverage sample was used for the measurements and test was done in duplicates. All measurements were recorded at ambient temperature and when the readings were stable.

Color

The color of the beverage samples was measured at room temperature using a HunterLab MiniScan Ez (Hunter Associates Laboratory Inc., Reston Virginia). The colorimeter used L* which measure lightness (0=black and 100=white), a* which measure red and green hues (+60= red color and -60= green color), and b* which indicates yellow and blue hues (+60=yellow color and -60= blue color) along with an illuminant A/10° (average incandescent light) to determine the color of the beverage surface. The MiniScan Ez was calibrated using standards black and white. Six samples were measured in triplicate (PO, BPO, AFM, 18-217, 18-381, and 50/50). The samples were poured into clear petri dishes, topped with a lid and placed on a white surface for measurement.

Proximate Analysis

Four grain samples were tested; unroasted SP 217-X, MMR Genetics 381/73 and roasted SP 217-X, MMR 381. Samples were ground using a Cyclone Sample Mill (Model 3010-030, UDY Corporation, Fort Collins, CO) with a 1 mm screen size. All samples were tested in duplicate.

Moisture Content

The moisture contents of the grain samples were measured in duplicate using AOAC 930.15 approved method (AOAC 2000). The procedure determines the moisture loss on a dry matter basis when heated under specific conditions. Two grams were placed in an aluminum sample pan and heated in a Precision Thelco, (Model 18, Thermo Fisher Scientific Inc., Waltham, MA) set at 135°C for 2 h. After heating, samples were immediately removed and placed in a desiccator to cool to room temperature. The following formula was used to calculate moisture content.

$$\% \text{ Moisture} = 100\% - \frac{(\text{wt. of sample after oven drying})100}{\text{original wt. of sample}}$$

Crude Protein Content

The protein content of the sorghum grain samples was measured in duplicate using AOAC 990.03 approved method, nitrogen combustion using a LECO FP 2000 instrument (Leco Corporation, St. Joseph, MI) (AOAC 2000). Nitrogen freed by combustion at high temperature in pure oxygen is measured by thermal conductivity detection and converted to equivalent protein by using a 6.25 conversion factor.

Crude Fat Content

The crude fat content of the sorghum samples was measured in duplicate using AOAC 920.39 approved method using a Fat Extractor (Model 1474, Labconco, Kansas City, MO) (AOAC 2000). This method determines crude fat by ether extraction with a subsequent solvent evaporation. Crude fat was reported as a percentage of the original sample weight.

Ash Content

The ash content of the sorghum grain samples was determined in duplicate using AOAC 942.05 approved method (AOAC 2000). Two gram samples were weighed in porcelain crucibles and placed in a 600°C preheated ash furnace (Neytech, model 85A). After 2 hours the samples were transferred directly to a desiccator, cooled and weighed. Ash content was reported as a percentage of the whole sample.

Crude Fiber Content

The crude fiber content was determined in duplicate using the Ankom Method, based on the AOAC 962.09 approved method (AOAC 2000). The Ankom Crude Fiber solvent solubilizes non-fiber components of the grain. The sample is subsequently filtered, rinsed, and dried to determine fiber content. Crude fiber content was calculated using the following formula and reported as a percentage of the original sample weight.

$$CF_{OM}\ (\text{DM basis}) = \frac{(W_4 - (W_1 \times C_2)) \times 100}{W_2 \times DM}$$

Where : W_1 = Bag tare weight
W_2 = Sample weight
W_3 = Weight after extraction process
W_4 = Weight of Organic Matter (OM)(Loss of weight on ignition of bag and fiber residue)
C_2 = Ash corrected bland bag (Loss of weight on ignition of bag/original blank bag)

