LEANDRO DE MORAIS CARDOSO

SORGHUM: VARIABILITY OF NUTRIENTS AND BIOACTIVE COMPOUNDS AND THEIR HEAT PROCESSING STABILITY

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ciência da Nutrição, para obtenção do título de *Doctor Scientiae*.

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Dedico *aos meus pais Maria e Antônio Paulo, às minhas irmãs Graciliane, Simone e Paloma e à minha namorada Soraia.*

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BIOGRAFIA

Leandro de Morais Cardoso, filho de Antônio Paulo de Morais Cardoso e Maria Emília Cardoso, nasceu no município de Curvelo, estado de Minas Gerais, em 21 de agosto de 1984.

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Pleiteou o título de *Doctor Scientiae* em novembro de 2014.

“Feliz aquele que transfere o que sabe e aprende o que ensina.”

Cora Coralina

RESUMO

CARDOSO, Leandro de Morais, D.Sc., Universidade Federal de Viçosa, novembro de 2014. **Sorghum: variability of nutrients and bioactive compounds and their heat processing stability.** Orientadora: Helena Maria Pinheiro Sant'Ana. Coorientadoras: Hércia Stampini Duarte Martino e Rita de Cássia Gonçalves Alfenas.

O sorgo é o quinto cereal mais produzido no mundo e considerado uma fonte de nutrientes e de compostos bioativos, especialmente 3-deoxiantocianidinas, taninos, vitamina E, policosanóis e outros compostos antioxidantes, que modulam beneficamente parâmetros relacionados com doenças não transmissíveis. Antes da utilização para consumo humano, o sorgo precisa ser processado, o que pode alterar os seus nutrientes e compostos bioativos. Este estudo teve como objetivo avaliar a variabilidade de nutrientes e compostos bioativos em sorgo (*Sorghum bicolor* L.) e a estabilidade deles à extrusão e processamento em forno convencional, utilizando calor seco. Cem genótipos de sorgo foram selecionados a partir de uma coleção com alta variabilidade genética da Embrapa Milho e Sorgo (Sete Lagoas, MG, Brasil) e analisados quanto aos teores de carotenoides e vitamina E (tococromanois) por cromatografia líquida de alta eficiência (CLAE) com detector de arranjo de diodos (DAD). A variabilidade genética de carotenoides e tococromanois em 100 genótipos de sorgo foi avaliada pela técnica de agrupamento de Tocher. Os cem genótipos de sorgo apresentaram alta variabilidade nos teores de tococromanois (280,7-2.962,4 µg/100g, base úmida), sendo que 23% dos genótipos foram classificados como fonte de vitamina E. Os carotenoides totais variaram entre 2,12 e 85,46 µg/100g nos cem genótipos de sorgo. De acordo com a variabilidade genética para carotenoides e tococromanois, os 100 genótipos foram divididos em sete grupos geneticamente distintos entre si. A partir de 100 genótipos de sorgo, três foram selecionados (SC319; B.DLO357 e SC391) e submetidos a três tipos de tratamento: F1) Farinha crua: grãos moídos em um moinho analítico micro-rotor (850 µm); F2) Calor seco em forno convencional/moagem (Forno/moagem): grãos inteiros submetidos ao calor seco em forno convencional (CSFC) (121 °C, 25 min) e, subsequentemente, moídos em um moinho analítico micro-rotor (850 µm); F3)

Extrusão/moagem: grãos extrudados em uma extrusora com parafuso de dupla rosca. Os grãos dos três genótipos processados foram caracterizados quanto ao teor de carotenoides, 3-deoxiantocianidinas, flavonas e flavanonas, determinados por CLAE-DAD; tococromanois e grau proantocianidinas, analisados por CLAE com detecção por fluorescência; e fenólicos totais e atividade antioxidante, determinados por espectrofotometria. As diferenças entre os efeitos dos diferentes processamentos foram avaliadas pela ANOVA, seguida do teste de Duncan (α = 5%). A retenção do total de tococromanois e equivalente de α-tocoferol diminuiu após a extrusão (69,1-84,8% e 52,4-85,0%, respectivamente), mas aumentou após processamento por CSFC (106,8-114,7% e 109,9-115,8%, respectivamente). A retenção dos carotenoides do sorgo diminuiu após a extrusão (30,7-37,1%) e CSFC (58,6-79,2%). Os teores de flavanonas e flavonas diminuíram após a extrusão (100%) e CSFC (31,7-61,6%). As 3-DXAS foram estáveis após CSFC, mas foram susceptíveis à extrusão (70,7-93,9%). As proantocianidinas foram identificadas apenas no genótipo SC391 e reduziram em após ambos os processamentos (CSFC: 39,2% e extrusão: 52,1%). Os fenóis totais diminuíram no genótipo SC319 submetido ao CSFC (8,3%) e em todos os genótipos extrudados (13,6-14,9%). O CSFC aumentou a capacidade antioxidante de todos os genótipos, enquanto que a extrusão reduziu a capacidade antioxidante de dois genótipos. Em conclusão, o perfil de tococromanois e carotenoides apresentaram ampla variação e os genótipos apresentaram alta variabilidade genética para carotenoides e tococromanois. O sorgo apresentou-se como uma fonte de tococromanois, que aumentaram após o CSFC e diminuíram após a extrusão. O teor de carotenoides em sorgo diminuiu após o CSFC e a extrusão. A estabilidade diferencial dos principais flavonoides em sorgo foi observada após o CSFC e a extrusão, o que implica que diferentes técnicas de processamento podem ser selecionadas para minimizar a perda de polifenóis bioativos em sorgo, dependendo da composição de flavonoides.

ABSTRACT

CARDOSO, Leandro de Morais. D.Sc. Universidade Federal de Viçosa, November de 2014. **Sorghum: variability of nutrients and bioactive compounds and their heat processing stability**. Adviser: Helena Maria Pinheiro Sant'Ana. Coadvisers: Hércia Stampini Duarte Martino and Rita de Cássia Gonçalves Alfenas.

Sorghum is the fifth most produced cereal in the world. This cereal is a source of nutrients and bioactive compounds, especially 3-deoxyanthocyanidins, tannins, and polycosanols, which beneficially modulate parameters related to non-communicable diseases. Sorghum needs to be processed prior to use for human consumption, which may change its antioxidant compounds. This study aimed to evaluate the variability of nutrients and bioactive compounds in sorghum (*Sorghum bicolor* L.) and their stability to extrusion and dry heat in a conventional oven. One hundred sorghum genotypes were selected from a core collection with high genetic variability from Embrapa Maize and Sorghum (Sete Lagoas, MG, Brazil) and the content of carotenoid and vitamin E were analyzed by high performance liquid chromatography (HPLC) with diode array detector. The genetic variability of carotenoids and tocochromanols in 100 sorghum genotypes were assessed by Tocher's clustering technique. The one hundred sorghum genotypes showed high variability in tocochromanol content (280.7-2,962.4 µg/100g in wet basis) and 23% of the genotypes were classified as source of vitamin E. Also, the total carotenoid content varied from 2.12 to 85.46 µg/100g in the one hundred sorghum genotypes. According to the genetic variability for carotenoids and tocochromanols, the 100 genotypes were divided into seven groups genetically distinct from each other. From 100 genotypes, three sorghum genotypes were selected (genotype SC319; genotype B.DLO357 and genotype SC391) and submitted to three types of treatment: *F1) Raw flour*: grains ground in a micro-rotor analytical mill (850 µm); *F2) Dry heat in a conventional oven/milling (Oven/ milling)*: whole grains subjected to dry heat in a conventional oven (DHCO) (121 °C, 25 min) and subsequently, ground in a micro-rotor analytical mill (850 µm); *F3) Extrusion/milling*: grains extruded in a co-rotating twin-screw. The grains of the three processed genotypes were characterized according to the content

of carotenoids, 3-deoxianthocyanidins, flavones and flavanones, that were determined by HPLC with diode array detector; the vitamin E content and the degree of polymerization of proanthocyanidins, that were analyzed by HPLC with fluorescence detection; and the total phenolics compounds and the antioxidant activity, that were determined by spectrophotometry. Data normality on the stability of antioxidant compounds was assessed using the Shapiro-Wilk test and the differences between treatments were evaluated by ANOVA, followed by Duncan test to compare the treatment averages, at 5% probability. The retention of the total tocochromanols and α-tocopherol equivalent decreased after extrusion (69.1-84.8% and 52.4-85.0%, respectively) but increased after DHCO (106.8-114.7% and 109.9-115.8%, respectively). Sorghum carotenoids were sensitive to extrusion (30.7-37.1%) and DHCO (58.6-79.2%). The content of flavanones and flavones decreased after extrusion (100%) and DHCO (31.7 to 61.6%). The 3-DXAs were stable in DHCO, but were susceptible to extrusion (70.7 to 93.9%). Proanthocyanidins were identified only in the genotype SC391 and reduced after both treatments (DHCO: 39.2% and extrusion: 52.1%). Phenols decreased in the genotype SC319 submitted to DHCO (8.3%) and in all extruded genotypes (13.6-14.9%). The DHCO increased the antioxidant capacity in all genotypes, whereas extrusion reduced the antioxidant capacity in only two genotypes. In conclusion, the tocochromanols profile in sorghum varied widely and the genotypes presented high genetic variability for carotenoids and tocochromanols. Sorghum was a source of tocochromanols, which increased after DHCO and decreased after extrusion. The carotenoid content in sorghum decreased after DHCO and extrusion. The differential stability of the major flavonoids in sorghum was observed under DHCO and extrusion treatments, implying that different processing techniques can be selected to minimize losses of bioactive polyphenols in sorghum depending on the flavonoid composition.

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1. BACKGROUND

Sorghum is one of the most resistant crops to drought and due to the increase in world population and reduction of water availability, it is an important crop for future use (TAYLOR et al., 2006). This cereal is the fifth most produced in the world and provides up to 70% of the calories ingested by individuals from countries in Africa, Asia and Central America. In the United States, South America and Australia, sorghum grain is used primarily for livestock feed and in a growing number of ethanol plants. Sorghum has also recently appeared in gluten-free food products in the U.S. (CIACCI, et al., 2007), such as tortillas and breakfast cereals.

In Brazil, sorghum is mainly used for animal feed (FOOD AND AGRICULTURAL ORGANIZATION, 2010). The Brazil is the 7th largest producer of sorghum (FOOD AND AGRICULTURAL ORGANIZATION, 2012), but it has few commercial cultivars of sorghum for human consumption. The Embrapa Maize and Sorghum is a major contributer to selecting sorghum genotypes that have high nutritional and functional value and that are appropriate for the climate and soil conditions in Brazil, as well as those suitable for development of products intended for human consumption. In partnership with Embrapa Tropical Agroindustry, Embrapa Food Technology, Embrapa Temperate Climate, Universidade Federal de Minas Gerais, Universidade Federal de São João Del Rei and Universidade Federal de Viçosa, the Embrapa Maize and Sorghum has developed the research project entitled "Sorghum for human consumption: characterization of genotypes in the compounds of interest for nutrition and human health, along with development of gluten-free products". One of the lines of this project includes the characterization of sorghum genotypes with regards to their functional potential and evaluation of retention in products developed with sorghum, which encompasses the focus of this thesis.

Sorghum has nutritional value and chemical composition similar to wheat and corn. Some cultivars have a high content of bioactive compounds (3-deoxiantocianidinas, tannins and others) and minerals (iron, zinc and others). The chemical and nutritional composition and functional values are determined by the sorghum genetic and environmental factors (KAYODÉ et

al., 2005) and can present a wide variability (CARDOSO ET AL., 2014; MARTINO et al., 2012; PINHEIRO-SANT'ANA, GUINAZI, OLIVEIRA, DELLA LUCIA, REIS & BRANDÃO, 2011). However, the variability of nutrients and bioactive compound, for example carotenoids and vitamin E, has not been evaluated in representative sorghum panels.

Sorghum must be processed prior to use for human consumption, which may change its bioactive compound profile (DLAMINI et al., 2007; OCHANDA et al., 2010; AFIFY et al., 2012; CARDOSO et al., 2014). Most of the studies published to date showed chemical changes resulting from processes traditionally used in African and Asian countries (fermentation, germination and maceration) (DUODU et al., 2002; DICKO et al., 2005; DICKO, GRUPPEN, ZOUZOUHO, et al., 2006; AFIFY et al., 2011; AFIFY, EL-BELTAGI, EL-SALAM, et al., 2012; JOOD et al., 2012; WU et al., in press). The effects of processing reflect the culture of the population and the needs of Western industries have been little studied. The main processes include dry heat in a conventional oven, widely used domestically, and extrusion, an important industrial technique (ATHAR et al., 2006; DING et al., 2006). In addition, a small number of bioactive components in sorghum were evaluated in these studies (AWIKA, 2003; DLAMINI et al., 2007; MORAES, 2011; WU et al., in press).

This research sought to develop studies to assess the variability and stability of nutrients and bioactive compounds in sorghum. It was speculated that sorghum has high variability with respect to carotenoid and vitamin E contents and profiles, and that its nutritional and functional values are modified by dry heat in a conventional oven and extrusion. It was expected that these studies contribute to the advancement in research on sorghum, providing input on possible methods for its use in food. Furthermore, it was expected to contribute to the realization of studies evaluating the effects of sorghum on other parameters related to human health, such as the intestinal microbiota, diabetes, hormones, gene expression and others.

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2. OBJECTIVES

2.1 General Objective

To evaluate the variability of nutrients and bioactive compounds in sorghum (*Sorghum bicolor* L.) and their stability when submitted to extrusion and dry heat in a conventional oven

2.2 Specific objectives

- Summarize the recent findings concerning nutrients and bioactive compounds of sorghum and its potential impact on human health;
- To evaluate the variability of carotenoids and vitamin E in one hundred sorghum genotypes;
- To evaluate the effects of extrusion and dry heat in a conventional oven on nutrients and bioactive compounds (carotenoids, vitamin E, 3-deoxyanthocyanidins, flavones, flavanones, proanthocyanidins and phenolic compounds) of three sorghum genotypes.

3. RESULTS

3.1 Article 1: Sorghum (*Sorghum bicolor* L.): nutrients, bioactive compounds, and potential impact on human health

This article has been accepted for publication in the Journal Critical Reviews in Food Science and Nutrition, as the acceptance letter below.

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February 20, 2014

To whom it may concern:

This is to certify that the paper referenced , BFSN-2013-1135.R1, "SORGHUM (SORGHUM BICOLOR L.): NUTRIENTS, BIOACTIVE COMPOUNDS AND POTENTIAL IMPACT ON HUMAN HEALTH" coauthored by Leandro de Morais CARDOSO (first author) was accepted January 21, 2014 for publication in the Journal Critical Reviews in Food Science and Nutrition of which I am Editor in Chief.

Sincerely

Fergus M. Clydesdale Distinguished Professor, Director of the Food Science Policy Alliance and Editor in Chief of Critical Reviews in Food Science and Nutrition

ABSTRACT

Sorghum is the fifth most produced cereal in the world and is a source of nutrients and bioactive compounds for the human diet. We summarize the recent findings concerning the nutrients and bioactive compounds of sorghum and its potential impact on human health, analyzing the limitations and positive points of the studies and proposing directions for future research. Sorghum is basically composed of starch, which is more slowly digested than that of other cereals, has low digestibility proteins and unsaturated lipids, and is a source of some minerals and vitamins. Furthermore, most sorghum varieties are rich in phenolic compounds, especially 3-deoxyanthocyanidins and tannins. The results obtained in vitro and in animals have shown that phenolics compounds and fat soluble compounds (polycosanols) isolated from sorghum benefit the gut microbiota and parameters related to obesity, oxidative stress, inflammation, diabetes, dyslipidemia, cancer, and hypertension. The effects of whole sorghum and its fractions on human health need to be evaluated. In conclusion, sorghum is a source of nutrients and bioactive compounds, especially 3-deoxyanthocyanidins, tannins, and polycosanols, which beneficially modulate, in vitro and in animals, parameters related to noncommunicable diseases. Studies should be conducted to evaluate the effects of different processing on protein and starch digestibility of sorghum as well as on the profile and bioavailability of its bioactive compounds, especially 3-deoxyanthocyanidins and tannins. Furthermore, the benefits resulting from the interaction of bioactive compounds in sorghum and human microbiota should be studied.

Keywords: nutritional value; 3-deoxyanthocyanidins; tannins; noncommunicable diseases; oxidative stress; cancer

ABBREVIATIONS: ABCA1: ATP-binding cassette transporter A1; *COX-2:* cyclooxygenase-2; DP: degree of polymerization; GPx: glutathione peroxidase activity; HDL-c: high-density lipoprotein-cholesterol; HIF-1α: hypoxia-inducible factor 1α; HMG-CoA: 3-hydroxy-3-methylglutaryl CoA; IGF-1R: insulin-like growth factor 1; IL-1β: interleukin-1β; LDL-c: low-density lipoprotein-cholesterol; MUFA; monounsaturated fatty acids; NQO:

NADH:quinone oxyreductase; NF- κβ: nuclear factor-κβ; PPAR-γ: peroxisome proliferator-activated receptor gamma; PUFA: polyunsaturated fatty acids; RES: reactive electrophilic species; RNS: reactive nitrogen species; ROS: reactive oxygen species; SOD: superoxide dismutase; TBARS: thiobarbituric acid reactive substances; TNF-α: tumor necrosis factor-α; VEGF: vascular endothelial growth factor.

INTRODUCTION

Sorghum (*Sorghum bicolor* L.) is a cereal of the family *Poaceae*, native to Africa, and was domesticated between 3,000 and 5,000 years ago (U.S. Grains Council, 2004). It is the fifth most produced cereal in the world, and is preceded by wheat, rice, maize, and barley (Food and Agricultural Organization, 2010). Around the world, there are over 7,000 varieties of sorghum (Kangama & Rumei, 2005).

The sorghum crop is extremely important in Asia, Africa, and other semi-arid regions of the world, where it is mainly used in human feeding (Afify, et al., 2011; Dillon, et al., 2007; Elkhalifa, et al., 2005). In Western countries such as the United States, Australia, and Brazil, sorghum is developed and cultivated primarily for animal feeding (Taleon, et al., 2012). However, due to its high nutritional and functional potential, several studies on sorghum for human consumption have been conducted in these countries (Ciacci, et al., 2007; Dykes, et al., 2005; Ferreira, et al., 2009; Maunder, 2005; Schober, et al., 2005; Taylor, J. R. N., et al.).

The sorghum grain has three distinct anatomical structures called the pericarp, endosperm, and germ. Some varieties have a fourth structure called the testa, located between the pericarp and the endosperm (Earp, et al., 2004). The proportion and chemical composition of sorghum's anatomical structures depend on the variety and growing conditions (Waniska & Rooney, 2000). Generally, the pericarp (outer coating) and the testa are composed of non-starch polysaccharides, phenolic compounds (3-deoxyanthocyanidins, tannins, and phenolic acids, among others), and carotenoids. The starch, proteins, B-complex vitamins, and minerals are located in the endosperm (storage tissue). The germ (embryo) is composed of lipids, fat-soluble

vitamins, B-complex vitamins, and minerals (Earp, et al., 2004; Food Security Department, 1999; Slavin, 2004; Waniska & Rooney, 2000).

Sorghum is an excellent source of bioactive compounds that can promote benefits to human health. The results of in vitro and animal studies have shown that compounds isolated from sorghum, mainly phenolics, promote beneficial changes in parameters related to noncommunicable diseases such as obesity, diabetes, dyslipidemia, cardiovascular disease, cancer, and hypertension (Awika, et al., 2009; Farrar, et al., 2008; Kamath, V., et al., 2007; Kim, J. & Park, 2012; Moraes, et al., 2012a; Muriu, et al., 2002; Shih, C.-H., et al., 2007; Woo, et al., 2012; Yang, L., et al., 2009).

Given the high beneficial potential of sorghum on human health and the absence of a critical review about the subject, we summarize the recent findings concerning the nutrients and bioactive compounds of sorghum and its potential to modulate parameters related to human health, analyzing the limitations and positive points of recent studies and proposing directions for future research.

METHODOLOGY

Original articles published between 2005 and 2013 in scientific indexing bases (PubMed, Biological Abstracts, CAB Abstracts, Food Science and Technology Abstracts, LILACS, Scielo, MEDLINE, and Science Direct) were researched. In addition, some relevant scientific papers published in previous years were included.

The article search was performed by matching the terms sorghum and *Sorghum bicolor* L. with a group of terms related to chemical composition and nutritional value (starch, amylose, amylopectin, proteins, kafirins, dietary fibers, minerals, vitamins, mineral bioavailability, starch digestibility, phenolic compounds, phenolic acids, tannins, flavonoids, 3-deoxyanthocyanidins, flavones, flavanones, sterols, polycosanols, stilbenes, antioxidant profile, phytates, protease inhibitors: trypsin, chymotrypsin and amylase, and lectins) and another group related to chronic noncommunicable diseases (obesity, weight loss, dyslipidemia, cardiovascular disease, cholesterol, triacylglycerol, high-density lipoprotein-cholesterol (HDL-c), low-density lipoprotein-cholesterol (LDL-c), diabetes, fasting glucose, glycemic response, fasting

insulin, insulinic response, cancer, oxidative stress, inflammation, interleukins, gut microbiota, and hypertension). The articles were screened and selected based on the title and abstract presented.

CHEMICAL COMPOSITION AND NUTRITIONAL VALUE

The chemical composition and nutritional value of whole sorghum are similar to rice, corn, and wheat. The energy value of 100g of sorghum grains varies between 296.1 and 356.0 kcal (Martino, et al., 2012; U.S. Department of Agriculture, 2012). The main components of sorghum are the polysaccharides (starch and non-starch), followed by proteins and lipids (Martino, et al., 2012; U.S. Department of Agriculture, 2012).

Polysaccharides

The content and composition of starch, the main polysaccharide of sorghum, are influenced by the genetic characteristics and growing conditions of the grain (Hill, et al., 2012). In some varieties, starch ranges between 32.1 and 72.5 g/100g and is composed mainly of amylopectin (81.0-96.5%) and amylose (3.5-19.0%) (Shegro, et al., 2012; Udachan, et al., 2012). The proportion of amylose and amylopectin affects the rheological proprieties (gelatinization, retrogradation, and gelling) and digestibility of the sorghum starch (Sang, et al., 2008; Singh, H., et al., 2010).

Sorghum has the lowest starch digestibility among cereals due to the strong association between the starch granules and proteins and tannins (Barros, et al., 2012; Mkandawire, et al., 2013; Rooney & Pflugfelder, 1986). Overall most of the starch granules are slowly digestible (30.0-66.2%) and the remainder is rapidly digestible (15.3-26.6%) or resistant (16.7-43.2%) (Mkandawire, et al., 2013; Sang, et al., 2008). The non-starch polysaccharides of sorghum (6.0 to 15.0 g/100g) include insoluble fibers (75.0-90.0%), mainly arabinoxylans, and soluble fibers (10.0-25.0%) (Martino, et al., 2012; Taylor, J. R. N. & Emmambux, 2010; U.S. Department of Agriculture, 2012).

Proteins

Sorghum proteins are classified as prolamins and not prolamins. Prolamins correspond on average to 79% (77-82%) of the total proteins (7 to 15g/100g) and the remainder is albumins, globulins, and glutelins (Afify, et

al., 2012b; Belton, et al., 2006; Martino, et al., 2012; U.S. Department of Agriculture, 2012). The kafirins are the major prolamins of the sorghum and comprise three major classes: α-kafirins (66-84%), β-kafirins (8-13%) and γ-kafirins (9-21%) (Belton, et al., 2006; Mokrane, et al., 2010). Sorghum kafirins are stored in the endoplasmic reticulum in spherical protein bodies. The β and γ-kafirins are located in the peripheral protein bodies region while α and δ-kafirins are encapsulated in the inner region (Wu, Yongrui, et al., 2013). This conformation determines the digestibility of sorghum proteins.

Overall, the digestibility of sorghum proteins, especially after cooked, is lower than cereals like wheat and maize (Afify, et al., 2012b; Duodu, et al., 2003; Mokrane, et al., 2010; Moraes, et al., 2012b). The kafirins of sorghum are resistant to peptidase due to the formation of intramolecular disulfide bonds; this is the main cause of the low digestibility (Belton, et al., 2006). However, in varieties rich in tannins, the complexation of the kafirins with this phenolic compound can reduce the protein digestibility up to 50% (Taylor, J., et al., 2007). Furthermore, other exogenous factors (interaction of the proteins with non-protein components such as starch, non-starch polysaccharides, phytic acid, and lipids) and endogenous factors (nature and organization of proteins inside the grain) contribute to this low digestibility (Belton, et al., 2006; da Silva, et al., 2011a; Duodu, et al., 2002; Duodu, et al., 2003; Ezeogu, et al., 2008; Ezeogu, et al., 2005).

Despite the reduction in protein digestibility of sorghum after cooking in wet heat, processing such as fermentation and germination may increase the digestibility up to 2 times (Afify, et al., 2012b; Correia, et al., 2008; ELKhier & Abd-ALRaheem, 2011; Pranoto, et al.; Wedad, et al., 2008). However, major recent efforts to improve the protein digestibility of sorghum aim to reduce the amount of kafirins, especially β and γ forms, and to increase the glutenins and albumins (da Silva, et al., 2011b; Goodall, et al., 2012; Kumar, et al., 2012; Taylor, J. & Taylor, 2011; Wu, Yongrui, et al., 2013). The genetically modified varieties have in vitro digestibility from 23 to 102% higher than control varieties (da Silva, et al., 2011a; Kumar, et al., 2012; Taylor, J. & Taylor, 2011).

The α-kafirins are the last proteins to be digested in the intestine and, because of their high abundance, the indigestibility reduces their nutritive

value (Wu, Yongrui, et al., 2013). The β and γ-kafirins are rich in cysteine, which forms disulfide bonds, and are therefore assumed to block the accessibility of α-kafirins to hydrolytic enzymes (Duodu, et al., 2003; Wong, et al., 2009). Thus, the higher digestibility in modified varieties can be attributed to increased enzyme susceptibility of the major storage protein, α-kafirin, because of changes in protein body morphology as well as to a reduction of the formation of disulfide bonds between β and γ-kafirins (Henley, et al., 2010; Kumar, et al., 2012; Mehlo, et al., 2013; Oria, et al., 2000).

