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Resistant starch content among several sorghum (*Sorghum bicolor*) genotypes and the effect of heat treatment on resistant starch retention in two genotypes

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ABSTRACT

The resistant starch (RS) contents in 49 sorghum genotypes and the effects of heat treatment using dry and wet heat on the grain and flour from two sorghum genotypes were investigated. The results showed a wide variation in the RS contents of the genotypes analyzed. The RS mean values were grouped into six distinct groups and ranged from 0.31 ± 0.33 g/100 g to 65.66 ± 5.46 g/100 g sorghum flour on dry basis. Dry heat causes minor losses in the RS content with retentions of up to 97.19 ± 1.92% of this compound, whereas wet heat retained at most 6.98 ± 0.43% of the RS. The SC 59 and (SSN76)FC6608 RED KAFIR BAZINE (ASA N23) cultivars, which have an average RS content of 65.51 g/100 g, were appropriate for human consumption, and the use of dry heat is presented as a better alternative for the preservation of RS in heat-treated grains.

1. Introduction

Sorghum (*Sorghum bicolor*) is the world's fifth largest produced cereal, behind only corn, rice, wheat, and barley (Food and Agriculture Organization of the United Nations Statistics Division, 2014), and is the most important grain for more than 750 million people in Africa, Asia and Latin America (Food and Agriculture Organization of the United Nations, 1999). Its origin is most likely in Africa, although there are indications of its remote presence in India. Sorghum can be classified into four groups: grain sorghum, milo sorghum, sweet sorghum and broomcorn sorghum (Ribas, 2003).

Although the production of sorghum is substantial, in most countries, sorghum is primarily used as animal feed (Mehmooda, Orhanb, Ahsanc, Asland, & Gulfraza, 2008). However, studies show that there is increased interest in its use in human food, which has made sorghum a potential source for the production of drinks and food. This interest is mainly due to its low production cost and nutritional characteristics, including protein digestibility (Lemlioglu-Austin, Turner, McDonough, & Rooney, 2012), antioxidant activity (Cardoso et al., 2014), the presence of resistant starch (Niba & Hoffman, 2003), dietary fiber (Dicko, Gruppen, Traoré, Voragen, & Van Berkel, 2006) and bioactive compounds (Khan, Yousif, Johnson, & Gamlatha, 2013), which enable its use as a functional ingredient.

The term "resistant starch" (RS) was first used by Englyst, Wiggins, and Cummings (1982) to designate the starch that was not hydrolyzed during incubation with digestive enzymes. To be considered resistant to digestion, the starch must arrive in the large intestine intact where it is fermented by the intestinal microbiota (Yue & Waring, 1998); thus, its effects can be compared to fiber.

Several in vivo studies have shown that RS has physiological effects that are potentially beneficial to health. Liu and Xu (2008), based on a study using mice, suggested that RS

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administration can slow the growth and/or development of neoplastic lesions in the colon. In a study on the effect of the consumption of corn and rice RS by rats with induced diabetes, Brites, Trigo, Carrapiço, Alviña, and Bessa (2011) found a reduction in the total cholesterol in rats fed bread enriched with RS. In addition, in the same study, the group of animals fed with RS showed a decrease in fecal pH and better postprandial glycemic response. A study by Shen, Zhang, Dong, Ren, and Chen (2015) evaluated the effects of sorghum RS on changes in body weight, blood lipids and the population of intestinal microbiota in the colons of overweight and obese rats fed a high-fat diet. RS was obtained by heating aqueous sorghum isolated in autoclave, following by cooling and hydrolysis of non-resistant starch by α-amylase. Then the sediment was separated by centrifugation, washed and dried. The results indicated that sorghum RS helps the body prevent and treat obesity through various mechanisms, including the synthesis and secretion of leptin and adiponectin and improvements in intestinal microbiota.

Willis, Eldridge, Beiseigel, Thomas, and Slavina (2009) reported on cakes made using different fiber sources, and the products with added RS were able to maintain satiety for a longer time (180 min) than those with corn bran, barley, oats, and polydextrose.