Total Phenolic Count

Total phenolics were determined by the Folin- Ciocalteu method (Caboni and others 1997). This method is not specific for individual phenolics, but the protocol is good at estimating total phenolic count. Six samples were tested in duplicates, these samples included AFM, PO, BOP, 18-381, 18-217, and 50/50. Samples were prepared following the brewing procedures previously mentioned. 20 µl of the test sample was mixed with 1200 µl H_2O in a microcentrifuge tube. 100 µl Folin-Ciocalteu reagent (Fluka Analytical, Switzerland) was added to the tube and 4 min. later 300µl of sodium carbonate (20% w/v) solution was added to the mixture. Finally, 380µl H_2O was added last to all test samples and the mixtures were allowed to stand for 2 h at 22°C. Test samples were transferred to cuvettes and absorbance was measured at 760nm. Gallic acid (GA) was used for the standard and the results were expressed in ppm of GA equivalent (GAE) per 8 fl. oz (w/v) of beverage. The following equation was used to calculate phenolics based on the gallic acid standard curve.

$$Y = 0.0011x + 0.0161$$

$$R^2 = 0.9537$$

Antioxidant Capacity

Oxygen Radical Absorbance Capacity (ORAC) analysis was performed on six samples (18-217, 18-381, 50/50, PO BOP and AFM) in triplicate at the University of Nebraska Lincoln. Samples were prepared by combining 18 g of the respective sample and brewing it in 8 fl oz. (236.6 g) distilled water for 4 min. The antioxidative capacity was determined by the ORAC method as described by Huang and others (2002). A stock solution of standard was prepared by dissolving 0.01 g of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) in 10 mL of 75 mM potassium phosphate buffer, pH 7.4, with standard dilution concentrations ranged from 0.46–62.50 µg/mL. The fluorescent probe, fluorescent (8.16×10^{-5} mM), was incubated with the standards and samples for 10 min, of which 3 min was with shaking. After incubation, the reaction was activated by adding 153 mM 2, 2'-azobis (2-amidinopropane) hydrochloride, i.e., the radical initiator. All samples/ standards were prepared in 96 well plates and monitored with a BMG Labtech FLUOstar Optima microplate reader (Durham, NC). The fluorescence was measured every 1.5 min at an excitation and emission wavelength of 485 nm and 520 nm, respectively, until the decreasing fluorescence values plateaued. From this data, the area under the curve (AUC) and net AUC were calculated and compared against a standard curve prepared with Trolox.

Sensory Analysis

A total of 97 untrained panelists participated in the sensory evaluation of three brewed sorghum tisanes. These panelists consisted of 53 females and 44 males between the ages of 18-70. Prior to beginning the sensory study, all panelists signed an informed consent statement (Appendix A). Panelists were pre-screened for potential food allergies (Appendix B). The sensory evaluation was conducted at Kansas State University in the Food Science Laboratory at Call Hall. Six of the products developed were evaluated in this study (PO unsweetened/sweetened, AFM unsweetened/sweetened, BOP unsweetened/sweetened). Thirty-seven consumers evaluated the three samples unsweetened and 60 consumers tried the three samples sweetened. Beverage samples were prepared by steeping 48 g of MMR 381/73 2009, 48 grams SP 217-X 2009 and 48 grams of the herbal and fruit blend with 64 fl. oz of boiling water for four minutes. The samples were divided into two, and 40 grams of German rock cane sugar was added to 946.4 ml (32 fl.oz, 1 quart) of each sample. Beverage samples were presented in odorless clear plastic cups at 82° C with a three digit random number code. All samples were presented at one time with no predetermined sampling order, thus eliminating any bias. Distilled water was provided for cleansing the palate before sampling and for use in between each sample. Evaluation was conducted using a 9- point hedonic scale to determine the degree of liking for the beverage products (9=like extremely, 5= neither like nor dislike, 1= dislike extremely). The samples were rated for aroma, appearance, flavor, mouth feel, and overall acceptability (Appendix C).

Statistical Analysis

Data from the Total Phenolic Content (TPC), color and sensory analysis were analyzed using SAS, Software Release 9.1.3 (SAS, Institute Inc., Cary, NC, 2003). When treatment effects were found significantly different for the TPC and color analysis, the least square means with Bonferroni groupings were used to differentiate treatment means. A level of significance was observed at $\alpha < 0.05$. Analysis for the consumer study was performed by doing an Analysis of Variance using Ordinal Logistic Regression to test the significance of the sugar and variety factors on the five response variables. The mean and standard error of the Oxygen Radical Absorbance Capacity, pH and proximate analysis were analyzed using Microsoft Excel (Microsoft, Roselle, IL).

