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Effects of endosperm texture and cooking conditions on the in vitro starch digestibility of sorghum and maize flours

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Abstract

The effects of endosperm vitreousness, cooking time and temperature on sorghum and maize starch digestion in vitro were studied using floury and vitreous endosperm flours. Starch digestion was significantly higher in floury sorghum endosperm than vitreous endosperm, but similar floury and vitreous endosperm of maize. Cooking with 2-mercaptoethanol increased starch digestion in both sorghum and maize, but more with sorghum, and more with vitreous endosperm flours. Increasing cooking time progressively reduced starch digestion in vitreous sorghum endosperm but improved digestibility in the other flours. Pressure-cooking increased starch digestion in all flours, but markedly more in vitreous sorghum flour; probably through physical disruption of the protein matrix enveloping the starch. Irrespective of vitreousness or cooking condition, the *alpha*-amylase kinetic constant (k) for both sorghum and maize flours remained similar, indicating that differences in their starch digestion were due to factors extrinsic to the starches. SDS-PAGE indicated that the higher proportion of disulphide bond-cross-linked prolamin proteins and more extensive polymerisation of the prolamins on cooking, resulting in polymers of $M_r > 100$k, were responsible for the lower starch digestibility of the vitreous sorghum endosperm flour.

Keywords: Vitreous endosperm; Floury endosperm; Starch digestibility; Sorghum; Maize; Cooking; Kafirin; Zein

1. Introduction

Grain sorghum (*Sorghum bicolor* L. Moench) is a very important source of dietary energy in Asia and Africa where it serves as principal staple for people in the semi-arid regions (ICRISAT/FAO, 1996). Sorghum, like other cereals, is rich in starch (≥70% with an approximately 75:25 amylopectin/amylose ratio) and should therefore be an optimal crop for industrial application in those parts of the world where it is grown (Horn et al., 1992; Zhan et al., 2003). Nevertheless, sorghum has remained industrially under-utilized. Attempts to optimise sorghum's use have resulted in an increase in the proportion of sorghum used as food, and in its exploitation as a cheap alternative source of fermentable extract in brewing (Goode and Arendt, 2003) and bioethanol processes (Zhan et al., 2003). Sorghum is also a potentially attractive energy source for the livestock industry (Rowe et al., 1999). While all sorghum starch is potentially digestible and technologically equivalent to maize starch, experience with livestock feeding (Rowe et al., 1999) and brewing (Goode and Arendt, 2003) suggests that starch in sorghum flour may be substantially less digestible. This constitutes a barrier to increased industrial utilization of sorghum.

The main protein constituents of sorghum grain, the kafirins (Taylor and Schüssler, 1986) exist in both monomeric and polymeric forms (El Nour et al., 1998; Oria et al., 1995). Polymeric kafirins are mainly formed by intermolecular disulphide cross-linking (Chandrashekar and Mazhar, 1999; Oria et al., 1995). Polymeric kafirins occur more in the vitreous endosperm fraction (Kumari and Chandrashekar, 1994), probably because the cysteine-rich γ- and β-kafirins abound in that part of the sorghum grain (Mazhar and Chandrashekar, 1995). Research suggests that they may be substantially less digestible than their

Abbreviations: 2-ME, 2-mercaptoethanol; R_{10}, initial velocity of hydrolysis; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; SEM, scanning electron microscopy); HI, hydrolytic index; HMW, high molecular weight.

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monomeric counterparts (Duodu et al., 2002; Oria et al., 1995). Sorghum kafirins polymerize during cooking and the extent of polymerization appears to be greater than in maize (Duodu et al., 2003). There are indications that polymerized kafirin may impede sorghum starch granule gelatinisation and subsequent digestibility (Chandrashekar and Kirleis, 1988; Hamaker and Bugusu, 2003; Zhang and Hamaker, 1998). Additionally, the monomeric kafirin content and composition influences the extent of kafirin polymerization in sorghum (Oria et al., 1995). This suggests the possibility that differences in sorghum endosperm texture and protein composition may influence the starch digestion dynamics.

It is known that differences in moist heat processing methods can also cause variations in the digestion of starch in cereal flours (Abdelgadir et al., 1996; Mangala and Tharanathan, 1999; Kotarski et al., 1992). Thus, the aim of the present work was to determine and compare the influence of endosperm texture and cooking conditions and the possible role of the endosperm proteins on in vitro starch hydrolysis in sorghum and maize flours.