Generally, sorghum proteins are rich in glutamic acid and nonpolar amino acids (proline, leucine, and alanine) and have lysine as the main limiting amino acid (De Mesa-Stonestreet, et al., 2010; Mokrane, et al., 2010; Moraes, et al., 2012b). In addition, they may be deficient in 5 other essential amino acids (methionine, cysteine, isoleucine, valine, and threonine) (Moraes, et al., 2012b). However, varieties obtained through breeding programs have 52-115% more lysine than conventional varieties (da Silva, et al., 2011b; Henley, et al., 2010; Kumar, et al., 2012; Taylor, J. & Taylor, 2011). Improved lysine contents were attributed to decreased levels of kafirin proteins and increased levels of lysine-rich, non-kafirin proteins in the grain endosperm (Shewry, 2007).

Unlike the major prolamins of wheat (gliadin), rye (secalin), and barley (hordein), the kafirins do not trigger an allergic response or an autoimmune reaction in humans (De Mesa-Stonestreet, et al., 2010). In addition to the qualitative evidence based on the type of proteins found in sorghum, there is genetic evidence that it has characteristics that do not allow the expression of toxic peptides related to gliadin (Pontieri, et al., 2013). Thus, sorghum is an effectively safe cereal for consumption by people with celiac disease.

Lipids

Sorghum has a reduced lipid content (1.24 to 3.07 g/100g), which is mainly composed of unsaturated fatty acids (83-88%) (Afify, et al., 2012a; Martino, et al., 2012; U.S. Department of Agriculture, 2012). In most of the varieties of sorghum the polyunsaturated fatty acids (PUFA) are higher than monounsaturated fatty acids (MUFA) (Afify, et al., 2012a; Hadbaoui, et al., 2010; Mehmood, et al., 2008). The major fatty acids of sorghum are linoleic

(45.6-51.1%), oleic (32.2-42.0%), palmitic (12.4-16.0%), and linolenic acids (1.4-2.8%) (Afify, et al., 2012a; Hadbaoui, et al., 2010; Mehmood, et al., 2008).

Minerals and vitamins

Sorghum is a source of minerals (phosphorus, potassium, and zinc) whose content varies according to the place of cultivation (Table 1) (Martino, et al., 2012; Shegro, et al., 2012; Silva, et al., 2012; U.S. Department of Agriculture, 2012). The bioavailability of most minerals of sorghum is still little known. However, it is known that zinc availability varies between 9.7% and 17.1% and iron availability ranges from 6.6% to 15.7% (Afify, et al., 2011; Kruger, et al., 2013). Studies have been conducted in order to increase the content and bioavailability of iron and zinc through biofortification, fortification, and genetic improvement of sorghum (Ashok Kumar, et al., 2013; Kruger, et al., 2013; Tripathi, et al., 2010; Tripathi & Platel, 2013).

Table 1: Average content of minerals (mg/100g) in sorghum grown in Brazil, United States and Ethiopia.

Minerals	Brazil [1] (n = 8)	United States [2] (n = 1)	Ethiopia [3] (n = 31)
Calcium	10.7	28.00	31.13
Iron	1.64	4.40	6.14
Potassium	-	350.00	188.80
Manganese	0.06	-	1.58
Sodium	nd	6.00	23.00
Potassium	217.87	287.00	289.34
Zinc	1.65	-	2.42
Magnesium	102.77	-	116.8
Copper	0.51	-	-
Sulfur	79.20	-	-
Aluminum	nd	-	-
Cadmium	nd	-	-
Chrome	nd	-	-
Lead	nd	-	-

[1] Martino, et al. (2012); [2] U.S. Department of Agriculture (2012); [3] Shegro, et al. (2012); nd: not detected, -: not analyzed.

Information on the content of vitamins in sorghum is scarce. However, it is worth noting that it is a source of some B-complex vitamins (thiamine, riboflavin, and pyridoxine) and fat-soluble vitamins (D, E, and K) (Cardoso, et al., 2014; Martino, et al., 2012; Ochanda, et al., 2010; U.S. Department of Agriculture, 2012).

BIOACTIVE COMPOUNDS

Phenolic compounds and their bioavailability

The phenolic compounds are the main bioactive compounds of sorghum and are present in all varieties of this cereal (Dykes & Rooney, 2006). Almost all classes of phenolics are found in sorghum (Awika & Rooney, 2004; Dykes, et al., 2005); however, the classes of phenolic acids, tannins, and flavonoids are major.

The profile and content of phenolic compounds in sorghum are more diverse and higher than those observed in wheat, barley, rice, maize, rye, and oats (Ragaee, et al., 2006). Sorghum varieties resistant to biotic and abiotic stresses were found to have on average higher contents of proanthocyanidins, 3-deoxyanthocyanidins, and flavan-4-ols than susceptible varieties (Dicko, et al., 2005).

The bioavailability of phenolic compounds after dietary intake has been a topic of increasing research in recent years (Crozier, et al., 2010; Faria, et al., 2013; Hole, et al., 2012; Prior & Wu, 2006; Yang, M., et al., 2011). However, the results obtained in humans are scarce and controversial. In addition, information about the bioavailability of phenolic compounds of sorghum, including tannins and 3-deoxyanthocyanidins, is not available yet.

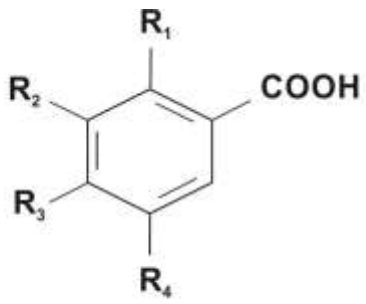
The main difficulty in analyzing the bioavailability of phenolic compounds is the absence of standardized methods capable of identifying their metabolites. For example, the analysis of phenolic compounds in urine provides a more realistic bioavailability but does not include the possibility that their metabolites were sequestered by body tissues (Crozier, et al., 2010). This limitation underestimates the bioavailability of phenolic compounds in food.

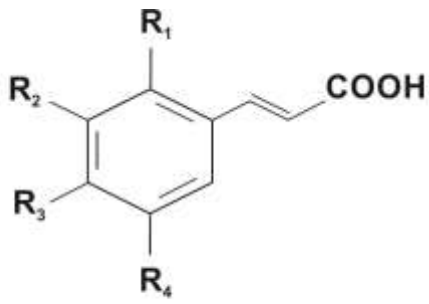
Different factors affect the bioavailability of phenolic compounds in humans, including environmental factors, food processing, food matrix, and interaction with other compounds and polyphenols (D'Archivio, et al., 2010; Fernandes, et al., *In press*). There is a need for extensive investigation about the alterations of phenolic compounds by the gastrointestinal tract and their subsequent absorption and metabolism, including distribution in tissues (D'Archivio, et al., 2010; Lafay & Gil-Izquierdo, 2008). Studies have shown that catabolites of phenolic compounds not absorbed in the small intestine pass into the large intestine where they can affect the colon microbiota (Crozier, et al., 2010; Faria, et al., 2013).

Knowledge of the bioavailability of phenolic compounds in sorghum, including dietary factors able to modulate it, is essential for analysis of their functional potential in humans and, in the long term, for the implementation of therapeutic measures for health professionals.

Phenolic acids

The phenolic acids are classified as hydroxybenzoic acid derivatives and hydroxycinnamic acid derivatives (Figure 1). These acids exhibit high antioxidant activity in vitro and thus may promote benefits to human health (Kamath, V. G., et al., 2004).





Hydroxybenzoic acids

	R_1	R_2	R_3	R_4
Gallic acid	H	OH	H	OH
Gentisic acid	OH	H	H	OH
Salicylic acid	OH	H	H	H
p-hydroxybenzoic acid	H	H	H	OH
Syringic acid	H	OCH_3	OH	OCH_3
Protocatequinic acid	H	OH	OH	H

Hydroxycinnamic acids

	R_1	R_2	R_3	R_4
Caffeic acid	H	OH	OH	H
Feluric acid	H	OCH_3	OH	H
o-coumaric acid	OH	H	H	H
p-coumaric acid	H	H	H	H
Sinapic acid	H	OCH_3	OCH_3	OCH_3
Vanillic acid	H	OCH_3	OCH_3	H

Figure 1. Structure of the major phenolic acids present in foods, including in sorghum.

The content of phenolic acids in some sorghum varieties ranged between 135.5 and 479.40 µg/g (Afify, et al., 2012c; Chiremba, et al., 2012), with major amounts of the protocatechuic (150.3 to 178.2 µg/g) and ferulic (120.5 to 173.5 µg/g) acids and small amounts of the p-coumaric (41.9 to 71.9 µg/g), syringic (15.7 to 17.5 µg/g), vanillic (15.4 to 23.4 µg/g), gallic (14.8 to 21.5 µg/g), caffeic (13.6 to 20.8 µg/g), cinnamic (9.8 to 15.0 µg/g), and p-hydroxybenzoic (6.1 to 16.4 µg/g) acids (Afify, et al., 2012c; Svensson, et al., 2010).

The phenolic acids in wines, fruits, and vegetables have a good bioavailability. In these matrices, the majority of phenolic acids are free or as conjugated forms that can be hydrolyzed in the upper intestinal tract (Hole, et al., 2012). On the other hand, phenolic acids in cereals, including in sorghum, are mostly bound to arabinoxylans chains or lignin (Abdel-Aal, et al., 2012; Dykes & Rooney, 2006; Hole, et al., 2012). These bound phenolic acids are not hydrolyzed by human digestive enzymes that decrease their bioavailability, but are fermented by the microbiota of the colon (Hole, et al., 2012; Saura-Calixto, 2010).

Knowledge about techniques for improving the bioavailability of phenolic acids in sorghum is incipient. The microorganisms and processing of grains can play a key role in improving this bioavailability. The study results demonstrated that cereal fermentation with specific probiotic strains and cooking processes can significantly increase the content-free phenolic acids (Hole, et al., 2012; Lafay & Gil-Izquierdo, 2008; N'Dri, et al., 2013; Saura-Calixto, 2010), thereby improving their bioavailability. The effects of other types of processing on the profile of phenolic acids in sorghum need to be studied.

Tannins (proanthocyanidins)

Tannins, secondary metabolites found in many plant species, are phenolic compounds that often act as a defense mechanism against pathogens and predators (Kaufman, et al., 2013). Overall, these compounds are absent in other major cereals, such as rice, wheat, and maize, but are present in sorghum varieties that have pigmented testa (Awika, 2003; Dykes & Rooney, 2006; Wu, Yuye, et al., 2012). The presence and content of condensed tannins in sorghum are controlled by the genes *S* and *Tannin1,*

among others (Hahn, D. H. & Rooney, L. W., 1986; Wu, Yuye, et al., 2012).

The tannins in sorghum vary as to the type, content, and distribution of the individual oligomers and polymers. They are classified as type I (no significant levels), type II (tannins that are extractable only in acidified methanol) and type III (tannins that are extractable in methanol and acidified methanol) (Hahn, D. & Rooney, L., 1986; Price, et al., 1978). Almost all of the tannins in sorghum are condensed and constituted by oligomers or polymers of catechins (flavan-3-ols and/or flavan-3,4-diols) (Awika & Rooney, 2004; Wu, Yuye, et al., 2012). In general, most sorghum tannins have a high molecular weight and a degree of polymerization (DP) higher than 10 (69-81%) (Awika, et al., 2003).

The content of tannins in sorghum varies between 0.2 and 48.0 mg/g and is highest in sorghum with black testa (Afify, et al., 2012c; Awika, et al., 2003; Dykes, et al., 2013; Martínez, et al., 2009; Schons, et al., 2011). However, this content, as well as the activity of tannins in sorghum, can be affected by the season (Mkandawire, et al., 2013). Therefore, the effect of the environment on tannins should be considered during the selection and breeding of tannin containing sorghum genotypes when the aim is to achieve health benefits (Mkandawire, et al., 2013).

The tannins reduced the availability of minerals, proteins, and starch of the sorghum (Al-Mamary, et al., 2001; Barros, et al., 2012; Taylor, J., et al., 2007). This reduction correlates not only with the content of tannins in the grain, but also with DP (Kaufman, et al., 2013; Mkandawire, et al., 2013). It is attributed mainly to the tannins with higher molecular weight or more complex tannin structures (DP > 10) (Barros, et al., 2013; Mkandawire, et al., 2013; Osman, 2004). Polymeric tannins, for example, are the major contributors to resistant starch formation due to their stronger interaction with starch, especially amylose (Barros, et al., 2013).

Despite the anti-nutritional effect, tannins are 15–30 times more effective than simple phenolics in radical scavenging ability (Hagerman, et al., 1998). Thus, tannins have been extensively studied for health-promoting capabilities (Beecher, 2004). The functional benefits of sorghum are attributed mainly to oligomers, which have been extensively studied (Beecher, 2004). The oligomers of tannins in foods contribute up to 19% of

the antioxidant capacity of the diet and promote benefits to human health due to immunomodulatory, anticancer, antioxidant, antirradical, anti-inflammatory, vasodilatory, cardioprotective, anti-thrombotic, and anti-UV actions (Dixon, et al., 2005; Floegel, et al., 2010; Sharma, S. D., et al., 2007; Waniska & Rooney, 2000).

The oligomeric and polymeric proanthocyanidins are not absorbed by animals and humans (Crozier, et al., 2010; Serrano, et al., 2009). But minor quantities of dimers of B1 and B2 procyanidins are detected in human plasma (Donovan, et al., 2007; Holt, et al., 2002). However, so far, it is unclear whether this low bioavailability is also observed in tannins in sorghum. Most tannins in foods pass unaltered to the large intestine where part of them are catabolized by the colonic microbiota yielding a diversity of phenolic acids (Selma, et al., 2009). The biological effects of tannins are generally attributed to their more readily absorbed colonic breakdown products, the phenolic acids, although there is a lack of detailed study in this area (Crozier, et al., 2010).

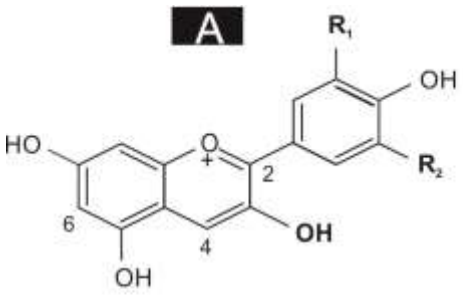
As to phenolic acids, processing can improve the digestibility of tannins in sorghum. The processing of grain sorghum in dry heat (95 °C for 20 min and 121 °C for 30 minutes) can depolymerize the condensed tannins in sorghum (Barros, et al., 2012), which can increase their bioavailability. The thermal processing can be a strategy to increase the bioavailability of tannins with a minimum reduction in the content of these compounds. Thus, the functional potential of sorghum rich in tannins can be maintained or even increased. Furthermore, the nutritional value of the grain may increase due to higher digestibility of starch and proteins resulting from the reduction of polymeric tannins. The depolymerization of tannins through other types of processing needs to be studied.

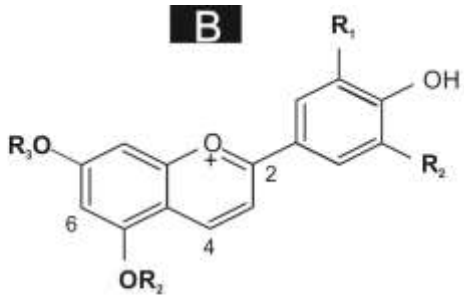
Flavonoids

Most flavonoids of the sorghum are located in the outer layers of the grain. Thus, differences in the color and thickness of the pericarp and presence of the testa influence the concentration and profile of flavonoids (Awika, et al., 2005; Dykes, et al., 2009). In turn, the physical characteristics of the sorghum are determined by genetic and environmental factors (Taleon, et al., 2012).

Three classes of flavonoids are in large quantities in sorghum: anthocyanins, flavones, and flavanones. Sorghum anthocyanins belong to the class of 3-deoxyanthocyanidins and correspond up to 79% of the flavonoids' content (Dykes & Rooney, 2006; Shih, C.-H., et al., 2007; Taleon, et al., 2012). They have a differentiated structure due to the absence of a hydroxyl group at position C-3 and therefore they are more stable than other anthocyanins (Awika, 2008; Awika & Rooney, 2004; Dykes, et al., 2009; Shih, C.-H., et al., 2007).

The main 3-deoxyanthocyanidins of the sorghum are non-methoxylated forms (luteolinidin and apigeninidin) (Awika, et al., 2004) (Figure 2). However, small quantities of methoxylated 3-deoxyanthocyanidins (5-methoxy-luteolinidin, 7-methoxy-luteolinidin, and 7-methoxy-apigeninidin) are observed in the sorghum as well as methoxylated 3-deoxyanthocyanins (5-methoxy-luteolinidin 5-glucoside, 5-methoxy-luteolinidin 7-glucoside, 7-methoxy-luteolinidin 5-glucoside, and 7-methoxy-apigeninidin 5-glucoside) and other non-methoxylated 3-deoxyanthocyanins (apigeninidin 5-glucoside and luteolinidin 5-glucoside) (Cardoso, et al., 2014; Taleon, et al., 2012; Wu, X. & Prior, 2005; Yang, L., et al., 2009). The content of sorghum 3-deoxyanthocyanidins correlates with its color and antioxidant activity (Awika & Rooney, 2004). Varieties with pericarp and black testa have 3 to 4 times more total 3-deoxyanthocyanidins (5.4 to 6.1 mg/g) than red and brown varieties (1.6 to 2.8 mg/g) (Awika, et al., 2004).





Anthocyanins in foods

	R_1	R_2
Cyanidin	OH	H
Pelargonidin	H	H
Peonidin	OCH_3	H
Malvidin	OCH_3	OCH_3
Delfinidin	OH	OH
Petunidin	OCH_3	OH

3-Deoxyanthocyanins in sorghum

	R_1	R_2	R_3
Apigeninidin	H	H	H
5-methoxy-apigeninidin	H	H	CH_3
Apigeninidin 5-glycoside	H	Gcl	H
7-methoxy-apigenindin 5-glycoside	H	Glc	CH_3
Luteolinidin	OH	H	H
Luteolinidin 5-glycoside	OH	Glc	H
5-methoxy-luteolinidin	OH	CH_3	OCH_3
7-methoxy-luteolinidin	OH	H	CH_3
5-methoxy-luteolinidin 7-glycoside	OH	CH_3	CH_3
7-methoxy-luteolinidin 5-glycoside	OH	CH_3	CH_3
5-methoxy-luteolinidin 5-glycoside	OH	CH_3	CH_3

Figure 2: Structure of common anthocyanins in foods (A) and 3-deoxyanthocyanidins in sorghum (B).

The total flavones of the sorghum vary from 0 to 386 µg/g (on average, 87 µg/g), with a prevalence of aglycone forms of luteolin and apigenin (Dykes, et al., 2011; Dykes, et al., 2009). The main flavanones of sorghum are the aglycone forms of eriodictyol and naringenin (Dykes, et al., 2011; Dykes, et al., 2009). The smallest contents are found in white varieties and the largest contents are observed in those with lemon-yellow pericarp (474 to 1780 µg/g) (Dykes, et al., 2011).

Information about the bioavailability of the 3-deoxyanthocyanidins, flavones, and flavanones in sorghum is not available in the literature so far. In other foods, including cereals, flavones and flavanones are highly available and are rapidly absorbed and excreted (Crozier, et al., 2009; Donovan, et al., 2007). The aglycone of flavones and flavanones, the forms most prevalent in sorghum, are rapidly absorbed (Spencer & Crozier, 2012; Urpi-Sarda, et al., 2012).

The bioavailability of anthocyanins in foods is relatively low compared to flavones and flavanones (Yang, M., et al., 2011) and is influenced by the nature of the sugar and also the structure of the anthocyanidin aglycone (Wu,

X., et al., 2005). Unlike flavonoids, where glycosides are hydrolyzed, anthocyanin glycosides are rapidly and efficiently absorbed in the small intestine (Fernandes, et al.; Prior & Wu, 2006). This fact indicates that the main aglycone forms present in sorghum can have low bioavailability.

The anthocyanins in foods appear to be rapidly absorbed and eliminated, reaching only low maximal concentrations in plasma and urine (Fernandes, et al.). In studies with animals and humans, the quantities of anthocyanins excreted in urine were less than 0.1% of intake (Crozier, et al., 2010; McGhie, et al., 2003; Prior & Wu, 2006; Wu, X., et al., 2005). Although anthocyanin bioavailability in foods appears low, it could have been underestimated due to the fact that some major metabolites have been ignored or not analyzed due to analytical limitations (Manach, et al., 2005). The difficulty in overcoming those analytical problems may contribute significantly to the low bioavailability of anthocyanins (Fernandes, et al.).

Between 60 and 90% of the anthocyanins may disappear from the gastrointestinal tract within 4 h after a meal (Prior & Wu, 2006). What happens to the bulk of the anthocyanins that disappear is not clear. Degradation accounts for a part of this disappearance, but differs for the various aglycones and may be modified further by the nature of the aglycone glycosylation, which further complicates our understanding of this process (Prior & Wu, 2006). Special attention is given to the contribution of the gastric mucosa to anthocyanin absorption as the result of the high content of intact anthocyanins (20–25%) detected in plasma few minutes after intake (Fernandes, et al.).

The contribution of intestinal tissue and the microbiota impact on absorption and anthocyanin bioavailability is also highlighted (Faria, et al., 2013; Fernandes, et al.). It has been suggested that this is likely due to the spontaneous degradation under physiological conditions or following microbial metabolism (Fernandes, et al.; Woodward, et al., 2009). In fact, colonic microbiota hydrolyzes glycosides into aglycones and degrades them to simple phenolic acids (Fernandes, et al.), which can be further fermented in the colon (Williamson & Clifford, 2010).

Stilbenes

Stilbenes are a small family of phenolic compounds derived from the

phenylpropanoid pathway that have numerous implications in plant disease resistance and human health (Chong, et al., 2009). The total content of stilbenes correlates with the color of the grain and is present in smaller quantities in white varieties. White sorghum contains traces of trans-piceid (up to 0.1 mg/kg) and trans-resveratrol is absent while in red sorghum, these two classes are present (Bröhan, et al., 2011).

Polycosanols and phytosterols

Polycosanols and phytosterols are associated with the lipid fraction of the sorghum (Leguizamón, et al., 2009; Wang, et al., 2008; Zbasnik, et al., 2009). Thus, these compounds have been studied mainly in lipids extracted from dry sorghum obtained after alcohol production.

One of the main components of the long-chained lipids extracted from sorghum grain kernels are polycosanols (33.4–44%) (Hwang, Keum Taek, et al., 2005; Hwang, Keum T, et al., 2004). Sorghum can be a major source of polycosanols that have physiological benefits. Total polycosanols content in unpolished sorghum grain was 74.5 mg/100g in the dry kernel while the content in the polished grain was 9.8 mg/100g in the dry kernel (Hwang, Keum Taek, et al., 2005; Hwang, Keum T, et al., 2004).

The content of sorghum phytosterols (4.13 to 24.45 µg/g, dry weight basis) is affected by growing conditions (Chung, et al., 2013). Sorghum grains are a relatively rich source of phytosterols when compared with fruits, vegetables, and other cereal grains commonly found in the food supply. Of the more than 200 sterols in vegetables, 3 have been identified in sorghum (sitosterol: 44.8 to 48.2%; campesterol: 26.1 to 38.0% and stigmasterol: 17.3% to 25.6%) (Delgado-Zamarreño, et al., 2009; Leguizamón, et al., 2009; Singh, V., et al., 2003; Wang, et al., 2007; Ye, et al., 2010).

Phytochemicals with anti-nutritional activity

Like other cereals, sorghum has phytochemicals with anti-nutritional activity. Phytates are major anti-nutritional compounds identified in sorghum (Abdel-Rahman & Osman, 2011; Afify, et al., 2011; Schons, et al., 2011). In addition, some varieties have protease inhibitors (trypsin, chymotrypsin, and amylase) and lectins (Abdel-Rahman & Osman, 2011; Neucere, 1982; Raimi, et al., 2012). These phytochemicals decrease the digestibility of proteins and carbohydrates, and mineral bioavailability.

POTENCIAL IMPACT OF SORGHUM ON THE HUMAN HEALTH

The potential functional benefits to human health associated with the consumption of compounds isolated from sorghum, and especially the whole grain, are still unknown. The results of in vitro and animal studies have shown that phenolics or fat soluble compounds isolated from sorghum beneficially modulate the gut microbiota and parameters related to noncommunicable diseases such as obesity, diabetes, dyslipidemia, cardiovascular disease, cancer, and hypertension (Tables 2 and 3). The main mechanisms of action of the compounds isolated from sorghum on parameters related to noncommunicable diseases, as found in results of in vitro and animal studies, are presented in Figure 3.

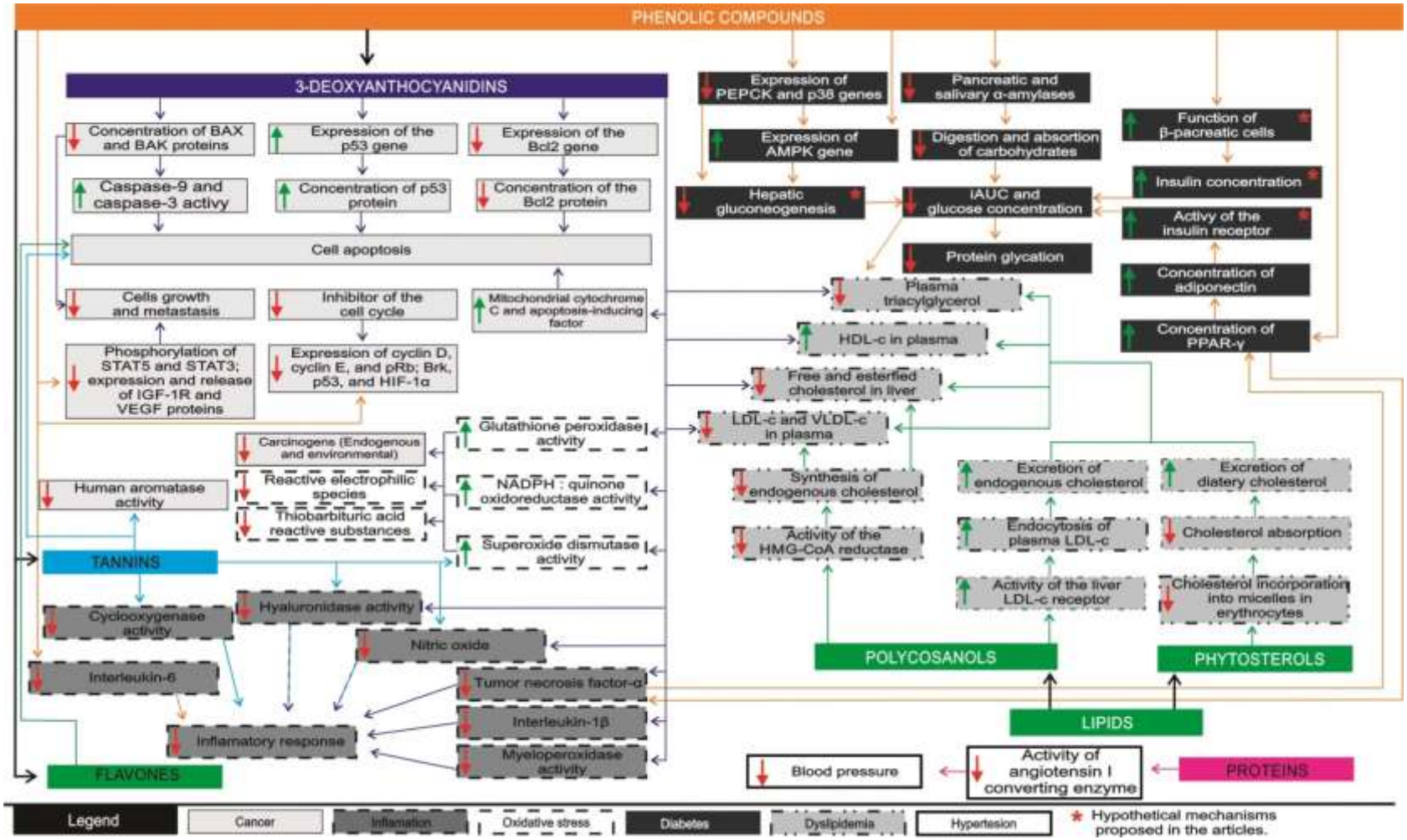


Figure 3: Main mechanisms of action of the sorghum on parameters related to noncommunicable diseases (diabetes, dyslipidemia, inflammation, cancer, oxidative stress and hypertension), proposed based on results of in vitro and animal studies

Table 2: Description of the in vitro studies about the effects of the fractions isolated from sorghum on parameters related to chronic noncommunicable diseases.

