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Transgenic sorghum with suppressed synthesis of kafirin subclasses: Effects on flour and dough rheological characteristics

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ABSTRACT

Arising from work showing that conventionally bred high protein digestibility sorghum types have improved flour and dough functionality, the flour and dough properties of transgenic biofortified sorghum lines with increased protein digestibility and high lysine content (TG-HD) resulting from suppressed synthesis of several kafirin subclasses, especially the cysteine-rich γ-kafirin, were studied. TG-HD sorghums had higher flour water solubility at 30 °C ($p < 0.05$) and much higher paste viscosity (41% higher) than their null controls (NC). TG-HD doughs were twice as strong as their NC and dynamic rheological analysis indicated that the TG doughs were somewhat more elastic up to 90 °C. CLSM of doughs and pastes indicated that TG-HD had a less compact endosperm protein matrix surround the starch compared to their NC. The improved flour and dough functional properties of the TG-HD sorghums seem to be caused by reduced endosperm compactness resulting from suppression of synthesis of several kafirin subclasses which modifies protein body and protein matrix structure, and to improved protein-starch interaction through hydrogen bonding specifically caused by reduction in the level of the hydrophobic γ-kafirin. The improved flour functionality of these transgenic biofortified sorghums can increase their commercial utility by complementing their improved nutritional quality.

1. Introduction

Sorghum's inferior protein quality with respect to the indispensible amino acid lysine and the low digestibility of its protein in wet cooked foods are major factors that limit its utilization when compared to other major cereals (Henley et al., 2010). Another protein related drawback of sorghum with respect the production of dough-based foods is that kafirin, its prolamin protein, does not form a viscoelastic, gas-holding dough in an aqueous system, unlike wheat gluten (Oom et al., 2008; Goodall et al., 2012). The Africa Biofortified Sorghum (ABS) consortium has developed transgenic sorghum lines with improved nutritional quality with respect to protein quality and availability, mineral availability and provitamin A (Biosorghum, undated). Particular ABS lines have been produced with substantially improved lysine content and protein digestibility resulting from suppression of synthesis of specific kafirin subclasses. The Protein Digestibility Corrected Amino Acid Score (PDCAAS) of these protein biofortified sorghums is double that of their null controls (Henley et al., 2010).

A wide range of food products, including porridges, couscous, flatbreads and biscuits have been made with early generation lines of the transgenic protein biofortified sorghums, which were tannin containing (Taylor and Taylor, 2011). Their quality was similar to that of their null controls (also tannin-containing), although their flour properties were not studied. However, Kruger et al. (2012) showed that these transgenic HD sorghums, which also expressed a low phytate trait, give higher extract (starch solubilisation) and free amino nitrogen in brewing, which indicated that they do have improved starch and protein functionality.

With specific respect to dough and bread quality of high protein digestibility sorghums, Goodall et al. (2012) working with conventionally bred high protein digestibility, high lysine (HD) sorghum showed that when it was composited with wheat flour, the dough had greater resistance to extension and time to dough breakage than normal sorghum-wheat composite dough; and also the HD sorghum-wheat composite had similar strain hardening to

Abbreviations: ABS, Africa Biofortified Sorghum; CLSM, confocal laser scanning microscopy; DSC, differential scanning calorimetry; N, null control sorghum; RNAi, RNA interference; TG-HD, transgenic sorghum with high protein digestibility; 2-DE, two dimensional gel electrophoresis; WAI, Water Absorption Index; WSF, Water Soluble Fraction.

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wheat dough. Furthermore, composite HD sorghum-wheat breads had higher loaf volume than the normal sorghum-wheat composite. Recently, we showed that sorghum lines with combined waxy and high protein digestibility traits, which were produced by conventional breeding, using the same high protein digestibility germplasm as Goodall et al. (2012), had improved flour quality compared to normal sorghum lines (Elhassan et al., 2015). Specifically, they exhibited higher paste viscosity and formed much softer and less sticky pastes and had much higher flour solubility. At 30 °C, flour solubility was similar to commercial wheat bread flours. The improvements in flour quality were attributed to the less dense (floury-type endosperm), high amylopectin content and unique endosperm protein composition of the lines.

In this present study, the flour quality of improved transgenic high protein digestibility, high lysine ABS sorghum lines was investigated. The lines were non-tannin, white tan-plant (so-called food grade) types (Da Silva et al., 2011b) and hence potentially more suitable as flour for dough-based food making.

2. Materials and methods

2.1. Sorghum lines

Crushed whole grain of two non-tannin, white tan-plant, transgenic high protein digestibility (TG-HD) sorghum lines (TG-HD-1 and TG-HD-2) and their normal digestibility null controls (NC1 and NC2) was obtained. The lines were produced through the ABS consortium by DuPont Pioneer, Johnston, Iowa in single controlled field trial.

