Resistant-type starch in sorghum foods – Factors involved and health implications

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Keywords:

endosperm, glycaemic response, kafirin, starch digestibility, tannins

ABSTRACT

Sorghum grain has a higher content of resistant starch (RS) than other cereals and seems to be more slowly digestible. However, people consume foods where the grain has been processed by thermal and other treatments. This review addresses whether sorghum foods generally have unusually high levels of RS and slowly digestible starch (SDS), what intrinsic factors are responsible and how processing conditions affect RS and SDS, and the health-related implications of sorghum food consumption. With non-tannin type sorghums, if the endosperm structure is little disrupted during food processing, as with conventional wet cooking, then the food can exert positive health effects on glycaemic response related to its high RS content. Thermally-induced cross-linking of the kafirin matrix proteins appears to be responsible for the low starch digestibility. However, when non-tannin sorghum is processed using high-shear technologies like extrusion cooking, the endosperm and starch granule structure is disrupted, rendering the starch fully digestible. Regarding tannin-type sorghums, the tannins have a strong inhibitory effect on starch digestibility, notably by binding with the starch, which can improve glycaemic response and other health related parameters. Future research on RS in sorghum foods must focus on the mechanisms responsible and interactions between intrinsic- and processing-related factors.

1. Introduction

Resistant starch (RS) is considered as the fraction of starch that resists digestion in the small intestine and reaches the large intestine intact [1]. As such, RS is today widely regarded as a type of dietary fibre [2]. Slowly digestible starch (SDS), in contrast, is completely digested over an extended time, 20-120 minutes, in the small intestine [3]. RS and SDS are associated with several important human nutritional and health benefits, most notably hypoglycaemic and hypolipidemic effects, which in turn are associated with anti-diabetic, anti-cardiovascular disease and anti-obesity outcomes [1,4,5], and in the case of RS acting as a prebiotic [6].

There is clear evidence that the raw grain of the cereal sorghum (*Sorghum bicolor* (L.) Moench) normally has a higher content of RS in comparison to the grains of other cereal species, as comprehensively reviewed [7,8,9]. Also, there is evidence that sorghum starch is slowly digestible [10]. However, people actually consume cereal foods where the raw grain has been processed. Cereal grains are essentially always subjected to a thermal treatment to bring about some degree of starch granule disruption and additionally almost always some physical disruption of the grain kernel structure, often involving partial or near complete removal of the bran layers and germ. As RS and SDS are associated with important human health benefits and the high content of RS in sorghum grain and the likely high content SDS, two questions can be posed:

1. Do sorghum foods have a higher content of RS and SDS than those of identically processed foods of other cereal grains?
2. If so, does consumption of sorghum foods confer any health benefits that can reasonably be attributed to their higher content of RS and SDS?

A 2019 survey identified seven human subject studies involving measurement of glycaemic response to consumption of sorghum foods [11]. Four of the studies showed a lower glycaemic response and three showed no effect. For example, a study of glycaemic response to various foods made from sorghum and wheat showed that coarse and fine semolina, flakes and pasta prepared from sorghum all had a significantly lower glycaemic index and glycaemic load than comparable products prepared from wheat [12]. However, a study involving just flaked wholegrain breakfast biscuits from three fundamentally different types of sorghum showed that only those made from brown (tannin-type) had more RS and SDS than an essentially identical product from wheat, and further there was no significant difference in postprandial glucose levels, nor response pattern between any of the products [13].

That the scientific literature is apparently contradictory is not at all surprising because there are many confounding factors that can affect starch digestion, both intrinsic to the sorghum and extrinsic with regard to the type and extent of processing and also to the type and rigour of assay procedure employed [11]. Intrinsic factors include: starch molecular structure, notably the proportion of amylose; starch granule structure; grain endosperm cell matrix composition and structure; endosperm cell wall structure; amount and type of dietary fibre; type and content of polyphenols, and notably the presence, content and type of condensed tannins. Extrinsic processing factors include: the amount of water added; processing temperature; degree of disruption of endosperm cell and starch granule structure, and extent of removal of bran layers. General extrinsic starch digestibility assay factors include: whether *in vitro*, animal model or human subject assay was employed, and in the case of the latter whether the subjects were healthy or had a disease condition such as Type-2 diabetes.

This review therefore attempts to answer the questions as to whether sorghum foods generally have unusually high levels of RS and SDS, and if so what are the intrinsic factors responsible and how do extrinsic processing conditions interact with the sorghum factors. The review also examines what are the health-related implications of consumption of sorghum foods related to RS and SDS.

2. Resistant and slowly digestible starch in raw and processed sorghum

Englyst et al. [14] first used the term resistant starch (RS) to refer to starch that was made resistant to α-amylase digestion by food processing. Later, Englyst et al. [15] proposed a classification system for starch fractions in food as rapidly digestible starch (RDS) (starch fraction digested within 20 minutes), slowly digestible starch (SDS) (starch fraction digested between 20 and 120 minutes) and RS (starch fraction that is not digested). RS is therefore defined as the portion of starch in foods that is not digested by amylase enzymes in the small intestine but passes on to the colon where it is available to be fermented by microorganisms [16]. Five types of resistant starch are identified:

- RSI (type I resistant starch): Physically inaccessible starch [15]. This refers to starch located in starch granules within the endosperm (storage tissue) of grains, which issurrounded by structures such as protein matrix and endosperm cell walls. These present a physical barrier and limit the accessibility of the starch-degrading enzymes.
- RSII (type II resistant starch): This refers to native or raw granular starch with ungelatinized starch granules and consists of B- or C-type polymorphs [16].
- RSIII (type III resistant starch): This is retrograded amylose or retrograded starch as found in cooked and cooked starchy foods.
- RSIV (type IV resistant starch): These are chemically modified starches such as cross-linked starch.