Related pathology	Cell type (if any)	Sorghum fraction used and preparation form	Main results	References
Diabetes	Not applied	Extract of Sumac sorghum bran rich in 3-deoxyanthocyanidins and high antioxidant activity (10% bran diluted in ethanol 50%)	↓ Glycation of proteins in approximately 60%	Farrar, et al. (2008)
	Not applied	Extracts of five sorghum varieties (10% grain diluted in ethanol 70%)	↓ Activity of α-glucosidase of *B. stearothermophilus* ↓ Activity of human pancreatic and salivary α-amylases.	Kim, J.-S., et al. (2011)
Inflammation and cancer	Not applied	Extracts of bran of sorghum varieties rich in tannins and 3-deoxyanthocyanidins (10% bran diluted in ethanol 50%)	↓ Hyaluronidase activity	Bralley, et al. (2008)
	Skin sarcoma cells (Hs 63.T) LPS-induced	Extracts of sorghum rich in phenolic compounds (5% sorghum diluted in methanol)	↓ Nitric oxide, IL-6, TNF-α ↓ DNA synthesis	Hwang, J.-M., et al. (2013)
	LPS-induced peripheral blood mononuclear cells	Extracts of white, bronze, red and black sorghum (8.3% to 25% bran diluted in ethanol)	↓ IL-1β and TNF-α in blood mononuclear cells (black sorghum rich in 3-deoxyanthocyanidins)	Burdette, et al. (2010)
Oxidative stress and cancer	Hepatoma (Hepa1c1c7) and colon cancer (HT-29) cells	Extracts of red and black sorghum rich in 3-deoxyanthocyanidins	↑ NQO activity in Hepa1c1c7 cells ↓ Proliferation of HT 29 cells	Yang, L., et al. (2009)
	Cancer cells of esophagus (OE33) and colon (HT-29)	Extracts of 8 varieties of sorghum (with or without tannins)	↑ NQO activity (black and white varieties without tannins) ↓ Cell proliferation (black and white varieties without tannins)	Awika, et al. (2009)
	Hepatoma cells (Hepa1c1c7 cell)	Extract of black sorghum rich in 3-deoxyanthocyanidins (10% bran diluted in methanol 80%)	↑ NQO activity	González-Montilla, et al. (2012)
Cancer	Leukemia cells (HL-60)	Extract of sorghum rich in 3-deoxyanthocyanidins	↑ Cell apoptosis	Shih, C.-H., et al. (2007)
	Breast cancer cells (MCF-7)	Extract of red sorghum rich in 3-deoxyanthocyanidins (5% sorghum diluted in methanol HCl 1%)	↑ Cell apoptosis	Suganyadevia, et al. (2011a)

Table 2: continued

Related pathology	Cell type (if any)	Sorghum fraction used and preparation form	Main results	References
	Not applied	Extracts of sorghum rich in 3-deoxyanthocyanidins or tannins	↑ Human aromatase activity	Hargrove, et al., (2011)
	Cancer cells of colon (HT 29) and liver (HEP G2)	Extract of red sorghum bran rich in 3-deoxyanthocyanidins (5% sorghum diluted in methanol HCl 1%)	↓ Cell proliferation	Suganyadevia, et al. (2011b)
	Leukemia cells (HL-60)	Extract of red sorghum bran rich in 3-deoxyanthocyanidins	↑ Activation of BAK and BAX, release of mitochondrial cytochrome C and apoptosis-inducing factor into the cytoplasm, and activation of caspase-9 and caspase-3 ↑ Cell apoptosis	Woo, et al. (2012)
	Breast cancer cells (MDA-MB 231 and MCF-7)	Extract of phenolic compounds of sorghum	↓ Phosphorylation of STAT5 and STAT3, and the expression or release of insulin-like growth factor 1 and VEGF proteins ↑ Expression of cyclin D, cyclin E, and pRb; Brk, p53, and hypoxia-inducible factor 1α	Park, Jin Hee, et al. (2012)
	Malignant cells of colonocytes	Extracts of white (rich in flavones), red and black sorghum (rich in 3-deoxyanthocyanidins)	↑ Luciferase and caspase-3 activity	Yang, L., et al. (2012)
	Breast cancer cells (MCF-7)	Extract of red sorghum bran rich in 3-deoxyanthocyanidins	↑ p53 gene expression ↓ Bcl-2 gene expression ↓ Cell proliferation	Suganyadevia, et al. (2013)
Hypertension	Not applied	Hydrolyzed proteins (α-kafirins) of sorghum	↓ Angiotensin I converting enzyme activity	Kamath, V., et al. (2007)

Oxidative stress

The chronic and excessive production of free radicals is crucial in the development of noncommunicable diseases (Hotamisligil, 2006; Lee, S., et al., 2011). The activity of components isolated from sorghum against oxidative stress has been demonstrated in vitro (Table 2). These functional benefits are attributed to the phenolic compounds and are most evident when extracts from black or red sorghum were used (Burdette, et al., 2010; Moraes, et al., 2012a).

Phenolic compounds isolated from sorghum regulate the expression of phase II enzymes (Awika, et al., 2009; González-Montilla, et al., 2012; Yang, L., et al., 2009). These enzymes modulate the defense system against oxidative stress by continuously converting highly reactive electrophilic species (RES) into non-toxic and excretable metabolites (González-Montilla, et al., 2012; Takabe, et al., 2006).

The main effect of sorghum on phase II enzymes is to increase the NADH:quinone oxyreductase (NQO) activity (Figure 3). This effect is attributed to sorghum 3-deoxyanthocyanidins and depends on their profile but not on the content (Awika, et al., 2009; Lewis, 2008; Suganyadevia, et al., 2011b; Yang, L., et al., 2009). Recent studies demonstrated that apigeninidin and luteolinidin did not show any significant NQO inducer activity (Awika, et al., 2009; Yang, L., et al., 2009). On the other hand, their 7-methoxylated forms were strong NQO inducers (Awika, et al., 2009; Yang, L., et al., 2009).

Varieties of black sorghum may exert greater effects on NQO due to the rich profile and high content of 3-deoxyanthocyanidins (Awika, et al., 2009; Lewis, 2008; Suganyadevia, et al., 2011b; Yang, L., et al., 2009). However, sorghum varieties with different pericarp color can also induce the activity of NQO. For example, white sorghum (KARI-Mtama), which has low levels of pigments, extractable phenolics, and antioxidant capacity, has relatively strong NQO inducers (Awika, et al., 2009; Yang, L., et al., 2009). This fact demonstrated that sorghum is a source of other phytochemicals, pigmented or not, that might act synergistically with 3-deoxyanthocyanidins and produce high inducer activity. Conversely, sorghum tannins have a very

poor ability to induce NQO and can inhibit the NQO activity caused by other phenolic compounds (Awika, et al., 2009).

The effects of sorghum on the oxidative stress in vivo are little known (Table 3). The superoxide dismutase activity (SOD) increased in normolipidemic rats fed with black sorghum bran (rich in 3-deoxyanthocyanidins) (Lewis, 2008). This increase appears to be strictly related to the action of 3-deoxyanthocyanidins present in the bran. Furthermore, white (rich in phenolic acids), brown (rich in tannins) or black (rich in 3-deoxyanthocyanidins) sorghum brans suppressed the glutathione peroxidase activity (GPx) (Lewis, 2008). However, in the single animal study done so far using whole grains, normolipidemic rats that consumed different sorghum varieties (white, brown rich in tannin, and red without tannin) showed no change in the SOD activity (Moraes, et al., 2012a). The absence of significant changes in the SOD activity (Moraes, et al., 2012a) may reflect the lower content of bioactive compounds in whole sorghum grain compared with the bran. Thus, the amount of bioactive compounds consumed by the rats treated with whole grain may have been lower than those fed sorghum bran.

On the other hand, the normolipidemic animals fed with whole red sorghum evaluated by Moraes, et al. (2012a) had lower concentrations of thiobarbituric acid reactive substances (TBARS) in their livers. This reduction suggested that whole sorghum inhibited the RES and that it can reduce oxidative stress through other mechanisms not evaluated in this study, including the increase of other antioxidant enzymes (i.e., catalase, GPx and SOD) and total antioxidant capacity. Furthermore, the under or overexpression of genes and proteins related to the oxidative system also may have contributed to these results.

Table 3: Description of the *in vivo* studies about the effects of the isolated fractions from sorghum on parameters related to chronic noncommunicable diseases.

Related pathology	Animal	Fraction and doses used	Duration of the study	Observed effects (effective treatment)	References
Obesity	Male New Zealand white rabbits	Low or high tannin sorghum added to the diet (60% of the diet)	4 weeks	↓ Weight gain, feed conversion ratio, and activities of the α-amylase, trypsin and lipase (high tannin sorghum) ↑ Food consumption, fecal nitrogen excretion (high tannin sorghum)	Al-Mamary, et al. (2001)
	Male New Zealand white rabbits	White or black (high tannin) sorghum grain (approximately 35%) added to the diet	5 weeks	↓ Weight gain (high tannin sorghum)	Muriu, et al. (2002)
Dyslipidemia and cardiovascular risk	Mice	30% of whole sorghum (30%) added to the diet	4 weeks	↑ Fecal excretion of bile acid and plasma HDL-c	Cho, et al. (2000)
	Hamster	Sorghum lipids (0.5, 1.0 and 5.0%) added to the diet	4 weeks	↓ Non-HDL cholesterol in plasma, esterified cholesterol and cholesterol absorption in liver (0.5 to 5.0%) ≡ Plasma HDL-c, phospholipids and free cholesterol in liver (0.5 to 5.0%); ↑ Hepatic triacylglycerol (5%) and fecal excretion of sterols (cholesterol) (0.5 to 5.0%)	Carr, et al. (2005)
	Hamster	Sorghum lipids (0.5, 1.0 and 5.0%) added to the diet	4 weeks	↓ Non-HDL cholesterol in plasma, free and esterified cholesterol in liver; ≡ Hepatic phospholipid (5%); Hepatic gene expression of NPC1L1, SRB1, SREBP2, HMGR, LDLR, and CYP7A1 ↑ Fecal excretion of sterols (cholesterol) and liver triacylglycerol; ABCA1 gene expression	Hoi, et al. (2009)
	Hyperlipidemic rats	Extracts of phenolic compounds in dichloromethane and ethyl acetate.; Oral intake of 50 and 300 mg/kg	2 weeks	↓ Triacylglycerol, LDL-c and total cholesterol in plasma (50 and 300 mg/kg of extract in ethyl acetate) ↑ HDL-c in plasma (50 and 300 mg/kg of extract in ethyl acetate)	Chung, et al. (2011b)
Diabetes, dyslipidemia and cardiovascular risk	Diabetic rats	Extracts of phenolic compounds in acetonitrile. Oral intake of 100 and 250 mg / kg	2 weeks	↓ Glycaemia, triacylglycerol and total cholesterol in plasma (250 mg/kg) ↑ Insulin, urea, uric acid and creatinine in plasma (250 mg/kg)	Chung, et al. (2011a)
	Diabetic rats	Extract of phenolic compounds Oral intake of 400 and 600 mg / kg	2 weeks	↓ Triacylglycerol, total cholesterol, LDL-c, glycaemia, area under the curve for glucose in plasma (400 and 600 mg/kg), PEPCK and p38 expression ≡ Glutamic oxaloacetic transaminase and glutamic pyruvic transaminase in plasma; insulin, GLUT4.	Kim, J. and Park (2012)

Table 3: Continued

Related pathology	Animal	Fraction and doses used	Duration of the study	Observed effects (effective treatment)	References
Dyslipidemia, cardiovascular risk and diabetes	Mice	Oral administration of 0.5% and 1% sorghum methanolic extract in mice fed a with high-fat diet	6 weeks	↓ Triacylglycerol, total cholesterol, LDL-c, glycaemia, insulin, area under the curve for glucose in plasma; adiponectina expression (0.5 to 1%) ↓ Tumor necrosis factor-α (1%). ≡ HDL-c, alanine and aspartate amino-transferase ↑ PPAR-γ expression (1%)	Park, Ji Heon, et al. (2012)
	Mice	Modified AIN-93 M diet (37) supplemented with 0%, 1% and 5% grain sorghum lipid	3 weeks	↑ *Bifidobacterium* (mainly a phylotype related to *B. animalis*) and HDL-c ↓ *Coriobacteriaceae* (mainly unclassified phylotypes yet) and non-HDL-c	Martínez, et al. (2009)
Inflammation	Mice	Application of ethanolic extract of white, bronze, red and black sorghum in the ear of the mice	-	↓ Ear edema of mice, generated induced inflammation (bronze and black sorghum) and myeloperoxidase activity (black sorghum)	Burdette, et al. (2010)
	Rats	Oral administration of 0.62 a 5g/kg of golden gelatinous sorghum rich in phenolic compounds	2 weeks	≡ Albumin, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphates, creatinin, total protein, albumin, blood urea nitrogen, chloride, sodium, potassium, and calcium; cell structure and formation (liver, kidney, spleen, lungs, and heart) ↓ Nitric oxide in Raw264.7 cells ↓ Inducible nitric oxide synthase and anti-cyclooxygenase (COX)-2, 12-O-tetradecanoylphorbol-13-acetate-induced ear edema	Shim, et al. (2013)
Inflammation, oxidative stress, diabetes, dyslipidemia	Normolipidemic Wistar rats	Whole sorghum (21.2 to 26.4%) added to the hyperlipidic diet	5 weeks	↓ TBARS in liver, tumor necrosis factor-α in plasma (red sorghum without tannin) ≡ Glucose, fructosamine, cholesterol, HDL-c, triacylglycerol, alanine and aspartate amino-transferase, superoxide dismutase, IL-8 and IL-10	Moraes, et al. (2012a)
Cancer	Normolipidemic rats	Diets containing 6% of bran from white (contains phenolic acids), brown (contains tannins), or black sorghum (contains 3-deoxyanthocyanidins)	10 weeks	↓ Number of aberrant crypts (black and brown sorghums) ↑ Superoxide dismutase activy (black sorghum) and glutathione peroxidase activity (three genotypes)	Lewis (2008)
	Mice	Application of methanolic extract of sorghum rich in phenolic compounds by subcutaneous injection		↓ Cell growth and metastasis in breast cancer cells (MDA-MB 231 and MCF-7)	Park, Jin Hee, et al. (2012)

Cancer

Most cancers originate from DNA damage caused by carcinogens (toxics, mutagenic, and carcinogenic agents) that make up reactive intermediates, such as reactive oxygen species (ROS), reactive nitrogen species (RNS), and other reactive electrophilic metabolites (Sharma, S., et al., 2010; Shih, P.-H., et al., 2007). The carcinogen rate in humans is strongly dependent on the activity of the phase I (cytochrome P-450) and II of the enzyme systems, which also removed endogenous and environmental carcinogens (Takabe, et al., 2006). The benefits of sorghum on phase II enzymes cited in the previous section, especially on NQO reductase, demonstrate its chemoprevention (Table 2 and Figure 3). However, it is not possible to infer its effects in humans due to the lack of studies.

Elimination of tumors in early stages is considered an integral part of anticancer effects. The results of the study have demonstrated that phenolic compounds from sorghum, especially 3-deoxyanthocyanidins, act directly against cancer cells due to the increase of the apoptosis and inhibition of the growth and metastasis of cancer cells of skin melanoma, colon, esophagus, liver, breast, and bone marrow (Awika, et al., 2009; Hwang, J.-M., et al., 2013; Park, Jin Hee, et al., 2012; Shih, C.-H., et al., 2007; Suganyadevia, et al., 2011a; Woo, et al., 2012; Yang, L., et al., 2009). Sorghum 3-deoxyanthocyanidins are more cytotoxic to cancer cells than the respective analogous anthocyanidins present in other foods (cyanidin and pelargonidin) (Shih, C.-H., et al., 2007). In addition to 3-deoxyanthocyanidins, apoptosis of the colon cancer cells resulted from estrogenic activity of the flavones of sorghum (Yang, L., et al., 2012).

The mechanisms by which sorghum's phenolic compounds induce in vitro apoptosis included the overexpression of genes and apoptotic proteins (BAX e BAK proteins and p53 gene expression), increase in enzymes' activity (caspase-9 and caspase-3 activy), and inhibition of anti-apoptotic factors (Bcl2 gene, anti-apoptotic Bcl2 proteins, mitochondrial cytochrome C, and apoptosis-inducing factor) (Park, Jin Hee, et al., 2012; Suganyadevia, et al., 2011b, 2013; Woo, et al., 2012; Yang, L., et al., 2012). Moreover, the sorghum phenolics inhibit the growth and metastasis of cancer cells by reducing the phosphorylation of STAT5 and STAT3, and the expression or

the release of insulin-like growth factor 1 (IGF-1R) and *vascular endothelial growth factor* (VEGF) and increasing cell cycle inhibitors (expression of cyclin D, cyclin E, and pRb; Brk, p53, and HIF-1α - hypoxia-inducible factor 1α) (Park, Jin Hee, et al., 2012; Suganyadevia, et al., 2011a; Woo, et al., 2012). All of these events can inhibit cellular DNA synthesis, as was recently observed in skin melanoma cells (Hwang, J.-M., et al., 2013).

Furthermore, in addition to 3-deoxyanthocyanidins, sorghum has tannins that may have anticancer activity. Studies have shown that tannins isolated from other foods affect regulatory enzymes, blocking signal transduction pathways and inducing apoptosis; thus, they have attracted wide attention for cancer treatment (Huang, et al., 2009). However, sorghum tannins still need to be better studied. In an recent study, sumac sorghum bran extract rich in tannins inhibited human aromatase (CYP19) activity in vitro more strongly than black sorghum bran extract rich in 3-deoxyanthocyanidins (Hargrove, et al., 2011). This suggests that the tannins found in sumac sorghum are more potent inhibitors than the 3-deoxyanthocyanidins found in black sorghum, inhibiting and precipitating aromatase (Hargrove, et al., 2011). This enzyme is key to the synthesis of estrogen and is an important target for chemotherapy of breast cancer dependent on this hormone (Dowsett, et al., 2010).

The anticancer effects of sorghum in vivo have been little studied (Table 3). Recently, cellular growth and metastasis in breast cancer cells (MDA-MB 231 and MCF-7) in rats were reduced after the application of subcutaneously methanolic extract of sorghum rich in phenolic compounds (Park, Jin Hee, et al., 2012). The results of the single study that evaluated the anticancer effects of sorghum showed that whole grains of the black and brown varieties (rich in 3-deoxyanthocyanidins and tannins, respectively) reduced the number of aberrant crypts of mice (Lewis, 2008). Furthermore, sorghum rich in tannins increased the colonocytes apoptosis.

Obesity and inflammation

Obesity is a pandemic that correlates with various noncommunicable diseases. The results of the studies demonstrate that sorghum rich in tannins reduces weight gain in animals (rats, pigs, rabbits, and poultry) (Al-Mamary, et al., 2001; Muriu, et al., 2002). The lower weight gain is undesirable in

animals for slaughter, but can provide benefits against obesity in humans.

The lower weight gain in animals fed with sorghum rich in tannins results in part from the complexation of this compound to sorghum starch that helps lower caloric intake. Starch is the major component of cereals and the main source of calories in cereal products (Margareta Leeman, et al., 2006). A recent study demonstrated that polymeric tannins from sorghum can naturally modify starch by interacting strongly with amylose forming resistant starch (Barros, et al., 2013). Resistant starch cannot be digested in the small intestine and thus reaches the large intestine, delivering the health benefits of dietary fiber (Fuentes-Zaragoza, et al., 2010). Furthermore, sorghum tannins can inhibit starch digestion by inhibiting saccharase and amylase enzymes (Mkandawire, et al., 2013; Nyamambi, et al., 2000; Osman, 2004).

Another important factor that may also have contributed to this lower weight gain was the complexation of tannins with proteins as well as digestive enzyme inhibition (trypsin, chymotrypsin, and lipases) (Ali, et al., 2009; Barros, et al., 2013; Frazier, et al., 2010; Nyamambi, et al., 2000; Osman, 2004; Rahman & Osman, 2011; Taylor, J., et al., 2007). Proteins rich in proline bind more sorghum tannins than other proteins. In addition, a protein containing more proline repeats will bind more tannin than one with fewer such repeats (Medugu, et al., 2010). Despite the evidence in animals, it is unknown whether sorghum (rich in tannins or not) modulates human weight. It is highlighted that the high consumption of sorghum rich in tannins can reduce the bioavailability of iron and zinc (Towo, et al., 2006).

Obesity is characterized by a chronic low-grade inflammation (Gregor & Hotamisligil, 2011). Until recently, the role of fat itself in the development of the obesity and its consequences was considered to be a passive one and adipocytes were considered to be little more than storage cells for fat (Greenberg & Obin, 2006; Gregor & Hotamisligil, 2011). However, it is known that adipocytes and obesity play an important role on inflammatory mediators that signal this process. The discovery that obesity itself results in an inflammatory state in metabolic tissues opened a research field that examines the inflammatory mechanisms in obesity (Greenberg & Obin, 2006). This remarkable understanding allows a more clear definition of the role that adipocytes play in health and in obesity and how inflammatory

mediators act as signaling molecules in this process (Gregor & Hotamisligil, 2011).

In an in vitro study, the extracts of sorghum rich in 3-deoxyanthocyanidins inhibited the secretion of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and nitric oxide by human mononuclear cells activated with bacterial lipopolysaccharide (Burdette, et al., 2010) (Figure 3). These effects were not observed in varieties with tannins. However, in another study, sorghums rich in tannins were more effective than those rich in 3-deoxyanthocyanidins in inhibiting hyaluronidase, an important enzyme associated with inflammation (Bralley, et al., 2008). The greater inhibitory effect of tannins can be attributed to their ability to complex the enzymes (competitive inhibition). However, in this study the tannins inhibited hyaluronidase through a competitive binding (Bralley, et al., 2008) and this indicates that the tannins bind to binding sites of this enzyme.

The evaluation in vivo of the anti-inflammatory effects of sorghum is still incipient but the results are promising. The addition of whole red grains without tannins or its lipid fraction to a hyperlipidemic diet reduced the expression of TNF-α in rats (Moraes, et al., 2012a; Park, Ji Heon, et al., 2012). The functional benefits in humans due to the consumption of whole sorghum and its fractions are still unknown, but they may result in part from the increased expression of adiponectin, which inhibits this inflammatory marker (Park, Ji Heon, et al., 2012). However, the sorghum extract of sorghum rich in tannins reduced the formation of edema in rats via the down-regulation of cyclooxygenase-2 (COX-2) expression, resulting in lower vascular permeability and edema with infiltration of neutrophils (Burdette, et al., 2010; Shim, et al., 2013). Thus, the results of in vitro and animal studies suggest that the anti-inflammatory effects of sorghum stem from its action on enzymes while 3-deoxyanthocyanidins act mainly on cytokines.

Dyslipidemia

In vitro and animal studies have shown that the lipidic and phenolic fractions from sorghum modulate parameters related to dyslipidemia and the risk of cardiovascular disease. These benefits result from the action of phytosterols, polycosanols, and phenolic compounds, which may modulate absorption, excretion, and synthesis of cholesterol.

The supplementation of the diet with sorghum lipids reduced the hepatic and plasma cholesterol of normolipidemic hamsters (Carr, et al., 2005; Hoi, et al., 2009) (Table 2). The phytosterols are one of the major bioactive compounds from sorghum lipid fraction able to inhibit the cholesterol absorption (Figure 3). Studies demonstrated that phytosterols isolated from other foods inhibited cholesterol absorption in humans, leading to increased fecal excretion and reduced plasma LDL-c concentration (Amiot, et al., 2011, 2013). These compounds reduce the amount of cholesterol captured in the gut enterocytes by inhibiting its incorporation into micelles, thereby lowering cholesterol absorption (Jesch & Carr, 2006).

The lipid fraction may also affect cholesterol absorption by altering the gut microbiota (Martínez, et al., 2009). The addition of sorghum lipid fraction to the diet of hamsters increased the *Bifidobacterium spp* (mainly a phylotype related to *B. animalis*) and HDL-c, and reduced the family *Coriobacteriaceae* (mainly yet unclassified phylotypes) and non-HDL-c (Martínez, et al., 2009). The correlation between the family *Coriobacteriaceae* and both non-HDL-c and cholesterol absorption suggest that this family could have a negative impact on cholesterol homeostasis by increasing cholesterol absorption. On the other hand, *Bifidobacterium* correlated with HDL-c and had no association with cholesterol absorption (Martínez, et al., 2009). The mechanisms by which these bacteria affect cholesterol metabolism remain an important field of future research.