The TG-HD sorghum lines have suppressed expression of certain kafirin subclasses by means of RNAi (RNA interference) technology, as described by Da Silva et al. (2011a). The lines were T2 self-ed seeds. TG-HD-1 and -2 were 75% pure with respect to the ABS032 gene construct which suppresses synthesis of α-kafirin A1 and α-kafirin B1and B2 (corresponding to the 19 and 22 kDa α-kafirin classes, respectively), γ-kafirin 1 and 2, and δ-kafirin 2.

2.2. Milling

A laboratory hammer mill fitted with a 250 μm opening screen was used to mill the sorghum samples into whole grain flour. The milled flours were then sealed in zip-lock type polyethylene bags and kept at 8–10 °C until used for analysis.

2.3. Extraction of kafirins

For analysis of the kafirin composition of the sorghum lines, total kafirin was extracted essentially as described by Taylor et al. (2005). The TG and NC whole grain flours were extracted with 70% (w/w) ethanol plus 0.35% (w/w) glacial acetic acid and 0.5% (w/w) sodium metabisulphite at 70 °C with vigorous stirring for 1 h. The supernatant was collected after centrifugation at 1000 × g at 25 °C for 5 min. The alcohol was allowed to evaporate from the solute and the precipitated protein washed with cold distilled water (<10 °C). The recovered protein was separated by filtration and air dried at 25 °C.

2.4. Two-dimensional gel electrophoresis (2-DE)

Kafirin composition was analysed by 2-DE as it separates proteins by their isoelectric point in addition to molecular size. The dry kafirin preparations were dialyzed against distilled water for 36 h at 10 °C, using dialysis tubing with a 12–14 kDa cut off (Visking ex. Labretoria, Pretoria, South Africa) with frequent changes of water and then freeze dried. Isoelectric focusing was carried out using 7 cm ZOOM® immobilised pH gradient strips with a range of pH 3–10 (Invitrogen, Carlsbad, CA). The strips were focused on a gradient at 200 V for 15 min, 450 V for 15 min, 750 V for 15 min and 2000 V for 70 min using a ZOOM® IPGRunner™ System according to the manufacturer's instructions. The strips were then equilibrated in the equilibration buffer, which contained dithiothreitol (as disulphide bond reducing agent) for 15 min and then in buffer containing the alkylating reagent iodoacetamide (125 mM) for 15 min. SDS-PAGE was carried out using Novex NuPAGE® 4–12% polyacrylamide gradient gels (Invitrogen).

2.5. Protein content

Dumas combustion was used to determine the protein content (N x 6.25) following AACC method 46-30 (AACC International, 2000).

2.6. Starch amylose content

The Megazyme amylose/amylopectin assay kit (Megazyme Ireland International, Bray, Ireland) was used to determine starch amylose/amylopectin ratio.

2.7. In vitro pepsin protein digestibility

In vitro protein digestibility of the flours was determined according to the pepsin digestibility method of Hamaker et al. (1986) as modified by Da Silva et al. (2011a).

2.8. Differential scanning calorimetry (DSC) of flour thermal behaviour

The method described by Beta et al. (2000) as detailed by Elhassan et al. (2015) was used.

2.9. Flour WAI and WSF

The method of Anderson et al. (1970) was used to determine Water absorption index (WAI) and water soluble fraction (WSF) of the flours at 30 °C and 60 °C. These temperatures were selected to represent normal dough mixing temperature and an elevated dough mixing temperature, just below the sorghum starch gelatinization temperature (Delcour and Hoseney, 2010).

2.10. Flour pasting properties

A Physica MCR 101 Rheometer (Anton Paar, Ostfildern, Germany) using a cup and a stirrer was used to determine flour pasting properties as detailed by Elhassan et al. (2015).

2.11. Gel strength (texture)

A TA-XT2 type texture analyser (Stable Micro Systems, Godalming, UK) was similarly used as detailed by Elhassan et al. (2015) to determine flour gel texture properties.

2.12. Dough stress-relaxation behaviour

The relaxation properties of the sorghum dough were determined according to the method of Singh et al. (2006) as modified by Falade et al. (2014). A texture analyser (EZ-L, Shimadzu, Kyoto, Japan) was used. Sorghum dough was prepared with 1 g flour and 0.9 g water. Homogeneous discs of doughs of diameter 19 mm and height 7 mm were made using a syringe. To compress the dough disc, a plastic rod (43 mm diam. and 10 mm height) was used at a