PAMELLA CRISTINE ANUNCIAÇÃO

# EFFECT OF SORGHUM (SORGHUM BICOLOR L.) ON METABOLIC VARIABLES IN ADULTS

Thesis submitted to the Federal University of Viçosa, as part of the requirements of Program in Science of Nutrition for obtaining the title of *Doctor Scientiae*.

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*I dedicate to my parents Adélcia and Geraldo, and to my love Júlio.*

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## BIOGRAPHY

Pamella Cristine Anunciação, daughter of Geraldo Leocádio da Anunciação and Adélcia do Rosário Ferreira da Anunciação, was born on May 25, 1986, in Macaé, Rio de Janeiro.

In September 2004, she joined the Nutrition course in Federal University of Ouro Preto (MG), graduating Nutricionist in July 2009.

In September 2009, she joined the Lato Sensu postgraduate in Clinical Nutrition in Gama Filho University (Belo Horizonte, MG). In 2011, obtained the title of Specialist in Clinical Nutrition.

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*“The only way to do a great job is loving what you do!”*

*(Steve Jobs)*

**SUMMARY**

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## LIST OF ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| 3-DXA | 3-deoxyanthocyanidins |
| 5-MeO-LUT | 5-methoxy-luteolinidin |
| 7-MeO-API | 7-methoxyapigeninidin |
| ANOVA | Analysis of variance |
| AP | Apigeninidin |
| BF% | Body fat percentage |
| BHT | Butylated hydroxytoluene |
| BMI | Body mass index |
| CRP | C-reactive protein |
| DAD | Diode array detector |
| DBP | Diastolic blood pressure |
| DEXA | Dual-energy X-ray absorptiometry |
| DM | Diabetes mellitus |
| DRI | Dietary Reference Intakes |
| EDTA | Acid etilen diamino tetraacetic acid |
| EER | Estimated energy requirement |
| ELISA | Enzyme-linked immunosorbent assay |
| FACT | Food Action Rating Scale |
| FBG | Fasting blood glucose |
| GAE | Gallic acid equivalents |
| GI | Glycemic index |
| GPx | Glutathione peroxidase |
| HC | Hip circumference |
| HDL-C | High-density lipoprotein cholesterol |
| HPLC | High-performance liquid chromatography |
| iAUC | Incremental area under the curve |
| IL-6 | Interleukin-6 |
| IL-10 | Interleukin-10 |
| KCAL | Kilocalories |
| LDL-C | Low density lipoprotein cholesterol |
| LUT | Luteolinidin |

| | |
|---|---|
| NCDs | Non-communicable diseases |
| pH | Hydrogen potential |
| RI | Refractive index |
| PCR | Polymerase chain reaction |
| SAD | Sagittal abdominal diameter |
| SBP | Systolic blood pressure |
| SCFA | Short chain fatty acid |
| SD | Standard deviation |
| TAC | Total antioxidant capacity |
| TC | Total cholesterol |
| TG | Triglycerides |
| TNF-α | tumor necrosis factor-α |
| VFA | Volatile fatty acids |
| WC | Waist circumference |
| WHtR | Waist-to-height ratio |

## RESUMO

ANUNCIAÇÃO, Pamella Cristine, D.Sc., Universidade Federal de Viçosa, março de 2017. **Effect of sorghum (*Sorghum bicolor* L.) on metabolic variables in adults.** Orientadora: Helena Maria Pinheiro Sant'Ana. Coorientadoras: Rita de Cássia Gonçalves Alfenas, Hércia Stampini Duarte Martino e Valéria Aparecida Vieira Queiroz.

O sorgo apresenta-se como uma possibilidade de consumo aos cereais convencionais por ser uma excelente fonte de compostos bioativos, que contribuem para sua elevada capacidade antioxidante. Os produtos à base de sorgo podem, portanto, ser úteis para reduzir o risco de doenças crônicas não transmissíveis. Este estudo teve como objetivo avaliar o efeito do consumo de preparações à base de sorgo extrusado nas variáveis metabólicas em indivíduos adultos. Para avaliar o efeito do consumo de sorgo extrusado na resposta glicêmica de uma refeição subsequente (Artigo 1), 10 adultos eutróficos e normoglicêmicos participaram de 4 sessões experimentais em que foram consumidas umas das 3 bebidas contendo diferentes genótipos de sorgo ou uma bebida sem sorgo (controle). As bebidas foram preparadas homogeneizando-se os ingredientes (farinha de sorgo, leite em pó, cacau em pó, adoçante) em 200 mL de água mineral. Trinta minutos após a ingestão de uma das bebidas (todas contendo 25g de carboidrato disponível), os voluntários consumiram uma solução glicosada. A resposta glicêmica foi monitorada nos tempos 0 (antes da ingestão da solução glicosada), 15, 30, 45, 60, 90 e 120 minutos (após o consumo da solução glicosada). Os resultados mostraram que a ingestão da bebida contendo sorgo P 3DXA (rico em proantocianidinas e 3-desoxiantocianidinas) resultou em iAUC pós-prandial menor do que as outras bebidas contendo sorgo, e as bebidas contendo sorgo minimizaram o pico da glicemia pós-prandial. Foram desenvolvidas preparações à base de sorgo e trigo (cereal matinal e bebidas) para oferecimento aos voluntários do estudo de intervenção (Artigo 2). As preparações foram avaliadas quanto à aceitação sensorial, ocorrência e concentração de compostos bioativos (3-desoxiantocianidinas, flavonas, flavanonas, fenólicos totais e vitamina E) e capacidade antioxidante. O cereal matinal à base de sorgo integral teve maior aceitação que o cereal à base de trigo integral. O cereal de sorgo apresentou maior concentração de 3-desoxiantocianidinas (100% maior), compostos fenólicos totais (98,2% maior) e capacidade antioxidante (87,9% maior) que o cereal matinal de trigo. Flavonas e flavanonas não foram detectadas em ambos os cereais. A concentração de vitamina E foi 78,6% maior no cereal matinal à base de trigo integral. Para avaliar o efeito do consumo de preparações à base de sorgo extrusado no controle de variáveis metabólicas (Artigo 3),

participaram do estudo 24 homens com excesso de peso. O estudo *crossover* randomizado consistiu de 2 períodos de 8 semanas com pelo menos 4 semanas de *washout*, em que os voluntários foram alocados em 2 tratamentos: consumo de preparações à base de sorgo ou à base de trigo. Foram avaliados composição corporal, perfil lipídico, glicêmico e resistência à insulina, marcadores de extresse oxidativo, vitaminas séricas e carotenoides, e ingestão calórica e de macronutrientes, ao início e ao final de cada período de intervenção. O consumo de sorgo extrusado por 8 semanas levou à redução no peso corporal, perímetro da cintura, percentual de gordura corporal e aumentou níveis de glutationa peroxidase plasmática. O consumo de trigo extrusado reduziu o perímetro da cintura, porém aumentou a glicemia de jejum. Para avaliar o efeito do consumo de sorgo na composição da microbiota intestinal (Artigo 4), durante a segunda etapa do estudo de intervenção, ao início e ao final de 8 semanas, foi coletada uma amostra de fezes dos 22 participantes e foi coletada amostra de sangue por punção venosa para determinar os marcadores de resposta inflamatória. As amostras de fezes foram coletadas em frascos estéreis, mantidas sob refrigeração e entregues em no máximo 12 horas. Alíquotas foram armazenadas a -80ºC até o momento das análises. O potencial hidrogeniônico (pH) fecal foi mensurado em pHmetro digital imediatamente após o preparo da amostra. Os ácidos orgânicos foram determinados por cromatografia líquida de alta eficiência com detecção por índice de refração. A composição da microbiota intestinal foi determinada por PCR com a finalidade de caracterizar os filos Proteobacteria, Firmicutes e Bacteroidetes. Os marcadores inflamatórios foram analisados por ELISA, utilizando kit específico ((HCYTOMAG-code 60K, Millipore). Após as 8 semanas de intervenção, não houve alteração no pH fecal, na concentração dos ácidos orgânicos e na composição da microbiota intestinal em ambos os grupos. Também não houve alteração nos marcadores inflamatórios avaliados. Sugere-se que a ingestão de preparações contendo sorgo pode ser uma medida eficaz para reduzir a glicemia pós-prandial da refeição subsequente, melhorar o controle glicêmico, auxiliar na redução de peso e na melhora do estresse oxidativo. O consumo de cereais matinais à base de sorgo integral deve ser encorajado, uma vez que tem boa aceitação sensorial e é uma fonte de compostos bioativos que podem promover benefícios para a saúde humana. Mais estudos a respeito do efeito do consumo de sorgo na composição da microbiota intestinal devem ser realizados com tamanho amostral maior ou utilizando técnicas mais específicas.

## ABSTRACT

ANUNCIAÇÃO, Pamella Cristine, D.Sc., Universidade Federal de Viçosa, March, 2017. **Effect of sorghum (*Sorghum bicolor* L.) on metabolic variables in adults.** Advisor: Helena Maria Pinheiro Sant'Ana. Co-advisors: Rita de Cássia Gonçalves Alfenas, Hércia Stampini Duarte Martino and Valéria Aparecida Vieira Queiroz.

Sorghum presents as a possibility of consumption to conventional cereals because it is an excellent source of bioactive compounds, which contribute to its high antioxidant capacity. Sorghum-based products can therefore be useful in reducing the risk of chronic noncommunicable diseases. This study aimed to evaluate the effect of the consumption of extruded sorghum-based preparations on the metabolic variables in adult subjects. To evaluate the effect of extruded sorghum consumption on the glycemic response of a subsequent meal (Article 1), 10 eutrophic and normoglycemic adults participated in 4 experimental sessions in which one of the 3 drinks containing different sorghum genotypes or a non-sorghum drink were consumed (control). Drinks were prepared by homogenizing the ingredients (sorghum flour, milk powder, cocoa powder, and sweetener) in 200 ml of mineral water. Thirty minutes after ingestion of one drink (all containing 25g of available carbohydrate) volunteers consumed a glucose solution. The glycemic response was monitored at times 0 (before glucose solution intake), 15, 30, 45, 60, 90 and 120 minutes (after consumption of the glucose solution). Results showed that ingestion of the P 3DXA drink (containing proanthocyanidins and 3-deoxyanthocyanidins) resulted in lower postprandial iAUC than the other sorghum containing drinks. Sorghum-containing drinks minimized the postprandial blood glucose peak compared to non-sorghum drink. Sorghum and wheat-based preparations (breakfast cereal and drinks) were developed for volunteers consumption in the intervention study (Article 2). These preparations were evaluated for sensory acceptance, occurrence and concentration of bioactive compounds (3-deoxyanthocyanidins, flavones, flavanones, total phenolics and vitamin E) and antioxidant capacity. Sorghum breakfast cereal was more widely accepted than whole wheat cereal. The sorghum cereal presented higher content of 3-deoxyanthocyanidins (100% higher), total phenolic compounds (98.2% higher) and antioxidant capacity (87.9% higher) than wheat breakfast cereal. Flavones and flavanones were not detected in both cereals. Vitamin E content was 78.6% higher in whole-wheat breakfast cereal. To evaluate the effect of the consumption of sorghum-based preparations in the control of metabolic variables (Article 3), 24 overweight men participated in the study. The randomized crossover study consisted of 2 periods of 8

weeks with at least 4 weeks of washout, in which volunteers were allocated in 2 treatments: consumption of sorghum or wheat-based preparations. Body composition, lipid and glycemic profile, insulin resistance, oxidative stress markers, serum vitamins and carotenoids, and caloric and macronutrient intake were evaluated at the beginning and at the end of each intervention period. The consumption of extruded sorghum for eight weeks led to a reduction in body weight, waist circumference, percentage of body fat and increased levels of glutathione peroxidase. To evaluate the effect of sorghum consumption on the composition of the intestinal microbiota, during the second stage of the intervention study, at the beginning and at the end of 8 weeks, a stool sample was collected from 22 participants and a blood sample was collected by venipuncture to determine the markers of inflammatory response. Stool samples were collected in sterile vials, kept under refrigeration and delivered in a maximum of 12 hours. Aliquots were stored at -80°C until analysis. The fecal hydrogen potential (pH) was measured in digital pHmeter immediately after sample preparation. Organic acids were determined by high performance liquid chromatography with refractive index detection. The composition of the intestinal microbiota was determined by PCR with the purpose of characterizing Proteobacteria, Firmicutes and Bacteroidetes. Inflammatory markers were analyzed by ELISA using specific kit ((HCYTOMAG-code 60K, Millipore). The consumption of extruded wheat reduced waist circumference, but increased fasting blood glucose. After 8 weeks of intervention, there was no change in faecal pH, organic acid concentration and microbiota composition in both groups. There was also no change in the inflammatory markers evaluated. It is suggested that the ingestion of sorghum-based preparations may be an effective measure to reduce postprandial blood glucose from the subsequent meal, improve glycemic control, assist in weight reduction, and improve oxidative stress. Consumption of whole grain sorghum-based cereals should be encouraged as it has good sensory acceptance and is a source of bioactive compounds that promote human health benefits. Further studies on the effect of sorghum consumption on the composition of the intestinal microbiota should be performed with a larger sample size or using more specific techniques.

## 1. INTRODUCTION

Obesity is one of the most serious public health problem because of its high prevalence worldwide. More than 1.9 billion adults were overweight and over 600 million were obese in 2014 (WHO, 2016). In Brazil, 18.9% of adults are obese (BRASIL, 2017). Increased body weight is related to increased risk of mortality and development of chronic non-communicable diseases (NCDs) (WHO, 2011). In addition, an association between obesity and vitamin deficiency has been postulated. Overweight individuals are more predisposed to vitamin deficiency, especially the fat-soluble vitamins, folic acid, vitamin B12 and vitamin C, as compared to individuals of normal weight (VALDÉS *et al.*, 2017).

Obesity, especially visceral, is associated with a low-grade pro-inflammatory state and oxidative stress (MATSUDA e SHIMOMURA, 2013), which can act synergistically, causing metabolic changes in adipose tissue and at the systemic level. The chronic state of subclinical inflammation favors insulin resistance, a central event in the generation of cardiometabolic risk. In the condition of oxidative stress, there is an increase in the expression of genes regulated by redox signaling (MATSUZAWA-NAGATA *et al.*, 2008), favoring the production of inflammatory cytokines, which, in turn, induce the production of free radicals (CHUNG *et al.*, 2009).

In this context, there is an interest in the role of the intestinal microbiota in the occurrence of obesity and metabolic disorders. Different compositions of the microbiota, especially related to feeding, may increase the production of proinflammatory cytokines by altering the expression of host genes and inducing pathogenic state capable of facilitating the development of NCDs (MORAES *et al.*, 2014).

Increased consumption of whole grains helps reduce the risk of developing chronic diseases associated with obesity (CONNOLY *et al.*, 2016). Whole grains are rich in fermentable carbohydrates such as dietary fiber, starch resistant and oligosaccharides and a proposed protection mechanism is the effect on intestinal microbiota (SLAVIN, 2003). Phytochemicals present in cereals, such as phenolic compounds, act to protect the body against oxidative stress and its effects, due to their antioxidant properties (TAYLOR *et al.*, 2014). Sorghum presents as a possibility of consumption to conventional cereals because it is an excellent source of bioactive compounds, such as phenolic acids, flavonoids, tannins, anthocyanins, vitamin E, as well as dietary fiber, which contribute to its high antioxidant capacity (AWIKA e ROONEY, 2004).

Due to increased interest in healthy and functional foods and concern for environmental sustainability, new opportunities arise for the use of sorghum as a raw material for human consumption (VÁZQUEZ-ARAÚJO *et al.*, 2012). Sorghum can be used as an ingredient in the preparation of porridge, cereal bar and various bakery products (GONZÁLEZ *et al.*, 2002; QUEIROZ *et al.*, 2012; TRAPPEY *et al.*, 2014). Sorghum-based products have shown good acceptability (ANUNCIAÇÃO *et al.*, 2017; CARSON *et al.*, 2000, GONZÁLEZ, 2005). Therefore, sorghum has sensory potential to replace traditional cereals and is considered an excellent option for the food industry. Sorghum-based products may serve as a strategy for the obesity control and thus be useful in reducing the risk of chronic non-communicable diseases (AWIKA e ROONEY, 2004).