- RSV (type V resistant starch): These are amylose-lipid complexes such as stearic acid-complexed high-amylose starch.

The distinction between SDS and RS is made in terms of their probable digestion in the small intestine. While RS is regarded as totally indigestible, the digestion of SDS is slow, but nonetheless complete [15]. With SDS, there is a slow increase in levels of postprandial blood glucose and these levels are sustained over time.

RS and SDS are determined using enzyme digestion methods involving enzymes such as pepsin and pancreatin followed by starch-degrading enzymes such as α-amylase and amyloglucosidase. The resulting glucose is determined spectrophotometrically and converted to starch using a conversion factor. These assays are mostly based on the methods of Englyst et al. [17] and Goni et al. [18] and today the Megazyme assay kit for RS is commonly used.

Table 1 shows some reported contents of different starch fractions in raw and cooked sorghum and sorghum fractions, including both non-tannin (essentially tannin free) and tannin types. The reported levels of RS and SDS in raw sorghum varied greatly. For example, [19] reported RS values for raw whole grain sorghum of different genotypes ranging from as low as 0.3% to as high as 65.7%. As indicated in the Introduction, there are several intrinsic factors that could be responsible, notably whether the sorghums were non-tannin or tannin types and the relative proportions of amylose and amylopectin. These factors are discussed in detail in the next section.

Examination of the data indicates that overall, raw sorghum tends to have higher RS content than other tropical cereals such as maize and temperate cereals. For example, the 1.8% RS reported for raw sorghum [20] was higher than the RS contents (0.2–0.55%) of temperate cereals (hard wheat, soft wheat, barley and rye). Giuberti et al. [21] reported a similar trend with a higher RS content (27.5%) of raw sorghum compared to 19.1% for maize and a range of 5.3-14.3% for temperate cereals (wheat, triticale, barley and oats). However, as noted in the Introduction, people consume processed, normally cooked, cereal foods. Here there is evidence that the higher content of RS in raw sorghum may be retained or even increased in the processed sorghum food and not in the same food produced from other cereals [22,23]. The factors responsible are discussed in detail in the next section.

3. Factors affecting starch digestibility in raw and processed sorghum

3.1 Tannins and other polyphenols

The interaction of sorghum phenolics with starch has become a subject of keen interest. This is because sorghum is an important source of phenolic compounds and that the interaction between sorghum polyphenols and starch affects starch digestibility with implications for potential anti-diabetic properties.

The chemistry of sorghum phenolics has been reviewed extensively [24,25,26,27]. To summarise, in sorghum, the major phenolic compounds are phenolic acids and flavonoids. The phenolic acids consist of various benzoic and cinnamic acid derivatives. The flavonoids are the most abundant group of phenolic compounds in sorghum and they exist in monomeric and polymeric forms. The major monomeric flavonoids in sorghum include flavones, flavanones, flavanols and the 3-deoxyanthocyanin pigments. The polymeric forms of flavonoids in sorghum are exclusively condensed tannins (proanthocyanidins), which are polymers of mainly flavan-3-ols and their derivatives. Sorghum is almost unique among cereals in that some sorghum varieties contain condensed tannins. Where interaction between phenolic compounds in sorghum and starch is concerned, the condensed tannins have received the most attention.

Research indicates that although monomeric flavonoids can interact with starch, this does not seem to have any significant impact on starch digestibility [28]. In contrast, there is growing evidence that sorghum tannins can interact strongly with starch to form indigestible complexes. Lemilioglu-Austin et al. [29] reported that addition of phenolic acetone extracts from tannin sorghum bran decreased starch digestibility, estimated glycaemic index and increased RS contents of high-amylose maize starch porridges. More recent research has revealed that the binding of sorghum condensed tannins to starch is quite specific in two main ways. Firstly, the high-molecular-weight sorghum condensed tannins are the most effective at binding to starch. Barros et al. [30] reported that significantly higher levels of RS were formed upon cooking high-amylose maize starch with sorghum phenolic extracts containing mainly high-molecular-weight proanthocyanidins. The ability of the proanthocyanidins to bind with amylose increased with increasing proanthocyanidin molecular weight. In effect, high-molecular-weight proanthocyanidins bound more effectively to amylose and this led to formation of a higher proportion of RS. Secondly, sorghum tannins bind more readily with amylose and linear portions of amylopectin rather than with amylopectin itself [28,31].

It is proposed that the binding between sorghum condensed tannins and starch (specifically amylose) is likely through hydrogen bonding and hydrophobic interactions [26,28]. This mechanism of interaction is facilitated by structural features of the sorghum proanthocyanidins, which cause hydroxyl groups to be more exposed for enhanced hydrogen bonding with amylose. In turn, the helical nature of amylose with its hydrophobic core facilitates hydrophobic interactions with the aromatic rings of the sorghum proanthocyanidin [26,28].