Results and Discussion

pH Measurements

The samples had mean pH measurements between 2.86 – 6.40. This pH range is largly due to the fruit and herb mixtures that were used in three of the samples (Table 3.2). The acidity of the fruit being used decreased the pH significantly compared to the 50/50, 18-217 and 18-381 which were all around a neutral pH. The lower pH's of BOP, PO, and AFM may be contributing to their brighter color along with making the tisanes have more of a tart taste, which could be affecting consumer acceptability.

Table 3.2-Comparison of pH for various sorghum tisanes.

Sample ID[A]	pH ± Standard error[B]
BOP	2.86±0.04
PO	3.16±0.00
AFM	3.21±0.03
18-217	6.31±0.08
18-381	6.32±0.00
50/50	6.41±0.04

[A]18-217= 18g Sp 217-X; 18-381=18g MMR-381; 50/50= 9g Sp 217-X and 9g MMR 381; AFM=6g Sp 217-X, 6g MMR-381 and 6g Angel Falls Mist Herbal Tea; BOP= 6g Sp 217-X, 6g MMR-381 and 6g of Blood Orange Pear Herbal Tea; PO= 6g Sp 217-X, 6g MMR-381 and 6g Pineapple Orange Herbal Tea

[B] Results are expressed as the mean ± std error of duplicate readings

Color

All samples had high L* values indicating they were more white than black. However, the tisanes without the fruit and herbs added to them were lighter in color (Table 3.3). All samples within the L* measurement showed to be significantly different. The samples infused with fruit and herbs had higher red hues as seen by the elevated a* values which measures green to red. All samples were significantly different for the a* value except samples 50/50 and 18-217. All b* values were positive showing that all samples had more yellow hues than blue. The samples with fruit and herbs however, had more yellow in them compared to the samples with just grain. Samples 18-217, 18-381, and 50/50 showed no significant difference between the samples along with AFM and PO showing no significant difference for the b* measurement.

Table 3.3-Comparison of L*a*b* values of various sorghum tisanes

Treatment [A]	L*[B]	a*[C]	b*[D]
18-217	56.72±0.45[c]	6.22±0.13[d]	11.19±0.19[c]
18-381	59.38±0.27[a]	2.50±0.22[e]	8.98±0.64[c]
50/50	57.80±0.23[b]	4.95±0.33[d]	11.26±0.38[c]
AFM	43.55±0.17[e]	31.75±0.92[b]	25.68±1.12[b]
BOP	34.66±0.51[f]	40.40±1.37[a]	38.84±4.27[a]
PO	46.09±0.31[d]	27.21±0.74[c]	22.50±0.99[b]

[A] 18-217= 18g Sp 217-X; 18-381=18g MMR-381; 50/50= 9g Sp 217-X and 9g MMR 381; AFM=6g Sp 217-X, 6g MMR-381 and 6g Angel Falls Mist Herbal Tea; BOP=6g Sp 217-X, 6g MMR-381 and 6g of Blood Orange Pear Herbal Tea; PO= 6g Sp217-X, 6g MMR-381 and 6g Pineapple Orange Herbal Tea

[B] L*= Black to white;

[C] a*=green to red;

[D] b*=blue to yellow

[abcdef] Means with different superscripts within a column are significantly different ($P<0.05$).