There are still few studies that evaluated in humans the effects of long-term consumption of sorghum and its functional potential on chronic non-communicable diseases (KHAN *et al.*, 2014; POQUETTE *et al.*, 2014; STEFOSKA-NEEDHAM *et al.*, 2017). No studies have been found to evaluate the chronic consumption of extruded sorghum in the control of metabolic variables and the effect on the composition of the intestinal microbiota. Therefore, studies are needed to develop and characterize chemically sorghum-based products, as well as to evaluate their sensorial acceptance, and to investigate the functional effects of sorghum consumption on the control of metabolic variables in adult individuals.

In this context, our hypothesis was that sorghum-based products may show good sensory acceptance and their consumption may contribute to bioactive compounds intake, weight loss, an improvement in glycemic control, inflammatory and oxidative stress parameters and intestinal microbiota composition, and reducing the food intake.

This thesis is composed of four articles. The article 1 discuss the effect of consumption of a drink containing sorghum on glycemic response of a subsequent meal. The article 2 compared the sensorial acceptance and the bioactive compound content of sorghum and wheat breakfast cereals. In article 3 we evaluated the effect of sorghum consumption on weight loss and oxidative stress marker in overweight men, and in the article 4, analyzed the effect of sorghum consumption on inflammatory markers, fecal pH, organic acid production and in intestinal microbiota composition.

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## 2. OBJECTIVES

### 2.1. General objective

To evaluate the effect of sorghum (*Sorghum bicolor* L.) consumption on the metabolic variables in adult individuals.

### 2.2. Specific Objectives

- To evaluate the effect of the consumption of a drink containing extruded sorghum on the glycemic response of a subsequent meal in eutrophic and normoglycemic subjects;
- To compare the sensory acceptance and the content of bioactive compounds of sorghum and wheat breakfast cereals;
- To evaluate the effect of the extruded sorghum consumption (drinks and breakfast cereal) on body composition, lipid profile, glycemic profile, insulin resistance, oxidative stress markers, serum vitamins (retinol and tocopherols) and carotenoids concentration and in caloric and macronutrient intake in overweight men;
- To evaluate the effect of extruded sorghum intake on inflammatory markers, fecal pH, short chain fatty acid production and the intestinal microbiota composition in overweight men.

## 3. RESULTS

### 3.1. Article 1: Consumption of a drink containing extruded sorghum reduces glycaemic response of the subsequent meal.

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ORIGINAL CONTRIBUTION

# Consumption of a drink containing extruded sorghum reduces glycaemic response of the subsequent meal

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**Abstract**

*Purpose:* Glycaemic control is essential to prevent the manifestation of diabetes in predisposed individuals and the development of associated comorbidities. It is believed that sorghum may modulate the glucose response. In this study, we investigated the effect of extruded sorghum consumption, and the profile of bioactive compounds, on postprandial glycaemia of a subsequent meal in normal weight and normoglycaemic subjects.

*Methods:* This was a randomized, single-blind, crossover designed study. After a 12 h overnight fasting, ten subjects reported to the laboratory to participate in four experimental sessions, and consumed one of three sorghum test drinks: sorghum P 3-DXAs (with proanthocyanidins—P and rich in 3-deoxyanthocyanidins—3-DXAs); 3-DXAs (without proanthocyanidins and rich in 3-DXAs); and control (low in 3-DXAs and without proanthocyanidins); or a non-sorghum drink. 30 min later, the subjects consumed a glucose solution (25 g glucose). Glycaemic response was monitored at times 0 (before glucose solution), 15, 30, 45, 60, 90, 120 min (after glucose solution consumption). The incremental areas under the glycaemic curve (iAUC) were calculated by the trapezoidal method.

*Results:* Intake of P 3-DXAs drink before the glucose solution resulted in a postprandial iAUC lower than the other sorghum test drinks. Sorghum drinks minimized the postprandial glycaemia peak.

*Conclusion:* Sorghum drinks consumption, especially the P 3-DXAs drink, 30 min before the glucose solution resulted in lower iAUC compared to the non-sorghum drink, leading to a lower glycaemic response.
**Keywords** Diabetes mellitus, Glycaemic response, Sorghum, Food and beverages

## Introduction

Diabetes mellitus (DM) is an endocrine and chronic metabolic disorder characterized by hyperglycaemia, which results from defective insulin secretion and/or action [1]. Glycaemic control is essential to prevent the manifestation of diabetes in predisposed individuals and the development of comorbidities related to this disease [2]. It is believed that inclusion of sorghum (*Sorghum bicolor L. Moench)* in the diet can maintain constant blood glucose [3–5]. However, in some regions of the world humans do not commonly consume this cereal, either due to cultural reasons, lack of knowledge of the cereal or the low availability of sorghum-based products.

Sorghum is produced and used for human consumption in Asia, Africa and other semi-arid regions of the world. In contrast, in Australia, USA and Brazil this cereal is mainly used for animal feed production [6]. A recent study showed that whole sorghum-based products present low glycaemic index [7]. This effect is related to the synergic action of its nutrients (e.g. calcium, magnesium, zinc and resistant starch) and bioactive compounds, particularly phenolic compounds and dietary fibre. It is noteworthy, however, that this effect may vary depending on the nutrient profile, type of preparations and grain processing conditions [7]. Genetic and environmental factors such as soil and climate determine the profile of sorghum vitamins and carotenoids [8].

The results of recent studies suggest that chronic consumption of phenolic compound extracts from sorghum improve glucose metabolism in animals [3–5]. In addition, acute or chronic consumption of whole sorghum-based preparations (e.g. fermented products, baked preparations and muffins) reduced postprandial glycaemia in humans and animal models [9, 10]. However, to date we found no studies that evaluated the effect of extruded sorghum consumption on postprandial glucose. Expanded extrudes, such as snacks or breakfast cereal, are very popular due to their ease of consumption. Furthermore, extruded sorghum flour may be indicated for use in ready-to-eat porridge or even for the preparation of drinks.

Although the effect of sorghum on immediate postprandial blood glucose has been investigated [10], its impact on blood glucose after consumption of a subsequent meal is

unknown. There is evidence that consumption of meals with low glycaemic index and rich in fibre not only lowers blood glucose acutely, but can also improve glucose and insulin after the subsequent meal (second meal) [11]. This effect may occur due to a reduction in the rate of carbohydrate digestion and absorption after the first meal and reduce the release of short-chain fatty acids, thereby improving insulin sensitivity of the subsequent meal [12]. The objective of this study was to evaluate the effect of consuming an extruded sorghum-containing beverage on postprandial glycaemia of a subsequent meal in normal weight and normoglycaemic subjects.

## Methods

### Subjects

The sample size was calculated according to Mera et al. [13], considering the incremental area under the curve of glycaemic response (iAUC) as the main variable [14]. A statistical power of 90 % and an expected difference of 10 % in the baseline values were adopted, totalling a sample of 10 individuals.

During recruitment, the volunteers filled out a form with personal information, data related to the inclusion criteria, family and individual medical history. The following eligibility criteria were considered: normoglycaemic subjects (capillary fasting blood glucose levels ≥70 and ≤99 mg/dL), normal weight (body mass index ≥18.5 and ≤24.9 kg/m2, body fat percentage ≥20 and ≤30 % for women and ≥12 and ≤20 % for men), no family history of diabetes or glucose intolerance and no use any medications that alter blood glucose.

The study protocol was in accordance with the Declaration of Helsinki and was approved by the Human Ethical Committee in Scientific Research (CAAE:13630513.0.0000.5153) of the Universidade Federal de Viçosa, Brazil. All subjects received oral and written information of the study protocol and signed a consent form.

### Protocol

This is a randomized crossover study, in which after a 12-h overnight fasting period, subjects reported to the laboratory to participate of four experimental sessions, in a random order. In each session, one of three sorghum drinks or the non-sorghum drink was consumed within 15 min. 30 min later, subjects consumed the glucose solution (subsequent meal). There was a washout period of at least 1 day between sessions.

Participants stayed in the laboratory for the following 120 min for postprandial glycaemia assessments (Fig. 1). At the end of the experimental session, all subjects received a standardized meal.

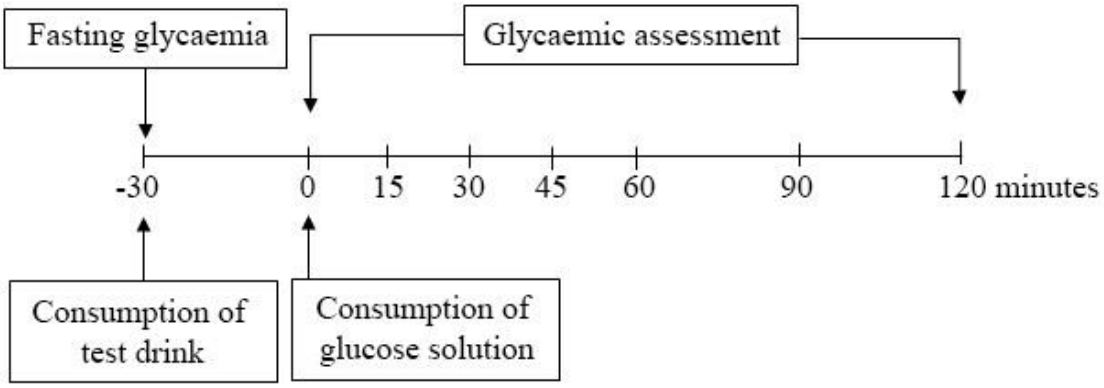


**Fig. 1** Experimental design of the study. After a 12-h overnight fasting period, subjects consumed one of three sorghum drinks or a non-sorghum drink. After 30 min, they consumed a glucose solution. Finger-stick blood samples were collected in fasting state, and 15, 30, 45, 60, 90 and 120 min after consumption of the glucose solution.

**Anthropometric and body composition evaluations**

Measurements were carried out by a single trained nutritionist following standard procedures. Height and weight were, respectively, measured [15] using a stadiometer fixed to the wall (SECA 206®, graduation 0.1 cm) and a platform digital scale (Toledo Brazil 2096PP®, graduation 50 g). Body composition was assessed by skinfold thickness [16], using a scientific adipometer Lange skinfold calliper (precision 0.1 cm). The body fat percentage was estimated by the sum of bicipital, tricipital, subscapular and suprailiac skinfolds [17].

**Test drinks**

Four drinks were prepared by homogenizing all the ingredients in 200 mL of mineral water (Table 1): (1) drink P 3-DXAs, containing extruded grains of sorghum genotype SC319 (with proanthocyanidins—P, and rich in 3-deoxyanthocyanidins— 3-DXAs); (2) drink 3-DXAs, containing extruded grains of genotype BDLO357 (without proanthocyanidins, and rich in 3-DXAs); (3) control drink, containing extruded grains of genotype SC391 (low in 3-DXAs, and without proanthocyanidins); (4) non-sorghum drink, which was prepared using the same ingredients of the sorghum drinks, except the sorghum flour. The control drink was our positive control since it contained a sorghum

genotype having smaller quantities or absence of bioactive compounds compared to the other sorghum drinks. The non-sorghum drink was our negative control since it did not contain sorghum or any other cereal. These drinks were necessary to compare the effect of the bioactive compounds profile on postprandial glycaemia.

**Table 1:** Ingredients of test drinks.

| Ingredients | Non-sorghum drink | Sorghum P 3-DXAs Drink | Sorghum 3-DXAs Drink | Sorghum Control Drink |
|---|---|---|---|---|
| Extruded sorghum (g) | - | 23 | 23 | 23 |
| Powdered skim milk (g) | 27 | 20 | 20 | 20 |
| Anhydrous glucose (g) | 11 | - | - | - |
| Cocoa powder (g) | 4 | 4 | 4 | 4 |
| Soy oil (mL) | 0.4 | - | - | - |
| Sweetener (mL) | 0.05 | 0.05 | 0.05 | 0.05 |

P 3DXAs: sorghum with proanthocyanidins and rich in 3-deoxyanthocyanidins; 3DXAs: sorghum without proanthocyanidins and rich in 3-DXAs; control: low in 3-DXAs, and without proanthocyanidins.

For the preparation of sorghum-containing drinks, the sorghum genotypes (SC319, SC391 and BDLO357) belonging to the collection of the Embrapa Milho e Sorgo (Sete Lagoas, MG, Brazil) were extruded [18]. The glucose solution was prepared by diluting 25 g of anhydrous glucose (Vetec, Rio de Janeiro) into 200 mL of spring water.

The drinks were designed to provide 25 g of available carbohydrate. All drinks except the glucose solution had equal amounts of macronutrients (Table 2). The content of nutrients and bioactive compounds of the drinks was estimated from the results of previous analysis of the chemical composition of extruded sorghum flour [19] and information obtained on the labels of other ingredients (Table 2).

**Table 2:** Nutrient and bioactive compounds content in 200 ml of drinks with and without sorghum.

| | Non-sorghum Drink[1] | P 3DXAs Drink[2] | 3DXAs Drink[2] | Control Drink[2] |
|---|---|---|---|---|
| Total carbohydrate (g) | 25.60 | 28.81 | 28.50 | 28.38 |
| Available carbohydrate (g) | 25.00 | 25.16 | 25.25 | 25.27 |
| Glucose (g) | 25.00 | 11.78 | 11.71 | 11.51 |
| Total starch (g) | - | 13.38 | 13.54 | 13.76 |
| *Amylose (g)* | - | 3.45 | 3.73 | 2.05 |
| *Amylopectin (g)* | - | 9.92 | 9.80 | 11.71 |
| Resistant starch (g) | - | 0.68 | 0.05 | 0.24 |
| Total dietary fiber[1] (g) | 0.60 | 3.65 | 3.25 | 3.11 |
| Soluble fiber[2] (g) | NA | 0.34 | 0.21 | 0.29 |
| Insoluble fiber (g) | NA | 2.71 | 2.43 | 2.22 |
| Protein[1] (g) | 10.20 | 10.25 | 10.32 | 10.29 |
| Lipid[1] (g) | 1.24 | 1.23 | 1.24 | 1.32 |
| Ash[1] (g) | - | 0.24 | 0.38 | 0.44 |
| Zinc[2] (mg) | - | 0.82 | 0.64 | 0.41 |
| Magnesium[2] (mg) | - | 0.04 | 0.04 | 0.02 |
| Calcium (mg) | | 0.01 | 0.005 | 0.003 |
| Total phenolic[2] (mg GAC Eq.) | - | 1.38 | 0.81 | 0.51 |
| 3-deoxyanthocyanidins (µg/g) | - | 22.59 | 16.61 | 1.44 |
| Proanthocyanidins[2] (µg Cat Eq.) | - | 0.23 | - | - |

[1]Based on information obtained on the label of ingredients used to prepare the test drinks; [2]Obtained considering the previous results of the chemical composition of extruded sorghum flour and information obtained on the labels of the other ingredients used to prepare the test drinks. NA: Not available. Total carbohydrate (g) = Glucose (g) + Total starch (g) + Total dietary fiber (g). Available carbohydrate (g) = Total carbohydrate - Total dietary fiber (g)).

P 3DXAs: sorghum with proanthocyanidins and rich in 3-deoxyanthocyanidins; 3DXAs: sorghum without proanthocyanidins and rich in 3-DXAs; control: low in 3-DXAs, and without proanthocyanidins.

**Postprandial glycaemia assessment**

Capillary finger-stick blood samples were taken in the fasting state (0 min) and at 15, 30, 45, 60, 90 and 120 min after consumption of the glucose solution. Glucose was measured using the glucometer Accu Check Performa Nano (Roche, São Paulo, Brazil). The incremental positive area under the glycaemic response curve (iAUC) was calculated by the trapezoidal method (Food and Agriculture Organization, 1998) using the software SlideWrite 7.0®.

**Statistical analysis**

Data were expressed as mean and standard error of the mean (SE). Data normality was assessed by the Shapiro–Wilk test. One-way ANOVA was used to assess significant differences in iAUC. The two-way repeated measures ANOVA was applied to verify the interaction of time and treatment factors, followed by post hoc comparisons using Duncan's test when necessary. Statistical analyses were conducted using SPSS 20 for Windows (SPSS, Inc., Chicago, IL, USA), adopting a significance level (α) of 5 %.

**Results**

Ten subjects (1 man and 9 women) with mean fasting glycaemia of 4.88 ± 0.07 mmol/L, BMI of 22.4 ± 0.91 kg/m2 and 24.6 ± 1.46 % body fat participated in the study. The study had a statistic power of 80 %, considering an expected reduction of 10 % in the incremental area under the curve (mean ± SD = 47.85 ± 5.35). During the test days, participants were asked about tolerance to the drinks and they reported no gastrointestinal side effects.