Despite its importance, there are few studies related to the RS content in sorghum grain, and those that exist (Mkandawire et al., 2013) do not show the variability among the different genotypes or the effects of heat treatment on the RS retention.

Thus, the objective of this study was to determine the resistant starch content in sorghum genotypes to identify the genotypes with potential for use in breeding programs to develop sorghum varieties that can act as functional foods with health benefits. Additionally, this study determined the influence of heat treatments on the resistant starch content of sorghum genotypes with high RS levels.

2. Materials and methods

2.1. Samples

Seed samples of 49 genotypes of grain sorghum were selected (Table 1) from a high genetic variability genotype panel belonging to the collection of the Sorghum Breeding Program of Embrapa (Brazilian Agricultural Research Corporation) Maize and Sorghum. The seeds were planted in the experimental field of Embrapa – Maize and Sorghum (Nova Porteirinha, MG, Brazil), in June 2010. The experimental plots were composed of two rows that were three meters long, with a spacing of 0.50 m between rows. The fertilization consisted of the application of 300 kg/ha of 08–28–16 (NPK) fertilizer. The harvest for the experiments occurred in October 2010.

The sorghum grains came from a trial carried out in the field with three replications. A composite sample was created, for each genotype, with grains of these three repetitions. From this composite sample, three replications were taken for laboratory analyses.

Once harvested, the whole seeds were sent to Embrapa Maize and Sorghum in Sete Lagoas, Minas Gerais, where they were stored in a cold chamber at 10 ± 2 °C. The seeds were milled for 30 s in a Wiley mill IKA A11 Basic (Staufen, Germany) before the analysis was performed.

2.2. Determination of the resistant starch content

Quantification of the RS content was performed according to the method certificated by AACC (2001) and AOAC (2000) using the RS assay kit supplied by Megazyme International Ireland Ltd., Wicklow, Ireland. Briefly, enzymatic hydrolysis of non-resistant starch (NRS) was performed through the simultaneous action of pancreatic α-amylase (10 mg/mL) and amyloglucosidase (3 U/mL) by incubating the sample for 16 h at 37 °C. Subsequently, the NRS was separated by centrifugation, and the pellet containing the RS was purified with ethanol and solubilized with 2 mol/L KOH. The concentration of RS was measured at 510 nm, and the content was expressed as g/100 g sorghum flour on a dry weight basis. The results were obtained in analytical triplicate and are presented as the mean ± the standard deviation.

2.3. Effect of processing on the resistant starch content of two sorghum genotypes

To verify the effect of heat processing on the RS content in sorghum, strain SC 59 and hybrid sorghum BR 305 were planted in June 2013. These samples were selected based on having the highest RS content among the 49 sorghum genotypes studied (present results) and among hybrid sorghum grains (unpublished data). The seeds were planted in the experimental field of Embrapa – Maize and Sorghum (Nova Porteirinha, MG, Brazil). The experimental plots were composed of two rows that were three meters long with a spacing of 0.50 m between rows. The fertilization consisted of the application of 300 kg/ha of 08–28–16 (NPK) fertilizer. The harvest occurred in October 2013. Once harvested, the whole seeds were sent to Embrapa Maize and Sorghum in Sete Lagoas, Minas Gerais and were submitted to the following treatments:

The samples were processed as following: RAW Raw grains: the grains were milled without receiving heat treatment; CKG Cooked grains (wet heat): 20 g of grains was boiled in 200 mL of water for 1 h on a hot plate. The grains were dried in an oven for 2 days at 65 °C and were milled after cooling; CKF Cooked flour (wet heat): 20 g of raw flour was mixed with 100 mL of water and cooked on a heater plate for 8 min. Then, the samples were dried in an oven for 4 h at 65 °C; RTG Roasted grains (dry heat): 20 g of grains was roasted in an electric oven at 180 °C for 15 min and were milled after cooling; RTF Roasted flour (dry heat): 20 g of raw flour was roasted in an electric oven at 180 °C for 15 min.

