HOW DOES EXTRUSION CHANGE THE PHENOLIC PROFILE AND IMPACT PROTEIN DIGESTIBILITY IN SORGHUM FLOURS?

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ABSTRACT: In this work, the effect of thermoplastic extrusion on the phenolic compounds (PC) profile by UPLC-MS^E and on the solubility/polymerization of kafirins by SE-HPLC was evaluated in extruded whole sorghum, with or without tannins. Free (FPC) and bound (BPC) phenolic compounds were extracted separately. The characteristics evaluated are antioxidant activity (by the methods DPPH and FRAP), total phenolic content (by the methodology involving the Reagent of Folin Ciocalteu), total proanthocyanidins content (TPAC) and total flavonoid content (TFC). FPC and BPC ranged from 41.1 to 434.3 and 8.2 to 283.7 mg GAE/100 g (db), respectively. DPPH and FRAP analysis showed a strong correlation with TPC (0.99 and 0.95, $p< 0.05$, respectively). TPAC and TFC show the degradation of tannins in monomeric flavonoids, after extrusion. Globally, 58 FPC and 100 BPC were tentatively identified. PCA biplot indicated a clear distinction between PC profile in flour and extrudate in both genotypes. SE-HPLC showed an increase in protein solubility after extrusion thus it also improves protein digestibility. This work reveals that the extrusion improve the release of BPC, the breakdown of polyphenols and the depolymerization of kafirins in sorghum grains, promoting the use of this cereal as a potential functional food for humans.

Keywords: *Sorghum bicolor*, kafirins, tannins, metabolomics, properties changes.

INTRODUCTION

Sorghum (*Sorghum bicolor* L.) is a potential substitute for allergenic cereals due to its agronomic, nutritional and functional benefits, especially related to its great content of phenolic compounds (PC) (STEFOSKA-NEEDHAM *et al.*, 2015). However, sorghum PC, particularly tannins, are able to form insoluble complexes with proteins (kafirins), decreasing their digestibility by up to 50% (TAYLOR *et al.*, 2007). This work aimed to evaluate the effect of extrusion on the PC profile by UPLC-ESI-QTOF-MS^E and on the solubility/polymerization of kafirins by SE-HPLC in extruded Brazilian sorghum grains considering tannin-rich and tannin-free genotypes.

MATERIAL AND METHODS

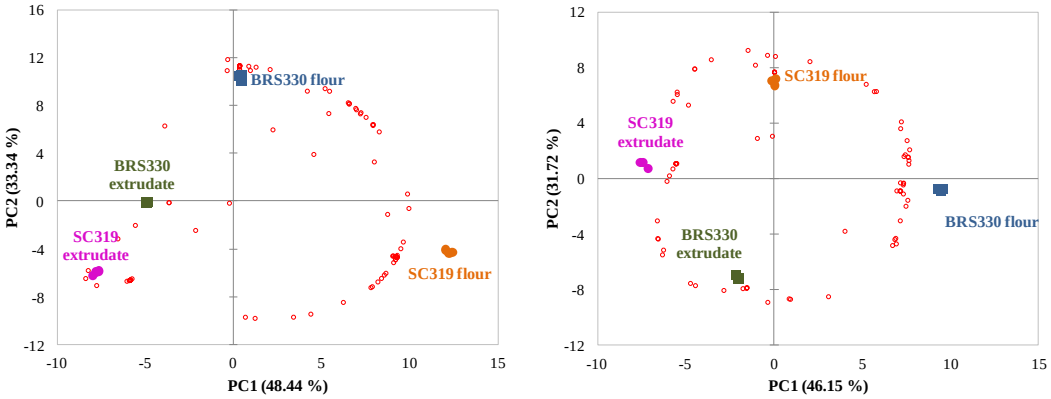
Two genotypes of sorghum: tannin-rich (SC319) and tannin-free (BRS330) were ground and extruded by using a single screw extruder fitted on a torque rheometer (19/20DN; Plast-Corder Lab-Station, Brabender, Germany) at 12% moisture, constant screw speed (280 rpm) at 50, 90 and 130 °C. Free (FPC) and bound (BPC) phenolic compounds were extracted separately (SANTOS *et al.*, 2019). Total phenolic content (TPC) was determined by Folin-Ciocalteu, antioxidant capacity was determined by DPPH and FRAP methods (SINGLETON *et al.*, 1999; SOMPONG *et al.*, 2011). Proanthocyanidin (TPAC) and flavonoid (TFC) contents were determined in microplates (RAO *et al.*, 2018). SE-HPLC analysis was used to compare the kafirins polymerization degree and phenolic profile was analyzed by UPLC-MS^E in negative mode (SANTOS *et al.*, 2019). Data were processed using Progenesis QI and statistical analysis was performed on XLSTAT and EZinfo 3.0 software.