Fasting glycaemia of subjects did not differ between study sessions (Table 3). The increase in postprandial glycaemia was influenced by time, treatment and the interaction of these factors (Fig. 2).

**Table 3:** Mean ± baseline blood glucose obtained 30 minutes immediately before the consumption of glucose solution.

| Intervention | Baseline blood glucose |
|---|---|
| P3DXAs drink | 90.30 ± 0.89[a] |
| 3DXAs drink | 88.70 ± 1.17[a] |
| Control drink | 88.40 ± 1.56[a] |
| Non-sorghum drink | 88.20 ± 1.25[a] |

Means of blood glucose followed by different letters in the column are statistically different at 5% of probability by one-way ANOVA.

After consumption of the glucose solution (subsequent meal), the incremental glycaemia of P 3-DXAs drink was lower ($p < 0.05$) than that obtained in response to the others sorghum drinks and to the non-sorghum drink for up to 30 min (Fig. 2). No differences were verified between the treatments at 45 and 90 min (Fig. 2).

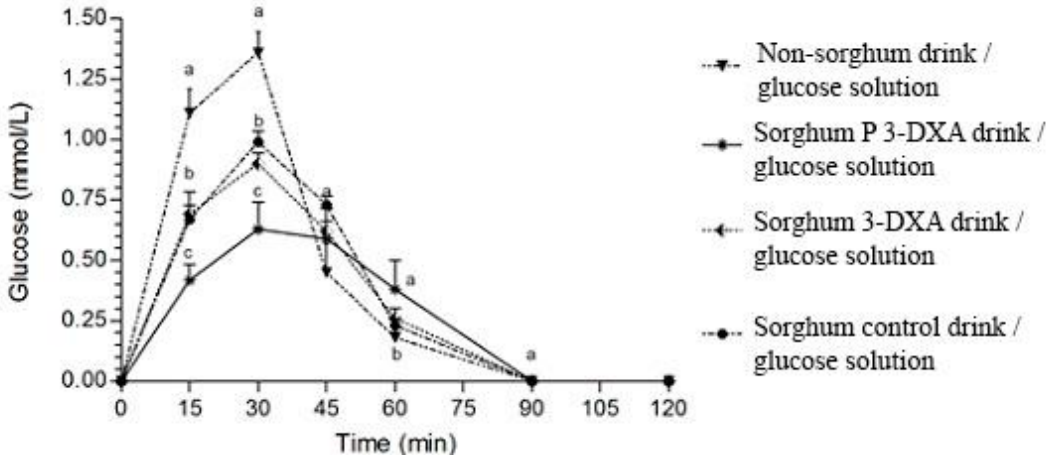


**Fig. 2** Mean ± SE of the postprandial glucose response (n = 10) obtained in the experimental sessions (first meal/second meal)—Session 1: non-sorghum drink/glucose solution; Session 2: sorghum P 3-DXAs drink/glucose solution; Session 3: sorghum 3-DXAs drink/glucose solution; Session 4: sorghum control drink/glucose solution. Different letters differ significantly from each other by the Duncan test at 5 % probability.

Compared to non-sorghum drink, sorghum drinks resulted in lower postprandial glycaemic response and reduced the peaks of glycaemia during the 120 min in which glycaemia was assessed (Fig. 2). Consumption of the sorghum drinks before the glucose solution resulted in a postprandial iAUC lower than the non-sorghum drink (Fig. 3).

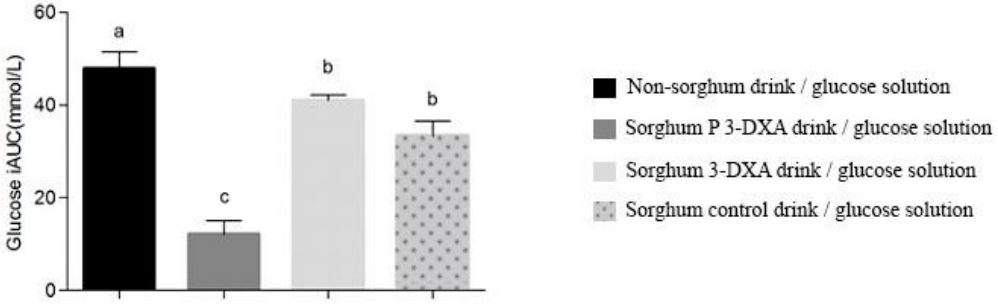


**Fig. 3** Mean ± SE of the postprandial incremental area under the glycaemic response curve (iAUC) after consumption of the drinks (first meal/second meal) in the experimental sessions: (1) non-sorghum drink/glucose solution; (2) sorghum P 3-DXAs drink/glucose solution; (3) sorghum 3-DXAs drink/glucose solution; (4) sorghum control drink/glucose solution (n = 10). Bars followed by different letters differ significantly from each other by the Duncan test at 5 % probability.

## Discussion

In the present study, the sorghum drinks resulted in lower postprandial glycaemia and iAUC in next 2 h after a glucose load (subsequent meal), reducing the peaks of

postprandial glycaemia. Among the sorghum drinks, P 3-DXAs drink was the one that led to the lower glycaemic response. Sorghum and non-sorghum drinks were consumed 30 min before assessing the glycaemic response to the glucose solution to ensure that the observed response would reflect the production and release of insulin stimulated by sorghum load. Monitoring glycaemic response in shorter time period (less than 30 min) would reflect the effect of stimulus of previous meal, not of the sorghum load [20].

Glycaemic control near normal levels is a major goal of nutritional intervention in diabetic and prediabetic patients [2]. Hyperglycaemic peaks may contribute to the development of diabetes complications, especially cardiovascular diseases [21]. It is recommended that therapeutic strategies be adopted to prevent the occurrence of blood glucose peaks [22]. In this context, the addition of sorghum to the diet is a viable alternative, which may favour glycaemic response in healthy people.

It is believed that the increased content of phenolic compounds, especially tannins and 3-deoxyanthocyanidins, dietary fibre and starch (total and resistant) in the P 3-DXAs drink, compared to others sorghum drinks and the non-sorghum drink, favoured the lower glycaemic response by the P 3-DXAs drink. These bioactive compounds may reduce the glycaemic response induced by a food [3, 4, 10, 23]. Although the P 3-DXAs drink has higher content of 3-deoxyanthocyanidins than the 3-DXAs drink, there is no direct evidence for 3-DXAs sorghum consumption and glycaemic properties in humans.

The sorghum phenolic compounds may contribute to improve glucose disorders in humans by modulating carbohydrate absorption. Sorghum tannins can reduce the activity of α-amylase (pancreatic and salivary) that digest starch [4, 23]. Thus, the rate of digestion and absorption of this carbohydrate, which is the main constituent of the sorghum drinks, may have been reduced, resulting in a lower glycaemic response.

The results of some studies suggest that the rate of carbohydrate digestion consumed in a meal may influence the glycaemic response of a subsequent meal [24, 25]. The consumption of slowly digested carbohydrate improves carbohydrate tolerance of the subsequent meal [24]. Marked insulin secretion and subsequent rebound relative hypoglycaemia may stimulate short-chain fatty acids release, which impairs downstream insulin signalling of the insulin receptor, leading to insulin resistance [26]. According to some authors, this effect occurs when the interval between the first meal and the subsequent meal is at least 4 h [24, 25]. However, it is still unknown whether this effect occurs when the interval between meals is shorter. Although not measured in the present study, sorghum ingestion may have stimulated lower levels of short-chain fatty acids

release, improving insulin sensitivity. In addition, the slow release of carbohydrate products due to sorghum slow digestion rate leads to a reduced GIP response, which is one of the main incretins enhancing insulin secretion in the presence of glucose [24, 25]. Therefore, the reduced GIP secretion may have contributed to reduce insulin response.

Furthermore, the tannins can interact with starch, especially amylose to form resistant starch, which is not digested in the small intestine [27, 28]. Consequently, there is a reduction in postprandial glycaemic response [28]. This effect was observed in a study in which normal weight and normoglycaemic subjects ate muffins containing sorghum compared to wheat muffins. The authors attributed this result to the higher content of resistant starch and slowly digestible starch and lesser content of rapidly digestible starch in muffins containing sorghum [10]. Studies demonstrated that sorghum contained the highest amount of resistant starch and the lowest amount of rapidly digestible starch compared with wheat, barley, oat, maize and rice [10, 29].

Sorghum can reduce the glycaemic response due to the action of its dietary fibre and resistant starch contents [9]. The dietary fibre, especially soluble fibres, reaches the large intestine intact, and thus delays the gastric emptying and slows down the rate of digestion and absorption of carbohydrates [30, 31].

Moreover, sorghum phenolic compounds and minerals can exert different stimuli on postprandial insulinemia [3, 4], reducing the glycaemic response. An increase in insulin concentration similar to glibenclamide (anti-diabetic medication) was observed in diabetic rats receiving phenolic extracts of sorghum, indicating better functioning of the β cells [3]. Therefore, this effect is of clinical relevance, especially for those type 2 diabetic patients whose insulin synthesis is reduced and that need some stimulation to increase insulin secretion. The consumption of sorghum can lead to better glycaemic response of these patients. Furthermore, it is suggested that the sorghum phenolic compounds can reduce glycaemia by inhibiting hepatic gluconeogenesis [4]. Our results indicated that the sorghum drinks had a higher content of phenolic compounds than the non-sorghum drink. However, in our study we did not evaluate the postprandial insulinemic response. In addition, more studies are needed to establish which sorghum compounds would be more effective to control glycaemia.

## Conclusion

Ingestion of the sorghum drinks 30 min before the glucose solution (subsequent meal) resulted in a lower postprandial glycaemic response compared to the non-sorghum

drink. The iAUC after consumption of P 3-DXAs drink followed by the glucose solution was lower than that obtained for the non-sorghum drink. Furthermore, compared to nonsorghum drink, sorghum drinks reduced postprandial glycaemia peaks. These results can be attributed to the higher tannins, total phenolic and resistant starch content, and the synergistic effect among these components, especially of the P 3-DXAs drink. Our results suggest that intake of preparations containing sorghum may be an effective strategy to reduce postprandial glycaemia of a subsequent meal, which may in turn lead to an improvement on glycaemic control.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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## 3.2. Article 2: Comparing sorghum and wheat whole grain breakfast cereals: sensorial acceptance and bioactive compound content

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# Comparing sorghum and wheat whole grain breakfast cereals: Sensorial acceptance and bioactive compound content

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**Abstract**

The sensory acceptance and the content of bioactive compounds of whole-sorghum and whole-wheat breakfast cereals were compared. Sensory acceptance was assessed using the Food Action Rating Scale. 3-Deoxyanthocyanidins, flavones and flavanones were determined by high-performance liquid chromatography (HPLC) with diode array detection, and vitamin E by HPLC with fluorescence detection. Total phenolics and antioxidant activity were determined by spectrophotometry. The sorghum breakfast cereal had better sensory acceptance (70.6%) than wheat breakfast cereal (41.18%). Sorghum had higher 3-deoxyanthocyanidin content (100% higher), total phenolic compounds (98.2% higher) and antioxidant activity (87.9% higher) than wheat breakfast cereal. Flavones and flavanones were not detected in both breakfast cereals. Total vitamin E content was 78.6% higher in wheat than in sorghum breakfast cereal. Thus, consumption of whole sorghum breakfast cereal should be encouraged, since it had good sensory acceptance and is a source of bioactive compounds that can promote benefits to human health.

**Keywords:** *Sorghum bicolor* L., Flavonoids, Tocopherols, Breakfast cereal

### 1. Introduction

Sorghum [*Sorghum bicolor* (L.) *Moench*] is a whole grain cereal that is better known to Western societies as an animal feed rather than a human food source (Stefoska-Needham, Beck, Johnson, & Tapsell, 2015). In countries, such as Australia, United States and Brazil, this cereal is mainly used for animal feed production. In contrast, sorghum is

produced and used for human consumption in countries of Africa, Asia and other semi-arid regions of the world (Taylor, Schober, & Bean, 2006).

The use of sorghum for human consumption in Western countries has increased due to its functional potential (Poquette, Gu, & Lee, 2014). Sorghum could be used as a substitute for conventional cereals due to its high bioactive compounds, minerals, dietary fiber, vitamin E and carotenoids content (Cardoso, Pinheiro, Martino, & Pinheiro-Sant'Ana, 2015a) and its potential to promote health and prevent diseases. This cereal can been used in the preparation of gluten-free products for individuals with celiac disease and other wheat intolerances (Stefoska-Needham et al., 2015). Furthermore, some sorghum genotypes contain tannins, which are bioactive compounds that could attract consumers interested in functional foods (Dlamini, Taylor, & Rooney, 2007).

Expanded extruded products, such as snacks and breakfast cereal, are very popular due to their crispness and ease of use. In the United States and other countries, including Brazil, these products are made typically with corn, although rice and wheat are also used. Although sorghum has a lower cost and is easier to produce than maize, until recently it had not been used for this purpose (Queiroz, Moraes, Martino, Paiva, & de Menezes, 2014). However, studies have been conducted in order to optimize the use of this cereal in the preparation of this type of product.

As far as we know, there are no studies on sensory analysis of whole-grain sorghum breakfast cereals compared to whole-grain wheat breakfast cereals. Sensory properties of a food product are important for its acceptance (Carson, Setser, & Sun, 2000). Sorghum-based products showed good acceptability (Carson et al., 2000; González, 2005; Shin, 1986). Therefore, sorghum has sensory potential to replace traditional cereals, being considered an excellent option for the food industry.

In addition, we found no studies that have assessed and compared the content of bioactive compounds in whole-grain sorghum and wheat breakfast cereals. Thus, the present study aimed to compare the sensory acceptance and the content of bioactive compounds of whole-grains sorghum and wheat breakfast cereals.

## 2. Materials and methods

### *2.1. Raw material, preparation and storage*

Sorghum grains (genotype SC319) were grown by Embrapa Milho e Sorgo in Nova Porteirinha, MG, Brazil, between May and September 2013. The whole grains were initially milled into flour using a disc mill model 3100 (Perten Instruments, Huddinge,

Sweden) set at position 2, added with 10% sucrose as fine granulated sugar and 0.5% of iodized salt (NaCl), and processed in a corotating intermeshing twin-screw extruder model Evolum HT 25 (Clextral, Firminy, France) at constant screw speed of 600 rpm and temperature profile of 30, 60, 90, 110, 110, 110, 120, 120, 130 and 140ºC, 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 2.0 mm in diameter each and 9 mm in length. The formulation 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 measuring 5.25 mm in 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 for moisture differences in the samples and provide a final moisture content of 12%. The samples were collected over 15–20 min and subsequently ground into particles measuring 212 µm.

The whole-grain wheat flour was acquired and extruded by SL Alimentos in Mauá da Serra, PR, Brazil. The wheat flour was added with 10% sucrose as fine granulated sugar and 0.5% of iodized salt (NaCl), which was processed in a co-rotating twin-screw model Evolum BC 72 (Clextral, Firminy, France) at constant screw speed of 200 rpm and temperature profile of 50, 81, 112, 118, 127 and 143ºC, from feeding to the outlet. The other conditions of extrusion were similar to sorghum.

The whole-grain sorghum and whole-grain wheat breakfast cereals (Fig. 1) were stored in polyethylene bags at 10 ± 2ºC until analyses.


**Fig. 1.** Whole-grain sorghum breakfast cereal (A) and whole-grain wheat breakfast cereal (B).

*2.2. Sensory acceptance*

The acceptance of sorghum and wheat breakfast cereals was evaluated by 51 untrained judges (21.6% male, 78.4% female) from the Federal University of Viçosa, Brazil, and surrounding areas. The study protocol was approved by the Human Ethical Committee in Scientific Research (CAAE: 13630513.0.0000.5153) of the Federal University of Viçosa.

The breakfast cereals (10–15 g) were served in plastic 50 ml cups coded with three digit numbers. Mineral water was provided for cleaning the mouth between analyzes of each product formulation. Along with the samples, each judge received a form to evaluate the acceptance of the extrudates. The Food Action Rating Scale (FACT) was used, being assigned score 9 to ‘‘I would eat it whenever I had the chance” and the score 1 for ‘‘Just eat that if I was forced” (Minim, 2013).

*2.3. Determination of bioactive compounds*

The occurrence and content of flavonoids (3-DXA, flavones and flavanones) and vitamin E (a, b, c and d-tocopherols and tocotrienols) were determined in sorghum and wheat breakfast cereals in five replicates. During all analyses, the samples and the extracts were protected from light (artificial and sunlight) and oxygen using amber glassware, aluminium foil and blackout curtains, and bottles with nitrogen gas environment.