After drying and cooling, the samples were milled in an IKA A11 basic analytical mill (Staufen, Germany) for 30 s and were stored in plastic bottles under refrigeration.

The resistant starch content was determined according to the methodology described in Section 2.2. The calculation of the apparent RS retention of samples was performed according to the equation proposed by Rodriguez-Amaya and Kimura (2004):

$$\text{Apparent retention }(\%) = \frac{\text{RS in processed food }(\text{g}/100\text{ g})}{\text{RS in non}-\text{processed food }(\text{g}/100\text{ g})}$$

2.4. Statistical analysis

Analysis of variance was performed for the RS content of the 49 analyzed genotypes, considering a completely randomized design with three replications. The significance of the contrast between the means was verified by the Scott-Knott test at 5% probability using the statistical program SISVAR (Ferreira, 2011).

In the study of the effect of processing on the RS content in two sorghum genotypes, analysis of variance was performed considering the completely randomized 2 × 5 factorial (genotype × handles) with three replicates. The significance of the contrast between the means was verified by a Tukey test at 5% probability using SISVAR (Ferreira, 2011).

Table 1
Pericarp color and origin of the 49 evaluated sorghum genotypes.

Genotype	Pericarp color	Origin	Genotype	Pericarp color	Origin
SC6	Brown	Ethiopia	01MN1589-B	Bronze	United States
ATF 14B	Bronze	n/a	SC53	Red	Sudan
SC1038	Yellow	Ethiopia	Lian Tang A	Brown	China
P898012	Gray	United States	SC323	Gray	Sudan
B.Tx626	Red	United States	(SN149)SA7000 CAPROCK (ASA N°88) Tx7000	Bronze	United States
SC562	Red	Sudan	SC115	Brown	Uganda
R.Tx431	Bronze	United States	SC108	Red	Ethiopia
SC391	Yellow	Egypt	SC1201	Brown	n/a
SC964	Brown	Uganda	N268B	Bronze	United States
P-721	Cream	United States	SC42	Brown	Ethiopia
LG70	Red	United States	SC49	Brown	Sudan
SC1356	Bronze	Sudan	ATF 13B	Bronze	n/a
SC1158	Bronze	Ethiopia	SC648	Brown	South Africa
B.TX399	Yellow	United States	SC63	Red	Sudan
SC566	Bronze	Nigeria	SC325	Brown	United States
EBA-3	Yellow	n/a	BR007B	Red	n/a
TX2911	Red	United States	SC672	Brown	Zimbabwe
SC135	Brown	Ethiopia	R.Tx2783	Red	United States
SC320	Cream	Chad	SC1328	Brown	Sudan
SC467	Red	India	SC655	Brown	South Africa
CSM-63	Gray	Mali	B.AZ9504	Brown	United States
(SN142)SA386 REDBINE-60 (ASA N98)	Bronze	n/a	SC319	Brown	Uganda
SC725	Brown	Japan	(SSN76)FC6608 RED KAFIR BAZINE (ASA N23)	Red	United States
B.DLO357	Red	United States	SC 59	Brown	Sudan
Dorado	Cream	n/a			

n/a: No information available.

3. Results and discussion

3.1. Resistant starch content of 49 sorghum genotypes

There was a wide variation on the RS content of the 49 sorghum genotypes; their means values were divided into six groups using the Scott-Knott test ($p < 0.05$) (Table 2). The method used in the analysis was able to detect both very low and high levels of RS, with values ranging from 0.31 ± 0.33 to 65.66 ± 5.46 g/100 g.

According to the frequency distribution (Fig. 1), the modal interval is Group c, which showed RS levels between 12.71 g/100 g and 20.31 g/100 g. The groups with the highest RS levels had the fewest genotypes. The 10 genotypes in Group a presented the lowest RS contents, ranging from 0.31 g/100 g to 3.86 g/100 g. This group has good potential for use in animal feed because a high indigestible starch content is an undesirable feature for animal weight gain.