Apart from interaction with starch, there is evidence that sorghum polyphenols, specifically condensed tannins interact strongly with endosperm proteins. Emmambux and Taylor [32] showed that condensed tannin extracts from condensed tannin sorghums interacted very strongly with sorghum kafirin protein to form a permanent haze in a buffered aqueous ethanol solution, suggesting an irreversible interaction. In contrast, monomeric phenolics such as ferulic acid and catechin as well as phenolic extracts from condensed tannin-free sorghum (essentially flavonoids) did not form complexes with kafirin. It was suggested that the condensed tannin-kafirin interaction may play a role in decreasing the protein digestibility of tannin-containing sorghum. It is also possible that condensed tannin-protein interaction could also decrease starch digestibility by forming complexes that act as a barrier and prevent access of starch hydrolyzing enzymes.

In vitro and *in vivo* studies show that sorghum condensed tannins can also limit starch digestibility by inhibiting starch hydrolysing enzymes. Mkandawire et al. [33] showed that tannin-containing extracts from sorghum inhibited α-amylase in a tannin concentration-dependent manner. Links et al. [34] reported from *in vitro* studies that condensed tannins extracted from sorghum displayed strong inhibitory effects against α-amylase and α-glucosidase. Links et al. [35] leveraged the strong affinity of condensed tannins for proteins to encapsulate sorghum condensed tannins in kafirin microparticles. The kafirin microparticle-encapsulated sorghum condensed tannins decreased blood glucose levels in healthy rats after ingestion of maltodextrin. This indicated that the tannins were slowly released in the small intestine where they inhibited the starch digestive enzymes, preventing a glucose spike.

3.1.1 Processing effects

There has been little research that directly investigates the effects of food processing on RS levels in tannin sorghum. De Carvalho Teixeria et al. [19] found that with dry roasting of tannin sorghum grain and flour, there was little loss in RS. However, when the grain or flour were wet cooked only approximately 7% of RS was retained. This probably accounts for some apparently contradictory findings about the RS content of processed tannin sorghum. Sorghum biscuits made from flaked then baked tannin sorghum contained some 28% RS, whereas the biscuits made from white and red non-tannin sorghum contained 21-23% RS [13]. However, with extrusion cooked composites of sorghum and maize, a red tannin sorghum composite contained significantly less RS but significantly more SDS than a white non-tannin sorghum composite [36].

3.2 Sorghum starch structure

The general literature indicates that sorghum starch has a higher gelatinization temperature than other cereal starches, e.g. starch gelatinization temperature ranges for sorghum of 68-78°C, for regular maize 62-72°C and for wheat of 51-60°C [37]. A recent review similarly reports a sorghum starch gelatinization temperature of 68.2-77.8°C [38]. It may be inferred that the high gelatinization temperature of sorghum starch is somehow related to its low digestibility. It has been proposed that the unit chain organization of amylopectin, i.e. lengths of the various chains and degree of branching, has a major influence on the temperature of starch granule gelatinization, with amylopectin inter-block chain length being positively correlated with onset gelatinization temperature [39]. However, our knowledge of sorghum starch fine structure is too fragmentary to indicate whether its low digestibility is related to its unit chain organization.

A major factor affecting starch digestibility is the relative proportion of amylose to amylopectin. The vast majority of sorghums have a normal proportion of amylose 20-30% [38]. High-amylose sorghum types exist, containing up to 55.8% amylose [40]. They were found to have a higher gelatinization temperature than normal types and were high in RS but also were high in fibre, which may have been a contributory factor to their high RS content. Waxy sorghums (high amylopectin type) are more common. In a comparative study of waxy, heterowaxy and normal sorghum starches, waxy sorghum starch with 0% amylose was found to have much higher proportion of RDS than heterowaxy (14% amylose) and normal sorghum starch (23.7% amylose) [41]. However, the heterowaxy starch had the highest proportion of RS.

There is evidence from a study of maize mutants that the proportion of SDS is related to amylose branched chain length [42]. The content of SDS was correlated with the weight ratio of both longer amylopectin branch chains (DP>13) and shorter branch chains. The high level of RS in the heterowaxy sorghum mentioned above was tentatively attributed to the fact that it had fewer amylopectin chains of DP>15 than the normal sorghum starch [39]. For sorghum, the proportions of amylopectin branch chain length have been variously reported as

DP 6-15 44-46%, DP 16-36 approx. 50%, DP>37 5-6% [39] and DP 6-12 28.5%, DP 13-24 54% and DP 25-36 12% [43]. Thus, one cannot conclude whether sorghum amylopectin generally belongs to a particular chain length category. Cultivation temperature is a factor that influences sorghum starch fine structure. When three sorghum lines were cultivated at a daily maximum temperature of 38°C, two of the three lines showed an increase in the proportion of long to short amylopectin branches and a reduction in branching compared to their controls cultivated at a maximum temperature of 32°C [44]. The generally high temperature of cultivation of sorghum is possibly a contributing factor to its high RS content.

3.2.1 Processing effects

Dry and wet heat processing of sorghum can have dramatically different effects on retention of RS. With two tannin sorghum genotypes that were very high in RS (>50% RS), roasting at 180°C of the whole grains or flour resulted in a ≥85% retention in RS, whereas boiling the whole grains or flour resulted in a ≥93% loss in RS [19]. Heat-moisture treatment (HMT), which involves heating with limited amount of water, up to 20-30%, at upwards of 100°C in an air-tight container, is a processing technique that can specifically modify starch properties [45]. Two studies have applied HMT to sorghum flours. Sun et al. [46] found a considerable increase in insoluble amylose content. Vu et al. [47] found that HMT at 100°C at 20% moisture for 4 h increased the content of RS from <6% to 22%. This was accompanied by some reduction in SDS. The increase in RS was attributed to enhanced amylose-lipid complexation as there was no change in starch crystallinity pattern.