*2.3.1. Flavonoids*

To extract the flavonoids, 20 ml of methanolic HCl solution 1% (v:v) were added to 2 g of sample and stirred for 120 min at 180 rpm. Then, the suspension was centrifuged at 2790g for 5 min and the supernatant collected, kept in amber bottle and stored in a freezer (-18 ± 1ºC) until analysis (Dykes, Seitz, Rooney, & Rooney, 2009).

The method described by Yang, Allred, Geera, Allred, and Awika (2012) and modified by Cardoso, Pinheiro, Martino, and Pinheiro-Sant’Ana (2015b) was used to identify and quantify the 3-deoxyanthocyanidins (luteolinidin, apigeninidin, 7-methoxyapigeninidin and 5-methoxy-luteolinidin), flavones (luteolin and apigenin) and flavanones (naringenin and eriodictyol). Flavonoids were determined 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. The following chromatographic conditions

were used: Kinetix C-18 column (150 x 4.6 mm id, 5 µm) equipped with a C-18 guard column (4 mm x 3 mm) (Phenomenex, Torrance, CA), column temperature 35ºC, injection volume of 20 µl, scan range 200–700 nm with detection at 480 nm for 3-deoxyanthocyanidins, 360 nm for flavones and 280 nm for flavanones. The mobile phase was composed of 2% formic acid in ultrapure water (line A) and 2% formic acid in acetonitrile (line B). The elution gradient of B was as follows: 0–3 min, 10% isocratic; 3–4 min, 10–12%, 4–5 min isocratic 12%; 5–8 min, 12–18%, 8–10 min, isocratic 18%; 10–12 min, 18–19%, 12–14 min, isocratic 19%; 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, isocratic 60%; 36–38 min, 60–10%, 38–45 min, isocratic 10%. To increase therepeatability of the retention time of the peaks it was used the following gradient: of 0–36 min flow 0.55 ml/min; 36–38 min, from 0.55 to 1.1 ml/min, 38–44 min, 1.1 ml/min; 44–45 min, 1.1 to 0.55 ml/min and the mobile phase was degassed with helium gas at 50 kPa before and during runs.

The identification of flavonoids was conducted by comparing the retention time and the absorption spectrum of the peaks of luteolinidin chloride (Sigma-Aldrich, St. Louis, MO, USA), apigeninidin chloride (Chromadex Santa Ana, CA, USA), luteolin, apigenin, naringenin and eriodictyol (Sigma-Aldrich, St. Louis, MO, USA), and samples analyzed under the same conditions. For quantification, analytical curves constructed from injection, in duplicate, of standard solutions with six different concentrations were used. The 5-MeO-LUT and 7-MeO-API were quantified using luteolinidin and apigeninidin standards, respectively, along with the appropriate molecular weight correction factor (Dykes et al., 2009). The compounds were expressed in µg/100 g of sample, as single compounds and as the sum of 3-DXAs.

*2.3.2. Vitamin E*

The occurrence and content of the eight components of vitamin E were carried out according to Pinheiro-Sant'Ana et al. (2011). Four milliliters (4 ml) of heated ultrapure water (80 ± 1ºC); 10 ml of isopropanol; 1.0 ml of hexane containing 0.05% BHT, 5 g of anhydrous sodium sulfate and 25 ml of extraction solvent mixture (hexane: ethyl acetate, 85:15, v/v) were added to approximately 5 g of sorghum or wheat flour. Subsequently, the suspension was homogenized using a micro grinder for 1 min and it was vacuum filtered on a Buchner funnel using filter paper, maintaining the residue in the extraction tube. The extraction step was repeated, adding to the residue 5 ml of isopropanol and 30

ml of the solvent mixture, with subsequent homogenization and vacuum filtration. Then, the extract was concentrated in a rotary evaporator at 70 ± 1ºC (2 min), transferred to a volumetric flask and made up to 25 ml with solvent mixture.

After extraction, an aliquot of 5.0 ml of extract were dried in nitrogen gas, recovered in 2.0 ml HPLC grade hexane (Tedia, Brazil) and filtered in filter units with porosity of 0.45 µm (Millipore, Brazil). Analyses were performed by injecting 15 µl of the extracts. The following chromatographic conditions were used: HPLC system (Shimadzu, SCL 10AD VP, Japan); fluorescence detector (290 nm excitation and 330 nm emission; Shimadzu, RF10AXL); Phenomenex Luna Si100 column (250 x 4 mm, 5 µm) coupled Si100 Phenomenex guard column (4 x 3 mm). The mobile phase was composed by hexane: isopropanol: glacial acetic acid (98.9:0.6:0.5 v/v/v); flow rate of 1.0 ml/min and run time of 22 min.

The identification of vitamin E compounds was conducted by comparing the retention time of the peaks of the commercial standards and samples analyzed under the same conditions. For quantification, analytical curves constructed from injection, in duplicate, of standard solutions prepared from commercial standards (Calbiochem®, EMD Biosciences, Inc. EUA) with six different concentrations were used.

*2.4. Determination of antioxidant activity*

The antioxidant activity was determined by spectrophotometry, using the DPPH radical method (1,1-diphenyl-2-picrylhydrazyl) (Bloor, 2001), in triplicate. Twenty milliliters (20 ml) of acetone solution at 70% was added to 2 g of sorghum or wheat extrudates. Then, the suspension was stirred at 180 rpm (2 h) and centrifuged at 2790g (5 min). The supernatant was transferred to an amber bottle and stored in a freezer (-18 ± 1ºC) until analysis.

In a test tube, protected from light, 100 µl of the extract was added to 1.5 ml of methanol solution of DPPH 0.1 mM and stirred by vortex for 30 s. After 30 min of standing, the absorbance of the solution was read in a spectrophotometer (Thermo Scientific, Evolution 606, USA) at 517 nm. The results were expressed as mmol Trolox equivalent/g sample. The 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (trolox) was obtained from Sigma-Aldrich (St. Louis, MO, USA).

*2.5. Determination of total phenolic*

The total amount of phenolic compounds was determined in triplicate using the Folin-Ciocalteu reagent (Singleton, Orthofer, & Lamuela-Raventos, 1999).

For analysis, 500 µl of the extract obtained in item 2.4 were added to 500 µl of Folin-Ciocalteu solution (20%) and 500 µl of sodium carbonate solution (7.5%). Then, the solution was stirred by vortex and incubated at room temperature for 30 min. The reading of absorbance was performed in spectrophotometer Evolution 606 (Thermo Scientific, USA) at 765 nm. Analytical curve of gallic acid (Sigma-Aldrich, St. Louis, MO, USA) (0.005–0.10 mg/ml) was used to quantify the phenolic compounds. The results were expressed in mg of gallic acid equivalents/g of sample (mg GAE/g).

*2.6. Experimental design and statistical analysis*

For sensory analysis, a randomized block design was used, with the blocks represented by the judges. Data normality was assessed using the Shapiro-Wilk test. The data were analyzed by ANOVA, followed by Tukey test. To compare the content of bioactive compounds between sorghum and wheat extrudates, the Student-t test was used. Statistical analyzes were performed using IBM SPSS Statistics software version 20.0 (Chicago, USA), adopting a significance level (a) of 5%.

## 3. Results and discussion

*3.1. Sensory acceptance*

Although little known for Brazilian people, the sensorial acceptance of whole-grain sorghum breakfast cereal (‘‘I would eat it frequently”) was higher ($p < 0.05$) than the whole-grain wheat breakfast cereal (‘‘I would eat it if it had available, but I do not force myself to eat it”) (Table 1).

**Table 1:** Medium Attitude Scale FACT for sorghum and wheat breakfast cereals.

| Formulations | Mean ± SD |
|---|---|
| Extruded sorghum | $6.67 \pm 1.58^{a}$ |
| Extruded wheat | $5.37 \pm 2.03^{b}$ |

Data expressed as mean ± standard deviation of the acceptance test responses. Means followed by different letters differ statistically at 5% probability by Tukey test.

Only the sorghum breakfast cereal was considered acceptable (Table 2) by presenting index of acceptance greater than 70% (Gularte, 2002). Although not evaluated in the present study, the most attractive colour (Fig. 1) and less dense texture of sorghum breakfast cereal may have contributed to its greater acceptance, as verified in other studies (González, 2005; Sanchez, 2004; Shin, 1986). Studies show that sorghum-based products may have better texture, greater expansion and lower density that wheat-based (Shin, 1986) and maize-based products (Sanchez, 2004).

**Table 2:** Distribution of percentage of rejection, indifference and acceptance of sorghum and wheat extruded.

| Formulations | % Rejection (score ≤ 4) | % Indifference (score = 5) | % Acceptance (score ≥ 6) |
|---|---|---|---|
| Extruded sorghum | 1.96 | 27.45 | 70.59 |
| Extruded wheat | 33.33 | 25.49 | 41.18 |

González (2005) demonstrated that excellent flavour, appearance and texture were obtained from whole grains brown tannin-sorghums, and could be an excellent choice for food processors. Sorghum with tannin also produced good extrudates, making it possible to add value to the product, due to its nutraceutical properties. Moreover, its reddish-brown appearance can be an advantage in special products (González, 2005). Therefore, the whole-grain sorghum breakfast cereal evaluated in the present study could be a good alternative for the food industry, since sorghum used also has a brown colour and the presence of tannins.

*3.2. Flavonoids*

We found four 3-DXAs (LUT, AP, 5-MeO-LUT and 7-MeO-AP) in the sorghum breakfast cereal (Fig. 2) and none in the wheat breakfast cereal (Table 3). Studies demonstrated that these are the main sorghum 3-DXAs and they are mainly found in grains with dark coloured pericarp (brown > red > yellow) (Awika, Rooney, & Waniska, 2004; Cardoso et al., 2015b; Dykes, Rooney, & Rooney, 2013). The maximum wavelength and retention time of 3-deoxyanthocyanidins in whole-sorghum breakfast cereal are presented in Table 4.

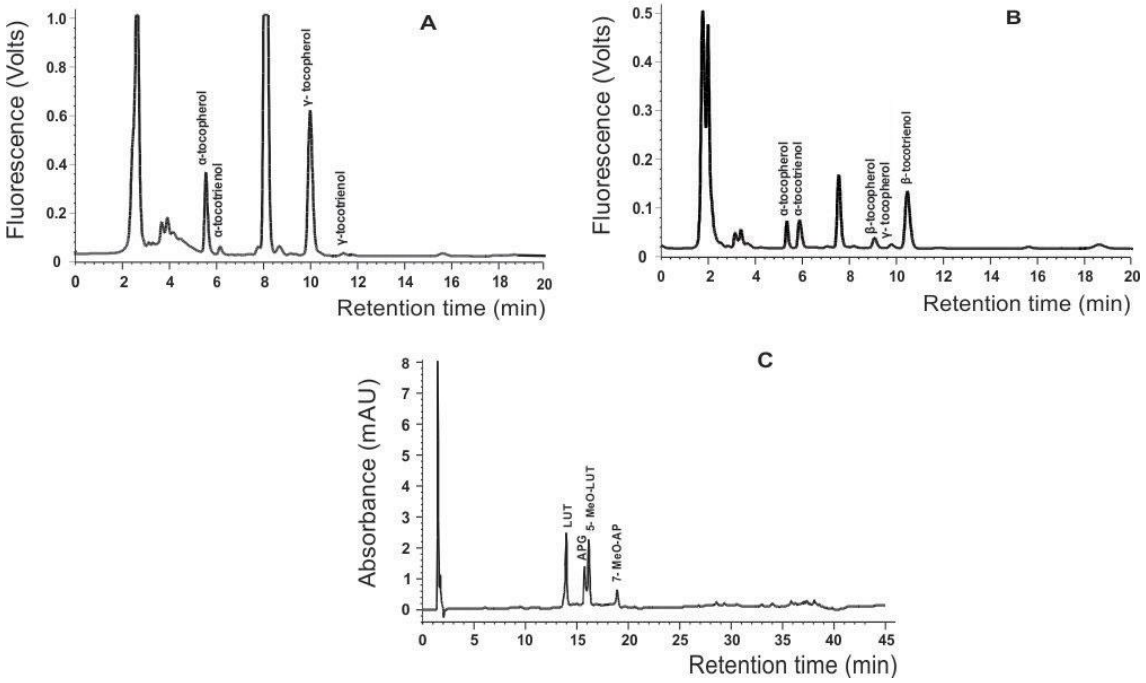


**Fig. 2.** HPLC analyses of vitamin E in sorghum (A) and in wheat (B), and 3-deoxyanthocianidins in sorghum breakfast cereal (C). LUT: Luteolinidin; APG: apigeninidin; 5-MeOLUT: 5-methoxy-luteolinidin; 7-MeO-AP: 7-methoxy-apigeninidin.

Apigeninidin was the most prevalent compound, comprising on average 36.18% of total DXAs (Table 3). Our result differs from the one verified by Cardoso et al. (2015b), in which 5-methoxyluteolinidin was more prevalent in extruded sorghum flour than apigeninidin. The difference in results can be attributed to environment interaction since the non-methoxylated 3-DXAs are more prone to degradation under weathering conditions (Taleon, Dykes, Rooney, & Rooney, 2012). Furthermore, sorghum used in the Cardoso et al. (2015b) experiment and in the present study were grown at different times.

**Table 3:** Profile and content of 3-deoxyanthocyanidins, vitamin E, phenolic compounds and antioxidant activity in whole-grain sorghum and wheat breakfast cereals.

| Compounds | Whole-grain sorghum breakfast cereal | Whole-grain wheat breakfast cereal |
|---|---|---|
| Total 3-DXAs (µg/100g) | 366.46±79.91[a] | nd[b] |
| *Luteolinidin* | 94.77±7.28[a] | nd[b] |
| *Apigeninidin* | 132.61±27.68[a] | nd[b] |
| *5-MeO-LUT* | 41.15±18.16[a] | nd[b] |
| *7-MeO-API* | 111.64±18.19[a] | nd[b] |
| Total vitamin E (µg/100g) | 796.90±33.72[b] | 3727.50±352.0[a] |
| *α-tocopherol* | 230.90±11.80[a] | 116.08±4.33[b] |
| *α-tocotrienol* | 70.62±2.94[b] | 463.52±20.02[a] |
| *β-tocopherol* | nd[b] | 83.70±3.90[a] |
| *β-tocotrierol* | nd[b] | 2997.60±289.50[a] |
| *γ-tocopherol* | 474.06±21.52[a] | 66.56±9.11[b] |
| *γ-tocotrienol* | 5.88±0.58[a] | nd[b] |
| *δ-tocopherol* | nd | nd |
| *δ-tocotrienol* | nd | nd |
| Total phenolic compounds (mg GAE/g) | 1.11±0.06[a] | 0.33±0.02[b] |
| Antioxidant activity (mmolTrolox/g) | 4.05±0.04[a] | 0.49±0.08[b] |

5-MeO-LUT: 5-methoxy-luteolinidin; 7-MeO-API: 7-methoxy-apigeninidin; 3-DXAs: 3-deoxyanthocyanidins; nd: not detected. The results were expressed as fresh matter as the average of five replicates ± standard deviation. Same letters on the line do not differ by t test at 5% probability.

**Table 4:** Maximum wavelength and retention time of 3-deoxyanthocyanidins in whole-sorghum breakfast cereal.

| Compounds | $\lambda_{max}$ (nm) | Retention time (min) |
|---|---|---|
| *Luteolinidin* | 239 | 13.4 |
| *Apigeninidin* | 240 | 14.7 |
| *5-Methoxy-Luteolinidin* | 240 | 15.0 |
| *7-Methoxy-Apigeninidin* | 240 | 17.4 |

The presence of 3-DXAs in whole-grain sorghum breakfast cereal may suggest that this cereal has potential to benefit human health, especially due to its antioxidant and anticancer properties (Awika et al., 2004; Cardoso, Pinheiro, Martino, & Pinheiro-Sant'Ana, 2015b). Extracts containing these compounds were able to inhibit the growth of cancer cells, reducing the oxidative stress and inflammation and improve the lipid profile (Awika, Yang, Browning, & Faraj, 2009; Cardoso et al., 2015b). Thus, the consumption of whole-grain sorghum breakfast cereal should be encouraged.

Despite being present in wheat (Hernandez, Afonso, Rodriguez, & Diaz, 2011) and whole-sorghum grains (Cardoso et al., 2015b), flavones and flavanones were not detected in breakfast cereals. In a previous study (Cardoso et al., 2015b), it was demonstrated that flavones and flavanones of sorghum are labile to extrusion cooking. This sensitivity to extrusion can also be the cause of the absence of flavones and flavanones in whole-wheat breakfast cereal in the present study.