In contrast, the products with high indigestible starch contents have high demand for human consumption. The genotypes in Group f, with RS contents varying from 65.36 to 65.65 g/100 g, are highly recommended for products for humans with functional appeal.

Table 2
Resistant starch content (g/100 g)[*] in different sorghum grain genotypes.

Genotype	Resistant starch (g/100 g)	Genotype	Resistant starch (g/100 g)
SC6	0.31 ± 0.33^{a}	01MN1589-B	12.71 ± 2.93^{c}
ATF 14B	0.69 ± 0.25^{a}	SC53	12.88 ± 1.97^{c}
SC1038	0.97 ± 0.12^{a}	Lian Tang A	12.88 ± 2.67^{c}
P898012	3.29 ± 0.49^{a}	SC323	14.58 ± 4.04^{c}
B.Tx626	3.34 ± 0.19^{a}	(SN149)SA7000 CAPROCK (ASA N°88) Tx7000	14.78 ± 1.45^{c}
SC562	3.35 ± 2.02^{a}	SC115	15.54 ± 4.59^{c}
R.Tx431	3.40 ± 1.19^{a}	SC108	15.58 ± 1.18^{c}
SC391	3.57 ± 0.05^{a}	SC1201	16.45 ± 0.93^{c}
SC964	3.82 ± 0.63^{a}	N268B	16.58 ± 1.37^{c}
P-721	3.86 ± 0.85^{a}	SC42	16.76 ± 3.81^{c}
LG70	5.91 ± 0.90^{b}	SC49	17.34 ± 0.66^{c}
SC1356	6.26 ± 2.72^{b}	ATF 13B	18.12 ± 6.15^{c}
SC1158	6.55 ± 0.44^{b}	SC648	18.12 ± 4.34^{c}
B.TX399	6.80 ± 1.30^{b}	SC63	18.19 ± 5.66^{c}
SC566	6.93 ± 3.09^{b}	SC35	19.29 ± 4.81^{c}
EBA-3	7.22 ± 1.80^{b}	BR007B	20.13 ± 2.81^{c}
TX2911	7.39 ± 2.13^{b}	SC672	20.31 ± 7.09^{c}
SC135	7.69 ± 3.98^{b}	R.Tx2783	21.46 ± 2.20^{d}
SC320	7.76 ± 2.92^{b}	SC1328	23.69 ± 0.67^{d}
SC467	8.02 ± 1.19^{b}	SC655	25.16 ± 1.25^{d}
CSM-63	8.88 ± 5.27^{b}	B.AZ9504	25.36 ± 4.51^{d}
(SN142)SA386 REDBINE-60 (ASA N98)	9.19 ± 0.53^{b}	SC319	31.30 ± 1.72^{e}
SC725	10.04 ± 2.89^{b}	(SSN76)FC6608 RED KAFIR BAZINE (ASA N23)	65.36 ± 5.48^{f}
B.DLO357	11.57 ± 0.87^{b}	SC 59	65.65 ± 2.93^{f}
Dorado	12.01 ± 6.24^{b}		

[*] The resistant starch contents (g/100 g) are the mean values ± standard deviation of three results. Different superscripted lowercase letters (a–f) indicate significant differences ($p < 0.05$) between the mean values of the resistant starch, as determined using the Scott-Knott test.