2. Experimental

2.1. Grains and preparation of materials

Grains of NK 283, a red tannin-free sorghum cultivar, were decorticated using a carborundum cone abrasive rice pearler (Miag, Braunschweig, Germany). Degermed pericarp-free grains were then used for preparing the vitreous and floury endosperm flours. The vitreous and floury sorghum endosperms were separated manually by carefully cracking the grains in a laboratory mortar with a pestle, then sieving to separate the coarse vitreous endosperm particles from the finer (powdery) floury endosperm. Floury endosperm particles were smaller than 200 μm, while the smallest vitreous endosperm particles were larger than 200 μm. This method of endosperm fractionation was effective and less laborious than manual dissection. Separation of floury and vitreous endosperm particles was confirmed using scanning electron microscopy (SEM) (data not shown). To enable comparison of rates of starch digestion, the vitreous and floury endosperm particles were milled separately to a common maximum particle size in a water-cooled coffee grinder to pass through a 175 μm sieve. SEM showed that after grinding, the cellular structure of the floury and vitreous endosperm particles remained essentially intact (data not shown).

The maize used in this study was a commercial sample of white maize purchased already degermed and decorticated. Separation of maize endosperms was as for sorghum. The floury and vitreous sorghum endosperm flours contained $5.13 \pm 0.04\%$ and $9.56 \pm 0.03\%$ crude protein (N×6.25) (dry basis) respectively, while the corresponding values for the two maize flours were $5.92 \pm 0.06\%$ and $7.77 \pm 0.04\%$ respectively. Protein content was determined by combustion analysis using a Leco FP-528 nitrogen/protein analyser (Leco Corporation, St Joseph, MI).

2.2. Starch digestion

Samples of flours equivalent to 100 mg starch were mixed vigorously in large test tubes (2.1×15 cm) with distilled water (5 ml) then boiled (10 min) in a water bath (96 °C). After cooling to 40 °C (in a water bath) the mixture was combined with diluted porcine pancreatic *alpha*-amylase (No A-3176, Sigma) solution (5 ml in 0.05 M tris–maleate buffer, pH 6.9) to give a final enzyme concentration of 2 U/ml. Contents of each test tube were mixed vigorously then incubated at 39 °C for 150 min, with mixing every 5 min. Samples (0.7 ml) were withdrawn at 10, 20, 30, 45, 60, 90 and 150 min. In experiments to study the effects of reducing agents, flour samples were boiled in distilled water containing 5 mM 2-mercaptoethanol (2-ME). All experiments were repeated three times.

The amount of starch digested was assayed as follows: samples (0.7 ml) collected in Eppendorf tubes were centrifuged at 7,200g (4 min). Aliquots (0.2 ml) of the supernatants were added to test tubes kept at 50 °C in a water bath. Soluble dextrins in supernatants were then digested to glucose using 10 U (0.3 ml) of *Aspergillus oryzae* glucoamylase (No A-9268, Sigma) solution in 0.2 M sodium acetate buffer (pH 4.5). Digestion was for 30 min at 50 °C. Glucose in digests was determined with the dinitrosalicylic acid reagent. Amounts of starch digested were calculated by multiplying the amount of glucose (in mg) by a factor of 0.9. Starch digestion was expressed in percentage of the amount of starch at the start of the reaction, as determined by the Megazyme Total Starch Assay Procedure (amyloglucosidase/*alpha*-amylase method) (Megazyme International Ireland, Bray, Ireland).

The non-linear model of Goni et al. (1997) was applied to describe the kinetics of starch hydrolysis. The model is described by the first order equation:

$$C = C_\infty(1 - e^{-kt})$$

where C corresponds to the percentage of starch hydrolysed at time t, C_∞ is the equilibrium percentage of starch hydrolysed after 150 min, k is the kinetic constant and t is the time (min). In these experiments t was chosen as 30 min, the time at which the starch hydrolysis curve approached steady state kinetics. Following Goni et al. (1997), the parameters C and k were estimated for each sample and each treatment based on data obtained during the in vitro hydrolysis procedure. Calculation of the samples' hydrolysis indices (HI%), the proportion of flour starch that is theoretically digestible, then followed as reported by both Goni et al. (1997) and Frei et al. (2003). First the area under the hydrolysis curve (AUC) was calculated using the equation (Goni et al., 1997; Frei et al., 2003):

$$\mathrm{AUC} = C_\infty(t_f - t_0) - (C_\infty/k)[1 - \exp[-k(t_f - t_0)]]$$