3.3 Amylase inhibitors

Alpha-amylase inhibitors have been isolated from sorghum that are inhibitory to human and other mammalian α-amylases [48,49]. Recent work, however, involving biomarker analysis of 14 diverse sorghum varieties indicated that the level of α-amylase/trypsin inhibitors is of the order of 60 times lower in sorghum grains than in commercial wheat [50].

3.3.1 Processing effects

The sorghum α-amylase inhibitors are very heat labile. Dry heating to 100°C completely eliminated α-amylase inhibition [51]. Thus, the α-amylase inhibitors in sorghum are unlikely to be a significant factor affecting its starch digestibility.

3.4 Endosperm cellular structure

The type of endosperm in sorghum strongly influences the rate of *in vitro* digestion of its starch even when cooked. Starch in the hard (corneous) endosperm is digested much less completely and more slowly than in the soft (floury) endosperm [52]. In contrast, with maize the rate of starch digestion was somewhat higher with hard endosperm than with the soft endosperm. A recent important finding was that intact endosperm cells isolated from white sorghum and red wheat greatly restricted *in vitro* starch digestion both in the raw and cooked state [53]. Furthermore, the values for raw intact cells were significantly lower compared to those for broken cells, and the values for cooked intact cells were very substantially lower than those for cooked isolated starch. The authors proposed that intact cereal endosperm walls are an effective barrier to amylase access. Additionally, it was proposed that the presence of an extensive endosperm protein matrix within cells, particularly in sorghum and non-catalytic binding of amylases to cell wall surfaces can limit enzymic starch hydrolysis within intact cells. The presence of incompletely gelatinised starch inside the cooked sorghum and wheat intact cells suggested that the swelling of the granules was limited by the intact cell structures. Granule swelling is requisite for rapid starch digestion [54].

The endosperm cell walls of sorghum are predominantly complex glucuronoarabinoxylans plus some cellulose and are probably strongly crosslinked by ferulic acid [55,56,57], like those of maize [55,57]. Hence, they are somewhat different from those of wheat, which comprise mainly arabinoxylans, mixed linkage 1,3;1,4-β-D-glucan and cellulose and are only ferulic acid crosslinked to a very limited extent [58]. Thus, it seems unlikely that the sorghum endosperm cell walls constitute a unique barrier to either amylase ingress or starch granule swelling.

3.4.1 Processing effects

There are very few human studies that take into account confounding intrinsic grain factors and provide comparative data between the same food product made from different cereals. One of the best is a comparison involving consumption of muffins made from white (non-tannin) sorghum and wheat wholegrain flours on glucose and insulin responses in healthy men [22]. The sorghum flour had substantially higher RS than the wheat flour. The sorghum muffins had substantially more SDS than the wheat muffins and contained some RS (3.6%), whereas there was only 0.5% RS in the wheat muffins. The mean incremental area under the curve (iAUC) for the plasma glucose response after consumption of the sorghum muffins was 25% less than after consuming the wheat muffins and the insulin response was 55% less.

Another well-designed investigation involved two related studies involving the consumption of flaked and breakfast cereal biscuits made from wholegrain sorghum and wheat [13,59]. The flaked biscuits were made from three different types of sorghum, white, red (both non-tannin) and brown (tannin-containing) and white wheat. The biscuits from all four cereal types did not differ between each other in RDS and SDS and only biscuits from the brown sorghum had a significantly higher RS content. The subjects in the first study were healthy men and women [13]. There was no significant difference in iUAC for plasma glucose between the cereal types but the insulin response was significantly higher for the biscuit made from red sorghum compared to the wheat sorghum biscuits. The second study was a longer term (3 months) dietary cross-over study involving overweight or slightly obese but otherwise healthy subjects comparing just the red sorghum biscuits with the wheat biscuits [59]. Consumption of both the sorghum and wheat flaked wholegrain biscuits resulted in improvements in several indices, including weight loss, lower body mass index, plasma glucose and total cholesterol. However, in contrast to [22] there were no significant differences in responses between the sorghum and wheat products. The contrasting findings point to the different processing technologies and their effects on the sorghum grain endosperm structure being responsible.

Making the flaked biscuits involved a roller flaking process after steam cooking [37]. It is significant that in numerous cattle feeding trials where non-tannin sorghum was processed using steam flaking, its starch digestibility was greatly increased compared to that of dry milled raw sorghum and to essentially the same level as steam flaked maize [60]. Similarly, several feeding trials with pigs have shown that if sorghum is more finely milled than maize, a similar feed-conversion ratio to that obtained with maize can achieved [60].