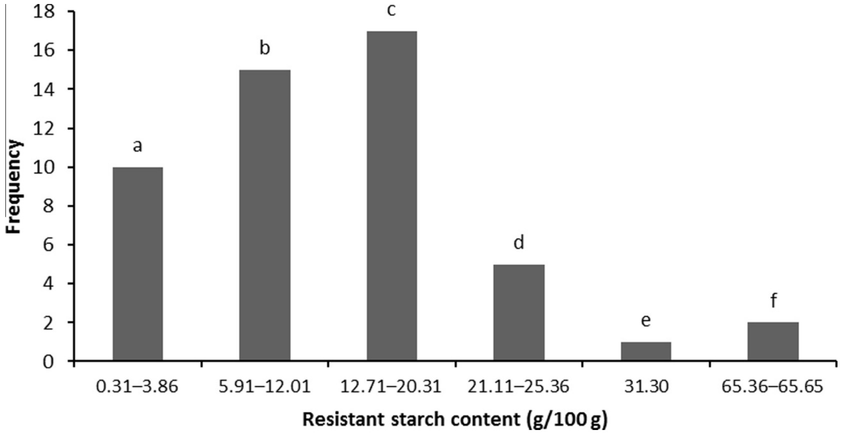

Fig. 1. Frequency distribution of the resistant starch content in 49 sorghum genotypes. Different superscripted lowercase letters (a–f) indicate significant differences ($p < 0.05$) between the RS contents of the genotypes, as determined using the Scott-Knott test.

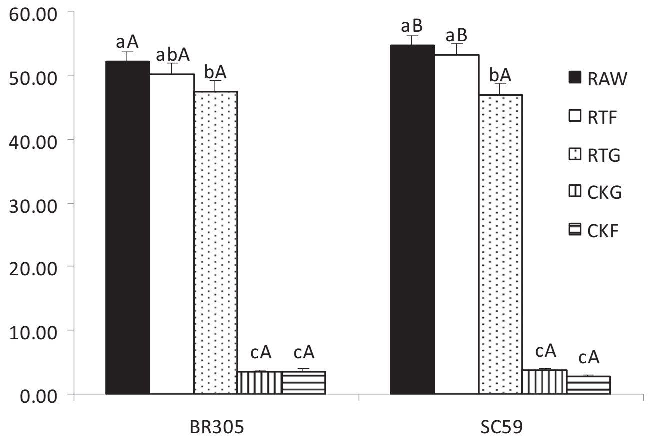

Fig. 2. Resistant starch content in two sorghum genotypes under different processing types. RAW: Raw grain; RTF: Roasted flour; CKF: Cooked flour; RTG: Roasted grain; CKG: Cooked grain. Different superscripted lowercase letters (a–c) indicate significant differences ($p < 0.05$) between the mean resistant starch content using the different processing methods for the same sorghum, as determined using the Tukey test. Different superscripted capital letters (A–B) indicate significant differences ($p < 0.05$) between the mean resistant starch content of the two sorghum genotypes using the same processing method, as determined using the Tukey test.

In a study by Niba and Hoffman (2003), the RS content observed in sorghum without heat treatment was 6.46 ± 2.0 g/100 g, whereas Saravanabavan, Shivanna, and Bhattacharya (2013) found values between 3.4 and 4.3 g/100 g of RS in three varieties of sorghum (pop, maldandi and red) that were not processed.

In the above works, the RS contents did not reach the highest values found in the sorghum genotypes in this study, demonstrating their potential for human nutrition.

Ragaee, Abdel-Aal, and Noaman (2006) reported RS contents of 1.77 ± 0.02 g/100 g in sorghum grains. Khan et al. (2013) observed RS contents of 0.42 ± 0.06 g/100 g, 2.21 ± 0.06 g/100 g and 2.95 ± 0.15 g/100 g in wheat flour, white sorghum flour and red sorghum flour, respectively, indicating that the indigestible portion of sorghum starch is higher than that of wheat. Only 6% of the samples in this study contained lower RS content than the red sorghum flour in the work of Khan et al. (2013).