*3.3. Vitamin E*

The whole-grain wheat breakfast cereal showed a higher total vitamin E content than the sorghum breakfast cereal ($p < 0.05$) (Table 3). As in unprocessed whole-grain sorghum (Cardoso, Pinheiro, da Silva, et al., 2015c; Cardoso et al., 2014; Martino et al., 2012), the sorghum breakfast cereal showed a higher content of γ-tocopherol (59.5% of total vitamin E), followed by α-tocopherol (28.9% of total vitamin E) (Table 3).

The α-tocopherol isomer has the highest in vivo biopotency, because its plasma concentration is maintained at significant levels in the body, while the other absorbed compounds are almost completely excreted (Martino et al., 2012; Traber, 2001). α-tocopherol and γ-tocopherol content was higher in whole-grain breakfast sorghum compared to the whole-grain wheat breakfast cereal in the present study ($p < 0.05$).

Tocotrienols were more predominant than tocopherols in whole-grain wheat breakfast cereal, especially the β-tocotrienol (80.4% of total vitamin E), as well as reported by others authors (Lampi, Nurmi, Ollilainen, & Piironen, 2008).

Tocopherols and tocotrienols naturally occur in cereals and are potent antioxidants with lipoperoxyl radical-scavenging activities (Jiang, 2014; Nielsen & Hansen, 2008). These vitamin E forms scavenge reactive nitrogen species, inhibit cyclooxygenase- and 5-lipoxygenase-catalyzed eicosanoids, and suppress proinflammatory signaling (Jiang, 2014). They are associated with a lower risk for cardiovascular diseases, cancer and dyslipidemia (Nielsen & Hansen, 2008). The content of vitamin E of sorghum and wheat breakfast cereals contributed to the antioxidant activity of these cereals.

*3.4. Phenolic compounds and antioxidant activity*

Sorghum breakfast cereal showed a higher content of phenolic compounds than wheat breakfast cereal (Table 3). It is important to note that the content of phenolic compounds in both cereals may be overestimated due to the action of interferents, such as proteins, nucleic acids and amino acids, which can react with Folin-Ciocalteu reagent (Granger, Gallagher, Fuerst, & Alldredge, 2011; Naczk & Shahidi, 2006).

Sorghum has the highest content of phenolic compounds among cereals (Cardoso et al., 2015b). Phenolic compounds are widely distributed in plants and their antioxidant activity and free radical scavenging ability have potential beneficial implications in human health (Hernandez et al., 2011).

The whole-grain sorghum breakfast cereal presented higher antioxidant activity than the extruded wheat ($p < 0.05$) (Table 3). The high sorghum antioxidant capacity, from the phenolic compounds, has been demonstrated by others studies (Awika et al., 2009; Cardoso et al., 2015b). Furthermore, it should be noted that the sorghum SC319 genotype contains tannins, which is the major antioxidant in sorghum (Dlamini et al., 2007). In addition, sorghum grains contain carotenoids and are a vitamin E source, which contribute to its antioxidant activity. Cardoso et al. (2014) observed that the antioxidant activity of sorghum flours correlated positively with the content of a-and c-tocopherols, total vitamin E, total phenolic compounds, luteolinidin, apigeninidin and total 3-DXAs. Consumption of whole-grain sorghum breakfast cereal should be encouraged since it contains high content of phenolic compounds and antioxidant activity that can beneficially modulate variables related to diabetes, obesity, dyslipidemia, oxidative stress and inflammation (Awika et al., 2009; Cardoso et al., 2015a; Yang et al., 2012).

## 4. Conclusion

The whole-grain sorghum breakfast cereal showed better sensory acceptance, higher 3-deoxyanthocyanidin and phenolic compounds content which contributes to its higher antioxidante capacity. The whole-grain wheat breakfast cereal presented higher vitamin E content than whole-sorghum breakfast cereal.

The consumption of whole-grain sorghum breakfast cereal should be encouraged since it had good sensory acceptance and is a source of bioactive compounds that can promote benefits to human health.

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## 3.3. Article 3: Sorghum consumption reduces body weight and increases concentration of oxidative stress marker in overweight men: a randomized controlled trial


### Abstract

The present study aimed to evaluate the effect of extruded sorghum intake on body composition and metabolic risk markers in overweight men during weight loss nutritional intervention. In a randomized controlled crossover study, 24 overweight men (25.6 ± 4.6 years) were randomly allocated into one of two treatments: sorghum-based preparations consumption or wheat-based preparations consumption. The study consisted of 2 periods of 8 weeks with at least 4 weeks of washout. Except on the weekends, subjects reported to the laboratory to consume sorghum or wheat-based breakfast cereal + milk/yogurt or a drink for breakfast. Energy restricted diets (-500 kcal/day) were prescribed based on estimated energy requirement. Anthropometric, clinical and metabolic risk variables were assessed at baseline and at the end of each intervention period. Sorghum consumption reduced body weight, body fat percentage, waist circumference and increased plasma glutathione peroxidase. Wheat consumption reduced waist circumference and increased blood glucose. Mean daily energy intake during the intervention period did not differ from habitual ingestion. However, the sorghum group increased daily carbohydrate and dietary fiber intake. Thus, the consumption of extruded sorghum can be used to control obesity and oxidative stress in overweight men.



**Keywords:** *Sorghum bicolor* L., body composition, obesity, bioactive compounds, glutathione peroxidase.


### Introduction

Obesity is a worldwide public health problem because of its high prevalence. More than 1.9 billion adults were overweight and over 600 million were obese in 2014 (World Health Organization, 2016). The excessive accumulation of visceral fat is related with low-grade inflammation and oxidative stress (Matsuda e Shimomura, 2013). Oxidative stress, an imbalance between the generation of free radical such as reactive oxygen/nitrogen species and the antioxidant defenses, is associated with the manifestation of obesity, diabetes and cardiovascular diseases (Khan *et al.*, 2015; Lee *et al.*, 2015). Our cells are protected against the harmful effects of reactive oxygen species by antioxidant

enzymes (e.g., superoxide dismutase, glutathione peroxidase and catalase) and other antioxidant substances (e.g., vitamin C, vitamin E, vitamin A, carotenoids and glutathione) (Matsuda e Shimomura, 2013).

Phytochemicals present in cereals, such as phenolic compounds, act to protect the body against oxidative stress and its effects, due to its antioxidant properties (Taylor *et al.*, 2014). Our group has previously conducted sorghum characterization studies (Cardoso, Pinheiro, Da Silva*, et al.*, 2015; Cardoso, Pinheiro, De Carvalho*, et al.*, 2015; Anunciação *et al.*, 2017) and verified that sorghum stands out as an excellent source of bioactive compounds including flavonoids, tannins, anthocyanins, vitamin E and carotenoids, which contribute to its high antioxidant capacity. There is now an increased interest in using sorghum in human nutrition since is gluten-free and due to other properties such as slow digestibility, cholesterol lowering, anti-inflammatory, anti-diabetes and anti-cancer properties (Dykes e Rooney, 2006; Yang *et al.*, 2009; Moraes *et al.*, 2012; Anunciacao *et al.*, 2016). Additionally, our previous study demonstrated that sorghum breakfast cereal has good acceptance and is a source of bioactive compounds (Anunciação *et al.*, 2017). Consumption of foods containing sorghum flour as an ingredient has the potential to enhance antioxidant status and beneficially modulate oxidative stress markers (Khan *et al.*, 2015).

The activity of components isolated from sorghum against oxidative stress has been demonstrated in vitro (Yang *et al.*, 2009) and in vivo (Moraes *et al.*, 2017). In addition, we identified one study that evaluated the acute effect of sorghum flour on oxidative stress markers in healthy subjects (Khan *et al.*, 2015). These functional benefits are attributed to the phenolic compounds of sorghum (Khan *et al.*, 2015). However, studies about the effect of extrused sorghum chronic consumption on weight loss and markers of oxidative stress in humans are lacking.

Thus, the present study aimed to evaluate the effect of extruded sorghum intake on body composition, serum levels of lipids and glucose, and oxidative stress markers in overweight men during a weight loss nutritional intervention.

## Methods

*Subjects*

Study participants were recruited through public advertisements. Eligibility criteria included: male; age 18-40 years; body mass index (BMI) 27.0-34.9 kg/m$^2$; waist circumference ≥ 90 cm; fasting capillary blood glucose 70-99 mg/dL; capillary

cholesterol < 240 mg/dL; capillary triglycerides < 150 mg/dL; absence of food allergies, acute and chronic diseases other than obesity.

Individuals with acute diseases and/or eating disorders (lactose or gluten intolerances) were not included. Other exclusion criteria were the use of medications known to affect appetite, glycaemia, and energy or lipid metabolism; alcohol consumers and/or smokers; use of dietary fiber supplement; recent changes in body weight (<5kg in the past three months); no consumption of the test food for more than six days (consecutive or not; the volunteers did not know they would be excluded because of that).

The study was approved by the Human Research Ethics Committee of the Federal University of Viçosa, Brazil (CAAE: 13630513.0.0000.5153). All volunteers were informed about the objectives of the study and provided written informed consent. The study presented a power (Mera *et al.*, 1998) of 80% to lead to a 7.5% body fat reduction ($\alpha = 0.05$, SD = 5.25).

*Study design*

This is a randomized controlled crossover 2 x 2, single blind, clinical trial. Twenty-four subjects were randomly allocated into one of two groups, when sorghum-based preparations (40g/d) or wheat-based preparations (38g/d) were consumed for breakfast for eight consecutive weeks. There was a four weeks washout period between treatments.

Subjects consumed the test preparations (breakfast cereal or a drink) at breakfast daily in the laboratory. On weekends, the preparations were provided to volunteers to be ingested in their own homes. For that, subjects received portions of each test preparation and a measuring cup indicating the exact amount of water or milk to be added (250 mL or 100 mL, respectively) to ensure appropriate dilution. The volunteers were instructed to consume the entire amount of test food provided.

Energy restriction (500 kcal/day) was based on the estimated energy requirement (EER) (Institute of Medicine, 2005), presuming an individual weight loss of 2 kg/month. The dietary intervention for both groups was conducted by nutritionists, and dietary prescription was based on Dietary Reference Intakes (DRIs) (Institute of Medicine, 2005). Subjects received a replacement foods list organized by food groups and guidelines regarding healthy food choices. They were asked to maintain their customary physical activity level.

*Test meal*

The test meal was a breakfast cereal or a drink. Sorghum (test meal) or wheat (control meal) breakfast cereals (Anunciação *et al.*, 2017), were served with milk (100 mL) or yoghurt (185 mL). All volunteers consumed milk or yogurt for the same number of times. The drink was prepared just before its consumption and consisted of water, milk powder, powdered drink (Clight®, two flavors: blackberry or peach) and sweetener (sucralose). These preparations had similar calories, macronutrients and dietary fiber content (Table 1).

The amount of sorghum consumed daily was based on a usual portion of breakfast cereal (40g) and on the amount of sorghum that the subjects could ingest in a meal based on previous tests. The amount of wheat was calculated to keep the same amount of milk or yoghurt that was provided with the sorghum portion, and provide similar concentration of calories, macronutrient and dietary fiber with the sorghum.

Table 1 – Nutrient and bioactive compounds content in test meals.

| Preparations | Sorghum breakfast cereal + milk | Wheat breakfast cereal + milk | Sorghum breakfast cereal + yoghurt | Wheat breakfast cereal + yoghurt | Sorghum drink | Wheat drink |
|---|---|---|---|---|---|---|
| Ingredients (g/serving)[1] | | | | | | |
| Extruded sorghum (g) | 40 | - | 40 | - | 40 | - |
| Extruded wheat (g) | - | 38 | - | 38 | - | 38 |
| Milk (mL) | 100 | 100 | - | - | - | - |
| Yoghurt (mL) | - | - | 185 | 185 | - | - |
| Powdered skim milk (g) | - | - | - | - | 25 | 25 |
| Powdered drink (g) | - | - | - | - | 2 | 2 |
| Sweetener (mL) | - | - | - | - | 0.05 | 0.05 |
| Chemical composition | | | | | | |
| Energy (Kcal) | 176.39 | 177.73 | 194.65 | 195.99 | 223.59 | 224.93 |
| Total carbohydrates (g) | 34.94 | 36.16 | 37.34 | 38.56 | 41.94 | 43.16 |
| Protein (g) | 7.63 | 7.76 | 9.80 | 9.93 | 12.43 | 12.56 |
| Fat (g) | 0.68 | 0.23 | 0.68 | 0.23 | 0.68 | 0.23 |
| Total dietary fiber (g) | 5.84 | 4.56 | 5.84 | 4.56 | 5.84 | 4.56 |
| Soluble fiber (g) | 0.46 | na | 0.46 | na | 0.46 | na |
| Insoluble fiber (g) | 5.37 | na | 5.37 | na | 5.37 | na |
| Resistant starch (g) | 1.18 | na | 1.18 | na | 1.18 | na |
| Total phenolic (mg GAE/g) | 1.15 | 0.12 | 1.08 | 0.11 | 1.07 | 0.03 |
| 3-DXAs (µg/g) | 7.64 | - | 4.71 | - | 4.20 | - |
| Proanthocyanidins (µg Cat Eq.) | 0.40 | na | 0.40 | na | 0.40 | na |

[1]Daily serving of sorghum breakfast cereal = 40g; wheat breakfast cereal = 38g; drinks = 250 mL. 3-DXAs: 3-deoxyanthocyanidins.
na: not analyzed

*Dietary intake assessment*

Before and after each intervention period, participants provided a 3-day food record (two nonconsecutive week days and one weekend day). The portion sizes of the food intake were converted into grams. Total calories, carbohydrates, lipids, proteins and dietary fiber consumed were analyzed using Avanutri software (version 5.5i, Brazil).

*Anthropometric and body composition measurements*

Anthropometric and body composition evaluations were performed by a single trained researcher. Body weight was assessed using an electronic platform scale (Model 2096 PP, Toledo Brazil), with a capacity for 150 kg and precision of 50 g. Height was measured using a stadiometer (Alturexata®, Brazil) fixed to the wall (Jellife, 1966). BMI ($kg/m^2$) was computed and classified according to WHO (2000).

Waist circumference (WC) was measured to the nearest 0.1 cm with a flexible band, at the midpoint between the last rib and the iliac crest (World Health Organization, 2000). WC ≥ 90 cm was adopted as a criterion to diagnose abdominal obesity. Hip circumference (HC) was measured with inelastic tape measure at the level of maximum protrusion of the gluteal muscles. Sagittal abdominal diameter (SAD) was measured with a portable, sliding beam, abdominal caliper (Holtain Kahn Abdominal Caliper®, Holtain Ltd., Dyfed, Wales, UK) at the midpoint between the iliac crests. Waist-to-hip ratio (WHR) was calculated as WC divided by HC and waist-to-height ratio (WHtR) as WC divided by height.

Body composition was assessed by dual-energy X-ray absorptiometry (DEXA) (GE Healthcare, Lunar Prodigy Advance), and the results were expressed as total body fat (%). Obesity was identified when body fat % was higher than 25% (Sociedad Espanola Para El Estudio De La Obesidad, 2000).

*Clinical assessments*

Blood pressure was assessed as recommended by the American Heart Association (AHA) (Pickering *et al.*, 2005).

Overnight fasting blood samples were collected from all of the volunteers at the beginning and the end (after 8 weeks) of each intervention period. Fasting blood glucose (FBG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were measured using enzymatic kits and colorimetric methods (glucose monoreagent, monoreagent cholesterol, triglycerides and HDL Direct monoreagent from Bioclin®, Brazil). Low-density lipoprotein cholesterol (LDL-C) levels were estimated in accordance with the Friedewald formula (Friedewald *et al.*, 1972). Fasting insulin was assessed by electrochemiluminescence and high-sensitivity C-reactive protein (hs-CRP), by nephelometry.

Serum retinol, carotenoids (α-carotene, β-carotene and lutein) and tocopherols (α- and γ-tocopherols) were assessed according to Turner e Burri (2012) with modifications. The vitamins were determined in a high performance liquid chromatography (HPLC) system (Shimadzu, SCL 10AT VP, Japan) equipped with diode array detector (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. The following chromatographic conditions were used: RP-18 column (Phenomenex Gemini, 250 mm x 4.6 mm, 5 µm), equipped with a C-18 guard column (Phenomenex ODS 4mm

x 3 mm). The injection volume was 40 µL and spectrum scan was 200-450 nm. Retinol, carotenoids and tocopherols were measured at 325, 450 and 292 nm, respectively. The mobile phase was composed of acetonitrile: dichloromethane: methanol (70:20:10) and flow rate was 1.0 mL/min.