3.5 Endosperm protein matrix effects

Zhang and Hamaker [23] provided convincing evidence that the sorghum endosperm protein inhibits starch digestion in wet-cooked foods. They found that the starch digestibility of several non-tannin sorghum genotypes was lower than a maize control when in the form of both cooked milled wholegrain and cooked refined flour, whereas the digestibilities of their isolated cooked starches were not different. Pre-treatment of the flours with the pepsin protease enzyme or sodium metabisulphite, a protein disulphide-breaking reducing agent, significantly increased the starch digestibility of the sorghum flours but had little or no effect on the maize flour. In fact, several studies have showed that when non-tannin sorghum flour is wet cooked there is a reduction in *in vitro* protein digestibility (Table 2). The reductions in protein digestibility ranged between 20% and 55%, whereas a more limited number of studies with maize showed either no reduction or a very small reduction [61]. This reduction in sorghum protein digestibility has been attributed primarily to disulphide crosslinking of the kafirin prolamin storage proteins [61]. It was suggested that this occurred between the cysteine-rich γ- and β-kafirin that are concentrated near the periphery of the kafirin protein bodies [62], or between the γ- and β-kafirins and α-kafirin, which could impede digestion of α-kafirin, the major prolamin as it is more centrally located in the protein bodies. This led to the hypothesis that the low starch digestibility of wet cooked non-tannin sorghum was as result of disulphide bond crosslinking of the kafirins occurring during cooking [7].

Work with transgenic sorghum lines provides strong support for the hypothesis that disulphide bonded crosslinking of kafirins involving the γ-kafirin does reduce protein enzymatic digestibility in wet cooked sorghum [63]. It was shown that isogenic white sorghum lines with suppressed synthesis of γ-kafirin 1 and 2 had substantially higher wet-cooked protein digestibility than their respective null controls that had the normal complement of kafirin classes. This was despite both the transgenic lines and null-controls having the same total content of kafirins. As to why the sorghum kafirin protein forms a barrier to starch digestion and the maize zein protein does not, Emmambux and Taylor [64] extracted these prolamins from raw, boiled and pressure-cooked sorghum. The work revealed that, as expected, the digestibility of kafirin from raw sorghum was considerably lower than that of zein from raw maize and that the kafirins extracted from the cooked sorghum had substantially lower digestibility than those from the raw sorghum, whereas there was only a marginal reduction in digestibility between zein from boiled and pressure cooked maize and raw maize. SDS-PAGE of isolated prolamins, which were then cooked, showed that when pressured cooked, kafirins were highly disulphide bond-polymerized with an M_r >100 kDa, whereas pressure cooking had little effect on zein.

There is also indirect evidence that the disulphide bonding of kafirins is the cause of low starch digestibility in wet cooked non-tannin sorghum foods. Some of this comes from fluorescence microscopy studies of the prolamin protein body-rich honeycomb-like matrix that surrounds the starch granules in the hard endosperm of sorghum and maize. Reconstructed 3-D images by Hamaker and Bugusu [65] indicated that the sorghum matrix protein behaves differently from maize, forming extensive extended web-like or sheet-like structures during cooking. Similar work by Ezeogu et al. [66], in slight contrast, suggested that when sorghum was wet-cooked the protein matrix collapsed and matted to a greater extent than with maize. Significantly, the work showed that the honeycomb matrix in sorghum cooked in the presence of mercaptoethanol expanded greatly, whereas the matrix in maize fragmented. This great expansion of the sorghum protein matrix paralleled a very large increase in free sulphydryl groups, due to disulphide bonding breakage. This finding is consistent with observed improvements in sorghum starch digestibility with addition of reducing agents, both *in vitro* cooked starch digestibility [23,67] and *in vivo* utilization of raw sorghum by broiler chickens [68]. This latter work, which involved study of several different non-tannin sorghum genotypes and over two experiments, showed that inclusion of sodium metabisulphite in the diet increased sorghum apparent metabolizable energy both on a grain dry matter basis and on a nitrogen corrected basis.

The fact that the two major α-kafirin polypeptides both have an additional cysteine residue compared to their zein homologues [69] may account for why kafirin polymerises by disulphide bonding to a much greater extent than zein. However, it is not clear how disulphide bond polymerization of the kafirins could reduce starch digestibility, especially as the protein bodies are surrounded by a matrix of other proteins [69]. In order to either restrict starch granule expansion or the access of amylases, some of the kafirins must actually be present in this matrix and/or disulphide bond crosslinking may also involve other types of proteins. Work by Ioeger et al. [70] provides support for both scenarios. They found that the protein of the sorghum floury-type (soft) endosperm, where the protein bodies are smaller or absent, had a higher content of γ-kafirin than the protein of the corneous endosperm. Moreover, the floury endosperm protein had a much higher content of both free sulphydryl groups and disulphide bonds than that of corneous endosperm but the corneous (hard) endosperm protein had a high proportion of disulphide bonds.

3.5.1 Processing effects

Sorghum processing involving wet cooking, i.e. under low shear, results in the foods having lower protein and starch digestibility than the same products produced from other cereals (Table 2). For example in porridge making, the protein matrix that surrounds starch granules in the corneous endosperm remains intact [65,66,71,72,73]. See Figure 1A. However, various workers have reported changes in the appearance of the matrix with wet cooking, such as the matrix forming convoluted sheets [71], the protein bodies becoming flattened [72], forming extended web- or sheet-like structures [65] and the matrix collapsing and matting [66]. Taken together, the findings indicate that with wet cooking the corneous endosperm matrix restricts the expansion of the starch granules as they take up water during gelatinization. It seems likely that the limited swelling of the starch granules is responsible for the lower starch digestibility of wet cooked sorghum through limiting amylase enzyme accessibility. The changes in the protein matrix structure during cooking are indicative of stretching of the matrix as a result of swelling of the starch granules. As indicated, that the protein matrix remains intact, seems to be due to disulphide crosslinking involving γ-kafirin, both within the protein bodies themselves and in the protein material between the protein bodies, as discussed above.