3.2. Effect of processing on the RS contents of sorghum genotypes

The RS contents in the two sorghum genotypes subjected to different heat treatments are depicted in Fig. 2. The RS content observed for the raw sorghum SC 59 was higher ($p < 0.05$) than the RS content of the hybrid BR 305. However, the cultivars had similar RS contents after processing. The type of sorghum did not influence ($p > 0.05$) the effect of processing on the RS content, except for RTF treatment. The RAW treatment presented the highest RS levels for both the BR 305 (52.26 ± 1.38 g/100 g) and SC 59 (54.83 ± 1.38 g/100 g) genotypes, followed by the RTF (50.11 ± 1.89 g/100 g for BR 305 and 53.29 ± 1.29 g/100 g for SC 59) and RTG (47.39 ± 1.78 g/100 g for BR 305 and 47.02 ± 1.60 g/100 g for SC 59) treatments. No significant difference ($p > 0.05$) was detected between the two dry heat treatments (RFT and RTG) in either genotype. The lowest results were observed for the wet heat treatments CKG (3.5 ± 0.29 g/100 g for BR 305 and 3.83 ± 0.29 g/100 g for SC 59) and CKF (3.57 ± 0.54 g/100 g for BR 305 and 2.86 ± 0.08 g/100 g for SC 59), with no significant difference ($p > 0.05$) between them. These findings indicated that the RS contents of samples subjected to dry heat were higher than those subjected to wet heat in both genotypes.

The SC 59 genotype showed a lower RS level in the 2013 test (54.8 g/100 g) than in the 2010 test (65.65 g/100 g), indicating the possibility of an influence from the cultivation environment.

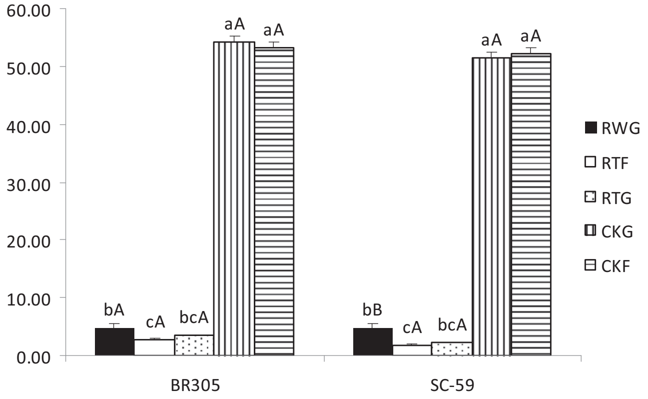

Fig. 3. Non-resistant starch content in two sorghum genotypes under different processing types. RAW: Raw grain; RTF: Roasted flour; CKF: Cooked flour; RTG: Roasted grain; CKG: Cooked grain. Different superscripted lowercase letters (a–c) indicate significant differences ($p < 0.05$) between the mean non-resistant starch content using the different processing methods for the same sorghum, as determined using the Tukey test. Different superscripted capital letters (A–B) indicate significant differences ($p < 0.05$) between the mean non-resistant starch content of the two sorghum genotypes using the same processing method, as determined using the Tukey test.

Table 3
Mean retention of the resistant starch (%) in sorghum genotypes after different treatments.

Treatment	Genotype	
	BR 305 RS retention (%)	SC 59 RS retention (%)
RTF	95.87 ± 2.95^{aA}	97.19 ± 1.92^{aA}
RTG	91.31 ± 3.45^{aA}	85.75 ± 2.38^{bB}
CKF	6.84 ± 0.83^{bA}	5.21 ± 0.12^{cA}
CKG	6.70 ± 0.46^{bA}	6.98 ± 0.43^{cA}

RTF: Roasted flour; CKF: Cooked flour; RTG: Roasted grain; CKG: Cooked grain.
Different superscripted lowercase letters (a–c) indicate significant differences ($p < 0.05$) between the mean RS retention (%) using the different processing methods for the same sorghum, as determined using the Tukey test. Different superscripted capital letters (A–B) indicate significant differences ($p < 0.05$) between the mean RS retention (%) of the two sorghum genotypes using the same processing method, as determined using the Tukey test.

Although there was an observed decrease, the SC 59 strain remained a major source of RS and, therefore, is promising for the development of cultivars with high concentrations of this bioactive compound.

In contrast to what was observed with the RS content, the NRS levels were high in the CKG (54.13 ± 0.64 g/100 g for BR 305 and 51.47 ± 0.99 g/100 g for SC 59) and CKF (53.33 ± 0.49 g/100 g for BR 305 and 52.18 ± 0.97 g/100 g for SC 59) treatments and low in the RAW (4.66 ± 0.31 g/100 g for BR 305 and 4.80 ± 0.79 g/100 g for SC 59), RTG (3.42 ± 0.52 g/100 g for BR 305 and 2.32 ± 0.05 g/100 g for SC 59) and RTF (2.80 ± 0.25 g/100 g for BR 305 and 1.84 ± 0.20 g/100 g for SC 59) treatments, indicating that wet heat promoted the conversion of RS to NRS (Fig. 3).