Proximate Analysis

The moisture, ash, fat, protein and fiber results observed from our unroasted samples where typical compared to the ranges seen in other sorghum samples (Waniska and Rooney 2000). In our study, approximately 9% moisture loss from roasting was observed for the two samples (Table 3.4). Crude protein, fat, fiber, and ash were not considerably affected by the roasting treatment. Little published data is available on roasting grains for use as a herbal infusion, however the procedures and moisture loss from roasting coffee beans is similar and can be used as a standard for sorghum roasting. Green coffee beans have an average moisture content of 12% before roasting and an average of 1-3% post roasting depending on the roasting procedure (Baggenstoss and other 2008). Furthermore, since moisture content is correlated to

quality of the coffee bean and final product characteristics, it can be assumed that moisture in sorghum will also have a considerable role in the final product characteristics of the tisane. This is also supported by the preliminary work that was done with different roasting time and temperatures.

Table 3.4-Mean and standard errors of proximate analysis results for various sorghum grain samples.

Sample ID[A]	% Moisture[B]	% Crude Protein[B]	% Crude Fat[B]	% Crude Fiber[B]	% Ash[B]
SP 217-X Roasted	1.35±0.01	9.23±0.03	2.90±0.04	1.81±0.00	1.17±0.05
SP 217-X Unroasted	10.59±0.01	9.50±0.00	3.23±0.07	1.96±0.01	1.24±0.00
MMR -381 Roasted	1.33±0.04	12.85±0.05	2.72±0.03	1.97±0.01	1.22±0.00
MMR -381 Unroasted	10.19±0.06	12.88±0.05	2.85±0.01	1.93±0.04	1.25±0.04

[A] Sp 217-X roasted: sorghum that has been milled, sifted and roasted at 212°C for 13 min.; Sp 217-X unroasted: raw sorghum that has been milled and sifted; MMR-381 roasted: raw sorghum that has been milled, sifted and roasted at 212°C for 15 min; MMR-381 unroasted: raw sorghum that has been milled and sifted.
[B] Means ± standard errors

Total Phenolic Count

Results for Total Phenolic Content (TPC) showed significant differences between all of the samples except between 18-217 and AFM, 18-217 and PO, and PO and 50/50 (Table 3.5). Sample 18-217 showed the highest TPC at approximately 55 mg gallic acid equivalent (GAE) compared to the 50/50 at 38.5 and 18-381 at 19.4. When roasted grain was compared to an unroasted sample for 18-217, a 72% loss was incurred for TPC (table 3.6). This comparison

shows how roasting may be affecting the phenolic content. The loss could be due to the heat from the roasting destroying some of the phenolic acids or from the bound and free forms that phenolic acids are found in. While no studies were found on this particular situation, one study was done that analyzed the effects of cooking, pH, and polyphenol level on carbohydrate and nutritional quality of sorghum (Sorghum bicolor (L.) Moench) (Knudsen and others 1988). The study demonstrated how acidification and cooking liberated polyphenols from their matrix, which were then allowed to react with glycine and proline residues in dietary proteins, thus resulting in significantly lower values for catechin equivalents and tannins in the acidified and cooked foods (Knudsen and others 1988). While this study was not conducted on sorghum in a beverage, it can help explain the lower TPC values of the roasted grain.

Samples infused with the fruit and herbal mixture showed enhanced TPC values due to the additional phenols found in the fruit and herbs in the mixture. BOP had the highest level at approximately 92 mg GAE/8 fl oz with AFM following at approximately 62 mg GAE/ 8 fl oz.(Table 9). These results show that BOP has approximately the same amount of phenols (mg/100mL) as oolong tea and about half has many phenols as black tea (Fukushima and Others 2009) Gaining more interest in the food industry, crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolics have the ability to retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food (Ozcan and Others 2009).

In addition, some red sorghum varieties have been shown to have higher antioxidant activities than the most important natural antioxidants, such as blackberries (Awika and others 2004, Dicko and others 2005). Furthermore, a positive correlation has been found between TPC and antioxidant activity in beverages and extracts from fruits and vegetables (Fukushima and