With cooking processes involving high shear, the endosperm protein matrix is disrupted, enabling full expansion of the starch granules, extensive starch solubilization and even total destruction of the starch granules. As a result, the starch in sorghum cooked under processes involving high shear seems to be as available to digestion as in other cereals cooked by such processes (Table 2). With hot air popping, rupturing of the heated kernel causes the superheated water within it to instantly vaporize [74]. The steam pressure literally explodes the gelatinized starch granules in the corneous endosperm into a starch foam, the sheets of which may be as thin as 1 μm (Fig. 1B). The explosive forces were shown to fragment both cell walls and the protein matrix, which became entrapped with the starch foam sheets [74]. With steam flaking, the effects on the starch granules are not as dramatic as with popping (Fig. 1C) but still considerably increased starch amylase susceptibility (Table 2). With a steam temperature of 99°C and a gap between the corrugated flaking rollers of 45 μm, steam flaking of sorghum grain was shown to rupture the starch granules with release of starch to form a continuous phase, especially with preconditioning (tempering) of the grain, in addition to causing swelling and distortion of the gelatinized granules (Fig. 1C) [75]. With extrusion cooking, the cereal flour is subjected to multiple effects [76]. There is wet heating under pressure and mechanical shear stress in the extruder barrel and steam-driven product expansion at the die. Jafari et al. [77] showed that with extrusion cooking of sorghum flour, feed moisture and extruder barrel temperature affected both the protein bodies and starch granules in the products. At high temperature (160°C) and low moisture (10%) (high shear conditions), most protein bodies disappeared and there was a general increase in random coil protein configuration with extrusion cooking. Similarly, under the more extreme extrusion conditions most starch granules were completely melted, forming fibres of starch.

4. Sorghum resistant starch and health

As stated, there are hardly any human studies on the effect of sorghum RS on health that provide good evidence of unique sorghum-related effects. The study by Poquette et al. [22] on whole grain sorghum muffins probably provides the clearest evidence that foods made from non-tannin sorghum which are processed under low shear-type conditions can have positive health benefits. The implications are that the muffins made from sorghum gave lower glycaemic and insulin responses than wheat muffins because of their higher content of SDS and RS, due to the sorghum endosperm structure not being severely disrupted during the flour milling, batter making and baking processes.

A study by Anunciação et al. [78] investigated the effects of consumption of beverages made with extruded sorghum of different types on glycaemic response in healthy individuals. It was found that the beverage made with tannin sorghum, which was also high in 3-deoxyanthocyanidin-type flavonoids, elicited the lowest glycaemic response after subsequent consumption of a glucose solution. This beverage also had the highest content of RS, 5.1% of total starch. However, consumption of beverages made from non-tannin sorghum high in 3-deoxyanthocyanidins or non-tannin sorghum low in 3-deoxyanthocyanidins elicited a similar but lower glycaemic response. Both had lower contents of RS, 0.4% and 1.7% of total starch, respectively. The implication is that the tannins even in sorghum that has been subjected to high shear processing reduce glycaemic response. However, it is not clear whether this is due to its higher content of RS due to tannins binding the starch and/or tannins binding with the endosperm proteins, both of which would render the starch resistant, or by the tannins inhibiting the digestive enzymes or a combination of all factors.

Most of the research on health benefits of sorghum RS are *in vivo* animal studies, and even with animal studies few effects observed can be attributed specifically to sorghum. Ge et al. [79] reported that RS from sorghum could indirectly convert the isoflavone daidzein to its metabolite equol, which has strong estrogenic activity and could relieve post-menopausal symptoms in rats such as atrophy of the ovaries. The mechanism responsible was that the fermentation of the RS by intestinal flora, specifically Lactobacillus and Clostridium XIVa, was instrumental in metabolic breakdown of daidzen to equol. In a follow up study, Ge at al. [80] reported that sorghum RS could regulate lipid metabolism in menopausal rats via the estrogenic activity of equol. This appeared to occur through a similar mechanism of stimulation of fermentation by high levels of sorghum RS, which leads to rapid conversion of daidzein to equol. Rats fed with soybean feed together with sorghum RS had increased levels of high-density lipoprotein cholesterol and reduced levels of glycerol, triglycerides, total cholesterol and low-density lipoprotein cholesterol. Thus, the sorghum RS could control body weight and quality of adipose tissue.

Pelpolage et al. [81] studied the effect of diets made with cooked and frozen white sorghum grains (refined and whole) on colonic fermentation in rats. The cooking and freezing was done to increase RS content. The rats that were fed sorghum showed lower levels of visceral fat, non-HDL cholesterol, total cholesterol and higher total faecal bile secretion and short chain fatty acids compared to rats fed with regular maize starch. The authors suggested that the relatively higher levels of RS in the sorghum diets stimulates microbial proliferation and enhances the breakdown of RS and production of short chain fatty acids. This then contributes to the observed beneficial effects such as low levels of visceral fat and non-HDL cholesterol.

Martinez et al. [82] studied the effect of feeding rats a high fat, high fructose diet incorporating flour from a high sorghum tannin hybrid with a high RS content. The sorghum had been dry heat treated, presumably to increase its RS content. They found that the rats had decreased triglycerides, uric acid, alanine aminotransferase, liver steatosis (increased buildup of fat in the liver) and lipogenesis, when compared to the high fat, high fructose diet. These rats also had improved insulin sensitivity, glucose tolerance and increased concentration of PPARα protein in the liver. However, similar to the work of Anunciação et al. [78], it is not clear whether the effects were due to the high content of RS or the tannins themselves having physiological effects or both factors.