Total antioxidant capacity was measured using the Antioxidant Assay Kit (Sigma®). Glutathione peroxidase (GPx) was measured by colorimetric method using a kit EGPX-100 (EnzyChrom).

*Statistical analysis*

Statistical analysis was performed using SPSS 20.0 software. The normality of the data was assessed by Shapiro-Wilk test. The average of each variable at the beginning and end of the intervention were compared using paired t test or Wilcoxon. The difference between the averages of the variables at the beginning and end of the intervention periods were compared using the Student t test or Mann Whitney. The difference between the average food intake, within the same treatment, was evaluated using the Student t test or Mann-Whitney test. A significance level (α) of 5% was adopted.

**Results**

Thirty-six overweight men (19-39 years of age) completed the first screening visit. However, 33 attended the initial assessments and 24 participants completed the study (Figure 1). The average age of participants was 25.6 ± 4.6 years.

There was a significant weight loss (-0.93 ± 2.08 kg, $p = 0.047$), and WC (-1.50 ± 2.42 cm, $p = 0.009$), waist-to-height ratio (-0.081 ± 0.02, $p = 0.019$) and body fat percentage (BF%) (-1.90 ± 1.82%, $p < 0.001$) reduction after sorghum consumption (Table 2). BF% reduction was higher in the sorghum group when compared to the wheat group (-1.90 ± 1.82% vs -0.23 ± 1.46, $p = 0.004$).

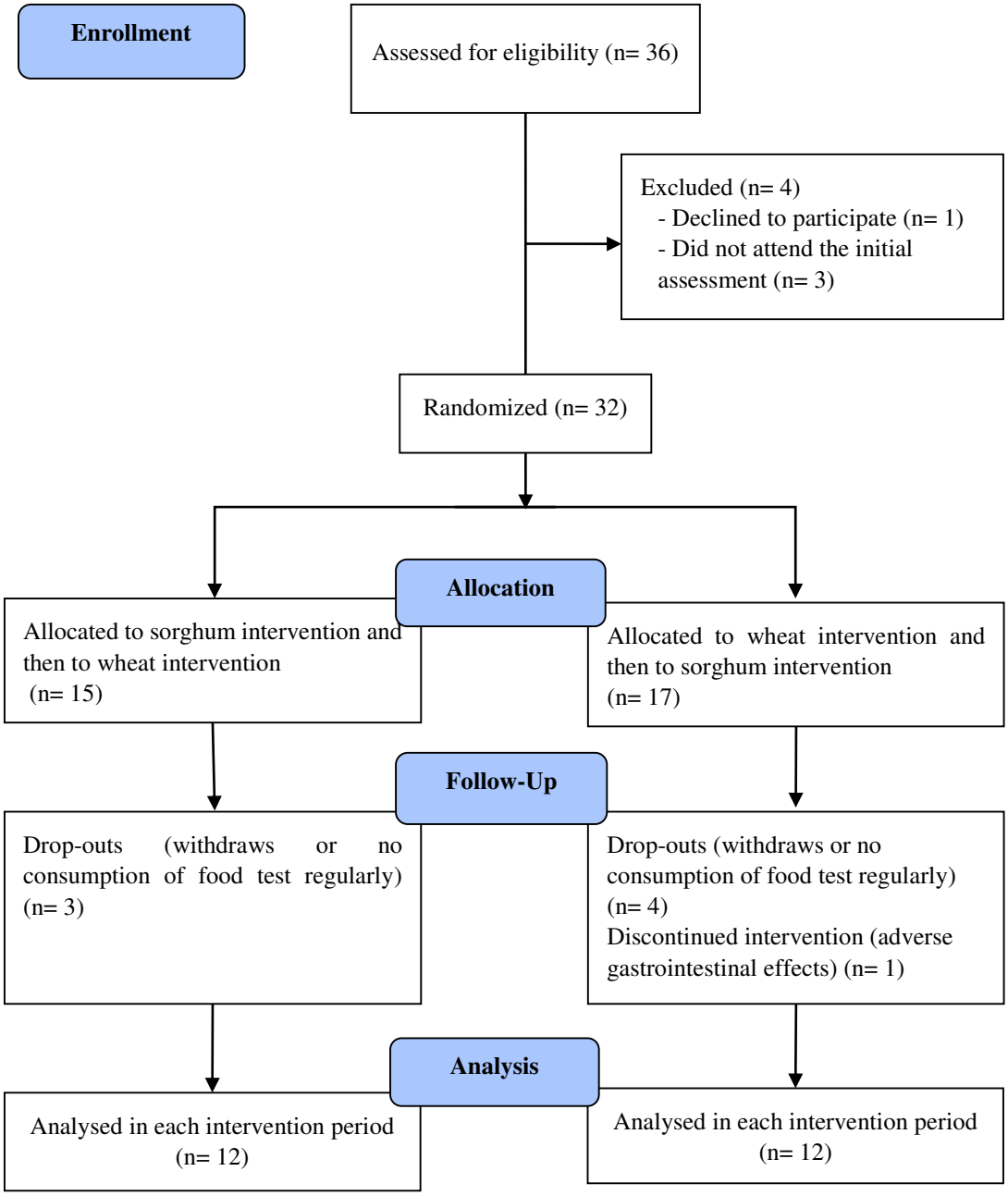


**Figure 1.** Screening fluxogram.

Table 2: Clinical and anthropometric variables of the participants at baseline and endpoint by treatment.

| Variables | Sorghum group (test) (n=24) | | | Wheat group (control) (n=24) | | | |
|---|---|---|---|---|---|---|---|
| | Baseline | Endpoint | $p^{(1)}$ | Baseline | Endpoint | $p^{(1)}$ | $p^{(2)}$ |
| Weight (kg) | 90.38±10.35 | 89.53±10.99 | 0.047 | 90.19±10.66 | 89.21±10.96 | 0.105 | 0.715 |
| BMI (kg/m²) | 29.07±1.49 | 28.79±1.84 | 0.047 | 29.01±1.80 | 28.7±2.13 | 0.108 | 0.768 |
| WC (cm) | 99.30±5.56 | 97.92±6.70 | 0.009 | 99.01±5.92 | 97.58±6.88 | 0.031 | 0.967 |
| SAD (cm) | 21.83±1.65 | 21.75±1.78 | 0.670 | 21.88±1.54 | 21.59±2.04 | 0.303 | 0.467 |
| WHtR | 0.56±0.03 | 0.55±0.04 | 0.019 | 0.57±0.04 | 0.56±0.04 | 0.023 | 0.785 |
| BF (%) | 32.09±5.85 | 30.38±6.23 | 0.000 | 31.58±5.39 | 31.34±5.57 | 0.870 | 0.004 |
| SBP (mmHg) | 129.10±14.78 | 126.14±10.69 | 0.364 | 130.26±7.82 | 130.74±10.11 | 0.200 | 0.362 |
| DBP (mmHg) | 83.62±10.69 | 80.43±9.34 | 0.095 | 83.32±7.57 | 83.11±6.55 | 0.750 | 0.261 |

Data are expressed as mean ± standard deviation. BMI: body mass index, WC: waist circumference, SAD: sagittal abdominal diameter, WHtR: waist-to-height ratio, BF: body fat percentage, SBP: systolic blood pressure, DBP: diastolic blood pressure.
(1) $p<0.05$ by paired t test or for Wilcoxon matched-pairs signed-rank test, as statistical within group differences (baseline vs. endpoint).
(2) $p<0.05$ by Student's t test or for Mann–Whitney test, as statistical significance between diet differences (sorghum vs. wheat)

No difference was observed in biochemical variables. However, an increase in fasting blood glucose (3.77 ± 5.91mg/dL, p = 0.007) was observed after the consumption of extruded wheat-based preparations (Table 3). Serum vitamins and total antioxidant capacity were not affected in the study. The consumption of extruded sorghum-based preparation for 8 weeks led to an increase in glutathione peroxidase (23.75 ± 41.09 U/L, p = 0.013) (Table 4).

Table 3: Biochemical variables of the participants at baseline and endpoint by treatment.

| Variables | Sorghum group (test) | | | Wheat group (control) | | | |
|---|---|---|---|---|---|---|---|
| | Baseline | Endpoint | $p^{(1)}$ | Baseline | Endpoint | $p^{(1)}$ | $p^{(2)}$ |
| Glucose (mg/dL) | 87.83±5.13 | 89.62±6.38 | 0.235 | 86.17±5.71 | 90.33±6.78 | 0.007 | 0.423 |
| Insulin (µUI/mL) | 6.31±2.83 | 7.19±2.81 | 0.103 | 6.89±2.71 | 6.82±3.42 | 0.930 | 0.313 |
| HOMA-IR | 1.38±0.66 | 1.59±0.68 | 0.116 | 1.41±0.66 | 1.51±0.81 | 0.563 | 0.533 |
| CRP (mg/L) | 0.51±1.13 | 0.28±0.38 | 0.329 | 0.14±0.13 | 0.17±0.13 | 0.364 | 0.585 |
| TG (mmol/L) | 119.04±63.60 | 117.62±52.74 | 0.876 | 126.21±61.79 | 127.21±62.70 | 0.935 | 0.984 |
| TC (mmol/L) | 173.96±33.31 | 175.33±32.86 | 0.756 | 173.87±29.48 | 175.37±32.44 | 0.753 | 0.916 |
| LDL-c (mmol/L) | 106.21±28.36 | 106.58±30.41 | 0.928 | 104.41±24.63 | 106.02±25.63 | 0.675 | 0.829 |
| HDL-c (mmol/L) | 43.92±6.86 | 45.20±8.37 | 0.363 | 44.21±8.21 | 43.92±7.36 | 0.856 | 0.395 |

n = 24 participants in each group. Data are expressed as mean ± standard deviation. HOMA-IR: homeostatic model of assessment of insulin resistance, CRP: high-sensitivity C-reactive protein, TG: triglycerides, TC: total cholesterol, LDL-c: low-density lipoprotein, HDL-c: high-density lipoprotein.
(1) $p<0.05$ by paired t test or for Wilcoxon matched-pairs signed-rank test, as statistical within group differences (baseline vs. endpoint).
(2) $p<0.05$ by Student's t test or for Mann–Whitney test, as statistical significance between diet differences (sorghum vs. wheat).

Table 4: Antioxidant variables of the participants at baseline and endpoint by treatment.

| Variables | Sorghum group (test) | | | Wheat group (control) | | | |
|---|---|---|---|---|---|---|---|
| | Baseline | Endpoint | $p^{(1)}$ | Baseline | Endpoint | $p^{(1)}$ | $p^{(2)}$ |
| α-tocopherol (µmol/L) | 22.94±8.04 | 22.60±6.44 | 0.846 | 22.10±7.68 | 22.46±6.49 | 0.788 | 0.846 |
| γ-tocopherol (µmol/L) | 9.45±0.89 | 9.62±0.79 | 0.391 | 9.36±0.81 | 9.36±0.61 | 0.984 | 0.537 |
| Retinol (µmol/L) | 4.95±1.77 | 5.30±1.19 | 0.352 | 4.94±1.80 | 5.46±1.22 | 0.254 | 0.763 |
| Lutein (µmol/L) | 9.19±8.62 | 8.94±7.00 | 0.981 | 6.74±4.53 | 8.57±9.96 | 0.943 | 0.921 |
| α-carotene (µmol/L) | 0.65±0.29 | 0.63±0.24 | 0.856 | 0.63±0.21 | 0.58±0.16 | 0.968 | 0.658 |
| β-carotene (µmol/L) | 1.35±0.59 | 1.23±0.43 | 0.602 | 1.22±0.30 | 1.28±0.52 | 0.841 | 0.316 |
| GPx (U/L) | 102.93±49.28 | 126.68±38.70 | 0.013 | 99.45±32.94 | 114.81±35.82 | 0.143 | 0.533 |
| TAC (mM Trolox) | 0.51±0.04 | 0.51±0.04 | 0.495 | 0.54±0.03 | 0.52±0.06 | 0.190 | 0.468 |

n = 24 participants in each group. Data are expressed as mean ± standard deviation. GPx: Glutathione peroxidase, TAC: total antioxidant capacity.
(1) $p<0.05$ by paired t test or for Wilcoxon matched-pairs signed-rank test, as statistical within group differences (baseline vs. endpoint).
(2) $p<0.05$ by Student's t test or for Mann–Whitney test, as statistical significance between diet differences (sorghum vs. wheat).

Mean daily energy, fat and protein intake did not differ from baseline in both groups (Table 5). However, there was an increase in carbohydrate (35.70 ± 59.79 g, p =

0.044) and fiber consumption (5.14 ± 6.62 g, p = 0.009) in the sorghum group. The increase in carbohydrate consumption positively correlated with fiber consumption (r = 0.650, p = 0.001).

Table 5: Average daily consumption of energy, macronutrients and dietary fiber.

| Variables | Sorghum group (test) | | | Wheat group (control) | | | |
|---|---|---|---|---|---|---|---|
| | Baseline | Endpoint | $p^{(1)}$ | Baseline | Endpoint | $p^{(1)}$ | $p^{(2)}$ |
| Energy (kcal) | 1523.83±187.81 | 1634.05±284.45 | 0.050 | 1760.32±350.53 | 1578.84±365.94 | 0.220 | 0.019 |
| Carbohydrate (g) | 187.71±46.99 | 216.07±42.73 | 0.044 | 232.07±42.81 | 214.16±49.05 | 0.353 | 0.038 |
| Dietary fiber (g) | 14.37±4.87 | 21.26±5.37 | 0.009 | 19.07±5.26 | 20.52±3.04 | 0.801 | 0.059 |
| Fat (g) | 48.24±10.02 | 48.60±37.88 | 0.898 | 52.76±12.35 | 41.05±12.55 | 0.116 | 0.211 |
| Protein (g) | 84.71±27.13 | 83.08±25.44 | 0.839 | 89.31±28.22 | 88.19±23.85 | 0.360 | 0.505 |

Data expressed in mean ± standard deviation; kcal: kilocalories.
(1) $p<0.05$ from paired t test or for Wilcoxon matched-pairs signed-rank test, as statistical within group differences (baseline vs. endpoint).
(2) $p<0.05$ from Student's t test or for Mann–Whitney test, as statistical significance between diet differences (sorghum vs. wheat)

**Discussion**

To date, this is the first study to evaluate the effect of chronic sorghum consumption on body composition, weight loss, oxidative stress markers and dietary intake in overweight individuals. Compared with the control preparations (wheat-based), the consumption of extruded sorghum-based preparations at breakfast for 8 weeks reduced body weight, BMI, WC and BF% of the participants. In addition, sorghum consumption increased the glutathione peroxidase and wheat consumption increased blood glucose.

Previous studies demonstrated that the consumption of 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, but can provide benefits in humans. To our knowledge, to date there are no studies evaluating the effect of chronic consumption of sorghum on weight loss in humans. The reduction in body weight and body fat percentage verified in the present study may be due to the higher content of dietary fiber, resistant starch and bioactive compounds (especially tannins) of sorghum compared to wheat. Weight loss inversely correlated with fiber consumption (r = -0.593, p = 0.020). Whole grain sorghum, with high fiber and slowly digestible starches, may increase satiety in humans due, in part, to its effects on the reduction of the glycemic

index (GI) of foods (Stefoska-Needham *et al.*, 2015), although we did not observe a reduction in caloric intake in the present study. Also, Wee and cols. (Wee *et al.*, 1999) showed that the consumption of low GI diets favors fat instead of carbohydrate oxidation, leading to body fat reduction, which may have contributed to weight loss in our study.

The weight loss in sorghum group may result in part from the complexation of tannins to sorghum starch that helps lower caloric intake. Barros et al. (Barros *et al.*, 2014) demonstrated that polymeric tannins from sorghum can naturally modify starch by interacting strongly with amylose forming resistant starch. Resistant starch cannot be digested in the small intestine and thus, it reaches the large intestine, delivering the health benefits of dietary fiber.

In the present study, waist circumference was reduced in both sorghum and wheat groups. Others authors also observed reduction in waist circumference after whole grains consumption associated with reduced-energy diet in overweight adults (Maki *et al.*, 2010; Charlton *et al.*, 2012). About 73% of the subjects reduced WC in the present study. Of these, 37.5% no longer have abdominal obesity. Excess abdominal adiposity is strongly associated with metabolic disturbances such as insulin resistance and hypertriglyceridemia (Despres, 2006).