others 2009). Genes for plant color, pigmented testas, spreader genes, and pericarp thickness contribute to the high phenolic counts seen in some varieties of sorghum (Dykes and others 2005). Therefore, the darker pericarp color seen for sample 18-217 could explain its higher TPC compared to the lighter color of pericarp seen in 18-381. With up to 6% (w/w) of phenolic compounds, sorghum has the highest content compared to other cereals (Deshpande and others, 1986, Beta and others, 1999, Awika and Rooney 2004). Phenolic acids found in sorghum are divided in two categories: hydroxybenzoates and hydroxycinnamates. They can be found in free or bound form and are typically concentrated in the outer layers of the sorghum kernel. The most prevalent phenolic acids found in sorghum are ferulic acid and *p*-coumaric acid, however, gallic protocatechuic, p-hydroxybenzoic, vanillic, caffeic and cinnamic have also been identified in red sorghum varieties (Waniska 2000, Awika and others 2004). The TPC analysis, however, only accounts for total phenolics present in the sample, therefore further testing would need to be done on the samples to characterize the individual phenolic acids.

Table 3.5-Comparison of total phenolic content for various sorghum tisanes.

Sample ID [A]	Mg GAE/8fl. oz [B]
18-217	**54.97±11.24^{bc}**
18-38	**19.40±4.01^{e}**
50/50	**38.5±6.91^{d}**
AFM	**65.21±4.18^{b}**
BOP	**91.91±4.02^{a}**
PO	**42.24±2.25^{cd}**

[A]18-217= 18g Sp 217-X; 18-381=18g MMR-381; 50/50= 9g Sp 217-X and 9g MMR 381; AFM=6g Sp 217-X, 6g MMR-381 and 6g Angel Falls Mist Herbal Tea; BOP=6g Sp 217-X, 6g MMR-381 and 6g of Blood Orange Pear Herbal Tea; PO= 6g Sp 217-X, 6g MMR-381 and 6g Pineapple Orange Herbal Tea

[B]Results expressed in mean ± standard error mg gallic acid equivalence/ 8fl oz. of duplicate analysis.

[abcde]Means with different superscripts within a column are significantly different ($p<0.05$).

Table 3.6-Means of roasted and unroasted sorghum and the effects on total phenolic content

SAMPLE 18-217[A]	*PHENOLICS MG/1G*[B]
Raw Grain	**10.8**
Roasted Grain	**3.0**
% loss	**72.2**

[A]Raw grain= Sp 217-X; Roasted grain= Sp 217-X roasted for 13 min at 415°F; % loss= 100-roasted grain phenolics/raw grain phenolics

[B]Total Phenolic Content expressed as the mean Gallic Acid Equivalence mg/1g

Oxygen Radical Absorbance Capacity (ORAC)

Sample 18-217 showed the highest ORAC value at 483μM Trolox/8 fl. Oz compared to 18-381 which resulted in 284 μM Trolox/236.6 mL (8 fl.oz, 1 cup). Comparing the 50/50 sample to AFM, BOP and PO, it is evident that the fruit and herbal mixture enhances the ORAC values for BOP by approximately 339 μM Trolox/236.6 mL (8 fl.oz, 1 cup) while only increasing the values for PO by 81μM Trolox/236.6 mL (8 fl.oz, 1 cup) and lastly showing that AFM does not significantly impact the ORAC value at all (Table 3.7). Roasting the grain samples showed to decrease the ORAC value by 90%, which is a very significant loss (Table 3.8). However, this loss may be correlated to the high temperature from the roasting degrading the phenolic acids or allowing the free and bound forms of the acids to interact with proteins. Processing, especially cooking of food, is a factor that has been shown to impact antioxidant capacity (Papas 1996). In general, cooking is regarded as being destructive to antioxidant compounds (Krishnaswamy and Raghuramula 1998).