5. Conclusions

A blanket assertion that sorghum foods exert positive health effects such as lower glycaemic and insulin responses than the same foods prepared from other cereals because they have a higher resistant starch content is not supported. One needs to distinguish between the type of sorghum from which the food was produced, e.g. non-tannin or tannin sorghum, and the processing technology used to produce the food.

Concerning non-tannin type sorghum, the evidence suggests that if the endosperm structure is not severely disrupted during food processing, then the food can exert positive health effects on glycaemic response. The sorghum endosperm structure appears to render the starch less digestible, specifically through cross-linking of the kafirin matrix proteins. This is probably caused by thermally-induced disulphide bonded crosslinking reducing starch granule disruption during gelatinization and also possibly by limiting the accessibility of digestive enzymes. This disulphide bonded endosperm protein cross-linking seems to be unique to sorghum. However, if non-tannin sorghum is processed using high-shear technologies such as extrusion cooking, flaking and popping, it appears that the endosperm and starch granule structure is disrupted to such an extent that the starch is fully digestible.

Regarding tannin-type sorghums, the situation is very complex. There is clear evidence that tannins have a strong inhibitory effect on starch digestion. Specifically, they bind with the starch and render it resistant to digestion, and probably as a consequence reduce the glycaemic response of tannin sorghum foods. However, it is likely that this is not the only mechanism involved as sorghum tannins also strongly bind the sorghum kafirin endosperm proteins, which would also contribute to the starch being resistant, and additionally the tannins inhibit amylase digestive enzymes, which will reduce the glycaemic response and they probably also have other physiological effects. Furthermore, the effects of processing tannin sorghum on resistant starch content may be related to whether the processing technology is predominantly dry or wet, with the former better at conserving the resistant starch present in the raw grain.

In order to advance our knowledge concerning resistant starch and slowly digestible starch in sorghum foods and their related positive health effects, research will have to focus on elucidating the relative importance of the different biochemical mechanisms that are potentially involved and the myriad of interactions between intrinsic sorghum-related factors and extrinsic food processing factors.

Conflict of interest

The authors declare no conflicts of interest.

6. References

[1] S. Lockyer, A.P. Nugent, *Nutr. Bull*. **2017**, *42*, 10.

[2] J.M. Jones, *Nutr. J*. **2014**, *13*, 1.

[3] U. Lehmann, F. Robin, *Trends Food Sci. Technol*. **2007**, *18*, 346.

[4] M. Miao, B. Jiang, S.W. Cui, T. Zhang, Z. Jin, *Crit. Rev. Food Sci. Nutr.* **2015**, *55*, 1642.

[5] M. Meenu, B. Xu, *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 3019.

[6] B.A. Ashwar, A. Gani, A. Shah, I.A. Wani, F.A. Masoodi, *Starch-Stärke.* **2016**, *68*, 287.

[7] J.R.N. Taylor, M.N. Emmambux, *Cereal Chem*. **2010**, *87*, 263

[8] A. Stefoska-Needham, E.J. Beck, S.K. Johnson, L.C. Tapsell, *Food Rev. Int*. **2015**, 31, 401.

[9] L. de Morais Cardoso, S.S. Pinheiro, H.S.D. Martino, H.M. Pinheiro-Sant'Ana, *Crit. Rev. Food Sci. Nutr*. **2017,** *57*, 372.

[10] M. Benmoussa, B. Suhendra, A. Aboubacar, B.R. Hamaker, B.R., *Starch-Stärke* **2006**, 58, 92.

[11] J.R.N. Taylor, J. Kruger, in Sorghum and Millets: Chemistry, Technology and Nutritional Attributes, 2nd ed. (Eds: J.R.N Taylor, K.G. Duodu), Woodhead Publishing, Duxford, UK, **2019**, Ch. 7.

[12] M.P.R. Prasad, B.D. Rao, K. Kalpana, M.V. Rao, J.V. Patil, *J. Sci. Food Agric*. **2015,** 95, 1626.

[13] A. Stefoska-Needham, E.J. Beck, S.K. Johnson, J. Chu, L.C. Tapsell, *Mol. Nutr. Food Res*. **2016**, *60*, 1118.

[14] H.N. Englyst, H.S. Wiggins, J.H. Cummings, *Analyst*, **1982**, *107*, 307.

[15] H.N. Englyst, S.M. Kingman, J.H. Cummings, *Eur. J. Clin. Nutr*. **1992**, *46(Supp 2)*, S33.

[16] D.F. Birt, T. Boylston, S. Hendrich, J-L. Jane, J. Hollis, J., L. Li, J. McClelland, S. Moore, G.J. Phillips, M. Rowling, K. Schalinske, M.P. Scott, E.M. Whitley, *Adv. Nutr*. **2013**, *4*, 587.

[17] H.N. Englyst, J. Veenstra, G.J. Hudson, *Brit. J. Nutr*. **1996**, *75*, 327.

[18] I. Goni, L. Garcia-Diz, E. Manas, F. Saura-Calixto, *Food Chem*. **1996**, *56*, 445.