In addition to affecting the absorption of exogenous cholesterol, the sorghum lipidic fraction affects the synthesis and excretion of endogenous cholesterol. In one of the first in vitro studies about this subject, the sorghum lipid fraction inhibited in a dose-dependent manner the 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase activity, a key enzyme in cholesterol synthesis (Cho, et al., 2000). However, the ability to reduce the in vivo cholesterol synthesis through HMG-CoA reductase requires further investigation. The polycosanols are one of the compounds present in the sorghum lipid fraction that can reduce the HMG-CoA reductase activity. These compounds, isolated from other food matrices, decrease the activity of this enzyme as well as increase the LDL receptor activity (Marinangeli, et al., 2010).

Sorghum lipid fraction promotes the excretion of gut neutral sterols (i.e., cholesterol and its metabolites) and thus decreased the concentration of plasma cholesterol in normolipidemic hamsters (Hoi, et al., 2009). It is not yet known whether sorghum bioactive compounds, including polycosanols and phytosterols, affect cholesterol metabolism through mechanisms similar to those proposed for compounds isolated from other plants. Therefore, possible metabolic pathways affected by the sorghum lipid fraction, including the expression of genes and proteins, are not still understood and poorly studied.

A single study published to date evaluated the effects of the sorghum lipid fraction on the molecular level of cholesterol metabolism in hamsters. This study observed the overexpression of a gene related to the synthesis of HDL-c (ABCA1 - ATP-binding cassette transporter A1) (Hoi, et al., 2009). However, no changes were observed in the expression of genes related to cholesterol absorption (Niemann-Pick C1 like 1); cholesterol synthesis; (sterol regulatory element binding protein-2 and HMG-CoA reductase); and excretion of LDL-c and endogenous cholesterol (scavenger receptor class B type 1, low density lipoprotein receptor, cholesterol 7a-hydroxylase) (Hoi, et al., 2009). This lack of effect can indicate that the intervention time used in the study (4 weeks) was not enough to cause changes in these genes.

In addition to the lipid fraction, sorghum phenolic compounds also affect the metabolism of cholesterol. However, the mechanisms involved in these functional benefits have not been elucidated. The results of recent studies have demonstrated that the oral intake of freeze-dried extracts of phenolic compounds of sorghum (50 to 600 mg/kg for 14 days) also reduced the plasma concentration of cholesterol and triacylglycerol in rats (Chung, et al., 2011a; Chung, et al., 2011b; Kim, J. & Park, 2012). These functional benefits vary according to the sorghum variety and type of solvent used during preparation of the extracts (Chung, et al., 2011a; Chung, et al., 2011b).

Knowledge about the effects of whole sorghum on the lipid profile and the risk for developing cardiovascular disease in animals is incipient and in humans is nonexistent. In a study on mice, the addition of 30% whole sorghum to the diet increased the fecal excretion of bile acid and plasma

HDL-c (Cho, et al., 2000).

Diabetes

Recent results indicate that sorghum fractions modulate the glucose metabolism in animals due to the action of the phenolic compounds (Table 2 and 3). However, it is not known whether the components isolated from sorghum and especially the whole grain are beneficial to humans. In studies with mice, the intake of extracts of sorghum phenolic compounds reduced the area under the curve of glucose and glycaemia (Chung, et al., 2011a; Kim, J. & Park, 2012; Park, Ji Heon, et al., 2012). Due to its strong effect on plasma glucose and insulin, the studies in animals have shown that phenolic extracts of sorghum exhibited a hypoglycemic effect similar to glibenclamida, an antidiabetic medication used in the control group (Chung, et al., 2011a; Kim, J. & Park, 2012).

The mechanisms by which sorghum phenolic compounds act involve metabolic pathways before and after absorption of carbohydrates that can contribute to the prevention and treatment of glycemic disorders in humans (Figure 3). It was recently demonstrated that these extracts inhibited in vitro activity of the enzymes *B. stearothermophilus* α-glucosidase as well as human pancreatic and salivary α-amylase (Kim, J.-S., et al., 2011). Thus, the decrease in the rate of glucose digestion through inhibition of enzymes may be the first action mechanism of sorghum on human metabolism.

Study results suggest that phenolic compounds may also affect insulin-dependent pathways, including concentrations and sensitivity of this hormone in humans. The increase in insulin concentration was observed in diabetic mice that received extracts of phenolic compounds (Chung, et al., 2011a). This increase indicates better functioning of the β cells and it has clinical relevance, especially for Type 2 diabetics, whose insulin synthesis is decreased. Furthermore, oral administration of the sorghum phenolic extracts can prevent and act as an adjuvant factor in the treatment of diabetes through an improvement in insulin sensitivity. This hypothesis is based on the fact that the extract of phenolic compounds from sorghum have induced antidiabetic effects in mice fed with a hiperlipidic diet through a mechanism that increased adiponectin and decreased TNF-α via overexpression of PPAR-γ, leading to improved insulin sensitivity (Park, Ji Heon, et al., 2012).

Furthermore, it is suggested that phenolic compounds reduce blood glucose concentration by inhibiting hepatic gluconeogenesis due to the down expression of the PEPCK and p38 genes and overexpression of the AMPK gene (Kim, J. & Park, 2012). However, sorghum extract had no significant effect on glucose uptake by skeletal muscle determined by GLUT4 translocation and Akt phosphorylation (Kim, J. & Park, 2012). It is not known whether sorghum exerts an effect on protein expression during glucose hepatic production and glucose uptake by skeletal muscle.

In addition to acting in basic processes of diabetes, the ethanolic extracts obtained from sorghum bran rich in phenolic compounds and with high antioxidant activity inhibit the glycation of proteins up to 60% (Farrar, et al., 2008). However, sorghum bran extract with low antioxidant activity and content of phenolic compounds, as well as bran of rice, oats, and wheat, did not inhibit this process. The glycation products are associated with diabetes and insulin resistance and may increase the formation of reactive oxygen species and the activation of the nuclear factor-κβ (NF- κβ) (Yamagishi, 2011).

Despite numerous in vitro evidence about the potential of bioactive compounds from sorghum to modulate parameters related to diabetes, only one published study to date evaluated the effects of whole sorghum. However, in this study, normolipidemic rats that ingested from 1 to 5% of whole sorghum added to a hyperlipidemic diet showed no changes in blood glucose, glycated proteins, and triacylglycerol (Moraes, et al., 2012a). The absence of significant changes in the study cited above may have resulted from a consumption of bioactive compounds less than those obtained in in vivo studies that used sorghum fractions. Therefore, these results indicate that consumption of whole sorghum may not promote significant changes in a short period of time during consumption of a nutritionally unbalanced diet. It is remarkable that the results do not refute the possibility that sorghum can promote preventive and therapeutic effects on other markers not analyzed in this study, including gene expression and conjugated protein to balanced and unbalanced diets.

Hypertension

Recently there is an indication in the scientific literature that sorghum

can reduce blood pressure (Table 2). In this study, an isolate of sorghum α-kafirins inhibited in competitive and non-competitive ways the activity of the angiotensin I converting enzyme (Kamath, V., et al., 2007).

Gut microbiota

The human gut is populated by an array of bacterial species, which develop important metabolic and immune functions, with a marked effect on the nutritional and health status of the host (Clemente, et al., 2012; Laparra & Sanz, 2010). The functional benefits of phenolic compounds of foods on human health may result from direct action of the absorbed bioactive compounds (and their metabolites) or indirect effects mediated by non-absorbed compounds that modify the microbiota environment and, consequently, human metabolism or could act at the membrane border inducing signal transduction pathways (Fernandes, et al.). The probable effects of bioactive compounds sorghum on the gut microbiota are unknown. It is important to analyze these effects during interventions in humans.

There is scientific evidence that unabsorbed phenolic compounds and their metabolites contribute to the maintenance of gut health by the modulation of the gut microbial balance through the stimulation of the growth of beneficial bacteria and the inhibition of pathogen bacteria, exerting prebiotic-like effects (Cardona, et al., 2013; Clemente, et al., 2012; Larrosa, et al., 2009; Requena, et al., 2010). Among the compounds, tannins are of special interest due to their high abundance and because, even though they are not absorbed in the large intestine, they are metabolized by the colonic microbiota (Requena, et al., 2010). Furthermore, sorghum has resistant starch and dietary fiber, which can modify gut microbiota (Martínez, et al., 2010; Scott, et al., 2008).

Although a great range of health-promoting activities of dietary phenolic compounds has been widely investigated, further scientific investigation is still needed in relation to their effect on modulation of gut microbiota (Cardona, et al., 2013; Requena, et al., 2010). Several studies demonstrated the effects of phenolic compounds from foods, including tannins and anthocyanins, on gut microbiota increasing the *Bifidobacterium spp* and *Lactobacillus spp* and decreasing the *Bacteroides spp*, *Clostridium spp*, *Propionibacterium spp*, *Salmonella typhimurium,*

Streptococcus mutans, and *Escherichia Coli* (Dolara, et al., 2005; Duarte, et al., 2006; Hidalgo, et al., 2012; Lee, H. C., et al., 2006; Tzounis, et al., 2011). The effects of sorghum, including varieties rich in tannins and 3-deoxyanthocyanidins, on gut microbiota are a field still to be explored. To date only one study has evaluated the relationship between bioactive compounds in sorghum in the gut microbiota of hamsters (Martínez, et al., 2009).

FINAL CONSIDERATIONS

Sorghum has a high nutritional value and is basically composed of starch, which is more slowly digested than that of other cereals, low digestibility proteins (mainly kafirins), unsaturated lipids, and is a source of some minerals (phosphorus, potassium, and zinc) and some B-complex vitamins (thiamine, riboflavin, and pyridoxine) and fat-soluble vitamins (D, E, and K). Furthermore, some varieties, especially the red, brown, and black colors, have a high content of phenolic compounds, especially 3-deoxyanthocyanidins and tannins, which are beneficial to human health.

The results of in vitro studies have demonstrated that compounds isolated from sorghum, particularly 3-deoxyanthocyanidins, tannins, and lipids, play a strong modulatory effect on gut microbiota and processes related to noncommunicable diseases (obesity, diabetes, dyslipidemia, cardiovascular disease, cancer, and hypertension). However, knowledge about the sorghum-specific bioactive compounds that promote these functional benefits is incipient.

Studies are needed to determine the preventive and therapeutic effects of sorghum whole grain and its fractions on human health, including gene and protein expression. It should be noted that the response of the human organism to these compounds may be dependent on their bioavailability. Thus, evaluating the bioavailability of sorghum’s bioactive compounds is essential to determining the benefits of sorghum grains and bioactive compounds on human health.

The profile of bioactive compounds has been shown to be a determinant factor of the functional potential of sorghum varieties. In this context, the selection of varieties of sorghum and practical optimization

should be performed to ensure the accumulation of bioactive components that will maximize the benefits of sorghum in humans. Furthermore, the behavior assessment of bioactive compounds in different processing conditions is essential to define the manner of use in which sorghum promotes maximum benefits to human health.

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3.2 Article 2: Tocochromanols and carotenoids in sorghum (*Sorghum bicolor* L.): diversity and stability to the heat treatment

Tocochromanols and carotenoids in sorghum (*Sorghum bicolor* L.): Diversity and stability to the heat treatment

ABSTRACT

The content and stability (retention) to dry heat in a conventional oven (DHCO) and extrusion of tocochromanols and carotenoids in sorghum genotypes were evaluated. One hundred sorghum genotypes showed high variability in tocochromanol content (280.7-2,962.4 µg/100g in wet basis) and 23% of the genotypes were classified as source of vitamin E. The total carotenoid varied from 2.12 to 85.46 µg/100g in one hundred sorghum genotypes. According to the genetic variability for carotenoids and tocochromanols, the 100 genotypes were grouped into 7 groups. The retention of the total tocochromanols and α-tocopherol equivalent decreased after extrusion (69.1-84.8% and 52.4-85.0%, respectively) but increased after DHCO (106.8-114.7% and 109.9-115.8%, respectively). Sorghum carotenoids were sensitive to extrusion (30.7-37.1%) and DHCO (58.6-79.2%). In conclusion, the tocochromanols profile in sorghum varied widely and the genotypes presented high genetic variability for carotenoids and tocochromanols. Sorghum was a source of tocochromanols, which increased after DHCO and decreased after extrusion. The carotenoid content in sorghum decreased after DHCO and extrusion.

Keywords: *Sorghum bicolor* L., carotenoid stability, vitamin E stability, extrusion, dry heat in a conventional oven

CHEMICAL COMPOUNDS STUDIED IN THE ARTICLE

Lutein (PubChem CID: 5281243); Zeaxanthin (PubChem CID: 53477763); α-

tocopherol (PubChem CID: 14985); β-tocopherol (PubChem CID: 6857447); γ-tocopherol (PubChem CID: 92729); δ-tocopherol (PubChem CID: 92094); α-tocotrienol (PubChem CID: 5282347); β-tocotrienol (PubChem CID: 5282348); γ-tocotrienol (PubChem CID: 5282349); δ-tocotrienol (PubChem CID: 5282350).

INTRODUCTION

Sorghum (*Sorghum bicolor* L.) is a source of phenolic compounds (i.e., 3-deoxyanthocyanidins and tannins) which are partially responsible for its high antioxidant activity, and benefits on human gut microbiota and markers related to chronic diseases such as obesity and cancer (Cardoso, Pinheiro, Martino & Pinheiro-Sant'Ana, In press; Taylor, Belton, Beta & Duodu, 2013). In addition, sorghum contains carotenoids and is a source of vitamin E, which contribute to its antioxidant activity (Cardoso, Montini, Pinheiro, Pinheiro Sant'Ana, Martino & Moreira, 2014).

Tocochromanols (α, β, γ and δ tocopherols and tocotrienols) naturally occurs in cereals and is associated with a lower risk for cardiovascular disease, cancer and dyslipidemia manifestation (Nielsen & Hansen, 2008; Tiwari & Cummins, 2009). The isomeric composition and tocochromanols content in sorghum vary significantly (Cardoso et al., 2014; Martino et al., 2012; Pinheiro-Sant'Ana, Guinazi, Oliveira, Della Lucia, Reis & Brandão, 2011). However, this variability has not been evaluated in a representative sorghum panel.

Sorghum carotenoids are composed mainly of xanthophyll (70% of lutein and zeaxanthin) (Kean, Bordenave, Ejeta, Hamaker & Ferruzzi, 2011), which reduce the risk of cardiovascular disease, age-related macular degeneration and other health problems (Perera & Yen, 2007). Due to the high functional potential of carotenoids, studies have been conducted to increase the content of these compounds in sorghum (Fernandez et al., 2009; Lipkie et al., 2013).

Genetic and environmental factors (*i.e.*, soil and climate) determine the profile of carotenoids and vitamins in sorghum (Chung, Yong, Lee & Kim, 2013; Fernandez et al., 2009). However, sorghum needs to be processed

before consumption, which can modify the content of β-carotene and tocopherols (Afify, El-Beltagi, El-Salam & Omran, 2012; Ochanda, Onyango, Mwasaru, Ochieng & Mathooko, 2010). Knowledge of changes to the profile and content of carotenoids and tocochromanols in sorghum is limited and the effects of dry heat in a conventional oven and by extrusion must be studied.

Processing in a conventional oven is widely used at the domestic level and can reduce carotenoids and increase tocochromanols in sorghum (Cardoso et al., 2014). However, it is not known if these effects differ according to the sorghum variety. Extrusion is the primary processing method used in the food industry (Brennan, Brennan, Derbyshire & Tiwari, 2011; Riaz, Asif & Ali, 2009; Tiwari et al., 2009). The effects of these processing methods on carotenoids and tocochromanol contents in sorghum have not been investigated yet.

Considering the assumption that processing can affect the content and profile of bioactive compounds in sorghum, the present study aimed to evaluate the profile and content of carotenoids and tocochromanols in one hundred sorghum genotypes, as well as the stability of these compounds in three sorghum genotypes submitted to dry heat in a conventional oven and extrusion. Data from this study may provide information for the development of sorghum hybrids with high contents of carotenoids and tocochromanols, and the selection of processing techniques that minimize losses of these compounds.

MATERIALS AND METHODS

Raw sorghum

One hundred sorghum genotypes (*Sorghum bicolor* L.) were selected from a core collection with high genetic variability from Embrapa Maize and Sorghum (Sete Lagoas, MG, Brazil). The genotypes were selected based on the pericarp color of the grain, aiming to obtain a heterogeneous group with regard to this characteristic (Table 1).

The one hundred sorghum genotypes were grown in the experimental field of Embrapa Maize and Sorghum (Nova Porteirinha, MG, Brazil), between June and October 2010. The experimental plots were composed of two rows of three meters long, with spacing of 0.50 m between rows. The

fertilization at planting consisted of the application of 300 kg/ha of formulated 08-28-16 (NPK). After 25 days of planting, fertilization with 50 kg/ha N was performed.

Table 1: Pericarp color and origin of 100 sorghum genotypes.

Genotypes	Pericarp color	Origin	Genotypes	Pericarp color	Origin
01MN1589-B	Bronze	United States	SC175	Light brown	Ethiopia
Ajabsido	Gray	Sudan	SC192	Yellow	India
ATF13B	Bronze	Brazil	SC206	Yellow	India
ATF14B	Bronze	Brazil	SC21	Brown	Ethiopia
B.AZ9504	Brown	United States	SC214	Yellow	India
B.DLO357	Red	United States	SC22	Cream	Ethiopia
B.Tx2752	Bronze	United States	SC224	Brown	Ethiopia
B.Tx3042	Bronze	United States	SC319	Brown	Uganda
B.Tx399	Yellow	United States	SC320	Cream	Chad
B.Tx626	Red	United States	SC323	Gray	Sudan
B.Tx635	Cream	United States	SC325	Brown	United States
B.Tx642	Yellow	United States	SC328	Light brown	Uganda
B.Tx645	Red	United States	SC35	Yellow	Ethiopia
BR007B	Red	Brazil	SC373	Yellow	Nigeria
SA7000 Caprock	Bronze	United States	SC391	Yellow	Egypt
CSM-63	Gray	Mali	SC414_12	Bronze	n/a
Dorado	Cream	n/a	SC42	Brown	Ethiopia
EBA-3	Yellow	n/a	SC441	Yellow	Sudan
KAT83369	Cream	n/a	SC465	White	Arabia
LG70	Red	United States	SC467	Red	India
Lian Tang Ai	Light brown	China	SC49	Light brown	Sudan
N268B	Bronze	United States	SC502	Light brown	n/a
NSA440-Karper	Cream	n/a	SC51	Yellow	Sudan
P-721	Cream	United States	SC53	Red	Sudan
P898012	Gray	United States	SC562	Red	Sudan
R.Tx2783	Red	United States	SC566	Bronze	Nigeria
R.Tx431	Bronze	United States	SC574	Light brown	Pakistan
R.Tx435	Yellow	United States	SC58	Gray	Sudan
R9188	White	n/a	SC59	Light brown	Sudan
FC6608 Red Kafir Bazine	Red	United States	SC598	Bronze	Uganda
SA386 Redbine-60	Bronze	n/a	SC6	Brown	Ethiopia
SA5330-Martin	Bronze	n/a	SC60	Red	Sudan
SC1017	Cream	Ethiopia	SC603	Light brown	Tanzania
SC103	Brown	South Africa	SC63	Red	Sudan
SC1033	Bronze	Ethiopia	SC630	Red	Zambia
SC1038	Yellow	Ethiopia	SC641	Light brown	Uganda
SC108	Red	Ethiopia	SC648	Brown	South Africa
SC115	Brown	Uganda	SC655	Brown	South Africa
SC1155	Brown	Ethiopia	SC671	Yellow	Kenya
SC1158	Bronze	Ethiopia	SC672	Light brown	Zimbabwe
SC118	Light brown	Sudan	SC673	Cream	Zimbabwe
SC1201	Light brown	n/a	SC702	Light brown	Sudan
SC124	Brown	Ethiopia	SC720	Light brown	Kenya
SC1251	Cream	Sudan	SC725	Light brown	Japan
SC1271	Cream	Ethiopia	SC757	Cream	Botswana
SC1319	Brown	Ethiopia	SC782	Brown	India
SC1322	Light brown	Sudan	SC855	Yellow	Egypt
SC1328	Brown	Sudan	SC964	Light brown	Uganda
SC135	Brown	Ethiopia	Shan Qui Red	Red	China
SC1356	Bronze	Sudan	Tx2911	Red	United States

n/a: Information not available

Once harvested, the whole grains were sorted, packed in cool boxes and sent to laboratory. Subsequently, the whole grains were packed in polyethylene bags, covered with aluminum foil and stored at 18 ± 1 °C, until two months for analysis.

Based on the results of the analysis performed in 100 sorghum genotypes, three sorghum genotypes with the following features were selected: genotype SC319 (low content of carotenoids and vitamin E); genotype B.DLO357 (greatest content of vitamin E) and genotype SC391 (greatest content of carotenoids). These grains were grown between June and October 2011 using the same growing, post-harvest, and shipping conditions described previously. Subsequently, the whole grains were packed in polyethylene bags, covered with aluminum foil and stored at -18 ± 1 °C, for at most one week.

Standards and reagents

Carotenoid standards (lutein and zeaxanthin) (Sigma–Aldrich, St. Louis, MO, USA), tocochromanol standards (α, β, γ and δ-tocopherols and tocotrienols) (Calbiochem®, EMD Biosciences, Inc., USA) were used. Analytic grade reagents (VETEC, São Paulo, Brazil) were used for carotenoids and tocochromanols extraction. For analysis of these compounds, it was used HPLC grade reagents (acetone, hexane, isopropyl alcohol, glacial acetic acid) (Tedia, São Paulo, Brazil).

Processing of the sorghum grains

The whole grains of the one hundred sorghum genotypes were ground in a micro-rotor analytical mill (850 µm) (Marconi, MA 090, Brazil) and subsequently submitted to analysis in until 24 hours. The three sorghum genotypes (SC319, SC319, and B.DLO357) were subjected to the processes described below:

F1) Raw flour: The whole grains were ground in a micro-rotor analytical mill (850 µm) (Marconi, MA 090, Brazil). Subsequently, the flours were packed in polyethylene bags, covered with aluminum foil and stored at -18 ± 1 °C, for no more than 24 hours until analysis;

F2) Dry heat in a conventional oven/milling (Oven/ milling): The intact whole grains were packed in aluminum trays (34 cm x 62 cm x 5 cm) and

subjected to dry heat in a conventional oven (121 °C, 25 min). Subsequently, the grains were ground in a micro-rotor analytical mill (850 µm) (Marconi, MA 090, Brazil) and the flours were packed in polyethylene bags, covered with aluminum foil and stored at -18 ± 1 °C, for no more than 24 hours until analysis (Cardoso et al., 2014);

F3) Extrusion/milling: The whole grains were previously milled into flour which was processed in a co-rotating twin-screw model Evolum HT 25 (Clextral, Firminy, France) at constant screw speed of 600 rpm and temperature profile: 30, 30, 60, 90, 100, 100, 120, 120, 150 and 150 °C, from feeding to the outlet (Vargas-Solórzano, Carvalho, Takeiti, Ascheri & Queiroz, 2014). The screw diameter (D) was 25 mm and the total configured screw length (L) was 1000 mm, providing an overall L/D ratio of 40. The die had four round openings of 3.8 mm each in diameter and 9 mm in length. Dry sorghum flour was placed in the feeding zone by a twin-screw, loss-in-weight gravimetric feeder model GRMD15 (Schenck Process, Darmstadt, Germany), and monitored by Schenck Process Easy Serve software (Schenck Process, Darmstadt, Germany). Distilled water was injected between the first and second feeding zones through a port with 5.25 mm internal diameter from the start of the barrel using a plunger metering pump model J-X 8/1 (AILIPU Pump Co. Ltd., China) set to compensate moisture differences in the samples and provide a final moisture content of 12%. The samples were collected over 15–20 min. Subsequently, the flours were packed in polyethylene bags, covered with aluminum foil and stored at 18 ± 1 °C, for no more than 24 hours until analysis. Once obtained through the previously described processing, the moisture of the flours was determined by gravimetry after oven drying (Nova Etica, 4000, Brazil) at 105 °C. Next, the flours were packed in polyethylene bags and stored (- 20 ± 1 °C) in a freezer until analyzes.

Determination of carotenoids and tocochromanols

During analyzes of carotenoids and tocochromanols, the samples and the extracts were protected from light (sunlight and artificial) and oxygen using amber glassware, dark environment, and bottles with nitrogen gas environment and hermetically closed.

Carotenoids

The occurrence and content of carotenoids (lutein and zeaxanthin) were investigated. Carotenoids were extracted in acetone and transferred to petroleum ether according to Rodriguez-Amaya, Raymundo, Lee, Simpson and Chichester (1976), with modifications proposed by Cardoso et al. (2014).

For carotenoids analyzes, 25.0 mL of extract were evaporated under a flow of nitrogen gas, dissolved in 1.0 mL of hexane: isopropanol (90:10, v/v) and filtered in filter units with porosity of 0.45 µm. The analyzes were performed in a HPLC system (Shimadzu, SCL 10AT VP, Japan) equipped with high pressure pump (Shimadzu, LC-10AT VP, Japan), autosampler with loop of 500 µL (Shimadzu, SIL-10AF, Japan), diode array detector (DAD) (Shimadzu, SPD-M10A, Japan) and helium degassing system of the mobile phase (Shimadzu, DGU-2 A, Japan). The chromatographic condition used for the analysis was as follows (Panfili, Fratianni & Irano, 2004): HPLC system – DAD, scanning of the spectrum from 350–600 nm, detection at 450 nm, Luna Si100 column (250 x 4 mm i.d., 5 µm) fitted with Si100 guard column (4 mm x 3 mm) (Phenomenex, Torrance, CA), column at room temperature and injection volume of 50 *µ*L. The mobile phase consisted of hexane: isopropanol (95:5, v/v). The mobile phase flow was 1.3 mL/min, isocratic.

Carotenoids identification was based on the commercial standards retention times and UV-Vis spectra. The quantification was performed by comparing peak areas with that obtained in the analytical curve constructed from the injection, in duplicate, of six different concentrations of standard solutions (lutein: 0.03 - 2.43 µg, R^2: 0.9984, Limit of detection (LOD): 6.86 µg/mL, Limit of quantification (LOQ): 34.30 µg/mL; zeaxanthin: 0.04 - 1.37 µg, R^2: 0.9899, LOD: 4.79 µg/mL, LOQ: 23.95 µg/mL). The carotenoids were expressed in µg/100g in wet basis (wb), as single compounds and as the sum of lutein + zeaxanthin.