Cardiovascular risk associated with obesity can be reduced through weight reduction which subsequently decreases oxidative stress markers and increased antioxidant system (Bigornia *et al.*, 2010). Superoxide dismutase, catalase and GPx activities have been found to be inversely related to BMI in obese adults (Mittal e Kant, 2009). In obese women, serum GPx activity was significantly increased after weight reduction (Bougoulia *et al.*, 2006). In the present study, sorghum consumption led to an increase in glutathione peroxidase, which is one of the main enzymatic antioxidants (Lee *et al.*, 2015).

In a study, pasta containing red whole grain sorghum flour enhanced antioxidant status and improved markers of oxidative stress in healthy subjects. This effect may be attributed to its higher content of polyphenols (Khan *et al.*, 2015). Differing from our study, Lewis (2008) observed that white (rich in phenolic acids), brown (rich in tannins) or black (rich in 3-deoxyanthocyanidins) sorghum brans suppressed the glutathione peroxidase activity. Our results suggest that wholegrain extruded sorghum consumption can reduce oxidative stress by increasing antioxidant enzymes, such as glutathione peroxidase. Due to this higher antioxidant activity compared to wheat (Anunciação *et al.*, 2017), sorghum consumption has a potential to reduce oxidative stress that plays an

important role in the pathogenesis of many chronic diseases such as diabetes, atherosclerosis, some cancers, aging, arthritis, and neurological diseases (Awika e Rooney, 2004; Stefoska-Needham *et al.*, 2015).

Intervention studies investigating the effects of wholegrain intake on glucose and insulin metabolism have provided conflicting results. In the present study, consumption of wholegrain wheat preparations increased blood glucose. However, mean blood glucose maintained at adequate levels. Differing from our study, other studies verified that wholegrain consumption have no effect on glucose metabolism in overweight subjects (Brownlee *et al.*, 2010; Giacco *et al.*, 2013). The conflicting results may be due to differences in the methodologies used for analyzing glucose metabolism (as evaluation of postprandial blood glucose) (Giacco *et al.*, 2013) or to differences in to the amount and type of wholegrain products (whole-wheat bread, porridge oats, wholegrain crisps, among others) of experimental diets (Brownlee *et al.*, 2010).

The consumption of sorghum-based preparations increased the daily intake of fibers (~ 7 g/day) of the participants. Maki *et al*. (2010) also observed improvements in dietary fiber intake (~ 6g/day) with whole-grain oat cereal consumption for 12 weeks. Our results showed that total dietary fiber intake increased to approximately 21g/day in sorghum group, which falls within the range of 20 to 35 g fiber/day recommended by the Institute of Medicine (2005). Thus, the inclusion of sorghum in the diet may help meeting some nutrition recommendations. Although dietary fiber intake did not contribute to increased satiety, since no reduction in caloric intake was observed, the synergistic effect of the bioactive compounds of sorghum may have contributed to weight loss and increased glutathione peroxidase. It is noteworthy that volunteers consumed sorghum once a day, and in a free diet, people can consume more than 40 g of sorghum per day and have even more positive effects.

Sorghum consumption has a potential to reduce body weight and oxidative stress, which plays an important role in the pathogenesis of many chronic diseases. This effect may be attributed to the bioactive compounds content of sorghum. Therefore, whole grain sorghum may be useful in managing energy balance, favoring overweight control.

## Conclusion

Daily consumption of preparations containing 40 g of extruded sorghum for 8 weeks reduced body weight, WC, BMI, WHtR and BF% in overweight men. Consumption of wheat-based preparations reduced WC and consequently the WHtR. No

difference was observed in the biochemical markers in both groups. However, glutathione peroxidase increased after sorghum consumption. Sorghum increased dietary fiber intake. Thus, sorghum can be an important strategy for weight loss and antioxidant defense in humans.

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## 3.4. Article 4: Consumption of sorghum favors obesity control without affecting the intestinal microbiota and its metabolites in overweight individuals


### Abstract

It has been reported that sorghum consumption can change intestinal microbiota and may prevent dysbiosis in animals. However, sorghum's effect in the human intestinal tract is not well understood. This study aimed to evaluate the effect of whole sorghum consumption on fecal pH, short chain fatty acids (SCFA) production and intestinal microbiota composition in overweight men. Twenty-four overweight men were allocated into one of two groups: sorghum group (test) or wheat group (control). Fecal hydrogen potential (pH) was measured in digital pHmeter. SCFA were determined by high performance liquid chromatography with refractive index detection. The intestinal microbiota composition was determined by qPCR. Consumption of whole sorghum and wheat-based preparations did not affect fecal pH, SCFA content or the intestinal microbiota composition. However, further studies evaluating the effect of whole grain sorghum consumption on the human intestinal microbiota with a larger sample size or using a more specific method should be performed.



**Keywords:** Sorghum, short chain fatty acids, gut microbiota, obesity


### Introduction

Excess body weight and associated insulin resistance favor the occurrence of chronic low-grade inflammation, which can stem in part from the release of proinflammatory mediators by visceral fat mass. Intestinal microbiota may therefore contribute to the development of inflammation and insulin resistance, either by its role on energy homeostasis regulation and fat storage or by inducing chronic inflammation or both (De Bandt *et al.*, 2011)

Whole grains play an important role on digestive health due to its high dietary fiber, phytonutrients and other nutrients content (Jonnalagadda *et al.*, 2011). It has been reported a positive association between increased whole grain intake and reduced risk of developing chronic diseases (Jonnalagadda *et al.*, 2011; Connolly *et al.*, 2016). One of the hypothetical mechanisms behind these associations is the colonic fermentation of the fiber present in that cereal, leading to the production of short chain fatty acids (SCFAs): acetate, propionate and butyrate. These SCFAs show anti-inflammatory properties and

act as energy source for colonocytes (Ríos-Covián *et al.*, 2016). They can provide energy and are possibly involved in fat and glucose metabolism in the host (Wong e Jenkins, 2007), suggesting that they may have an impact on the occurrence of metabolic risk factors.

It has been demonstrated that whole grain wheat (Costabile *et al.*, 2008), high-fiber rye (Mcintosh *et al.*, 2003) and whole grain oat-based breakfast (Connolly *et al.*, 2016) have protective health effects because they can modulate markers of bowel health or the microbial ecology of the human gut. Bran from black and brown sorghum cultivars changed SCFA concentrations in animals, suggesting a possible change in gut microbiota (Turner *et al.*, 2010). Sorghum lipid extract acts as a "prebiotic" by improving the host cholesterol metabolism through effects on gut microbiota. *Bifidobacteria* significantly increased in hamsters fed grain sorghum lipid extract (Martínez *et al.*, 2009). However, sorghum's effect on human intestinal tract is not well understood.

The results of another animal study indicated that sorghum bran diets might prevent dysbiosis of predominant bacterial populations, decreasing microbial diversity commonly associated with ulcerative colitis (Ritchie *et al.*, 2015). The presence of bioactive compounds like 3-deoxyanthocianidins and condensed tannins may be the factors in these diets capable of changing the luminal environment and microbial populations (Ritchie *et al.*, 2015).

Sorghum SC319 genotype, used in the present study, is an excellent source of bioactive compounds, such as phenolic acids, flavonoids, tannins, 3-deoxyanthocianidins and vitamin E, which contribute to its high antioxidant capacity (Cardoso *et al.*, 2015; Anunciação *et al.*, 2017). Our group verified that this sorghum genotype has good acceptability (Anunciação *et al.*, 2017) and reduced the glycaemic response of the subsequent meal in healthy adults (Anunciação *et al.*, 2016). However, to date, we did not identify any study that evaluated the effect of chronic consumption of whole grain sorghum on human intestinal microbiota. Thus, the present study aimed to evaluate the effect of extruded sorghum intake on body composition, inflammatory markers, fecal pH, SCFA content and in the composition of the intestinal microbiota in overweight men.

## Methods

*Subjects*

Twenty-four overweight men (25.6 ± 4.6 years of age) were recruited through public advertisements. The study presented a power (Mera *et al.*, 1998) of 80% to lead to

a 7.5% body fat reduction ($\alpha = 0.05$, SD = 5.25). All volunteers were informed about the objectives of the study and provided written informed consent.

Inclusion criteria were: male; age 18-40 years; body mass index (BMI) 27.0-34.9 kg/m$^2$; waist circumference ≥ 90 cm; fasting capillary blood glucose 70-99 mg/dL; capillary cholesterol < 240 mg/dL; capillary triglycerides < 150 mg/dL; absence of acute and chronic diseases other than obesity and food allergies.

Individuals with eating disorders (lactose or gluten intolerances) were not included. Other exclusion criteria were the use of medications known to affect appetite, glycaemia, and energy or lipid metabolism; alcohol consumers and/or smokers; use of dietary fiber supplement; recent changes in body weight (<5kg in the past three months); no consumption of the test food for more than six days (consecutive or not).

The study was approved by the Human Research Ethics Committee of the Federal University of Viçosa, Brazil (CAAE: 13630513.0.0000.5153).

*Study design*

This is a randomized, single blind, clinical trial. Twenty-four subjects were randomly allocated into one of two groups: sorghum group (test) or wheat group (control).

Subjects daily attended the laboratory to consume the test preparations (breakfast cereal or a drink) at breakfast. On weekends, the preparations were provided to volunteers to be ingested in their own homes. For that, subjects received portions of each test preparation packages and a measuring cup indicating the exact amount of water or milk to be added (250 mL or 100 mL, respectively) to ensure appropriate dilution. The volunteers were instructed to consume the entire amount of test food provided.

Energy restriction (500 kcal/day) was based on the estimated energy requirement (EER) (World Health Organization, 2000), presuming an individual weight loss of 2 kg/month. The dietary intervention for both groups was conducted by nutritionists, and dietary prescription was based on Dietary Reference Intakes (DRIs) (World Health Organization, 2000). Subjects received a replacement foods list organized by food groups and guidelines regarding healthy food choices. They were asked to maintain their customary physical activity level.

*Test meal*

The test meal was a breakfast cereal or a drink. Sorghum (test meal) or wheat (control meal) breakfast cereals (Anunciação *et al.*, 2017), were served with milk (100

mL) or yoghurt (185 mL). All volunteers consumed milk or yogurt for the same number of times. The drink was prepared just before its consumption and consisted of water, milk powder, powdered drink (Clight®, two flavors: blackberry or peach) and sweetener (sucralose). These preparations had similar calories, macronutrients and dietary fiber content (Table 1).

The amount of sorghum consumed daily was based on a usual portion of breakfast cereal (40g) and on the volume of sorghum that the subjects could ingest in a meal based on previous tests. The amount of wheat (38g) was calculated to keep the same amount of milk or yoghurt which was provided with the sorghum breakfast cereal portion, and to provide similar concentration of calories, macronutrient and dietary fiber with the sorghum.

**Table 1.** Nutrient and bioactive compounds content in test meals.

| Preparations | Sorghum breakfast cereal + milk | Wheat breakfast cereal + milk | Sorghum breakfast cereal + yoghurt | Wheat breakfast cereal + yoghurt | Sorghum drink | Wheat drink |
|---|---|---|---|---|---|---|
| Ingredients (g/serving)[1] | | | | | | |
| Extruded sorghum (g) | 40 | - | 40 | - | 40 | - |
| Extruded wheat (g) | - | 38 | - | 38 | - | 38 |
| Milk (mL) | 100 | 100 | - | - | - | - |
| Yoghurt (mL) | - | - | 185 | 185 | - | - |
| Powdered skim milk (g) | - | - | - | - | 25 | 25 |
| Powdered drink (g) | - | - | - | - | 2 | 2 |
| Sweetener (mL) | - | - | - | - | 0.05 | 0.05 |
| Chemical composition | | | | | | |
| Energy (Kcal) | 176.39 | 177.73 | 194.65 | 195.99 | 223.59 | 224.93 |
| Total carbohydrates (g) | 34.94 | 36.16 | 37.34 | 38.56 | 41.94 | 43.16 |
| Protein (g) | 7.63 | 7.76 | 9.80 | 9.93 | 12.43 | 12.56 |
| Fat (g) | 0.68 | 0.23 | 0.68 | 0.23 | 0.68 | 0.23 |
| Total dietary fiber (g) | 5.84 | 4.56 | 5.84 | 4.56 | 5.84 | 4.56 |
| Soluble fiber (g) | 0.46 | na | 0.46 | na | 0.46 | na |
| Insoluble fiber (g) | 5.37 | na | 5.37 | na | 5.37 | na |
| Resistant starch (g) | 1.18 | na | 1.18 | na | 1.18 | na |
| Total phenolic (mg GAE/g) | 1.15 | 0.12 | 1.08 | 0.11 | 1.07 | 0.03 |
| 3-DXAs (µg/g) | 7.64 | - | 4.71 | - | 4.20 | - |
| Proanthocyanidins (µg Cat Eq.) | 0.40 | na | 0.40 | na | 0.40 | na |

[1]Daily serving of sorghum breakfast cereal = 40g; wheat breakfast cereal = 38g; drinks = 250 mL. 3-DXAs: 3-deoxyanthocyanidins.
na: not analyzed.

*Anthropometric and body composition measurements*

Anthropometric and body composition evaluations were performed by a single trained researcher. Body weight was assessed using an electronic platform scale (Model 2096 PP, Toledo Brazil), with a capacity for 150 kg and precision of 50 g. Height was measured using a stadiometer (Alturexata®, Brazil) fixed to the wall (Jelliffe, 1966). BMI ($kg/m^2$) was computed and classified according to WHO (2000).

Waist circumference (WC) was measured to the nearest 0.1 cm with a flexible band, at the midpoint between the last rib and the iliac crest (World Health Organization, 2000). WC ≥ 90 cm was adopted as a criterion to diagnose abdominal obesity. Sagittal abdominal diameter (SAD) was measured with a portable, sliding beam, abdominal caliper (Holtain Kahn Abdominal Caliper®, Holtain Ltd., Dyfed, Wales, UK) at the midpoint between the iliac crests. Waist-to-height ratio (WHtR) was calculated as WC divided by height.

Body composition was assessed by dual-energy X-ray absorptiometry (DXA) (GE Healthcare, Lunar Prodigy Advance), and the results were expressed as total body fat (%). Obesity was identified when body fat % was higher than 25% (Sociedad Española Para El Estudio De La Obesidad, 2000).

*Inflammatory markers*

Overnight fasting blood samples were collected from all of the volunteers at the beginning and the end (after 8 weeks) of intervention period. Inflammatory markers (interleukin-6, interleukin-10 and tumor necrosis factor-α) were assessed by enzyme-linked immunosorbent assay (ELISA) using the kit for Milliplex Map Human Cytokine/Chemokine Magnetic Bead (HCYTOMAG-code 60K, Millipore).

*Fecal samples*

At baseline and after 8 weeks of intervention, the subjects were asked to provide a fecal sample preferably within a few hours of collection; otherwise, the material was kept at 4ºC for up to 12 h. The subjects brought the sample to the laboratory in polystyrene containers, with ice cubes for temperature maintenance. The samples were weighed into microtubes and stored at -80ºC until analyses.

*Fecal pH*

The fecal hydrogen potential (pH) was measured in digital pHmeter T-1000 (Tekna, São Paulo, Brazil). One gram of feces was filled in falcon-type tube (15 mL capacity) and 10 mL of ultrapure water was added. After vortex homogenization, the pH was read.

*Determination of organic acids*

For the extraction of organic acids in fecal contents, around 500 mg of faeces were weighed in duplicate and stored at -80°C until analysis. The faeces were unfrozen at room temperature (23 ± 2°C) and homogenized with the addition of 1 mL of ultrapure water. The samples were centrifuged (Himac CT 15RE, Hitachi) at 12000 g for 10 min at 4°C. Then, supernatants were treated as described by Siegfried et al (1984).

Organic acids (acetic, succinic, formic, propionic, valeric, isovaleric, isobutyric and butyric acid) were determined by high performance liquid chromatography (HPLC) in a Dionex Ultimate 3000 Dual detector HPLC (Dionex Corporation, Sunnyvale, CA, USA) coupled to a refractive index (RI) Shodex RI-101. The following chromatographic conditions were used: Bio-Rad HPX-87H column (300 mm x 4.6 mm) equipped with a Bio-Rad Cation H guard column, column temperature 45°C, injection volume of 20 µL. The mobile phase was composed by concentrated sulfuric acid, EDTA and ultrapure water; flow rate of 0.7mL/min.