Wet heat caused a drastic reduction in the RS levels in sorghum grain and flour in both genotypes with retentions of only 6.70 ± 0.46% and 6.84 ± 0.83%, respectively, for the BR 305 hybrid, and 6.98 ± 0.43% and 5.21 ± 0.12%, respectively, for the SC 59 genotype (Table 3). In contrast, the dry heat did not cause high losses in the RS levels of the roasted flour, with retentions of 95.87 ± 2.95% and 97.19 ± 1.92% for the BR 305 and SC 59 genotypes, respectively. Although the RS contents in the roasted grains were significantly lower ($p > 0.05$) than the raw grains, the reduction in RS levels caused by this type of processing was much lower than that caused by wet heat, leading to 91.31 ± 3.45% retention in BR 305 and 85.75 ± 2.38% in SC 59.

Starch resistance may be due to several factors, which can be classified into three types: RS1 (physically inaccessible), RS2 (structurally resistant granules) and RS3 (retrograded starch) (Englyst et al., 1982). The reduced RS content after thermal processing may be associated with disruption of starch granules, which would increase the access of the enzyme to the starch, allowing for digestion. This fact is confirmed by the study of Freitas and Leonel (2008). However, the effect of heat on the sorghum RS content is not completely established.

The results of the present work indicated that heat processing affects the digestibility of starch probably by influencing its gelatinization, which allows a greater action of digestive enzymes. As observed by Alsaffar (2010), gelatinized starch is easier hydrolyzed than native starch granules by the amylolytic enzymes. This author states that the greater the proportion of water added during the heating, the greater the degree of gelatinization and the digestibility of starch. In the current study, dry heat reduced the RS content of samples up to 13.4% (RTG SC59), while the wet heat was able to decrease the RS content up to 94.7% (CKF SC59).

In a study about factors affecting the rate of hydrolysis of starch in food, Snow and O'Dea (1981) observed a reduction from 12 to 53 g/100 g of RS in rice submitted to cooking processing, while cooked oats the values decreased from 16 to 3 g/100 g. Muir and O'Dea (1992) also observed a reduction of RS in boiled oats and cooked bananas. These authors showed that the increase of the rate of hydrolysis caused by heat treatment occurred because the starch was gelatinized and then was more available for enzymatic action.

Niba and Hoffman (2003) demonstrated an increase from 6.5 g/100 g in unprocessed sorghum grains to 10.4 g/100 g in the samples heated in an autoclave at 120 °C. Saravanabavan et al. (2013) observed that the production of sorghum popcorn reduced the RS content in three varieties (pop sorghum, maldandi and red sorghum), leading to an increase in starch digestibility. Khan et al. (2013) did not observe a modification of the resistant starch content in pasta after cooking with the addition of white and red sorghum flour.

Studies with other products also showed the effect of thermal processing on the RS content. Shrestha et al. (2010) and Shrestha et al. (2015) observed a reduction in the RS content in corn extruded at temperatures up to 120 °C. Using wet heat with or without pressure, Eyaru, Shrestha, and Arcot (2009) reported reductions of up to 75 and 90%, respectively, for different plants.

4. Conclusions

The viability of the use of sorghum as a source of RS for human nutrition was confirmed because grains with different genotypes showed high levels of this component. Two genotypes stood out with over 65% RS. The 10 genotypes with extremely low levels of resistant starch (between 0.31 and 3.86 g/100 g) may be useful as animal feed.

Heat treatment influenced the RS content in grains and flours for both genotypes; wet heat caused a drastic reduction in the levels of this component. Dry heat led to a reduction of up to 15% of the RS content and was a better alternative for the processing of sorghum grain to maintain the high RS contents.

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