Tocochromanols

The occurrence and content of tocochromanols (α, β, γ and δ-tocopherols and tocotrienols) were investigated. Tocochromanols in sorghum were extracted using an extractor solution (hexane: ethyl acetate; 85:15 v/v), isopropyl alcohol, ultrapure water, hexane with 0.05% of BHT and anhydrous sodium sulfate (Pinheiro-Sant'Ana et al., 2011).

After extraction, aliquots of 5.0 mL of the extract were dried in nitrogen gas, dissolved in 2.0 mL of HPLC grade hexane and filtered in filter units with porosity of 0.45 µm. The analyzes were performed in a HPLC system (Shimadzu, SCL 10AD VP, Japan) composed of a high pressure pump with quaternary gradient valve for low pressure (Shimadzu, LC-10AD VP), autosampler with loop of 50 µL (Shimadzu, SIL-10AF), helium degassing system of the mobile phase (Shimadzu, DGU-2 A), and fluorescence detector (Shimadzu, RF10AXL). The chromatographic condition used for the analysis was as follows (Pinheiro-Sant'Ana et al., 2011): HPLC system; fluorescence detection (290 nm excitation and 330 nm emission); Luna Si100 column (250 x 4 mm i.d., 5 µm) fitted Si100 guard column (4 mm x 3 mm) (Phenomenex, Torrance, CA), column at room temperature, injection volume of 20 *µ*L. The mobile phase was composed by hexane: isopropanol: glacial acetic acid (98.9:0.6:0.5, v/v/v) and the flow was 1.0 mL/min, isocratic.

Identification of the tocochromanols found in sorghum was based on commercial standards retention times and co-chromatography. The quantification was performed by comparing peak areas with that obtained in the analytical curves constructed from injection, in duplicate, of six different concentrations of standard solutions (α-tocopherol: 1.02-104.21 ng, R^2 = 0.999; α-tocotrienol: 3.21-157.60 ng, R^2 = 0.997; β-tocopherol: 1.76-127.92 ng, R^2 = 0.999; β-tocotrienol: 2.83-149.69 ng, R^2 = 0.999; γ-tocopherol: 2.23-107.61 ng, R^2 = 0.989; δ-tocopherol: 2.08-13.61 ng, R^2 = 0.999 and δ-tocotrienol: 2.70-12.76 ng, R^2 = 0.998). The LOD and LOQ of the tocochromanols were 0.02-0.07 µg/mL and 0.11-0.36 µg/mL, respectively.

The α-tocopherol equivalents were calculated using the equation: (α-tocopherol × 1.0) + (β-tocopherol × 0.5) + (γ-tocopherol × 0.1) + (δ-tocopherol × 0.03) + (α-tocotrienol × 0.3) + (β-tocotrienol × 0.05) (U. S. Institute of Medicine, 2000). The tocochromanol content was expressed in µg/100g wb, as single tocochromanols and as total tocochromanols (sum of the tocochromanols).

True retention of compounds

For the calculation of retention of antioxidant compounds, all the processed flours were weighed on an analytical balance (GEHAKA, AG 200) before and after heat treatment. The true retention was calculated by the

equation of Murphy, Criner and Gray (1975).

Experimental design and statistical analysis

The profile of carotenoids and tocochromanols of 100 sorghum genotypes and the genetic variability of the genotypes for these characteristics were analyzed using a completely randomized design (CRD). The effects of the heat treatment on the carotenoids and tocochromanols of sorghum were evaluated using CDR, in a 3 x 3 factorial scheme (3 types of processing: milling, dry heat in a conventional oven, and extrusion; and 3 sorghum genotypes: SC319, SC391, and B.DLO357). All tests were performed in three repetitions.

The genetic variability of carotenoids and tocochromanols in 100 sorghum genotypes were assessed by Tocher's clustering technique using the average Euclidean distance as dissimilarity measure. Statistical analyzes were performed using the software Genes (Cruz, 1998). Data normality on the stability of carotenoids and tocochromanols in sorghum was assessed using the Shapiro-Wilk test and differences between treatments were evaluated by ANOVA. Duncan test was used to compare the treatment averages. These statistical analyzes were performed using the SAS package (Statistical Analysis System), version 9.2 (2008), at 5% probability.

RESULTS AND DISCUSSION

Tocochromanols and carotenoids in 100 sorghum genotypes

Tocochromanols profile

Sorghum showed a very diverse tocochromanol profile and content (Figure 1A, Table 2, and Supplementary Table 1). Eight tocochromanols were identified in sorghum, and most genotypes (69%) simultaneously showed four to six compounds. γ-Tocopherol was the most frequent compound (100% of the genotypes) followed by α-tocopherol (97% of the genotypes). β-Tocotrienol was the least tocochromanol incidence (12% of genotypes).

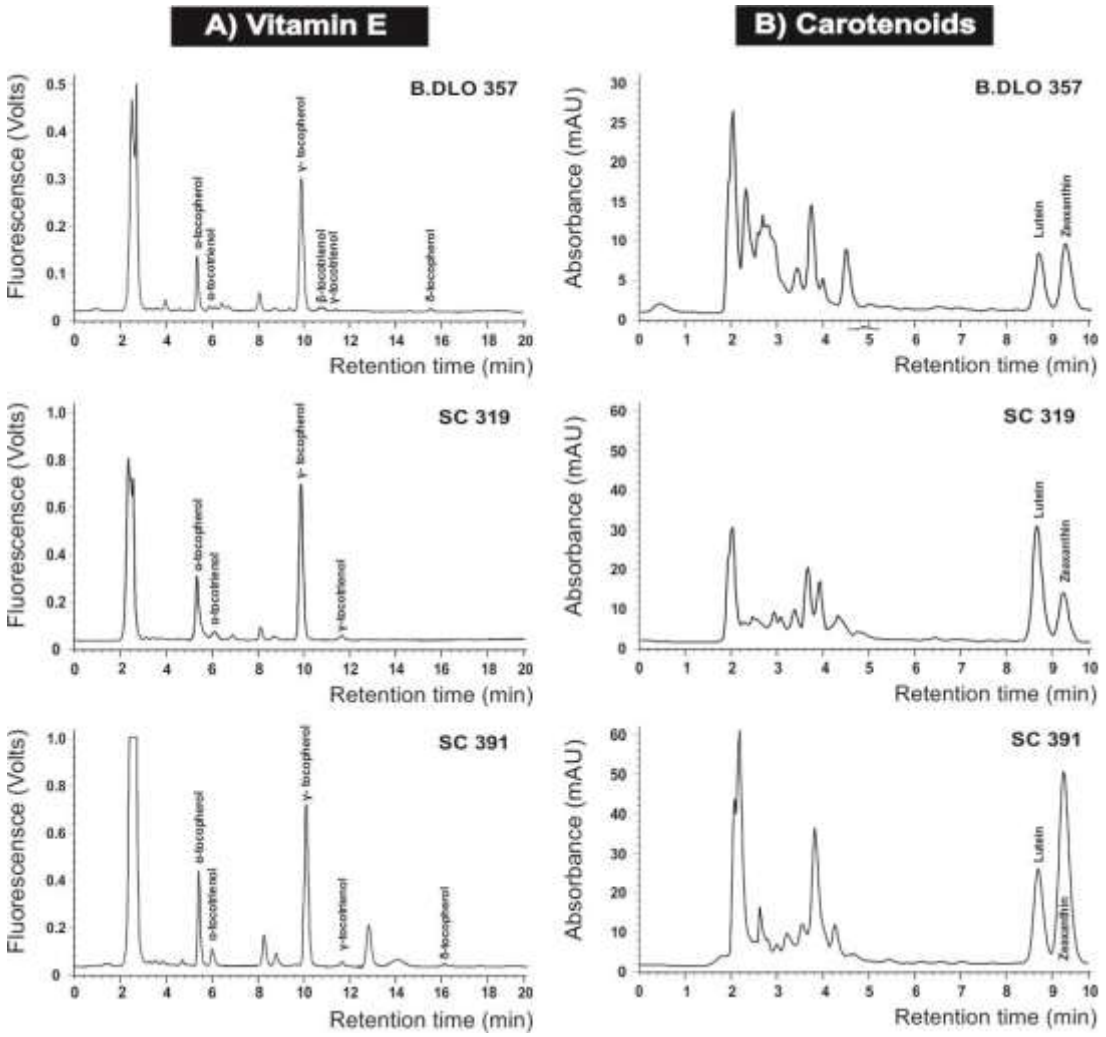

Figure 1: Profile of vitamin E (A) and carotenoids (B) in standards and three sorghum genotypes obtained by HPLC. Chromatographic conditions: according to materials and methods.

Table 2: Occurrence (%) and content of tocochromanols, α-tocopherol equivalent (Vitamin E) and carotenoids (µg/100g in wet basis) in 100 sorghum genotypes

Variables	Vitamin E										Carotenoids		
	α-T	α-T3	β-T	γ-T	β-T3	γ- T3	δ-T	δ- T3	Total	α-TE	Lutein	Zeaxanthin	Lutein + Zeaxanthin
Occurrence	99.0	85.0	53.0	100.0	12.0	88.0	69.0	54.0	100	--	100	100	--
Occurrence as majority component	2.0	0.0	1.0	97.0	0.0	0.0	0.0	0.0	--	--	8	92	--
Minimum	0.0	0.0	0.0	174.6	0.0	0.0	0.0	0.0	280.7	28.1	0.44	1.44	2.12
Maximum	1231.6	311.9	784.7	2109.0	850.5	270.5	379.8	484.2	2962.4	1359.2	63.37	58.85	85.5
Mean	406.7	120.4	58.1	1153.0	35.7	42.2	42.8	29.1	1888.0	590.5	6.81	15.48	22.29

Supplementary Table 1: Content of vitamin E and carotenoids (µg/100g in wet basis) in 100 sorghum genotypes

Genotypes	Vitamin E										Carotenoids		
	α-T	α-T3	β-T	γ-T	β-T3	γ- T3	δ-T	δ- T3	Total	α-TE	Lutein	Zeaxanthin	Sum
01MN1589-B	512.8 ± 6.6		36.7 ± 0.3	1719.7 ± 22.5		27.4 ± 0.3	92.5 ± 0.9	37.6 ± 0.2	2426.6 ± 30.9	705.9 ± 9.1	5.2 ± 0.1	7.6 ± 0.1	12.8 ± 0.1
Ajabsido	443.8 ± 21.6	136.7 ± 5.4	18.0 ± 0.5	1496.5 ± 73.4		39.4 ± 1.9	32.5 ± 0.7	22.2 ± 0.4	2189.0 ± 103.7	644.9 ± 30.8	4.8 ± 0.0	14.1 ± 0.0	18.9 ± 0.1
ATF13B	192.1 ± 2.4	114.2 ± 1.1		897.8 ± 11.8					1204.0 ± 15.3	366.6 ± 3.94	1.2 ± 0.0	8.9 ± 0.1	9.0 ± 0.1
ATF14B	822.2 ± 14.7	78.9 ± 1.4		1130.8 ± 20.2		37.5 ± 0.7	30.6 ± 0.4		2100.0 ± 36.9	959.9 ± 17.9	1.5 ± 0.0	8.1 ± 0.0	9.6 ± 0.0
B.AZ9504	433.0 ± 17.0	146.9 ± 7.1		1064.1 ± 15.0		20.2 ± 7.1	26.1 ± 0.2	17.5 ± 5.6	1707.8 ± 42.7	584.5 ± 17.8	12.1 ± 0.2	22.6 ± 0.2	34.7 ± 0.4
B.DLO357	1231.6 ± 4.0	120.9 ± 4.4		838.5 ± 10.9	119.2 ± 5.2	20.4 ± 0.2	49.9 ± 1.3		2380.5 ± 10.5	1359.2 ± 3.9	2.7 ± 0.0	5.5 ± 0.1	7.0 ± 0.1
B.Tx2752	306.8 ± 26.5	300.6 ± 24.6	80.2 ± 6.5	926.0 ± 79.5	543.9 ± 47.7	47.2 ± 3.0	66.4 ± 4.8	48.4 ± 3.4	2319.5 ± 196.4	558.9 ± 47.2	0.7 ± 0.0	1.4 ± 0.0	2.1 ± 0.0
B.Tx3042	482.3 ± 19.7	128.6 ± 3.9	33.0 ± 0.9	1263.5 ± 52.0		23.1 ± 0.9			1930.3 ± 77.5	663.7 ± 26.6	3.2 ± 0.1	9.7 ± 0.1	12.8 ± 0.1
B.Tx399	489.2 ± 10.3	168.9 ± 17.6		1407.0 ± 13.9		130.2 ± 3.2		37.1 ± 2.4	2233.4 ± 38.0	680.6 ± 16.2	4.8 ± 0.1	19.5 ± 0.1	24.5 ± 0.2
B.Tx626	661.5 ± 29.8	66.9 ± 16.4		982.1 ± 14.5		40.5 ± 1.1	39.1 ± 0.6		1790.1 ± 6.5	780.0 ± 24.7	0.4 ± 0.0	5.0 ± 0.1	5.5 ± 0.1
B.Tx635	10.7 ± 0.2	105.8 ± 2.5		958.0 ± 6.4		46.8 ± 0.9			1122.2 ± 6.9	138.3 ± 1.2	1.5 ± 0.0	18.7 ± 0.1	20.2 ± 0.1
B.Tx642	588.79 ± 18.7	175.0 ± 19.8		1669.9 ± 58.9		82.0 ± 7.3		21.5 ± 4.0	2537.2 ± 66.8	808.5 ± 20.7	2.9 ± 0.0	13.4 ± 0.1	16.4 ± 0.2
B.Tx645	721.8 ± 29.4	234.4 ± 8.2	22.5 ± 0.5	911.6 ± 36.7		41.9 ± 1.6	49.7 ± 1.1	27.2 ± 0.4	2009.1 ± 77.8	896.0 ± 35.6	0.67 ± 0.0	4.5 ± 0.1	5.2 ± 0.1
FC6608 Red Kafir Bazine	244.1 ± 55.2	135.9 ± 9.2		1063.6 ± 27.1		71.6 ± 0.4		18.7 ± 0.6	1533.9 ± 86.4	391.5 ± 59.5	1.5 ± 0.0	11.1 ± 0.1	12.6 ± 0.1
BR007B	938.3 ± 8.6	68.6 ± 0.5	13.4 ± 0.0	988.6 ± 9.1		12.6 ± 0.1	30.8 ± 0.1	40.5 ± 0.2	2092.7 ± 18.5	1065.4 ± 9.65	20.1 ± 0.2	6.9 ± 0.1	26.9 ± 0.5
SA7000 caprock	54.7 ± 1.6		461.1 ± 16.3	401.9 ± 13.0		50.1 ± 1.7		21.5 ± 0.0	989.0 ± 33.67	325.5 ± 11.2	3.8 ± 0.6	8.8 ± 1.1	12.6 ± 1.65
CAT83369	469.0 ± 47.0	169.7 ± 11.4		1328.2 ± 28.4		89.4 ± 0.3		23.4 ± 0.7	2079.6 ± 52.7	652.7 ± 44.8	2.8 ± 0.1	12.4 ± 0.1	15.5 ± 0.1
CSN-63	474.4 ± 18.1		22.9 ± 0.5	1607.9 ± 64.5		140.5 ± 5.6	137.0 ± 4.6	25.9 ± 0.5	2408.5 ± 93.9	650.8 ± 25.6	2.7 ± 0.0	10.9 ± 0.1	13.6 ± 0.2
Dorado	373.4 ± 14.8	113.1 ± 3.1	38.5 ± 1.1	1121.1 ± 44.4		63.4 ± 2.5		22.2 ± 0.1	1731.7 ± 65.9	538.7 ± 20.6	1.5 ± 0.0	9.0 ± 0.1	11.5 ± 0.1
EBA-3	67.8 ± 1.3			2109.0 ± 10.0				20.0 ± 0.2	2197.8 ± 12.1	278.8 ± 2.5	9.2 ± 0.2	22.5 ± 0.2	31.8 ± 0.3
LG70	195.1 ± 7.3	311.9 ± 10.9	81.3 ± 2.8	1306.8 ± 51.0	186.6 ± 8.2	66.4 ± 2.5	69.8 ± 1.8	395.5 ± 14.9	2613.2 ± 99.4	471.4 ± 17.6	2.3 ± 0.0	6.9 ± 0.0	9.2 ± 0.0
Lian Tang Ai	302.0 ± 13.6			1643.0 ± 74.9			43.9 ± 1.1		1989.9 ± 89.67	468.6 ± 21.1	9.1 ± 0.2	14.1 ± 0.1	23.2 ± 0.5
SA5330-Martin	558.2 ± 7.9	82.0 ± 0.7	28.9 ± 0.5	1497.5 ± 21.4		79.7 ± 1.1	51.6 ± 0.4		2298.9 ± 31.9	748.9 ± 10.5	1.2 ± 0.0	6.5 ± 0.0	7.5 ± 0.1
N268B	32.0 ± 9.1	52.5 ± 17.1		1597.2 ± 10.4	493.8 ± 0.5	18.6 ± 44.1			2194.1 ± 40.6	232.2 ± 6.5	13.9 ± 0.4	47.65 ± 0.5	61.6 ± 0.5
NSA440- Karper	284.1 ± 14.7	133.0 ± 31.6		910.7 ± 8.2		21.9 ± 3.5	36.2 ± 2.1	32.2 ± 1.5	1418.0 ± 54.6	416.1 ± 24.1	12.3 ± 0.1	30.8 ± 0.1	43.2 ± 0.2

Supplementary Table 1: Continued

Genotypes	Vitamin E										Carotenoids		
	α-T	α-T3	β-T	γ-T	β-T3	γ- T3	δ-T	δ- T3	Total	α-TE	Lutein	Zeaxanthin	Sum
P-721	529.5 ± 78.8	304.3 ± 43.0	28.7 ± 0.7	1558.5 ± 2.7		270.5 ± 16.0	99.1 ± 3.0	65.4 ± 6.3	2855.9 ± 106.1	793.0 ± 89.5	3.7 ± 0.1	9.4 ± 0.1	13.1 ± 0.1
P898012	124.1 ± 5.2	50.9 ± 0.9		1016.2 ± 44.9		14.5 ± 0.6			1205.7 ± 51.6	241.0 ± 9.0	2.6 ± 0.1	12.2 ± 0.1	14.7 ± 0.1
R.Tx2783	115.1 ± 4.4	103.1 ± 2.6	582.7 ± 22.2	812.7 ± 30.0		39.3 ± 1.4			1652.9 ± 61.3	518.6 ± 19.1	4.1 ± 0.0	27.6 ± 0.1	31.7 ± 0.1
R.Tx431				280.7 ± 13.0					280.7 ± 13.0	28.1 ± 1.3	7.6 ± 0.2	8.1 ± 0.0	15.6 ± 0.2
R.Tx435	321.2 ± 13.4	190.0 ± 6.7		1314.5 ± 55.8	850.5 ± 37.3	32.9 ± 1.3	52.8 ± 1.3		2761.9 ± 115.9	553.8 ± 22.9	6.6 ± 0.2	38.8 ± 0.2	45.4 ± 0.3
R9188	275.2 ± 9.8	90.1 ± 1.9	21.4 ± 0.3	1130.1 ± 41.2		13.5 ± 0.4			1530.5 ± 53.7	425.9 ± 14.7	1.2 ± 0.0	5.0 ± 0.1	7.1 ± 0.1
SA386 Redbine-60	596.7 ± 46.7	215.4 ± 2.2		788.5 ± 24.9		18.7 ± 4.8	37.4 ± 4.2		1656.6 ± 82.2	741.5 ± 49.9	3.1 ± 0.0	16.1 ± 0.2	19.5 ± 0.2
RSC648	540.1 ± 27.5	170.7 ± 1.9		1893.8 ± 15.5		19.8 ± 1.4			2624.3 ± 40.3	780.7 ± 28.0	7.3 ± 0.1	19.1 ± 0.1	26.4 ± 0.1
SC1017	420.9 ± 14.6	145.2 ± 3.7	18.0 ± 0.2	1506.1 ± 52.9		26.1 ± 0.8	44.6 ± 0.7		2160.9 ± 72.9	625.4 ± 21.1	9.8 ± 0.2	19.9 ± 0.2	29.7 ± 0.5
SC103	270.3 ± 8.3	88.5 ± 1.4	20.0 ± 0.2	1110.1 ± 35.2		13.3 ± 0.3			1503.1 ± 45.5	418.3 ± 12.4	7.5 ± 0.1	10.4 ± 0.1	17.7 ± 0.2
SC1033	366.67 ± 31.3	213.2 ± 17.0		1516.4 ± 65.8		125.2 ± 16.2	53.0 ± 3.67	46.2 ± 2.8	2320.2 ± 213.4	545.5 ± 42.7	10.9 ± 0.1	14.2 ± 0.2	25.1 ± 0.5
SC1038	902.0 ± 7.6	130.9 ± 0.7	19.9 ± 0.0	1434.0 ± 12.1		30.2 ± 0.2	31.7 ± 0.0	29.2 ± 0.1	2578.0 ± 20.8	1095.7 ± 9.1	7.8 ± 0.1	11.2 ± 0.1	18.9 ± 0.2
SC108	585.2 ± 23.8	222.1 ± 20.2		1373.8 ± 0.5	64.65 ± 0.8	22.5 ± 0.1	67.9 ± 0.5		2336.0 ± 43.9	794.5 ± 29.8	4.4 ± 0.1	12.5 ± 0.1	16.9 ± 0.2
SC115	209.5 ± 8.6	158.2 ± 5.4	27.6 ± 0.7	805.3 ± 33.9		65.2 ± 2.7	45.0 ± 1.0	27.7 ± 0.4	1338.4 ± 52.8	352.6 ± 14.1	4.6 ± 0.1	11.8 ± 0.1	16.4 ± 0.1
SC1155	518.1 ± 5.4	68.0 ± 0.6		922.5 ± 14.5		13.7 ± 0.7	34.0 ± 0.2	28.7 ± 0.2	1585.1 ± 8.8	631.8 ± 4.1	3.9 ± 0.1	15.3 ± 0.1	19.2 ± 0.1
SC1158	381.8 ± 19.6	180.6 ± 42.6	363.67 ± 7.9	1224.0 ± 15.1		29.5 ± 4.8	48.6 ± 2.8	43.5 ± 2.1	2271.4 ± 82.7	741.7 ± 36.4	4.5 ± 0.1	10.8 ± 0.1	15.1 ± 0.2
SC118	433.8 ± 8.1	126.1 ± 7.0		1219.1 ± 12.1		17.4 ± 0.2			1796.5 ± 12.8	593.6 ± 7.3	5.3 ± 0.2	6.1 ± 0.0	11.4 ± 0.2
SC1201	512.5 ± 19.5	251.67 ± 8.3		1519.9 ± 58.2		34.3 ± 1.2	33.5 ± 0.4		2351.6 ± 87.5	740.0 ± 27.8	2.1 ± 0.0	12.8 ± 0.0	14.9 ± 0.1
SC124	19.8 ± 0.2	94.9 ± 1.1	18.6 ± 0.2	1236.5 ± 19.67		57.5 ± 0.9			1426.9 ± 21.0	181.2 ± 2.6	17.1 ± 0.2	20.6 ± 0.2	37.7 ± 0.2
SC1251	399.1 ± 6.4	197.2 ± 3.0		1193.9 ± 20.5		99.2 ± 1.67	52.9 ± 0.7	53.2 ± 0.7	1995.3 ± 33.1	579.2 ± 9.7	0.9 ± 0.0	8.1 ± 0.1	8.0 ± 0.1
SC1271	261.8 ± 1.1	163.1 ± 0.5		1387.5 ± 7.3		40.4 ± 0.2	47.2 ± 0.2	23.2 ± 0.2	1922.9 ± 9.1	450.9 ± 2.2	1.7 ± 0.0	7.6 ± 0.1	9.3 ± 0.1
SC1319	422.9 ± 35.1	62.5 ± 0.6		1056.7 ± 2.5		8.4 ± 0.2	51.0 ± 1.4	25.1 ± 0.6	1626.5 ± 34.8	548.8 ± 35.4	16.5 ± 0.3	26.0 ± 0.1	43.5 ± 0.4
SC1322	228.1 ± 2.9	187.2 ± 1.0	29.3 ± 0.2	858.4 ± 11.1		67.1.±.0.9	46.4 ± 0.5	28.8 ± 0.1	1445.2 ± 17.5	386.1 ± 4.7	8.6 ± 0.1	19.3 ± 0.2	27.9 ± 0.3
SC1328	295.1 ± 4.0	153.5 ± 1.67		1595.6 ± 22.3		45.5 ± 0.6	95.1 ± 1.0		2184.8 ± 29.7	503.6 ± 6.8	6.9 ± 0.1	20.5 ± 0.2	27.3 ± 0.5
SC135	562.7 ± 9.5			886.5 ± 15.0		12.9 ± 0.2	58.3 ± 0.67		1520.3 ± 25.5	653.1 ± 11.1	25.3 ± 0.4	14.1 ± 0.1	39.5 ± 0.5
SC1356	478.8 ± 18.7	101.2 ± 2.6	165.7 ± 6.1	1430.5 ± 71.6		37.2 ± 1.4	60.2 ± 1.5		2273.6 ± 91.5	736.9 ± 27.2	6.4 ± 0.1	17.7 ± 0.1	24.1 ± 0.2