Samples expressed in μM Trolox/236.6 mL (8 fl.oz, 1 cup) are important in order to understand the antioxidant activity in which each sample contributes. An antioxidant is a compound that protects biological systems against the potentially harmful effects of processes or reactions that can cause excessive oxidation (Ozcan and others 2009). The antioxidant activity of phenolic compounds is mainly due to their redox properties which can absorb and neutralize free radicals or decompose peroxides. While many studies have been done on antioxidant activity the correlation between *in vitro* antioxidant activity and actual health benefits are largely unknown. Antioxidant activity does provide use information for screening plant materials with desirable compounds, but *in vitro* studies tell us nothing about the release, uptake, distribution or metabolism of the antioxidants in the human body. However in sorghum, phenol content has

been shown to correlate strongly with antioxidant activity (Awika 2003a, Awika and others 2003b). Furthermore, epidemiological studies suggest that the consumption of cereal grains, including sorghum can reduce the mortality rate from cardiovascular disease, which is thought to be linked to its antioxidant properties (Awika and Rooney 2004).

Table 3.7-Mean and standard errors of oxygen radical absorbance capacity values for various sorghum tisanes

Sample ID[A]	**μM Trolox**/236.6 mL (8 fl.oz, 1 cup) [B]
AFM	**431.30 ± 21.33**
BOP	**772.62 ± 18.96**
PO	**514.29 ± 23.70**
50/50	**433.70 ± 7.11**
18-217	**483.50 ± 14.22**
18-381	**284.40 ± 16.59**

[A]18-217= 18g Sp 217-X; 18-381=18g MMR-381; 50/50= 9g Sp 217-X and 9g MMR 381; AFM=6g Sp 217-X, 6g MMR-381 and 6g Angel Falls Mist Herbal Tea; BOP=6g Sp 217-X, 6g MMR-381 and 6g of Blood Orange Pear Herbal Tea; PO= 6g Sp 217-X, 6g MMR-381 and 6g Pineapple Orange Herbal Tea

[B] Results expressed in mean ± standard error μM Trolox equivalence/ 8fl oz. of triplicate analysis.

Table 3.8-Means of roasted and unroasted sorghum and the effects on the ORAC value.

Sample SP 217-X[A]	ORAC TE μM/1g[B]
Raw Grain	**268.5**
Roasted Grain	**26.8**
% loss	**90.1**

[A]Raw grain= Sp 217-X 2009; Roasted grain= Sp 217-X 2009 roasted for 13 min at 212°C; % loss= 100-roasted grain ORAC/raw grain ORAC

[B]Oxygen Radical Absorbance Capacity expressed as the mean Trolox equivalence μM/1g

Sensory Analysis

The analysis of the data from the consumer study indicated how each attribute was impacted by the variety of tea and the sugar level. Sugar significantly affected the overall acceptability, flavor, appearance and mouthfeel while the variety impacted the flavor, aroma and overall acceptability ($P < 0.05$). The addition of sugar enhanced the overall acceptability for all varieties.

Aroma

Within the aroma attribute, AFM was found to be significantly different from BOP with a higher liking score ($P < 0.05$) (Table 3.9). There was no significant difference seen between the AFM and PO but there was a significant difference seen between BOP and PO, with PO having a higher score.

Appearance

There was no significant difference seen between the samples as a function of variety or sugar level ($P < 0.05$). From the mean and standard errors it is obvious that all samples had relatively the same likeability for the appearance attribute (Table 3.9).

Flavor

The flavor attribute showed significant differences due to both the variety of tea and the sugar level ($P < 0.05$). AFM was significantly different from BOP and PO due to its higher likeability. However, PO was significantly different from BOP thus having a higher likeability for flavor ($P < 0.05$) (Table 3.9). There was no interaction seen for the sugar, thus, regardless of the sugar level AFM was liked more for the flavor attribute.

Mouthfeel

The mouthfeel attribute was significantly affected by sugar but was not impacted due to the variety of the tea. Sugar showed to enhance all the scores regardless of the variety, thus making the beverage more acceptable in mouthfeel when sugar was present.

Acceptability

The variety of the tea along with the sugar level significantly affected the acceptability attribute ($P < 0.05$). AFM was significantly different from BOP and PO due to its higher likeability score. PO was significantly different from BOP ($P < 0.05$), resulting in a higher overall acceptability. There was no interaction seen with sugar, resulting in higher scores and increased acceptability for all varieties of tea (Table 3.9). AFM had the highest overall acceptability compared to other varieties regardless of sugar level.