The following organic acids were used for the calibration of the standard curve: acetic, succinic, formic, propionic, valeric, isovaleric, isobutyric and butyric acid. All acids were prepared with a final concentration of 10 mmol/L, except isovaleric acid (5 mmol/L) and acetic acid (20 mmol/L).

*DNA extraction from fecal samples*

DNA was isolated following the procedures of the kit QIAamp Fast DNA Stool Mini kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. A sample of 200 ± 20 mg of faeces was used for each extraction. The purified DNA was stored at -80ºC until analyses.

*Quantitative real-time polymerase chain reaction (qPCR) analysis of gut microbiota*

DNA concentration was determined by absorbance at 260 nm (A260), and purity estimated by the determination of A260/A280 ratio in a Multiskan™ 1500 spectrophotometer (Thermo Fisher Scientifics; Waltham, MA, USA). The PCR was used to characterize the fecal microbiota using group-specific primers (Table 2). These oligonucleotides were purchased from the Alpha DNA e Diagnósticos Moleculares LTDA (Goiânia, GO, Brazil).

PCR detection and amplification of the 16S rRNA gene were performed with a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Califórnia, EUA) (Primer Express software).

Each microtiter plate well contained the following: QuantiNova SYBR® Green PCR Kit (Qiagen, Hilden, Germany), which contained all the nucleotides, polymerase, reaction buffer and SYBR green dye; forward and reverse primers at concentrations of 300nM and nuclease-free water to a total of 23 μL per well. To this solution, 2.0 μL of each sample or standard was added and the plate was briefly centrifuged (Labnet, model MPS1000) and placed in the thermocycler for analysis.

The amplification conditions for strains were as follows: initial denaturation of the DNA at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 10 seconds, primer annealing at optimum temperature for 20 seconds and extension at 72°C for 15 seconds. A melting curve analysis was made after amplification to distinguish the targeted from the non-targeted PCR product. The bacterial concentration in each sample was calculated by comparing the Ct (cycle threshold) values obtained from standard curves from Primer Express® software. A standard curve was made from serial dilutions of DNA isolated from each pure culture of the different reference strains, ranging from 20 ng to 0.032 ng of the 16S rRNA gene. A linear relationship was observed between cell numbers and Ct values ($r^2 = 0.99\text{-}0.96$). The different strains used were obtained from the American Type Culture Collection (ATCC) (*Bacteroides ovatus* ATCC 8483; *Escherichia coli* ATCC 11775) and from the Tropical Cultures Collection (*Lactobacillus delbrueckii* UFV H2b20 CCT 3744).

**Table 2.** PCR primers used in this study.

| Group | Primer sequences | Standard genomic DNA | References |
|---|---|---|---|
| *Total bacteria* | F- GCAGGCCTAACACATGCAAGTC | *Escherichia coli* | (Castillo *et al.*, 2006) |
| | R- CTGCTGCCTCCCGTAGGAGT | | |
| *Firmicutes* | F- ATGTGGTTTAATTCGAAGCA | *Lactobacillus delbrueckii* | (Guo *et al.*, 2008) |
| | R- AGCTGACGACAACCATGCAC | | |
| *Bacteroidetes* | F- CATGTGGTTTAATTCGATGAT | *Bacteroides ovatus* | (Guo *et al.*, 2008) |
| | R- AGCTGACGACAACCATGCAG | | |
| *Proteobacteria* | F- CATGACGTTACCCGCAGAAGAAG | *Escherichia coli* | (Friswell *et al.*, 2010) |
| | R- CTCTACGAGACTCAAGCTTGC | | |

Oligonucleotides used as primers (F, forward; R, reverse) for the quantification of 16S rDNA genes.

*Statistical analysis*

Statistical analysis was performed using SPSS 20.0 software. The normality of the data was assessed by Shapiro-Wilk test. The average of each variable at the beginning and end of the intervention were compared using paired t test or Wilcoxon. The difference between the averages of the variables at the beginning and end of the intervention periods were compared using the Student t test or Mann Whitney. A significance level (α) of 5% was adopted.

## Results

There was a significant weight loss (-1.25 ± 0.84 kg, p = 0.0015), and WC (-2.33 ± 1.74 cm, p = 0.012), SAD (-0.70 ± 0.67 cm, p = 0.034), waist-to-height ratio (-0.011 ± 0.012, p = 0.047) and body fat percentage (BF%) (-2.97 ± 1.91%, p = 0.013) reduction after sorghum consumption for eight weeks (Table 3). BF% reduction was higher in the sorghum group when compared to the wheat group (-2.97 ± 1.91% vs -0.16 ± 1.47%, p = 0.005). Inflammatory markers was not affected after sorghum consumption. However, wheat consumption increased IL-6 levels.

**Table 3.** Anthropometric variables and inflammatory markers of the participants at baseline and endpoint by treatment.

| Variables | Sorghum group (test) | | | Wheat group (control) | | | |
|---|---|---|---|---|---|---|---|
| | Baseline | Endpoint | p(1) | Baseline | Endpoint | p(1) | p(2) |
| Anthropometry | | | | | | | |
| Weight (kg) | 84.37±7.09 | 83.12±6.93 | 0.015 | 91.83±11.49 | 90.68±11.10 | 0.141 | 0.919 |
| BMI (kg/m²) | 28.16±0.96 | 27.74±0.98 | 0.018 | 28.83±2.10 | 28.49±2.38 | 0.121 | 0.907 |
| WC (cm) | 94.46±3.47 | 92.18±3.84 | 0.012 | 100.60±6.10 | 99.59±6.79 | 0.132 | 0.169 |
| SAD (cm) | 20.83±1.20 | 20.13±0.99 | 0.034 | 22.64±1.98 | 22.11±1.46 | 0.088 | 0.673 |
| WHtR | 0.54±0.04 | 0.53±0.01 | 0.047 | 0.57±0.04 | 0.56±0.05 | 0.064 | 0.346 |
| BF (%) | 29.15±4.53 | 26.18±4.97 | 0.013 | 31.77±6.68 | 31.61±6.91 | 0.739 | 0.005 |
| Inflammatory markers | | | | | | | |
| IL-6 (pg/mL) | 0.87±0.19 | 1.15±0.03 | 0.114 | 0.77±0.28 | 1.01±0.18 | 0.014 | 0.703 |
| IL-10 (pg/mL) | 1.50±1.01 | 1.73±0.88 | 0.753 | 0.96±0.49 | 1.17±0.62 | 0.247 | 0.848 |
| TNFα (pg/mL) | 7.54±5.10 | 9.02±5.34 | 0.060 | 6.41±2.60 | 7.02±2.74 | 0.237 | 0.274 |

n = 11 participants in each group. Data are expressed as mean ± standard deviation. BMI: body mass index, WC: waist circumference, SAD: sagittal abdominal diameter, WHtR: waist-to-height ratio, BF: body fat percentage, IL-6: interleukin-6; IL-10: interleukin-10; TNFα: tumor necrosis factor.
(1) $p<0.05$ by paired t test or for Wilcoxon matched-pairs signed-rank test, as statistical within group differences (baseline vs. endpoint).
(2) $p<0.05$ by Student's t test or for Mann–Whitney test, as statistical significance between diet differences (sorghum vs. wheat)

Fecal pH was not affected after the intervention (Table 4). In addition, we observed no significant changes in fecal concentrations of acetic, propionic and butyric acids after sorghum or wheat consumption for 8 weeks (Table 4).

**Table 4.** Effect of sorghum and wheat consumption on concentration of volatile fatty acids (VFA) of overweight subjects.

| | Sorghum group | | | Wheat group | | | |
|---|---|---|---|---|---|---|---|
| Variables | Baseline | Endpoint | p[2] | Baseline | Endpoint | p | p[3] |
| Organic acids | | | | | | | |
| Total VFA[1] | 28.35±11.83 | 31.82±11.78 | 0.592 | 29.65±10.41 | 27.42±12.87 | 0.527 | 0.436 |
| Acetic acid | 14.73±1.96 | 14.76±3.7 | 0.974 | 14.09±3.52 | 14.56±5.40 | 0.758 | 0.809 |
| Propionic acid | 6.32±2.72 | 6.05±3.12 | 0.829 | 7.14±3.99 | 6.18±2.90 | 0.267 | 0.641 |
| Butyric acid | 4.90±2.51 | 5.46±3.77 | 0.702 | 5.89±2.99 | 5.01±2.90 | 0.594 | 0.456 |
| Fecal pH | 6.73±0.37 | 6.68±0.55 | | 6.85±0.45 | 6.96±0.53 | | 0.254 |

[1]Total VFA (mmol/l), acetic acid, propionic acid, butyric acid, isobutyric acid, formic acid, succinic acid, valeric acid and isovaleric acid (mol/100 mol).
[2] $p<0.05$ from paired t test or for Wilcoxon matched-pairs signed-rank test, as statistical within group differences (baseline vs. endpoint).
[3] $p<0.05$ from Student's t test or for Mann–Whitney test, as statistical significance between diet differences (sorghum vs. wheat).

We observed no changes in the composition of the intestinal microbiota after 8 weeks of intervention in both groups (Table 5).

**Table 5.** Percentages of target species in samples relative to total bacteria content.

| | Sorghum group | | | Wheat group | | | |
|---|---|---|---|---|---|---|---|
| Target taxon | Baseline | Endpoint | p[1] | Baseline | Endpoint | p[1] | p[2] |
| *Bacteroidetes* | 108.89±40.00 | 106.51±24.66 | 0.878 | 73.96±51.99 | 106.39±53.13 | 0.080 | 0.115 |
| *Proteobacteria* | 0.19±0.25 | 0.20±0.21 | 0.812 | 1.30±1.67 | 3.56±11.00 | 0.508 | 0.075 |
| *Firmicutes* | 4.16±1.53 | 6.29±4.54 | 0.249 | 10.74±11.30 | 4.74±2.97 | 0.085 | 0.250 |

[1] $p<0.05$ from paired t test or for Wilcoxon matched-pairs signed-rank test, as statistical within group differences (baseline vs. endpoint).
[2] $p<0.05$ from Student's t test or for Mann–Whitney test, as statistical significance between diet differences (sorghum vs. wheat).

## Discussion

Sorghum is rich in dietary fiber, resistant starch and phenolic compounds that can benefit the gut microbiota and parameters related to obesity, oxidative stress, inflammation, diabetes, dyslipidemia, cancer, and hypertension (Cardoso *et al.*, 2017). In the present study, sorghum consumption for 8 weeks reduced body weight, WC, SAD, BF%, BMI and WHtR of the participants. This effect may be due to the higher content of dietary fiber, resistant starch and bioactive compounds (especially tannins) of sorghum compared to wheat. These sorghum compounds may increase satiety in humans (Stefoska-Needham *et al.*, 2015).

Studies have shown associations between the intake of whole grains and decreased inflammatory markers (Lefevre e Jonnalagadda, 2012). In the present study, wheat consumption for 8 weeks increased IL-6. Intervention studies, however, do not demonstrate a clear effect of the intake of whole grains on inflammation and it could therefore be that other components in the diet modulate the immune response (De Punder e Pruimboom, 2013).

Diet influences the colon luminal environment by affecting transit time and the production of microbial metabolites (SCFA, e.g. butyrate) that alter luminal pH (Walker *et al.*, 2011). Fermentation and SCFA production also inhibit the growth of pathogenic organisms by reducing luminal and fecal pH. Low pH reduces peptide degradation and the resultant formation of toxic compounds such as ammonia, amines, and phenolic compounds, and decreases the activity of undesirable bacterial enzymes (Slavin, 2013).

In the present study, fecal pH was not changed after the consumption of whole sorghum (40g/d) or wheat-based (38g/d) preparations for 8 weeks. In another study (Mcintosh *et al.*, 2003), intakes of both high-fiber rye and wheat foods (90g/d) during 4 weeks were equally effective in decreasing the pH compared with a low fiber foods, improving bowel health. This was associated with a significant ($p = 0.0001$) increase in butyrate with the high-fiber rye foods (Mcintosh *et al.*, 2003). The amount of dietary fiber consumed may explain the differences found in these studies. In the McIntosh et al. (2003) study, cereals provided 18 g of fiber/day, whereas in the present study cereals provided about 5 g/day.

Acetic acid, propionic acid, and butyric acid are the most abundant, representing 90–95% of the SCFA present in the colon. The main sources of SCFA are carbohydrates (Ríos-Covián *et al.*, 2016). Although no differences were observed between the evaluated groups in the present study, acetic, propionic, and butyric acid were the most prevalent SCFA in both groups. The consumption of whole grain sorghum or wheat-based preparations for 8 weeks did not alter the concentration of fatty acids in the evaluated men. Corroborating with our study, no significant changes in fecal concentrations of acetic, propionic and butyric acids were observed after consumption of whole grain wheat breakfast cereal (48g/d) for 3 weeks (Costabile *et al.*, 2008) or after whole grain oat-based breakfast cereal (45g/d) for 6 weeks (Connolly *et al.*, 2016).

Few studies, and none containing sorghum, have measured the ability of whole grain cereals to modulate bacterial relative abundance in human feces (Costabile *et al.*, 2008; Carvalho-Wells *et al.*, 2010; Connolly *et al.*, 2016). We observed no differences in

the relative population levels of intestinal bacteria in both intervention groups (sorghum and wheat). Costabile et al. (2008) also observed no significant changes in numbers of total bacteria and *Bacteroides spp*. in adults after consumption of whole grain wheat breakfast cereal. However, they observed increased numbers of lactobacilli after whole grain wheat intake. In another study, consumption of whole grain oat-based breakfast cereal for a 6-week period increased the numbers of lactobacilli and total bacterial population, and no significant changes in the population size of *Bacteroides* spp. were observed (Connolly *et al.*, 2016). Little or no change was observed in the numbers of total bacteria and *Bacteroides spp.* after consumption of maize-based whole grain breakfast cereal (Carvalho-Wells *et al.*, 2010).

Whole grain cereals when fermented possess potential prebiotic activity (Costabile *et al.*, 2008; Carvalho-Wells *et al.*, 2010). Whole grain sorghum contain a complex mix of bioactive compounds; however, more research is needed to determine the impact of sorghum consumption on the human gut microbiota, elucidating mechanisms by which these bioactive compounds affect the intestinal microbiota.

## Conclusion

The consumption of sorghum (40g/d) for 8 weeks reduced body weight, WC, BMI, WHtR and BF% in overweight men. However, consumption of sorghum or wheat (38g/d) for 8 weeks did not affect the fecal pH, SCFA production and population levels of intestinal bacteria. Therefore, consumption of sorghum favored obesity control without affecting the intestinal microbiota and its metabolites in overweight men. The benefits resulting from the interaction of sorghum bioactive compounds in human microbiota should be further studied.

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## 4. GENERAL CONCLUSION

Our results suggest that ingestion of sorghum-containing preparations may be an effective strategy to reduce the postprandial glycaemia of a subsequent meal, which in turn may lead to an improvement in glycemic control. Consumption of whole sorghum should be encouraged as it has good sensory acceptance and is a source of bioactive compounds that may promote human health benefits. In addition, sorghum may be considered an important strategy for reducing weight and improving oxidative stress in humans. Sorghum consumption favors obesity control without affecting the intestinal microbiota and its metabolites in overweight men. It is suggested that further studies be conducted to evaluate the effect of wholegrain sorghum consumption on the intestinal microbiota in humans.

## 5. FINAL CONSIDERATIONS

Although sorghum is still little known by the Brazilian population, the consumption of sorghum-based preparations as breakfast cereal should be stimulated, since it has good sensory acceptance in comparison to the wheat breakfast cereal. In addition, sorghum may have a beneficial effect on metabolic disorders due to its content of slowly digestible starches, resistant starch, dietary fibers and bioactive compounds (3-deoxyanthocyanidins, vitamin E, phenolic compounds, tannins) that contribute to its high antioxidant capacity and thus promote human health benefits.

Most studies that demonstrated the effect of sorghum bioactive compounds on parameters related to chronic noncommunicable diseases have used extracts or compounds isolated from sorghum and have been conducted in animals. Few studies on humans have been reported, so there is a need to study whole-grain sorghum or its fractions in the context of a healthy diet.

New studies evaluating the effects of chronic sorghum consumption are still needed. It is suggested that studies be carried out to evaluate the possible effects of chronic consumption of whole sorghum on the intestinal microbiota composition in a representative sample of overweight adults. In addition, it is suggested to evaluate the effect of whole sorghum consumption on other populations.