RESULTS AND DISCUSSION

As expected, the SC319 tannin-rich genotype showed higher TPC (free+bound) than BRS330 tannin-free in raw flour and in extrudates (data not shown). When extracts were separately analysed, extrusion increased FPC (+60%) and decreased BPC (-40%) in SC319; but reduced both (-40%; -90%, respectively) in BRS330. The hypothesis is that the high temperature applied in the thermoplastic extrusion led to the release of PC (in SC319 genotype); but also favors the decomposition of heat-labile PC (BRS330 genotype). The analysis of antioxidant activity (using the methods of DPPH and FRAP) showed a strong correlation with TPC (0.99 and 0.95, $p<0.05$, respectively). TPAC and TFC had negative strong correlation (0.96, $p<0.05$), which can be attributed to the degradation of proanthocyanidin leading to the formation of flavonoid monomers and dimmers.

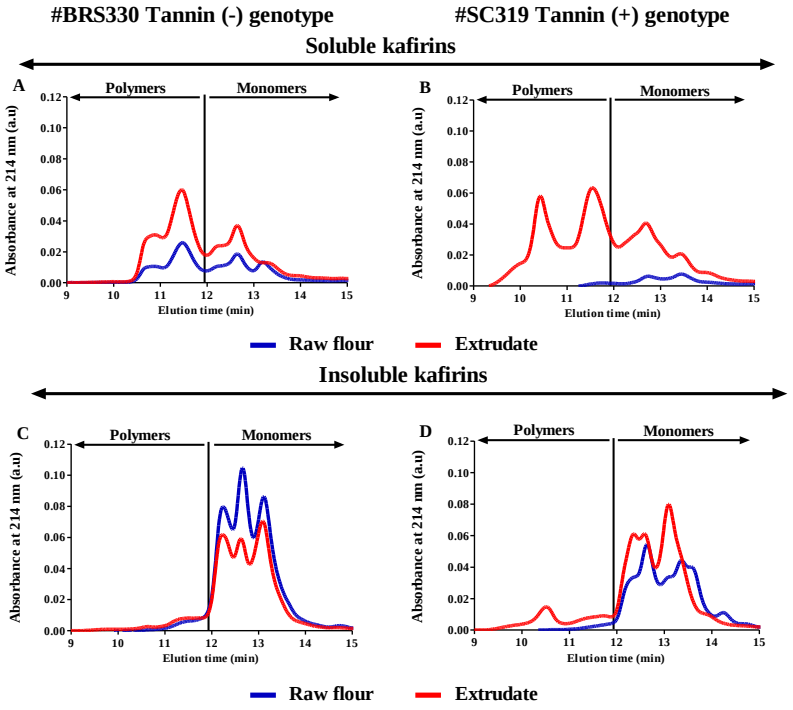
Globally, 58 FPC and 100 BPC were tentatively identified, in addition to 23 PC in both extracts; BPC presented higher abundance (58%) (data not shown). The identified PC belonged mainly to the flavonoid class (45%), followed by phenolic acids (29%), other polyphenols (12%) and lignans (2%). Moreover, 12% could not be assigned to a class being classified as unknown (data not shown). The PCA biplot was applied to investigate the degree of similarity or dissimilarity between the PC quantified relatively in each sample. The PCA biplot (Fig. 1) indicated a clear distinction between flour and extrudate in both genotypes, explaining in average 80% of the total variance observed, reaffirming the different patterns of the PC profile between genotypes and after extrusion.

Figure 1. Principal component analysis (PCA) of sorghum samples in (A) free and (B) bound extracts. The samples (symbols) are distributed according to relative intensity of identified PC (red circles). The PC1 axis is the first principal direction along which the samples show the largest variation; while the PC2 axis is the second most important direction and it is orthogonal to the PC1 axis.



SE-HPLC (Fig. 2) was applied to quantify and to monitor molecular size distribution of two solubilized protein fractions (alcohol-soluble and cross-linked kafirins). The BRS330 genotype showed an increase in extractible total kafirins content, mainly attributed to soluble proteins. In the SC319 genotype, total protein content increased above the double. Contrary of tannin-free genotype, SC319 showed a 49% increase of insoluble kafirins after the process.

Figure 2. Elution profiles obtained by SE-HPLC of soluble and insoluble kafirins from the sequential extraction of BRS330 (A and B) and SC319 (C and D) genotypes of sorghum samples.



This protein solubility increase may be associated with the loss of its strongly folded structure, leading to protein degradation and denaturation during the thermal process. It provides higher accessibility of the peptide chain to hydrolytic enzymes or aggregates, and thus increased protein digestibility (MERTZ *et al.*, 1984). However, the presence of tannins in genotype can significantly increase insoluble kafirins content, attributed to the formation of newly formed polymers showing irreversible interactions (cross-linking) favored by high temperatures during the extrusion process. This polymerization is clearly shown in Figure 2D, when a new peak is formed (elution time: 10.5 min). Another hypothesis would be due to the increase of the total area of the graph in Figure 2D, showing that extrusion provided the increase in extractability.

CONCLUSION

This work was essential to better understand the effect of extrusion, genotype and presence of tannin on phenolic content and composition in two sorghum genotypes. Extrusion proved to be an efficient thermal process in the release of PC previously bound to cell matrix and in kafirins solubilization. However, this effect can be strongly affected by the genotype due to the presence/absence of tannins.

ACKNOWLEDGMENTS

This work was funded by FAPERJ, CNPq, EMBRAPA, INRA and CAPES - Finance Code 001.

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