Supplementary Table 1: Continued

Genotypes	Vitamin E										Carotenoids		
	α-T	α-T3	β-T	γ-T	β-T3	γ- T3	δ-T	δ- T3	Total	α-TE	Lutein	Zeaxanthin	Sum
SC175	507.9 ± 7.1	93.8 ± 0.8	169.6 ± 2.5	1122.2 ± 15.8		18.9 ± 0.2	42.0 ± 0.5	18.8	1973.5 ± 26.5	734.4 ± 10.1	1.0 ± 0.0	11.4 ± 0.1	13.4 ± 0.1
SC192	406.8 ± 6.9	58.8 ± 0.5	27.2 ± 0.3	1358.1 ± 23.2			73.5 ± 0.9	18.9 ± 0.1	1943.2 ± 31.8	583.9 ± 9.5	0.8 ± 0.0	9.9 ± 0.0	10.8 ± 0.0
SC206	651.4 ± 24.3	216.1 ± 6.8	41.3 ± 1.1	1294.1 ± 48.4	35.5 ± 2.2	33.2 ± 1.2	57.5 ± 1.2	31.2 ± 0.4	2360.2 ± 85.5	869.8 ± 31.9	4.0 ± 0.1	4.8 ± 0.1	8.8 ± 0.1
SC21	61.9 ± 2.1		426.6 ± 16.8	367.9 ± 14.2					856.5 ± 33.2	312.1 ± 11.0	1.2 ± 0.0	9.4 ± 0.1	10.6 ± 0.1
SC214	470.8 ± 1.0	78.2 ± 1.0		1682.1 ± 7.8		18.1 ± 0.1	46.5 ± 0.4	28.5 ± 036	2324.0 ± 8.7	663.9 ± 1.6	10.5 ± 0.5	23.8 ± 0.1	34.2 ± 0.3
SC22	467.1 ± 4.7	136.5 ± 8.3		1092.4 ± 4.6		33.2 ± 0.8	26.6 ± 0.5	26.7 ± 0.4	1782.5 ± 9.1	618.1 ± 3.5	3.9 ± 0.1	27.4 ± 0.2	31.3 ± 0.2
SC224	377.0 ± 3.1	69.8 ± 0.4	126.2 ± 1.1	835.0 ± 7.8		14.1 ± 0.1			1422.0 ± 12.8	545.5 ± 4.9	2.7 ± 0.1	9.8 ± 0.1	12.5 ± 0.1
SC319	145.21 ± 1.9	86.3 ± 0.9		678.74 ± 9.5		1.9 ± 0.0			912.2 ± 12.4	351.9 ± 3.2	1.9 ± 0.1	10.7 ± 0.1	12.6 ± 0.1
SC320	507.7 ± 5.1		36.3 ± 0.5	1702.7 ± 18.6		27.1 ± 0.5	91.6 ± 0.7	37.2 ± 0.2	2402.65 ± 25.5	698.9 ± 7.5	3.4 ± 0.1	12.9 ± 0.1	16.3 ± 0.2
SC323	25.9 ± 0.7		470.3 ± 17.0	424.5 ± 16.1					920.8 ± 34.8	303.6 ± 11.4	9.5 ± 0.2	16.8 ± 0.1	26.1 ± 0.5
SC325	13.4 ± 0.3	132.2 ± 2.8		1198.7 ± 9.0		58.5 ± 1.1			1402.7 ± 10.6	172.9 ± 1.7	1.6 ± 0.0	5.4 ± 0.1	6.0 ± 0.1
SC328	520.9 ± 5.6	129.1 ± 3.1		872.2 ± 10.3		63.9 ± 1.2	41.2 ± 0.6	445.5 ± 43.2	2072.8 ± 36.6	648.1 ± 7.3	6.7 ± 0.1	13.3 ± 0.1	20.0 ± 0.1
SC35	865.8 ± 11.9	206.1 ± 2.2	31.5 ± 0.5	926.3 ± 11.0		76.9 ± 0.0	46.7 ± 0.5		2153.5 ± 26.0	994.7 ± 13.5	2.3 ± 0.1	6.9 ± 0.0	9.5 ± 0.1
SC373	44.7 ± 1.3	57.0 ± 2.9		519.5 ± 103.4		15.8 ± 5.4		22.8 ± 0.5	659.7 ± 107.1	113.7 ± 10.8	3.5 ± 0.1	38.7 ± 0.2	42.2 ± 0.2
SC391	559.4 ± 40.2	201.9 ± 3.2		739.2 ± 21.7		17.5 ± 4.4	35.0 ± 3.7		1553.1 ± 70.6	694.9 ± 43.5	63.4 ± 0.3	22.1 ± 1.1	85.5 ± 1.1
SC414_12	33.8 ± 0.3		556.1 ± 7.7	548.1 ± 7.5				18.0 ± 0.0	1156.9 ± 15.6	366.6 ± 4.0	13.4 ± 0.2	15.4 ± 0.1	28.8 ± 0.2
SC42	866.6 ± 12.5	47.8 ± 0.2	78.9 ± 3.7	726.2 ± 10.1			56.4 ± 0.5		1775.9 ± 33.8	1037.4 ± 18.7	7.2 ± 0.1	14.2 ± 0.0	21.4 ± 0.1
SC441	355.5 ± 24.2	213.7 ± 13.4	32.0 ± 1.8	839.9 ± 57.4		55.67 ± 3.8	41.6 ± 1.0	31.7 ± 1.5	1571.0 ± 104.0	521.3 ± 34.0	15.9 ± 0.5	10.0 ± 0.1	25.9 ± 0.3
SC465	189.7 ± 7.1	92.2 ± 2.2	16.4 ± 0.2	931.5 ± 36.2		13.9 ± 0.4	45.8 ± 0.9		1289.4 ± 47.0	320.1 ± 11.6	3.4 ± 0.0	8.8 ± 0.1	12.2 ± 0.1
SC467	39.8 ± 1.7	46.1 ± 8.1		1441.9 ± 9.7	111.6 ± 18.8			26.6 ± 0.7	1666.1 ± 18.9	203.5 ± 4.5	17.8 ± 0.2	58.9 ± 0.5	76.65 ± 0.7
SC49	560.9 ± 21.1	147.9 ± 3.9	21.9 ± 0.5	1733.5 ± 65.6		145.0 ± 5.4	74.1 ± 1.7		2683.2 ± 97.8	791.7 ± 28.9	5.5 ± 0.1	13.3 ± 0.1	18.8 ± 0.2
SC502	397.4 ± 5.4	98.8 ± 7.8		756.5 ± 5.1		30.4 ± 0.2	21.5 ± 0.6	28.7 ± 2.6	1333.2 ± 13.1	503.5 ± 7.6	7.3 ± 0.2	14.0 ± 0.1	21.3 ± 0.2
SC51	404.5 ± 15.0	91.6 ± 2.5		174.6 ± 6.4		53.5 ± 2.1	66.6 ± 1.7		790.6 ± 28.4	451.5 ± 17.4	3.7 ± 0.1	51.8 ± 0.2	55.5 ± 0.3
SC53	580.4 ± 22.6	149.8 ± 4.5	23.4 ± 0.5	830.2 ± 32.2	579.0 ± 23.7	23.4 ± 0.8	33.2 ± 0.4		2219.4 ± 84.6	750.0 ± 28.6	21.8 ± 0.0	20.2 ± 0.8	41.0 ± 0.4
SC547	555.9 ± 24.2	171.2 ± 5.8	23.8 ± 0.5	1874.1 ± 82.2		49.4 ± 2.1	40.8 ± 0.7	27.8 ± 0.3	2742.9 ± 115.8	807.8 ± 34.5	10.1 ± 0.1	9.6 ± 0.1	19.65 ± 0.2
SC562	566.0 ± 10.0	140.3 ± 3.5		947.8 ± 18.4		69.5 ± 1.6	44.8 ± 0.7	484.2 ± 49.4	2252.6 ± 61.5	704.5 ± 13.5	10.2 ± 0.1	17.2 ± 0.2	27.4 ± 0.5

Supplementary Table 1: Continued

Genotypes	Vitamin E										Carotenoids		
	α-T	α-T3	β-T	γ-T	β-T3	γ- T3	δ-T	δ- T3	Total	α-TE	Lutein	Zeaxanthin	Sum
SC566	598.1 ± 10.1	51.4 ± 0.4	19.1 ± 0.2	1570.0 ± 26.6		71.1 ± 1.2	70.7 ± 0.9		2381.3 ± 39.3	782.5 ± 12.0	2.2 ± 0.0	15.0 ± 0.1	18.1 ± 0.2
SC58	364.1 ± 34.4	72.65 ± 6.5		1518.0 ± 19.5		9.2 ± 0.1	61.7 ± 17.3		2025.6 ± 70.7	539.5 ± 38.0	3.8 ± 0.1	20.0 ± 0.1	24.8 ± 0.2
SC59	596.7 ± 41.6	215.4 ± 7.5		788.5 ± 29.1		18.7 ± 4.4	37.4 ± 3.7		1656.6 ± 77.7	741.5 ± 44.8	13.5 ± 0.5	15.1 ± 0.1	28.7 ± 0.3
SC598	313.6 ± 8.9	91.7 ± 28.7		1031.4 ± 13.1	310.8 ± 126.9	63.5 ± 0.8	36.3 ± 0.2	24.7 ± 0.1	1872.1 ± 80.7	460.9 ± 12.4	6.2 ± 0.1	13.0 ± 0.0	19.2 ± 0.1
SC6	620.9 ± 9.9	210.0 ± 2.9	20.7 ± 0.2	1594.7 ± 25.5		20.5 ± 0.5	43.5 ± 0.4		2511.0 ± 39.1	855.3 ± 13.4	8.5 ± 0.2	38.2 ± 0.2	46.7 ± 0.3
SC60	583.5 ± 21.9		28.1 ± 0.5	1977.8 ± 74.9		172.6 ± 6.5	168.5 ± 5.5	31.9 ± 0.5	2962.4 ± 109.4	800.4 ± 29.8	1.7 ± 0.0	3.5 ± 0.0	5.2 ± 0.1
SC603	43.5 ± 1.6		784.7 ± 35.9	708.2 ± 32.1					1536.1 ± 69.6	506.4 ± 22.8	3.2 ± 0.0	4.8 ± 0.0	8.0 ± 0.0
SC63	308.5 ± 11.5	301.6 ± 9.9	72.5 ± 2.5	1027.5 ± 38.0	137.5 ± 5.0	51.5 ± 1.8	379.1 ± 13.3	45.9 ± 0.0	2323.8 ± 83.6	556.2 ± 19.9	7.5 ± 0.1	14.0 ± 0.1	22.4 ± 0.1
SC630	448.7 ± 17.4	118.3 ± 3.2	17.5 ± 0.2	1386.8 ± 54.2		116.0 ± 4.5	59.5 ± 1.4	58.9 ± 1.6	2205.5 ± 82.5	633.4 ± 23.9	2.1 ± 0.1	9.1 ± 0.1	11.2 ± 0.1
SC641	345.8 ± 12.9	191.9 ± 5.9	31.2 ± 0.7	841.4 ± 31.4		58.5 ± 2.1	40.1 ± 0.6	29.4 ± 0.4	1537.9 ± 53.0	504.2 ± 18.1	7.5 ± 0.2	16.8 ± 0.1	24.2 ± 0.2
SC655	394.2 ± 7.0	97.0 ± 7.9		750.6 ± 9.8		30.2 ± 0.1	21.3 ± 0.7	28.5 ± 2.4	1322.7 ± 18.6	499.3 ± 9.5	9.5 ± 0.1	14.1 ± 0.1	23.4 ± 0.2
SC671	310.2 ± 12.3			973.3 ± 39.1			58.8 ± 1.5	43.7 ± 1.0	1385.9 ± 53.9	409.5 ± 16.5	1.6 ± 0.0	14.9 ± 0.1	16.4 ± 0.1
SC673	531.9 ± 23.6	62.1 ± 1.6	23.6 ± 0.67	1543.7 ± 68.8		22.2 ± 0.9			2183.5 ± 95.5	716.7 ± 31.5	1.8 ± 0.1	16.4 ± 0.1	18.5 ± 0.1
SC702	635.6 ± 8.5	117.3 ± 0.0	212.2 ± 2.7	1404.2 ± 18.8		23.7 ± 0.5	52.6 ± 0.3		2445.5 ± 31.5	918.8 ± 11.0	4.9 ± 0.1	9.4 ± 0.1	14.3 ± 0.1
SC720	209.0 ± 7.2	157.9 ± 4.5	27.5 ± 4.5	803.7 ± 28.4		65.1 ± 2.5	44.9 ± 0.7	27.7 ± 0.2	1335.7 ± 43.5	351.9 ± 11.6	6.9 ± 0.1	4.8 ± 0.1	11.7 ± 0.1
SC725	54.1 ± 0.9			1681.5 ± 3.5		37.5 ± 0.3		15.0 ± 0.2	1788.8 ± 3.9	222.2 ± 0.9	5.9 ± 0.1	33.4 ± 0.5	39.5 ± 0.4
SC757	498.2 ± 5.9	123.8 ± 9.5		948.5 ± 8.4		38.2 ± 0.3	26.9 ± 0.7	36.0 ± 67.1	1671.5 ± 82.0	630.0 ± 8.4	2.65 ± 0.1	9.7 ± 0.1	12.4 ± 0.1
SC772	309.1 ± 13.8	302.2 ± 12.4	72.6 ± 2.9	1029.5 ± 46.5	137.5 ± 47.7	51.6 ± 2.5	379.8 ± 16.5	45.9 ± 1.4	2328.4 ± 102.8	557.5 ± 24.5	15.1 ± 0.5	33.65 ± 0.2	48.7 ± 0.4
SC782	519.2 ± 29.8	159.4 ± 1.6		1768.3 ± 10.9		18.5 ± 1.2			2465.3 ± 40.6	743.8 ± 31.1	10.5 ± 0.2	10.9 ± 0.1	21.2 ± 0.2
SC855	687.1 ± 28.6	135.7 ± 4.5	23.9 ± 0.6	932.6 ± 38.65		32.9 ± 1.5	49.8 ± 1.2	37.5 ± 0.8	1899.7 ± 75.4	834.6 ± 34.1	4.4 ± 0.1	24.4 ± 0.2	28.8 ± 0.2
SC964	461.4 ± 19.1	117.9 ± 3.5	18.1 ± 0.3	1240.9 ± 51.6		93.2 ± 3.8	57.7 ± 1.5		1989.2 ± 79.9	631.6 ± 35.5	4.6 ± 0.1	11.6 ± 0.1	16.2 ± 0.1
Shan Qui Red	638.8 ± 9.0	117.9 ± 1.1	213.2 ± 2.9	1411.2 ± 19.9		23.8 ± 0.3	52.8 ± 0.4	23.7 ± 0.1	2481.4 ± 33.6	923.4 ± 12.8	5.7 ± 0.1	16.6 ± 0.1	22.3 ± 0.2
Tx2911	357.1 ± 13.1	217.1 ± 6.7	32.7 ± 0.8	835.4 ± 30.8		55.1 ± 1.0	40.5 ± 0.6	28.8 ± 0.3	1566.8 ± 54.3	523.4 ± 18.6	16.0 ± 0.2	14.6 ± 0.2	31.6 ± 0.3

The tocochromanol content (total, isolated compounds and α-TE) showed a high coefficient of variation (27-40%), suggesting a high genetic variability for these characteristics. Tocopherols were more prevalent in sorghum than the tocotrienols, resulting in a ratio tocopherols/tocotrienols between 1.47 and 117.91; average 13.25 (data not shown). This result was similar to one observed in a previous study wherein the prevalence of tocopherols was observed in sorghum (ratio = 6.23) (Cardoso et al., 2014). However, this ratio differed from the observed in wheat, oats, barley and rye, in which the ratio is less than 1, due to the prevalence of tocotrienols (Tiwari et al., 2009; Zieliński, Michalska, Piskuła & Kozłowska, 2006). γ-Tocopherol was the major tocochromanol in sorghum (61% of the total), followed by α-tocopherol (22% of the total).

The average content of tocochromanols in sorghum (1,888.0 μg/100g) was higher than the observed in commercial cultivars of wheat, oats, barley, and rye (Okarter, Liu, Sorrells & Liu, 2010; Tiwari et al., 2009). Considering the content of α-TE in 100 g of raw grain, 23% of sorghum genotypes were classified as sources of vitamin E (B.DLO357; SC1038; BR007B; SC35; SC42; ATF14B; SHAN QUI RED; SC702; B.Tx645; SC206; SC6; SC855; SA386 REDBINE-60; SC547; SC60; B.Tx642; SC108; P-721; SC49; SC566; RSC648; SC1158 e B.Tx626). These genotypes present between 750 and 1500 μg of α-TE, and therefore can supply from 5 to 10% of vitamin E recommendations for adult males (19-30 years) (U. S. Institute of Medicine, 2000). The B.DLO357 genotype presented α-TE content at least 24% greater than the other genotypes considered sources of vitamin E. It should be noted that, before being consumed by humans, the sorghum grains must be submitted to processing (*e.g.* fermentation, extrusion, and germination), which can modify the potential of these genotypes to meet the vitamin E recommendations.

Carotenoids profile

Zeaxanthin was the major carotenoid in most genotypes (92%) (Figure 1B and Table 2), which was similar to the observed by other authors (Cardoso et al., 2014; Fernandez, Hamblin, Li, Rooney, Tuinstra & Kresovich, 2008; Lipkie et al., 2013). The sum of lutein + zeaxanthin of the genotypes showed a high coefficient of variation (65.2%), with content

ranging from 2.12 to 85.5 µg/100g (average: 22.3 µg/100g). The high variability in the content of carotenoids in the present study is in agreement with the findings of other authors in grains with white to yellow endosperm (Fernandez et al., 2009; Kean, Ejeta, Hamaker & Ferruzzi, 2007). Information about the determinants of carotenoid content in sorghum and its fractions (pericarp, testa, endosperm, and germen) is incipient. The presence and content of carotenoids in the endosperm of sorghum can be modulated by nine genes involved in carotenoid synthesis or degradation and any of them could be responsible for the variation in the carotenoid concentrations in the endosperm (Fernandez et al., 2008).

The genotypes SC391 and SC467 (both with yellow endosperm) showed the highest carotenoid contents (85.50 µg/100g and 76.65, respectively). However, this content corresponded to 35-50% of that observed by Fernandez et al. (2009) in sorghum with the same endosperm color. The genotype N268B (white endosperm and yellow pericarp) showed carotenoid content (61.60 µg/100g) three times greater than that observed in cultivars with white endosperm (Cardoso et al., 2014; Fernandez et al., 2008). This suggests that the accumulation of carotenoids in genotype N268B occurs mainly in its pericarp. Thus, this genotype has potential for use in breeding programs seeking to increase the content of carotenoids in sorghum. In the medium term, the genotype N268B can be crossed with a genotype presenting yellow endosperm, aiming to produce hybrids that simultaneously accumulate higher quantities of carotenoids in the pericarp and endosperm.

Genetic variability

One hundred sorghum genotypes were clustered into seven different groups according to the genetic variability of tocochromanols and carotenoids (Table 3), indicating high variability for these characteristics. Due to the greater divergence in some genotypes, the Group 1 corresponded to 92 genotypes which were subdivided in 9 subgroups (1A to 1I) genetically distinct from each other.

Table 3: Grouping of 100 sorghum genotypes as the carotenoid and tocochromanol profile, according to Tocher clustering technique [A, B]

Group (Genotypes number)	**Subgroups** (Genotypes number)	**Genotypes**
1 (92)	A (77)	SC502; SC655; SC1155; SC103; SC224; SC465; SC465; ATF 13B; P898012; SC319; SC671; DORADO; SC118; FC6608 Red Kafir Bazine; SC115; SC757; B.Tx3042; SC720; SC641; SC1271; SC598; SC192; SC175; Ajabsido; SC1322; SC964; B.Tx626; CAT83369; SC673; SC22; B.AZ9504; SC1017; SC1356; SC58; SC441; SC1251; SC1328; SA386 Redbine-60; SC108; SC855; Tx2911; SC59; SC1201; SA5330-Martin; SC630; SC566; Lian Tang Ai; SC782; SC206; SC214; ATF14B; SC702; Shan Qui Red; B.Tx642; SC320; B.Tx399; 01MN1589-B; SC547; RSC648; SC1033; B.Tx645; SC1158; SC1319; NSA440- Karper; SC325; SC35; SC42; SC1038; B.Tx635; SC49; SC124; CSN-63; SC135; BR007B; SC6; SC725; EBA-3
	B (2)	SC328; SC562
	C (6)	SC414-12; SC323; SC21; SA7000 Caprock; SC603; R.Tx2783
	D (2)	SC373; SC51
	E (1)	R.Tx431
	F (1)	SC53
	G (1)	B.Tx2752
	H (1)	B.DLO357
	I (1)	SC60
2 (1)		SC772; SC63
3 (2)		**N268B**; R.Tx435
4 (1)		**SC391**
5 (1)		LG70
6 (1)		**SC467**
7 (1)		P-721

[A] Genotypes belonging to the same group or subgroup are genetically similar; [B] Underlined genotypes: sources of vitamin E; genotypes in bold: highest contents of carotenoids.

Genotypes with high content of carotenoids and sources of tocochromanols were included separately. Most genotypes considered sources of tocochromanols were included in the subgroup 1A. The genotype

considered the main source of tocochromanols (B.DLO357) formed a group that has a single element (1H). In contrast, the genotypes that showed the highest carotenoid contents were included in three groups (Groups 3, 4 and 6). This clustering technique facilitates the selection of divergent materials for generating improved hybrids, since they allow the selection of genotypes belonging to different heterotic groups which are appropriate for crossing (Cardoso et al., 2009). In this context, the genotype B.DLO357 (rich in tocochromanols) belonging to group 1A could be crossed with the genotype SC391 (high carotenoid content) of group 4.

Stability of tocochromanols and carotenoids

Moisture

Moisture of the control flours of the genotypes SC391 and B.DLO357 was similar (p>0.05) and higher than that of the control flour of the genotype SC319 (p<0.05). Both heat treatments did not significantly affect the moisture of sorghum flours (Table 4). Thus, changes in carotenoids and tocochromanols observed in this study did not result from changes in the grain moisture. Thereby all results were expressed in wet basis.

Table 4: Effect of heat treatments on moisture (mg/100g) of different sorghum genotypes [A, B]

Treatments	SC 319	SC 391	B.DLO 357
Control	11.77 ± 0.10 [aB]	12.73 ± 0.20 [aA]	12.28 ± 0.22 [aA]
Conventional oven	11.41 ± 0.37 [aB]	12.48 ± 0.13 [aA]	11.83 ± 0.17 [aB]
Extrusion	11.37 ± 0.24 [aA]	11.90 ± 0.37 [aA]	11.82 ± 0.30 [aA]

[A] Results were expressed in fresh basis as the mean of three repetitions ± standard deviation; [B] Means followed by the same small letter in the columns or capital letter in lines are not statistically different at 5% probability by Duncan test.

The lack of effect of the heat treatment in conventional oven on the moisture from the grains can be attributed to the low binomial time/temperature used and the fact that the grains have been subjected to this process intact and subsequently ground. During the extrusion of the sorghum grain, water was added to restore possible loss of moisture resulting from this processing. Thus, the setting of this parameter can be contributed to the absence of significant differences in the moisture.

Tocochromanols stability

The total content of tocochromanols in the three control genotypes differed significantly. The genotype B.DLO357 showed content at least 60% greater than the genotypes SC391 and SC319. Both thermal treatments changed the content and isomeric composition of tocochromanols (p<0.05) (Table 5). The stability (retention) of these compounds varied depending on the genotype (p<0.05). All these modifications affected the potential of the genotypes to meet the recommendations of vitamin E.

Extrusion reduced the tocopherol and tocotrienol contents of all sorghum genotypes. None of the tocopherols and tocotrienols presented a retention behavior that supports the conclusion that some of them were more or less sensitive to extrusion. However, in general, the tocopherols presented higher retention than the tocotrienols (on average, 75.4% and 80.1%, respectively). Due to this, an increase from, on average, 7.7 to 8.7 in the tocopherols/tocotrienols ratio was observed (data not shown).

The higher stability of tocopherols was recently demonstrated in sorghum grains submitted to different types of processing, including grain popping (non-extrusion) (Cardoso et al., 2014). However, this stability differed from that observed in other cereals such as wheat and oats, in which a reduction of tocopherols/tocotrienols ratio after extrusion was observed (Zieliński et al., 2006). Although differences in extrusion techniques may have led to these differences in stability, the factors that can have had contributed to the different behavior of tocopherols in sorghum as compared to other cereals still need to be evaluated.

Table 5: Effect of heat treatments on the content (µg/100g in wet basis) and retention (%) of tocochromanols and α-tocopherol equivalent (Vitamin E) in different sorghum genotypes [A, B, C]

Genotype	Treatments	α- Tocopherol		α-Tocotrienol		γ- Tocopherol		β-Tocotrienol		γ- Tocotrienol		δ- Tocopherol		Total vitamin E		α-tocopherol equivalents	
SC319	Control	180.0 ± 10.3 [h]		62.2 ± 4.2 [d]		442.1 ± 12.7 [g]		nd		4.2 ± 0.1 [e]		nd		689.6 ± 26.4 [h]		243.6 ± 10.3 [h]	
	Conventional oven	214.8 ± 14.8 [g]	**118.7 a**	52.4 ± 4.0 [d]	**84.2 b**	519.7 ± 36.0 [f]	**117.5 a**	nd		4.4 ± 0.2 [e]	**103.4 a**	nd		791.2 ± 42.3 [g]	**114.7 a**	282.4 ± 16.5 [g]	**115.8 a**
	Extrusion	81.0 ± 3.4 [i]	**44.8 f**	37.2 ± 1.0 [e]	**59.8 c**	354.7 ± 17.4 [h]	**80.2 d**	nd		3.5 ± 0.1 [f]	**82.9 c**	nd		476.5 ± 35.4 [i]	**69.1 e**	127.7 ± 8.1 [i]	**52.4 d**
SC391	Control	484.7 ± 20.2 [e]		147.1 ± 7.1 [b]		770.7 ± 27.4 [d]		nd		32.8 ± 1.1 [a]		43.2 ± 1.5 [a]		1478.3 ± 27.2 [e]		607.2 ± 15.3 [e]	
	Conventional oven	554.6 ± 14.6 [d]	**114.4 c**	133.6 ± 8.5 [c]	**90.8 a**	847.4 ± 34.3 [c]	**110.0 b**	nd		25.6 ± 2.1 [b]	**78.1 c**	30.6 ± 3.8 [c]	**71.0 c**	1591.8 ± 3.7 [d]	**107.7 b**	680.3 ± 12.7 [d]	**112.0 a**
	Extrusion	410.7 ± 25.6 [f]	**84.7 d**	127.1 ± 5.7 [c]	**86.4 b**	663.7 ± 27.7 [e]	**86.1 d**	nd		26.2 ± 4.8 [b]	**80.0 bc**	25.9 ± 0.3 [d]	**59.9 e**	1253.6 ± 26.0 [f]	**84.8 c**	516.0 ± 25.9 [f]	**85.0 b**
B.D [d] LO 357	Control	1100.2 ± 38.0 [b]		160.9 ± 9.6 [a]		904.4 ± 15.5 [b]		135.3 ± 16.3 [a]		16.8 ± 0.7 [c]		37.1 ± 2.1 [b]		2354.6 ± 41.1 [b]		1246.7 ± 18.7 [b]	
	Conventional oven	1220.4 ± 46.1 [a]	**116.1 b**	145.8 ± 6.8 [b]	**90.7 a**	1006.1 ± 65.4 [a]	**111.2 b**	88.6 ± 17.2 [b]	**65.5 a**	16.7 ± 0.7 [c]	**99.3 a**	37.9 ± 2.9 [b]	**102.1 a**	2515.5 ± 133.7 [a]	**106.8 b**	1370.3 ± 26.3 [a]	**109.9 a**
	Extrusion	758.8 ± 24.3 [c]	**69.0 e**	137.1 ± 5.4 [bc]	**85.2 b**	839.1 ± 33.4 [c]	**92.8 c**	44.8 ± 2.5 [c]	**33.1 b**	14.3 ± 0.4 [d]	**85.7 b**	33.3 ± 0.8 [c]	**89.9 b**	1827.4 ± 36.3 [c]	**77.6 d**	887.7 ± 20.8 [c]	**71.2 c**

[A] Contents are the mean ± standard deviation of three repetitions and were expressed in wet basis; [B] Retention are the mean of three repetitions; [C] Means followed by the same letter in the columns are not statistically different at 5% of probability by Duncan test; nd: not detected.