Table 3.9-Mean and standard errors of five sensory attributes for sorghum tisanes from three varieties with and without the addition of sugar.

Treatment ID[A]	Aroma[B]	Appearance[B]	Flavor[B]	Mouthfeel[B]	Overall Acceptability[B]
PO w/sugar	6.13±1.64	6.52±1.47	6.72±1.57	6.63±1.65	6.72±1.54
BOP w/sugar	5.07±1.76	6.72±1.62	5.66±1.96	6.14±1.79	5.93±1.96
AFM w/sugar	5.64±1.84	6.58±1.52	6.68±1.52	6.76±1.34	6.63±1.40
PO w/o sugar	6.47±1.70	6.66±1.53	5.24±2.21	5.61±1.81	5.79±2.03
BOP w/o sugar	4.95±1.70	6.77±1.55	4.26±2.01	5.36±1.75	4.79±1.81
AFM w/o sugar	6.10±1.74	6.51±1.35	5.31±1.87	5.72±1.82	5.72±1.76

[a] PO w/sugar= 6g Sp-217x, 6g MMR-381, 6g Pineapple Orange Herbal Tea and 40g German rock cane sugar/946.4 ml (32 fl.oz, 1 quart) of tea; AFM w/sugar=6g Sp-217x, 6g MMR-38, 6g Angel Falls Mist Herbal Tea and 40g German rock cane sugar/946.4 ml (32 fl.oz, 1 quart) of tea; BOP w/sugar=6g Sp-217x, 6g MMR-381, 6g of Blood Orange Pear Herbal Tea and 40g German rock cane sugar/ 946.4 ml (32 fl.oz, 1 quart) of tea; PO w/o sugar=6g Sp-217x, 6g MMR-381 and 6g Pineapple Orange Herbal Tea; AFM w/o sugar=6g Sp-217x, 6g MMR-38, and 6g Angel Falls Mist Herbal Tea; BOP w/o sugar=6g Sp-217x, 6g MMR-381, and 6g of Blood Orange Pear Herbal Tea.

[B] Results expressed in Mean ± standard error

An average of a 7 for overall acceptability is typically used by many food companies in order for a product to enter into the market. Similar to the sorghum tisane, green tea's health benefits have been know about for years but the diffusion of the beverage into the U.S. was not overly popular with consumers due to its bitter taste. However, with new research available its health benefits, the tea has been given considerable attention which has improved consumers overall acceptability of the product (Lee and Chambers 2010). Further sensory work characterizing desirable and non desirable characteristics of the sorghum tisanes and then

altering the beverages to the consumers preference would likely increase the acceptability scores and make it a suitable product for the market.

Likelihood of buying

To assess the likelihood of purchase by the consumer, individuals were asked to rate their purchase intent (not likely, likely, very likely) of the products. The response is in relation to all varieties tried. Among the 57 consumers who tried the varieties with sugar, 24% said they were not likely to buy this product, 39% said they were likely to buy it and 37% said they were very likely to buy it. Thirty-nine consumer tried the varieties unsweetened, of which, 23% said they would not likely buy the product, 56% said they would likely buy the product and 21% said they were very likely to buy the product. With 76% of the consumers who participated in this study saying they were likely or very likely to purchase this product, it can be assumed that the three sorghum tisanes have high market potential.

Conclusion

Overall, this research demonstrated that it is feasible to use sorghum to make an herbal infusion. Milling and roasting tests showed that granulation size and degree of roasting affect final product characteristics and may be contributing to phenol and antioxidant quantities. The total phenolic test showed the darker pigmented sorghum (SP 217-X) to contain a high amount of phenolics compared to the lighter pigmented hybrid (MMR 381). The Oxygen Radical Absorbance Capacity assay and the Total Phenolic Content assay both showed quantifiable amounts of antioxidants and phenols, with SP 217 x having higher amounts. Furthermore, it was confirmed that by adding fruit and herbal infusions to the grain, the phenol and antioxidant quantities increased while also increasing consumer acceptability. The consumer test additionally showed that the sugar addition improved sensory characteristics and overall consumer acceptability. These results can assist in the product development process for advancing sorghum use in the market place and potential use has a herbal infusion.