Extrusion decreased significantly the contents and retentions of total tocochromanols and α-TE. After extrusion, the genotype B.DLO357 remained with the highest contents of total tocochromanols and α-TE. However, the higher retentions were observed in the genotype SC391, suggesting a lower sensitivity of this genotype to extrusion. The retentions of total tocochromanols and α-TE in extruded sorghum genotypes were greatest than those found in other extruded cereals including wheat, barley, oats and rye which ranged from 6-37% (Zieliński et al., 2006). The impact of the extrusion on cereals differs according to characteristics inherent to the technique, such as temperature, moisture and time (Vargas-Solórzano et al., 2014). Thus, the characteristics used for the extrusion of sorghum can justify the lower losses of tocochromanols in the extruded sorghum. The sensitivity of sorghum tocochromanols compared to other cereals when subjected to the same extrusion conditions must be evaluated.

Dry heat in a conventional oven increased the tocopherols/tocotrienols ratio by 33.9% (on averaged, from 7.7 to 10.3) (data not shown). However, unlike the extruded grains, the higher tocopherols/tocotrienols ratio resulted from the significant increase in the retention of α and γ-tocopherols (114.4-118.7%), main sorghum tocochromanols, at the expense of reduction in the retention of tocotrienols (71.0-90.8%). The higher retentions of α and γ-tocopherols suggested a lower sensitivity of α and γ-tocopherols, since there was no reduction in retention after the processing.

Furthermore, since the dry heat in a conventional oven did not cause significant changes in the moisture of the grains (Table 2), the higher retentions after this treatment can be resulted from the increase in the tocopherols extractability due to their release from its binding sites (Hwang et al., 2012; Seybold et al., 2004). The increase in retention of sorghum tocopherols, including α and γ, after dry heat in a conventional oven was similar to that observed in other food matrices, including the sorghum genotype BRS310 (Cardoso et al., 2014; Hwang, Stacewicz-Sapuntzakis & Bowen, 2012; Seybold, Fröhlich, Bitsch, Otto & Böhm, 2004).

The increase of tocopherols in sorghum genotypes submitted to dry heat in conventional oven increased the contents and retentions of total tocochromanols and α-TE in all genotypes. Thus, this process increased the potential of sorghum to meet vitamin E recommendations. After this processing,

the genotype B.DLO357 showed the highest contents of total tocochromanols and α-TE, and remained as a source of vitamin E. However, the largest retentions were observed in genotype SC319 (p<0.05).

Stability of carotenoids

The total carotenoid content in the three control genotypes differed significantly. The genotype SC391 showed content at least 2.5 times greater than the genotypes B.DLO357 and SC319. Carotenoid content and retention in sorghum decreased after extrusion and dry heat in a conventional oven (Table 6).

Table 6: Effect of heat treatments on the content (µg/100g in wet basis) and retention (%) of carotenoids in different sorghum genotypes [A, B, C]

Genotype	Treatments	Lutein		Zeaxanthin		Lutein + Zeaxanthin	
SC319	Control	8.5 ± 1.1 [a]		13.0 ± 0.4 [c]		21.5 ± 1.2 [c]	
	Conventional oven	5.3 ± 0.5 [b]	**61.9 b**	8.9 ± 0.4 [d]	**68.5 b**	14.2 ± 1.7 [d]	**66.1 b**
	Extrusion	1.7 ± 0.2 [ef]	**20.0 e**	4.9 ± 0.4 [f]	**37.4 d**	6.6 ± 0.6 [g]	**30.7 e**
SC391	Control	6.0 ± 0.4 [b]		47.3 ± 1.9 [a]		53.3 ± 1.9 [a]	
	Conventional oven	4.2 ± 0.7 [c]	**69.5 a**	27.0 ± 0.4 [b]	**57.2 c**	31.2 ± 1.8 [b]	**58.6 c**
	Extrusion	2.5 ± 0.4 [d]	**41.0 c**	14.8 ± 2.1 [c]	**31.3 e**	17.2 ± 2.5 [d]	**32.4 e**
B.DLO 357	Control	1.9 ± 0.1 [e]		8.7 ± 0.7 [d]		10.6 ± 0.8 [e]	
	Conventional oven	1.4 ± 0.3 [f]	**73.7 a**	7.0 ± 0.4 [e]	**80.5 a**	8.4 ± 0.9 [f]	**79.2 a**
	Extrusion	0.6 ± 0.1 [g]	**31.7 d**	3.4 ± 0.5 [g]	**38.6 d**	3.9 ± 0.3 [h]	**37.1 d**

[A] Contents are the mean ± standard deviation of three repetitions and were expressed in wet basis; [B] Retention are the mean of three repetitions; [C] Means followed by the same letter in the columns are not statistically different at 5% of probability by Duncan test.

After extrusion, the genotype SC391 remained with increased carotenoid content (Lutein + Zeaxanthin) (p<0.05). However, the highest carotenoid retention in the extruded grains, which ranged from 30.7 to 37.1%, was observed in the genotype B.DLO357 (p<0.05). The retaining band observed in this study was similar to that observed in grains of popped sorghum (non-extrusion) (Cardoso et al., 2014). The low retention of carotenoids after extrusion indicates that these compounds are thermo sensitive, and probably sensitive to pressure and shearing as well (Cardoso et al., 2014; Waramboi, Gidley & Sopade, 2013).

The sensitivity of carotenoids in cereals depends on the extrusion conditions, including temperature, moisture and time of extrusion (Riaz et al.,

2009; Tiwari et al., 2009). Under high moisture conditions (above 40%), loss of carotenoids may be less than 20%. Under these conditions, the melting temperature and viscosity of the extrusion need to be lower, which reduces the degradation of carotenoids (Okarter et al., 2010; Riaz et al., 2009). Thus, the losses observed in this study are restricted to the condition tested in which it was used temperature zones ranging between 30 and 150 °C. The impacts of different extrusion conditions on the stability of sorghum carotenoids should be the objective of future studies.

After dry heat in conventional oven, the genotype SC391 remained with increased carotenoid content (Lutein + Zeaxanthin) (p<0.05). The retention of carotenoids in grains submitted to dry heat in conventional oven was affected by the genotype (p=0.032), suggesting that chemical characteristics of sorghum can affect the stability of its carotenoids. Furthermore, carotenoid retention in grains submitted to dry heat in conventional oven correlated directly with the total carotenoid of the genotype (R^2 = 0.981, p<0.048). Thus, the higher retention was observed in genotype B.DLO357.

The majority of carotenoids in foods form carotene-protein complexes, which reduce their bioaccessibility (Lipkie et al., 2013). Our speculative hypothesis, which should later be studied, is that the lower bioaccessibility of carotene-protein complexes reduces the thermal sensitivity of carotenoids and that the proportion of these complexes is inversely related to total carotenoid content. Thus, the genotype B.DLO357 may have a greater proportion of complexes and consequently a lower proportion of free carotenoids, which can be less sensitive to heat treatment. The retention in this genotype becomes higher than in other genotypes that have a greater carotenoid content.

CONCLUSION

The sorghum genotypes showed high variability in the profile and content of carotenoids and tocochromanols. Sorghum genotypes were a source of vitamin E and γ and α-tocopherols were their main tocochromanols. The genotypes analyzed showed low contents of carotenoids. Both heat treatments affected the content and retention of tocochromanols. Content and retention of tocopherols, total tocochromanols and α-tocopherol equivalents increased after dry heat in a conventional oven. The content and retention of tocopherols, tocotrienols, and consequently total tocochromanols and α-tocopherol equivalents decreased after extrusion. The content and retention of carotenoids

in sorghum decreased after extrusion and dry heat in a conventional oven.

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3.3 Article 3: Phenolic compounds profile in sorghum processed by extrusion and dry heat in a conventional oven

ABSTRACT

The content and stability to dry heat in a conventional oven (DHCO) and extrusion cooking of phenolic compounds profile in sorghum genotypes were evaluated. Flavanones and flavones decreased after to extrusion cooking (100%) and DHCO (31.7- 61.6%). The 3-DXAs were stable in DHCO but were susceptible to extrusion cooking (70.7-93.9%). Proanthocyanidins were identified only in the genotype SC391 and were reduced after both treatments (DHCO: 39.2% and extrusion cooking: 52.1%). Phenols decreased in the genotype SC319 submitted to DHCO (8.3%) and in all extruded genotypes (13.6-14.9%). The DHCO increased the antioxidant capacity in all genotypes, whereas extrusion cooking reduced antioxidant capacity in only two genotypes. In general, differential stability of the major flavonoids in sorghum was observed under DHCO and extrusion cooking, implying that different processing techniques can be selected to minimize loss of bioactive polyphenols in sorghum depending on flavonoid composition.

Keywords: *Sorghum bicolor* L., flavonoids, proanthocyanidins, antioxidant profile, extrusion cooking, dry heat in a conventional oven.

CHEMICALS STUDIED THIS ARTICLE

Luteolinidin (PubChem CID: 441701); 5-Methoxy-luteolinidin (PubChem CID: not available); Apigeninidin (PubChem CID: 159360); 7-Methoxy-apigeninidin (PubChem CID: not available); Luteolin (PubChem CID: 5280445); Apigenin (PubChem CID: 159360); Naringenin (PubChem CID: 932); Eriodictyol (PubChem CID: 440735).

INTRODUCTION

Sorghum (*Sorghum bicolor* L.) has the highest content of phenolic compounds among cereals. The profile of these compounds in sorghum is determined according to genetic factors that regulate the color and thickness of its pericarp, presence of pigmented testa, and secondary plant color (Joseph M. Awika, McDonough, & Rooney, 2005; Dykes, Seitz, Rooney, & Rooney, 2009; Taleon, Dykes, Rooney, & Rooney, 2012). Some biotic and abiotic stresses

also increase the accumulation of phenolic compounds in sorghum (Mamoudou H Dicko et al., 2005). The phenolic compounds of sorghum, including, proanthocyanidins, 3-deoxyanthocyanidins, and flavones, beneficially modulate the gut microbiota and variables related to noncommunicable diseases such as obesity, diabetes, dyslipidemia, cardiovascular disease, and cancer (Cardoso, Pinheiro, Martino, & Pinheiro-Sant'Ana, In press; Yang, Allred, Geera, Allred, & Awika, 2012).

Sorghum has to be processed prior to use for human consumption, which may change its antioxidant profile (Cardoso et al., 2014; Dlamini, Taylor, & Rooney, 2007). Thus, studies regarding effects of processing on the antioxidant profile of sorghum are essential to assess and maximize the benefits of this cereal on human health (Cardoso et al., 2014). Studies have shown that some processing methods (*e.g.* fermentation, germination and soaking in water) alter the profile and content of phenolic compounds in sorghum (Afify, El-Beltagi, El-Salam, & Omran, 2012; Dicko, Gruppen, Traore, van Berkel, & Voragen, 2005).

The knowledge on the effects of dry and wet heat on phenolic compounds in sorghum is limited (Cardoso et al., 2014; Dlamini et al., 2007). The effects of processing in a conventional oven on flavones, flavanones, and proanthocyanidins still need to be evaluated, as well as the influence of extrusion conditions different from those tested by others (Joseph M Awika, Dykes, Gu, Rooney, & Prior, 2003) on sorghum flavonoids. Extrusion cooking is a versatile and energy efficient process used to produce snacks and a wide variety of food products and the processing in a conventional oven is widely used at the domestic level. Thus, the present study sought to evaluate the stability of phenolic compounds (total, 3-deoxyantocyanidins, flavones, flavanones, and proanthocyanidins) and antioxidant capacity of three sorghum genotypes processed by extrusion and dry heat in conventional oven.

MATERIALS AND METHODS

Raw sorghum

Three sorghum genotypes from a core collection from Embrapa Maize and Sorghum (Sete Lagoas, MG, Brazil) were used: genotype SC319 (origin: Uganda; brown pericarp, with proanthocyanidins, and rich 3-deoxyanthocyanidins - DXAs); genotype B.DLO357 (origin: United States; red pericarp, rich in 3-DXAs, and without proanthocyanidins); genotype SC391 (origin: Egypt; yellow pericarp, low 3-DXAs, and without proanthocyanidins).

The grains were grown in the experimental field of Embrapa Maize and Sorghum (Nova Porteirinha, MG, Brazil), between June and October 2011.

Once harvested, the whole grains were sorted, packed in cool boxes and sent to Vitamins Analysis Laboratory. Subsequently, the whole grains were packed in polyethylene bags, covered with aluminum foil and stored at -18 ± 1 °C, for at most one week.

Standards and reagents

The standards of luteolinidin chloride, gallic acid, luteolin, apigenin, naringenin, eriodictyol, and trolox were obtained from Sigma–Aldrich (St. Louis, MO, USA). The apigeninidin chloride was obtained from Chromadex (Santa Ana, CA, USA). For the extraction and analyses of the phenolic compounds and their fractions, it was used analytic grade reagents purchased from VETEC (São Paulo, Brazil) (acetone, chloroform, methanol, and HCl) and Sigma–Aldrich (St. Louis, MO, USA) (vanillin, β-carotene, α-linoleic acid, tween 40, 2,2-diphenyl-1-picrylhydrazyl radical, and Folin-Ciocalteu). Furthermore HPLC grade reagents (methyl alcohol, ethyl acetate, acetone, acetonitrile, hexane, isopropyl alcohol, acetic acid and formic acid purchased from Tedia (São Paulo, Brazil).

Processing of sorghum flours

The three selected sorghum genotypes were subjected to the processes described below:

- *Raw flour*: The whole grains were ground in a micro-rotor analytical mill (850 µm) (Marconi, MA 090, Brazil). Subsequently, the flours were packed in polyethylene bags, covered with aluminum foil and stored at -18 ± 1 °C, for no more than 24 hours until analysis;

- *Dry heat in a conventional oven/milling (Oven/ milling)*: The intact whole grains were packed in aluminum trays (34 cm x 62 cm x 5 cm) and subjected to dry heat in a conventional oven (121 °C, 25 min). Subsequently, the grains were ground in a micro-rotor analytical mill (850 µm) (Marconi, MA 090, Brazil) and the flours were packed in polyethylene bags, covered with aluminum foil and stored at -18 ± 1 °C, for no more than 24 hours until analysis (Cardoso, et al., 2014);

- *Extruded grains/milling*: The whole grains were previously milled into flour which was processed in a co-rotating twin-screw model Evolum HT 25 (Clextral, Firminy, France) at constant screw speed of 600 rpm and temperature

profile: 30, 30, 60, 90, 100, 100, 120, 120, 150 and 150 °C, from feeding to the outlet (Vargas-Solórzano, Carvalho, Takeiti, Ascheri, & Queiroz, 2014). The screw diameter (D) was 25 mm and the total configured screw length (L) was 1000 mm, providing an overall L/D ratio of 40. The die had four round openings of 3.8 mm each in diameter and 9 mm in length. Dry sorghum flour was placed in the feeding zone by a twin-screw, loss-in-weight gravimetric feeder model GRMD15 (Schenck Process, Darmstadt, Germany), and monitored by Schenck Process Easy Serve software (Schenck Process, Darmstadt, Germany). Distilled water was injected between the first and second feeding zones through a port with 5.25 mm internal diameter from the start of the barrel using a plunger metering pump model J-X 8/1 (AILIPU Pump Co. Ltd., China) set to compensate moisture differences in the samples and provide a final moisture content of 12%. The samples were collected over 15–20 min. Subsequently, the extruded was ground in a micro-rotor analytical mill (850 µm) (Marconi, MA 090, Brazil) and stored in polyethylene bags, at -18 ± 1 °C, for no more than 24 hours until analysis.

Determination of flavonoids

The main 3-deoxyanthocianidins (luteolinidin – LUT, apigeninidin – AP, 7-methoxy-apigeninidin – 7-MeO-AP, and 5-methoxy-luteliolinidin – 5-MeO-LUT), flavones (luteolin and apigenin) and flavanones (naringenin and eriodictyol) in sorghum were analyzed from the extract prepared for determination of total phenols.

The method proposed by Yang, Allred, Geera, Allred, and Awika (2012) and modified by Cardoso, et al. (2014) was used to identify and quantify the flavonoids in the sorghum samples. Analyses were performed in a high performance liquid chromatography (HPLC) system (Shimadzu, SCL 10AT VP, Japan) equipped with diode array detector (DAD) (Shimadzu, SPD-M10A, Japan), high pressure pump (Shimadzu, LC-10AT VP, Japan), autosampler with loop of 500 µL (Shimadzu, SIL-10AF, Japan), and helium degassing system of the mobile phase (Shimadzu, DGU-2 A, Japan). The chromatographic conditions used for these analyses included a HPLC system, C-18 Kinetex column (150 × 4.6 mm i.d., 5 µm) fitted with C-18 guard column (4 mm x 3 mm) (Phenomenex, Torrance, CA), column temperature at 35 °C, injection volume of 15 μL and scanning of the spectrum from 200-700 nm. The 3-DXAs, flavones, and flavanones were measured at 485 nm, 340 nm and 280 nm, respectively.

The mobile phase consisted of 2% formic acid in ultrapure water (line A) and 2% formic acid in acetonitrile (line B). The elution gradient for B was as follows: 0–3 min, 10% isocratic; 3–4 min, 10–12%; 4–5 min, 12% isocratic; 5–8 min, 12–18%; 8–10 min, 18% isocratic; 10–12 min, 18–19%; 12–14 min, 19% isocratic; 14–18 min, 19–21%; 18–22 min, 21–26%; 22–28 min, 26–28%; 28–32 min, 28–40%; 32–34 min, 40–60%; 34–36 min, 60% isocratic; 36–38 min, 60–10%; 38–45 min, 10% isocratic. In order to increase the repeatability of the retention time of the peaks, it was used the following gradient flow 0–36 min, 1.0 mL/min isocratic; 36–38 min, 1.0–2.0 mL/min; 38–44 min, 2.0 mL/min isocratic; 44–45 min, 2.0–1.0 mL/min and the mobile phase was degassed with helium gas at 50 kPa during the runs.

Identification of the flavonoids was based on retention times and UV-Vis spectra of the commercial standards. Quantification of each compound was performed by comparing peak areas with that of analytical curves constructed from injection, in duplicate, of six different standard concentrations (see Table S1 in supplementary material). The 5-Meo-LUT and 7-MeO-AP were quantified using luteolinidin and apigeninidin standards, respectively, along with the appropriate molecular weight correction factor (Dykes, Seitz, Rooney, & Rooney, 2009). The R^2 of the calibration curve ranged from 0.9939 to 0.9992, limits of detection from 18.98 to 35.12 ng/mL, and limits of quantification from 94.90 to 175.60 ng/mL. The compounds were expressed in µg/g of sample, as single compounds and as sum of 3-DXAs, flavones, flavanones and flavonoids.

Content and degree of polymerization of proanthocyanidins

The proanthocyanidins presented in 1 g of sorghum flour was extracted in 20 mL of 70% acetone in water and stirred for 120 min. The suspension was centrifuged at 2790 *g* for 10 min and the supernatant collected and evaporated until complete dryness by rotary evaporation TE 211 (Tecnal, São Paulo, Brazil). Then, the dried extract was dissolved in 4 mL of HPLC methanol, packed in amber bottle and stored in a freezer (-18 ± 1 °C). The proanthocyanidins were evaluated by HPLC using a fluorescence detector with detection at 230 nm and emission at 321 nm (Langer, Marshall, Day, & Morgan, 2011). Aliquots of the extracts (10 mL) previously filtered (0.45 µm) were injected into a normal phase column Phenomenex Develosil diol (250 mm x 4.6 mm, 5 µm). The mobile phase was a binary gradient composed by acid acetonitrile (acetonitrile/acetic acid, 98:2, v/v) (line A) and aqueous solution of

acid methanol (methanol/water/acetic acid, 95:3:2, v/v/v) (line B). The flow of the mobile phase was 0.6 mL/min and gradient elution to B was as follows: 0-3 min, 7% isocratic; 3-57 min, 7-37.6%, 57-60 min, 37.6-100%, 60-67 min, 100% isocratic; 67-73 min, 100-7%, 73-83 min, 7% isocratic. The concentration of proanthocyanidins was expressed in µg of catechin equivalents (corrected for molecular weight)/g sample (µg CatEq/g).

Determination of total phenols

Sorghum flour (1 g) was extracted in 10 mL of methanol (w:v) and stirred (180 rpm, 2 hours). Then, the extract was centrifuged (2,790 *g*, 10 min) and the supernatant was collected and stored at -20 ± 1 °C (Dykes, Seitz, Rooney, & Rooney, 2009). Total phenols were determined using the Folin-Ciocalteu reagent (Singleton, Orthofer, & Lamuela-Raventós, 1999). Aliquots of 0.5 mL of the extract, added of 0.5 mL of sodium carbonate 7.5% and 0.5 mL of Folin-Ciocalteu (diluted to 20% in water) were stirred in vortex and incubated at room temperature (28 ± 1^{o}C, 30 min). Then, the absorbance was read in a spectrophotometer (Evolution 60S, Thermo scientific) at 765 nm. The quantification was performed by analytical curve obtained by the reading of the absorbance of solutions of gallic acid with different concentrations (0.01 to 0.10 mg/mL; y = 24.888x + 0.0246; R^2 = 0.996). The results were expressed in mg of gallic acid equivalents per gram of flour (mg GAEq/g).

Determination of antioxidant capacity

Approximately 1 g of sorghum flour was extracted in 20 mL of 60% methanol in water. Then, the suspension was stirred at 180 rpm (2 h) and centrifuged at 3,000 rpm (2490 *g*, 15 min). The supernatant was transferred to an amber bottle and stored in a freezer (-18 ± 1° C) until the time of the analysis. The antioxidant capacity was determined using the DPPH$^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl) (Bloor, 2001).

Aliquots of 0.1 mL of the extracts were added of 1.5 mL of methanol solution of DPPH 100 µmol, followed by manual shaking for 1 minute. After 30 min of rest, the absorbance was read in a spectrophotometer (Evolution 60S, Thermo scientific) at 517 nm. An analytical curve was constructed using 50–100 µmol/L of trolox solutions (R^2 = 0.9975). The antiradical activity (%) was calculated using the equation $[1 - (Abs_{sample} - Abs_{blank}) / (Abs_{control} - Abs_{blank})] \times 100$. The inhibited %DPPH for each sample was plotted against the concentration of trolox standards, and the antioxidant capacity of the samples

was calculated from the linear equation. Antioxidant capacity was expressed as µmol Trolox equivalent (TE)/g sorghum flour.

True retention of antioxidant compounds

For the calculation of the retention of antioxidant compounds, all the grains were weighed on an analytical balance (GEHAKA, AG 200) before and after heat treatment. The true retention was calculated by the equation of Murphy, Criner, and Gray (1975).

Experimental design and statistical analysis

The effects of the processing on phenolic compounds of sorghum were evaluated using a completely randomized design, in a factorial scheme 3 x 3 (3 genotypes and 3 types of processing). All tests were performed in three repetitions. Data normality was assessed using the Shapiro-Wilk test and differences between treatments were evaluated by ANOVA. Duncan test was used to compare the treatment averages. The association between variables was assessed by Pearson's correlation coefficient. Statistical analyzes were performed using the SAS package (Statistical Analysis System), version 9.2 (2008), at 5% probability.

RESULTS AND DISCUSSION

Flavonoids

3-Deoxyanthocyanidins

All investigated 3-DXAs (LUT, AP, 5-MeO-LUT and 7-MeO-AP) were identified in the three sorghum genotypes in different concentrations (Figure 1 and Table 1). Studies showed that these are the main sorghum 3-DXAs and may occur concomitantly in varieties with pericarp colors ranging from lemon yellow to black (Dykes, Peterson, Rooney, & Rooney, 2011; Dykes, Rooney, & Rooney, 2013; Dykes et al., 2009). LUT and 5-MeO-LUT were the main DXAs in the genotypes, comprising on average 32.1 and 30.7% of total DXAs, respectively. Recently, it was demonstrated that the profile of sorghum 3-DXAs depends on various factors, including the secondary plant color (Dykes et al., 2011). In general, the LUT and 5-MeO-LUT are more prevalent in plants with purple secondary color while plants with red secondary color have more AP and 7-MeO-AP (Dykes et al., 2013; Dykes et al., 2009).