RECOMMENDED FUTURE WORK

With minimal previous studies done on grain tisanes, future work should include performing more analytical tests on the chemical and physical properties of the grain to understand how roasting and milling are affecting the total phenolic content. Further sensory testing is needed to further capture the flavor characteristics of the herbal infusion so changes can be made to obtain a 7 overall acceptability score. Furthermore, additional research is needed to determine why various hybrids of sorghum perform so differently in an herbal infusion when processing procedures are kept the same. Further analysis is needed to determine what phenolic acids are present in the sample and if their bound or free form is affecting phenolic and antioxidant content. Lastly, understanding the potential health benefits of grain teas and how those processing parameters affect those properties is essential in order to produce the best quality products.

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Appendix A - INFORMED CONSENT STATEMENT FOR CONSUMER SENSORY ANALYSIS OF A BREWED GRAIN AND FRUIT TEA

The purpose of this project is to determine consumer preference of a brewed grain and fruit tea. Testing is expected to take less than 5 minutes. All ingredients in these products are food grade and approved by FDA. If you have no food allergies, there are no known risks or discomforts associated with consumption of these products. Your data will be treated as research data and will in no way be associated with you other than for identification purposes, thereby assuring confidentiality of your performance and responses.

1. I (print name)_____, agree to participate as a panelist in a sensory consumer testing conducted by Dr. Fadi Aramouni.

2. I understand that this study is part of a research project.

3. I understand that there will be a free ice cream certificate upon completion of the testing session.

4. I understand that I do not have to participate in this research and there will be no penalty if I choose not to participate.

5. I understand that I may withdraw from the research at any time.

6. If I have any questions concerning this study, I understand that I can contact Dr. Fadi Aramouni at 216 Call Hall (785-532-1668).

7. If I have any questions about my rights as a panelist or about the manner in which the study is conducted, I may contact the Committee on Research Involving Human Subjects, 103 Fairchild Hall, Kansas State University, Manhattan, KS 66506 (785-532-6195).

8. If you have any food allergies, you cannot participate in this study. Thank you for your willingness to help.

SIGNATURE:_____ DATE:_____

Appendix B - CONSUMER SCREENING FORM FOR A BREWED GRAIN AND FRUIT TEA

Please complete the information below:

Age:

□ 18-25 □ 26-30 □ 31-35 □ 36-40 □ 41-45 □ 46-50

□ 51-55 □ 56-60 □ 61-70 □ 71-80 □ 81-90 □ Over 90

Gender:

□ Male □ Female

Education Completed:

□ High School □ Some College □ B.S. □ M.S.

□ Ph.D. □ MD □ Other

The products you just tested are good sources of antioxidants and possess other health benefits, knowing this, how likely would you be to purchase one of these products?

□ Not Likely □ Likely □ Very Likely

Appendix C - CONSUMER BALLOT FOR A BREWED GRAIN AND FRUIT TEA

Instructions: You will be testing three samples of a brewed grain and fruit tea. Samples are presented in the order to be tasted. Make sure to use the ballot with the sample number that matches the number by the sample. Please be sure to answer the questions completely and honestly. Check the box that best describes your answer. Take a drink of water before you start and between samples.

SAMPLE: 294

Please check only one box that represents your response (X)

1. How much do you like or dislike the aroma of this sample?

Dislike Extremely				Neither Like nor Dislike				Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

2. How much do you like or dislike the appearance (color and clarity) of this sample?

Dislike Extremely				Neither Like nor Dislike				Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

3. How much do you like or dislike the flavor of this sample?

Dislike Extremely				Neither Like nor Dislike				Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

4. How much do you like or dislike the mouth feel?

Dislike Extremely				Neither Like nor Dislike				Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

5. Please rate your overall acceptability of this sample

Dislike Extremely				Neither Like nor Dislike				Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

Additional Comments:_____