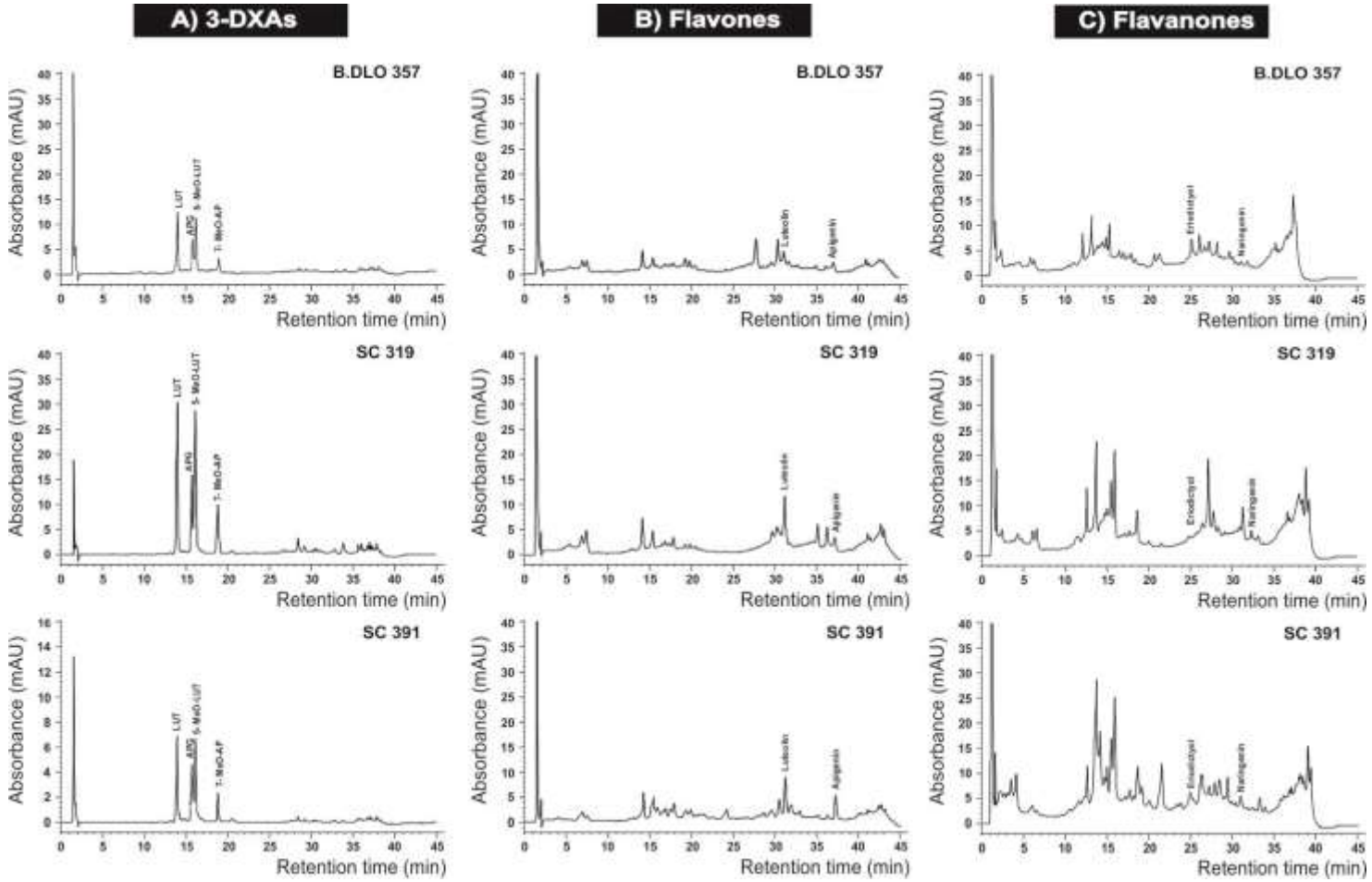

Figure 1: Profile of 3-deoxyanthocyanidins (A), flavones (B), and flavanones (C) in the three sorghum genotypes obtaining by HPLC. Chromatographic conditions: according to materials and methods. 3-DXAs: 3-deoxyanthocyanidins, 7-MeO-AP: 7-methoxy-apigeninidin; 5-MeO-LUT: 5-methoxy-luteolinidin.

Table 1: Effect of heat treatments on the content (µg/100g in dry basis) and retention (%) of 3-deoxyanthocyanidins in sorghum genotypes [A, B, C]

Genotype	Treatment	Luteolinidin		Apigeninidin		5-methoxy-luteolinidin		7-methoxy-apigeninidin		Total 3-deoxyanthocyanidins	
		Content	Retention	Content	Retention	Content	Retention	Content	Retention	Content	Retention
SC319	Control	103.6 ± 11.3 [aA]		64.8 ± 7.0 [aA]		120.0 ± 11.5 [aA]		54.0 ± 10.4 [aA]		342.4 ± 27.2 [aA]	
	Conventional oven	98.8 ± 14.6 [aA]	95.4 [aAB]	53.8 ± 5.0 [bA]	83.1 [aA]	100.0 ± 7.1 [aA]	83.3 [aC]	46.2 ± 5.1 [aA]	85.6 [aB]	298.8 ± 29.4 [aA]	87.3 [aB]
	Extrusion cooking	26.4 ± 0.7 [bA]	25.4 [bA]	14.2 ± 0.6 [cA]	21.8 [aA]	40.5 ± 3.4 [bA]	33.7 [bA]	19.4 ± 1.4 [cA]	35.9 [bA]	100.4 ± 5.9 [cA]	29.3 [bA]
SC391	Control	17.1 ± 2.7 [aC]		9.5 ± 0.8 [aB]		17.5 ± 2.3 [aC]		6.5 ± 1.0 [aB]		50.6 ± 5.7 [aC]	
	Conventional oven	17.4 ± 2.5 [aC]	104.2 [aAB]	10.3 ± 0.3 [aB]	91.8 [aA]	18.7 ± 2.0 [aC]	107.1 [aB]	7.0 ± 1.4 [aB]	107.3 [aA]	53.9 ± 6.0 [aC]	106.4 [aA]
	Extrusion cooking	2.6 ± 0.5 [bC]	15.0 [bB]	0.8 ± 0.1 [bB]	8.5 [bB]	2.2 ± 0.8 [bC]	12.4 [bB]	0.9 ± 0.1 [bC]	13.8 [bB]	6.4 ± 1.3 [bC]	12.7 [bB]
B.DLO 357	Control	64.6 ± 7.1 [aB]		59.0 ± 3.0 [aA]		60.5 ± 3.3 [aB]		41.8 ± 2.8 [aA]		226.0 ± 10.3 [aB]	
	Conventional oven	70.0 ± 5.6 [aB]	108.3 [aA]	53.4 ± 4.4 [aA]	90.4 [aA]	68.5 ± 5.5 [aB]	113.1 [aA]	40.1 ± 2.3 [aA]	95.8 [aB]	231.9 ± 11.8 [aB]	102.6 [aA]
	Extrusion cooking	4.6 ± 0.6 [bB]	7.1 [bC]	2.6 ± 0.2 [aB]	4.4 [bB]	4.7 ± 0.5 [bB]	7.7 [bC]	2.5 ± 0.1 [bB]	6.1 [bC]	14.4 ± 1.4 [bB]	6.4 [bB]

[A] Content are the mean ± standard deviation of three repetitions and were expressed in dry basis; [B] Retention are the mean of three repetitions; [C] Averages in columns followed by the same lower case letter in the same genotype or capital in the same treatment are not statistically different at 5% of probability by Duncan test.

Total 3-DXAs varied significantly between the control genotypes (p<0.05), representing between 24.5% (SC391) and 79.7% (SC319) of total flavonoids (data not shown). As reported in the literature (Joseph M Awika, Rooney, & Waniska, 2004; Dykes et al., 2013; Dykes et al., 2009), 3-DXAs content was higher in grains with dark colored pericarp (brown > red > yellow). Thus, the genotype SC319 had the highest total 3-DXAs, followed by the genotypes B.DLO357 and SC391. The contents of 3-DXAs in the three genotypes were similar for the grains with the same pericarp coloring, derived from plants with red/purple secondary color (Dykes et al., 2013; Dykes et al., 2009). The 3-DXAs content in genotype SC319 stood out when compared to grains with black pericarp, which are the main sources of these phenolic compounds. The content in this genotype was similar to observed in five hybrids of black sorghum (329-485 µg/g) and corresponded to 30-50% of the content presented in three black sorghum lines (676-1054 µg/g) (Dykes et al., 2013).

The content and retention of 3-DXAs in the genotypes remained constant after dry heat in a conventional oven. Thermal stability of 3-DXAs is an important target for the food industry, since improved chemical stability allows that this compound (isolated or in the grain) can be used in these processing conditions, without reducing its functional and technological potentials. Recent studies demonstrated that sorghum 3-DXAs have higher thermal stability than anthocyanins in general (Cardoso et al., 2014; Yang, Dykes, & Awika, 2014).

A differentiated structure of the 3-DXAs, which provides greater chemical stability, may have contributed to its higher thermal stability based on unknown mechanisms (Cardoso et al., 2014). Additionally, factors inherent to sorghum such as location of 3-DXAs in the grain and complexation with other matrix compounds may have contributed to this stability. The presence of other phenolic compounds, *e.g.*, phenolic acids, flavones, flavonols, or flavanones, in the grain could serve as copigments of anthocyanins, hence improving stability (Yang et al., 2014). It is important to study the thermal stability of 3-DXAs in order to determine the relationship between composition of 3-DXAs and their thermal stability (Yang et al., 2014).

The 3-DXAs (total and fractions) were highly sensitive to extrusion cooking, with retention between 4.4 and 29.3% (average 16.4%). Furthermore, it should be consider the processing has reduced the extractability of these

compounds by engine that is still unknown. Studies have shown that the anthocyanins in foods are sensitive to extrusion (10-77%) (Camire, Dougherty, & Briggs, 2007; Khanal, Howard, Brownmiller, & Prior, 2009; Mora-Rochin et al., 2010). In general, the non-methoxylated 3-DXAs were less sensitive to extrusion cooking than methoxylated forms (average retention, 18.3 *vs* 13.7%, respectively).

Despite the adverse effect of extrusion on anthocyanins in foods, including sorghum 3-DXAs, some characteristics of the extrusion cooking (*e.g.*, higher moisture and lower temperature) can minimize these losses (Durge, Sarkar, & Singhal, 2013). Thus, the impact of different extrusion cooking conditions on stability of the sorghum 3-DXAs should be the subject of future studies. Furthermore, the addition of compounds that enhance the stability 3-DXAs needs to be studied. In a recent study, the addition of 1% citric acid prior to extrusion of rice colored with anthocyanins increased the retention by 18% (Durge et al., 2013). Furthermore, the extrusion of the intact grain rather than your flour can contribute to minimize losses during this processing.

Flavones

Apigenin and luteolin were identified in all analyzed genotypes (Figure 1 and Table 2). Luteolin was the flavone most prevalent in genotypes SC319 and SC391 (78 and 60%, respectively). In genotype B.DLO357, luteolin and apigenin were distributed almost equally. The profile of sorghum flavones varies according to the secondary plant color. Apigenin is predominant in plants with bronze color while luteolin is the main flavone in plants with red/purple color (Dykes et al., 2011).

Total flavones varied significantly among the sorghum genotypes ($p<0.05$) (Table 2). The genotype SC391 (yellow pericarp) had the highest content of flavones, followed by the genotypes B.DLO357 (red pericarp) and SC319 (brown pericarp). The contents observed in this study varied within the range observed by other authors in plants with red/purple secondary color (3.5-47.1 µg/g) (Dykes et al., 2011; Dykes et al., 2009). Unlike the 3-DXAs, the main sources of flavones are sorghum grains of plants with bronze secondary color with lemon yellow or red pericarp (268-362 µg/g, 60-386 µg/g, respectively) (Dykes et al., 2011; Dykes et al., 2009).

Table 2: Effect of heat treatments on the content (µg/100g in dry basis) and retention (%) of flavones and flavanones in sorghum genotypes [A, B, C]

Genotype	Treatment	Luteolin		Apigenin		Total Flavones		Naringenin		Eriodictyol		Total Flavanones	
		Content	Retention	Content	Retention	Content	Retention	Content	Retention	Content	Retention	Content	Retention
SC319	Control	28.2 ± 2.0 [aA]		7.9 ± 0.3 [aB]		36.1 ± 2.3 [aB]		24.6 ± 4.2 [aC]		26.6 ± 2.3 [aB]		51.2 ± 0.3 [aC]	
	Conventional oven	13.4 ± 0.6 [bA]	47.5 [B]	3.8 ± 0.1 [bB]	47.9 [A]	17.2 ± 0.7 [bA]	47.6 [B]	12.4 ± 1.1 [bC]	49.6 [A]	15.7 ± 1.2 [bAB]	59.1 [A]	28.1 ± 0.2 [bC]	54.9 [aA]
	Extrusion cooking	nd	0	nd	0	nd	0	nd	0	nd	0	nd	0
SC391	Control	27.3 ± 0.8 [aA]		17.5 ± 0.3 [aA]		44.9 ± 1.1 [aA]		215.7 ± 11.1 [aA]		51.6 ± 5.3 [aA]		267.3 ± 1.3 [aA]	
	Conventional oven	12.8 ± 0.7 [bA]	46.7 [B]	4.6 ± 0.2 [bA]	26.4 [B]	17.4 ± 0.7 [bA]	38.8 [C]	78.6 ± 7.1 [bA]	26.3 [aB]	18.8 ± 2.3 [bA]	26.4 [C]	97.4 ± 0.8 [bA]	26.4 [cB]
	Extrusion cooking	nd	0	nd	0	nd	0	36.3 ± 4.2 [cA]	16.9 [bA]	nd	0	36.3 ± 0.4 [cA]	13.6 [dA]
B.DLO 357	Control	7.3 ± 0.3 [aB]		6.7 ± 0.2 [aB]		13.9 ± 0.4 [aC]		169.4 ± 8.5 [aB]		23.5 ± 3.1 [aB]		192.9 ± 0.7 [aB]	
	Conventional oven	5.9 ± 0.1 [bB]	80.5 [A]	3.5 ± 0.3 [bB]	52.8 [A]	9.4 ± 0.4 [bB]	71.3 [A]	67.8 ± 4.3 [bB]	40.0 [aA]	14.2 ± 1.9 [bB]	40.4 [B]	82.0 ± 0.5 [bB]	42.5 [bA]
	Extrusion cooking	nd	0	nd	0	nd	0	26.9 ± 3.9 [cB]	15.9 [bA]	nd	0	26.9 ± 0.3 [cB]	13.9 [dA]

nd: not detected. [A] Content are the mean ± standard deviation of three repetitions and were expressed in dry basis; [B] Retention are the mean of three repetitions; [C] Averages in columns followed by the same lower case letter in the same genotype or capital letter in the same treatment are not statistically different at 5% of probability by Duncan test.

Sorghum flavones were highly sensitive to dry heat processing and their retention varied between 28.8% and 68.3% (average retention of 51.6%). The retention correlated with total flavones present in the grain (R^2 = 0.872, p <0.019). Apigenin was more sensitive to dry heat in a conventional oven than luteolin (average retention, 42.4% and 58.2%, respectively). After extrusion cooking, flavones were not detected in the genotypes. To date, studies on stability of flavones in foods, particularly in cereals, are unavailable. To our knowledge, this was the first study to evaluate the stability of luteolin and apigenin in sorghum submitted to dry heat in a conventional oven and extrusion cooking.

Flavanones

Both investigated flavanones were identified in the sorghum genotypes (Figure 1 and Table 2). Naringenin was the main flavanone in genotypes B.DLO357 and SC391 (88.2 and 80.8%, respectively). Eriodictyol and naringenin were almost equally distributed in the genotype SC319 (47.5 and 52.5%, respectively).

Total flavanones varied among the sorghum genotypes (p<0.05). The sorghum pericarp color is a determinant of total flavanones (Dykes et al., 2011). In the present study, the genotype SC391 (yellow pericarp) presented the highest total flavanones concentration, followed by the genotypes B.DLO357 (red pericarp) and SC319 (brown pericarp). Unlike the genotypes B.DLO357 and SC319, the main flavonoids of the genotype SC391 were flavanones (60.2%).

The genotype SC391 was a good source of flavanones and its contents corresponded to at least 50% of that observed in fruits considered a source of this phenolic (*e.g.*, cunquate: 574 µg/g; lemon: 498 µg/g, and orange: 426 µg/g) (Bhagwat et al., 2013). However, this content was lower than that observed in eight varieties with lemon-yellow pericarp, which in general are the major sources of flavanones (474-1.780 µg/g) (Dykes et al., 2011). Flavanone contents in genotypes SC319 and B.DLO357 vary within the range observed for sorghum with the same pericarp color (68-241 µg/g) (Dykes et al., 2011).

Like the flavones, sorghum flavanones were highly sensitive to both processing methods. Dry heat in a conventional oven reduced the total flavanones retention by 44.6% on average (from 36.5% to 54.9%). These

losses are inversely correlated with total flavanones in the grain. In all genotypes, eriodictyol retentions were significantly lower ($p = 0.026$) than naringenin (averages of 42% and 52%, respectively). Similar to flavones, flavanones were not detected in extruded genotypes. The analysis of possible degradation products, effects of these processing conditions on influences on their extractability, and stability of these sorghum flavonoids under other processing conditions needs to be further evaluated.

Proanthocyanidins

The genotypes SC391 and B.DLO 357 did not contain proanthocyanidins. Total proanthocyanidins in the genotype SC319 (Table 3) was low when compared to a sorghum variety with high proanthocyanidin content (Hi Tannin) (20,500 µg CatEq/g) (Joseph M Awika et al., 2003). The proanthocyanidins in Hi Tannin sorghum was determined by high performance liquid chromatography, however, using conditions different from those adopted in this study.

Both processing methods reduced the total proanthocyanidins content in genotype SC319 by up to 52%. However, the effects of dry heat in a conventional oven and extrusion cooking on the sorghum proanthocyanidins profile were different. After dry heat in a conventional oven, the contents and retentions of monomers, dimers and trimers of proanthocyanidins remained unchanged, whereas those oligomers and polymers reduced significantly, similar to that observed by other authors (Joseph M Awika et al., 2003). The reduction of proanthocyanidins in sorghum was directly correlated with the DP ($p<0.05$), and therefore greater reductions were observed for oligomers (DP=4-9) and polymers (DP>10). During processing, these proanthocyanidin forms may have interacted with sorghum macromolecules (*e.g.*, carbohydrates and proteins), forming insoluble and less extractable complexes (Joseph M Awika et al., 2003). Studies have shown that this interaction is higher in proanthocyanidins with high DP (Barros, Awika, & Rooney, 2013; Barros, Awika, & Rooney, 2012).

Table 3: Effect of heat treatments on the content (µg Catechin Equivalent/g in dry basis) and retention (%) of proanthocyanidins in sorghum genotypes [A, B, C]

Degree of polymerization	SC319				
	Control	Conventional oven		Extrusion	
	Content	Content	Retention	Content	Retention
Monomers	16.79 ± 0.53 [b]	15.45 ± 1.05 [b]	*92.0*	49.87 ± 3.82 [a]	*297.1**
Dimers	18.97 ± 0.31 [b]	18.00 ± 0.81 [b]	*94.3*	55.52 ± 3.75 [a]	*292.7**
Trimers	31.37 ± 1.29 [a]	30.91 ± 1.12 [a]	*98.5*	30.17 ± 2.97 [a]	*96.2*
Oligomers					
4	nd	nd		nd	
5	nd	nd		nd	
6	nd	nd		nd	
7	65.57 ± 5.04 [a]	48.42 ± 2.81 [b]	*83.8*	35.58 ± 3.18 [c]	*54.3*
8	nd	nd		nd	
9	66.49 ± 6.20 [a]	49.9 ± 3.62 [b]	*24.9*	nd	*0**
Polymers	814.22 ± 60.04 [a]	453.66 ± 52.18 [b]	*55.7*	315.67 ± 19.08 [c]	*38.0**
Total proanthocyanidins	1013.4 ± 82.31 [a]	614.33 ± 61.58 [b]	*60.6*	486.82 ± 32.81 [c]	*48.0**

[A] Content are the mean ± standard deviation of three repetitions and were expressed in dry basis; [B] Retention are the mean of three repetitions; [C] Means followed by the same letter in the lines are not statistically different at 5% of probability by Duncan test; * Retention statistically different than conventional oven at 5% of probability by Duncan test.

The total proanthocyanidins and their oligomers and polymers decreased more after extrusion cooking than after dry heat in a conventional oven. The complexion of proanthocyanidins with other sorghum constituents during extrusion cooking may have contributed to these reductions. Different from dry heat in a conventional oven, extrusion cooking increased the content of proanthocyanidin monomers and dimers 3 fold. This suggests that extrusion cooking may help break down high molecular weight proanthocyanidins into compounds of lower molecular weight, as previously shown (Awika et al 2003). The increased retention of monomer and dimer proanthocyanidins is important since they naturally possess greater bioavailability and effectiveness against oxidative stress (Cardoso et al., In press; Ou, Percival, Zou, Khoo, & Gu, 2012).

Total phenols and antioxidant capacity

The effects of both processing methods on total phenols varied according to the sorghum genotype (p<0.05). The total phenolic content remained

constant in genotypes SC391 and B.DLO357 after dry heat in a conventional oven and reduced significantly in the genotype SC319. In extruded genotypes, the phenolic reduced between 11.8% (B.DLO357) and 20.0% (SC319) due to sensitivity of their flavanones and flavones. The retention in total phenolic compounds was significantly lower in genotype SC319 in both treatments (91.7% and 80.0%). These differences in phenolic retention among the sorghums were likely due to decreased extractability of proanthocyanidins, which were only present in the SC319 genotype (Table 3).

Table 4: Effect of heat treatments content of total phenolic compounds (µg gallic acid equivalents/g in dry basis), antioxidant capacity (µmol Trolox equivalent/g in dry basis) and their retentions (%) in different sorghum genotypes [A, B, C]

Genotype	Treatment	Total phenolics		Antioxidant capacity	
		Content	Retention	Content	Retention
SC319	Control	6.0 ± 0.1 ^{aA}		28.3 ± 0.5 ^{bA}	
	Conventional oven	5.5 ± 0.2 ^{bA}	91.7 ^{aB}	33.0 ± 2.0 ^{aA}	116.3 ^{aA}
	Extrusion cooking	4.8 ± 0.2 ^{cA}	80.0 ^{bB}	28.5 ± 1.1 ^{bA}	100.2 ^{bA}
SC391	Control	2.2 ± 0.1 ^{aC}		7.6 ± 0.4 ^{bC}	
	Conventional oven	2.1 ± 0.2 ^{abC}	96.9 ^{aB}	8.7 ± 0.5 ^{aC}	111.8 ^{aA}
	Extrusion cooking	1.9 ± 0.1 ^{bC}	86.4 ^{bA}	6.8 ± 0.3 ^{cC}	89.6 ^{bA}
B.DLO357	Control	3.5 ± 0.3 ^{aB}		17.1 ± 1.0 ^{bB}	
	Conventional oven	3.7 ± 0.2 ^{aB}	105.7 ^{aA}	19.6 ± 0.8 ^{aB}	114.6 ^{aA}
	Extrusion cooking	3.0 ± 0.1 ^{bB}	85.1 ^{bA}	15.6 ± 0.9 ^{cB}	84.6 ^{bA}

[A] Content are the mean ± standard deviation of three repetitions and were expressed in dry basis; [B] Retention are the mean of three repetitions; [C] Averages in columns followed by the same lower case letter in the same genotype or capital letter in the same treatment are not statistically different at 5% of probability by Duncan test.

The effects of processing on antioxidant capacity varied according to the sorghum genotype (p<0.05). Antioxidant capacity increased significantly in all sorghum genotypes after dry heat in a conventional oven (Table 4), as also demonstrated by other authors (Cardoso et al., 2014). Different factors may have contributed to this result, including the elevated thermal stability of 3-DXAs, which are important sorghum antioxidants, as well as increased

extractability of other antioxidants (*e.g.*, vitamin E) that act synergistically with phenolic compounds of sorghum increasing the antioxidant capacity (Cardoso et al., 2014).

As observed by other authors studying sorghum without proanthocyanidins (Cardoso et al., 2014; Dlamini et al., 2007), extrusion cooking reduced the antioxidant capacity in genotypes SC391 (10.4%) and B.DLO357 (15.4%). These losses were correlated with decreased of 3-DXAs ($p<0.05$). However, extrusion cooking did not affect the antioxidant capacity of genotype SC319, probably due to the increased concentration of lower molecular weight proanthocyanidins. This may have compensated for a possible loss resulting from the reduction of 3-DXAs and other phenolic compounds. Furthermore, it is expected that the reduction of the polymerization degree of proanthocyanidin increase the acceptability of in sorghum that contains this phenolic compounds. Bran infusions of tannin sorghum presented less sensory acceptance than infusions tannin-free due to astringency (Kobue-Lekalake, Taylor, & de Kock, 2012).

CONCLUSION

The effects of dry heat in a conventional oven and extrusion on phenolic compounds profile of sorghum varied according to the genotype, primarily due to differences in flavonoid profiles. In general, the effect of extrusion was more deleterious than those of dry heat in a conventional oven. Flavones and flavanones of sorghum were more sensitive to extrusion and dry heat in a conventional oven than 3-DXA and proanthocyanidins. Even though thermal degradation was a factor, part of the changes in phenolic contents may be attributed to reduced extractability after processing. The data suggest that, depending on phenolic profile of sorghum, processing techniques can be selected that to minimize losses of these bioactive compounds.

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4. FINAL CONSIDERATIONS

Sorghum has a high nutritional value and is basically composed of starch, which is more slowly digested than that of other cereals, low digestibility proteins (mainly kafirins), unsaturated lipids, and is a source of some minerals (phosphorus, potassium, and zinc) and some B-complex vitamins (thiamine, riboflavin, and pyridoxine) and fat-soluble vitamins (D, E, and K). Furthermore, some varieties, especially the red, brown, and black colors, have a high content of phenolic compounds, especially 3-deoxyanthocyanidins and tannins, which are beneficial to human health.

The results of in vitro studies conducted by other authors have demonstrated that compounds isolated from sorghum, particularly 3-deoxyanthocyanidins, tannins, and lipids, play a strong modulatory effect on gut microbiota and processes related to noncommunicable diseases (obesity, diabetes, dyslipidemia, cardiovascular disease, cancer, and hypertension). However, knowledge about the sorghum-specific bioactive compounds that promote these functional benefits is incipient.

Studies are needed to determine the preventive and therapeutic effects of sorghum whole grain and its fractions on human health, including gene and protein expression. It should be noted that the response of the human organism to these compounds may be dependent on their bioavailability. Thus, evaluating the bioavailability of sorghum's bioactive compounds is essential to determining the benefits of sorghum grains and bioactive compounds on human health.

The profile of bioactive compounds has been shown to be a determinant factor of the functional potential of sorghum varieties. In this context, the selection of varieties of sorghum and practical optimization should be performed to ensure the accumulation of bioactive components that will maximize the benefits of sorghum in humans. Sorghum genotypes from a core collection from Embrapa Maize and Sorghum showed high variability in the profile and content of carotenoids and tocochromanols. Furthermore, sorghum genotypes were a source of vitamin E and γ and α-tocopherols were their main tocochromanols. The genotypes analyzed showed low contents of carotenoids.

The behavior assessment of bioactive compounds in different processing conditions is essential to define the manner of use in which sorghum promotes maximum benefits to human health. In the presented study, the effects of dry

heat in a conventional oven and extrusion on phenolic compounds, carotenoids and vitamin E profile of sorghum varied according to the genotype. In general, the effect of extrusion was more deleterious than those of dry heat in a conventional oven. Flavones and flavanones of sorghum were more sensitive to extrusion and dry heat in a conventional oven than 3-DXA and proanthocyanidins. Content and retention of vitamin E (tocopherols, total tocochromanols and α-tocopherol equivalents) increased after dry heat in a conventional oven and decreased after extrusion. Carotenoids decreased after extrusion and dry heat in a conventional oven. The data suggest that, depending on bioactive compound profile of sorghum, processing techniques can be selected that to minimize losses of these bioactive compounds.