[19] N. de Carvalho Teixeira, V.A.V. Queiroz, M.C. Rocha, A.C.P. Amorim, T.O. Soares, M.A.M. Monteiro, C.B. de Menezes, R.E. Schaffert, M.A.V.T Garcia, R.G. Junqueira, *Food Chem*. **2016**, *197*, 291.

[20] S. Ragaee, E.M. Abdel-Aal, M. Noaman, *Food Chem*. **2006**, *98*, 32.

[21] G. Giuberti, A. Gallo, C. Cerioli, C. F. Masoero, *Anim. Feed Sci. Technol*. **2012**, *174*, 163.

[22] M.N. Poquette, X. Gu, S.O. Lee, *Food Funct*. **2014**, **5**, 894.

[23] G. Zhang, B.R. Hamaker, *Cereal Chem.* **1998**, *75*, 710.

[24] J.M. Awika, L.W. Rooney, *Phytochemistry* **2004**, *6*5, 1199.

[25] L. Dykes, L.W. Rooney, *J. Cereal. Sci*. **2006**, *44*, 236.

[26] J.M. Awika, in Gluten-free Ancient Grains - Cereals, Pseudocereals, and Legumes: Sustainable, Nutritious, and Health-Promoting Foods for the 21st Century (Eds: J.R.N. Taylor, J.M. Awika), Woodhead Publishing, Duxford, UK, Ch. 3.

[27] K.G. Duodu, J.M. Awika, in Sorghum and Millets: Chemistry, Technology, and Nutritional Attributes, 2nd ed. (Eds; J.R.N. Taylor, K.G. Duodu), Woodhead Publishing, Duxford, UK, **2019**, Ch. 8.

[28] D. Amoako, J.M. Awika, *Curr. Opin. Food Sci*. **2016**, *8*, 14.

[29] D. Lemlioglu-Austin, N.D. Turner, N.D., C.M. McDonough, L.W. Rooney, *Molecules*, **2012**, *17*, 11124.

[30] F. Barros, J.M. Awika, L.W. Rooney, *J. Sci. Food Agric*. **2014**, *94*, 1212.

[31] F. Barros, J.M. Awika, L.W. Rooney, *J. Agric. Food Chem*. **2012**, *60*, 11609.

[32] N.M. Emmambux, J.R.N. Taylor, *J. Sci. Food Agric.* **2003**, *83*, 402.

[33] N.L. Mkandawire, R.C. Kaufman, S.R. Bean, C.L. Weller, D.S. Jackson, D.J. Rose, *J. Agric. Food Chem*. **2013**, *61*, 4448.

[34] M.R. Links, J. Taylor, M.C. Kruger, J.R.N. Taylor, *J. Funct. Foods* **2015**, *12*, 55.

[35] M.R. Links, J. Taylor, M.C. Kruger, V. Naidoo, J.R.N. Taylor, *J. Funct. Foods* **2016**, *20*, 394.

[36] N.L. Mkandawire, S.A. Weier, C.L. Weller, D.S. Jackson, D.J. Rose, *LWT-Food Sci. Technol*. **2015**, *62*, 662.

[37] J.A. Delcour, R.C. Hoseney, Principles of Cereal Science and Technology, 3rd ed, AACC International Press, St. Paul, MN.

[38] S.R. Bean, L. Zhu, B.M. Smith, J.D. Wilson, J.D., B.P. Ioerger, M. Tilley, in Sorghum and Millets: Chemistry, Technology and Nutritional Attributes, 2nd ed. (Eds: J.R.N Taylor, K.G. Duodu), Woodhead Publishing, Duxford, UK, **2019**, Ch. 6.

[39] V. Vamadevan, E. Bertoft, K. Seetharaman, *Carbohydr. Polym*. **2013**, *92*, 1653.

[40] H. Hill, L.S. Lee, R.J. Henry, *Food Chem.* **2012**, *13*, 175.

[41] Y. Sang, S. Bean, P.A. Seib, J. Pedersen, Y.C. Shi, *J. Agric. Food Chem*. **2008**, 56, 6680.

[42] G. Zhang, Z. Ao, B.R. Hamaker, J. Agric. Food Chem. **2008**, *56*, 4686.

[43] R.C. Kaufman, J.D. Wilson, S.R. Bean, F. Xu, Y.C. Shi, *J. Cereal. Sci*. **2017**, 74, 127.

[44] E. Li, J. Hasjim, J., V. Singh, M. Tizzotti, I.D. Godwin, R.G. Gilbert, *Cereal Chem*. **2013**, *90*, 223.

[45] E. da Rosa Zavareze, E., A.R.G. Dias, *Carbohydr. Polym*. **2011**, *83,* 317.

[46] Q. Sun, Z. Han, L. Wang, L. Xiong, *Food Chem***. 2014**, *145*, 756.

[47] T.H. Vu, S. Bean, C.F. Hsieh, Y.C. Shi, *J. Sci. Food Agric*. **2017**, *97*, 4770.

[48] A.V.M. Kutty, T.N. Pattabiraman, *J. Agric. Food Chem*. **1986**, 34, 552.

[49] C. Bloch Jr, M. Richardson, *Protein Seq. Data Anal.* **1992**, 5, 27.

[50] S.T. Sagu, E. Landgräber, M. Rackiewicz, G. Huschek, H. Rawel, *Molecules* **2020**, *25*, 5982.

[51] V.H. Mulimani, D. Supriya, *Plant Foods Hum. Nutr.* **1993**, 44, 181.

[52] L.I. Ezeogu, K.G. Duodu, J.R.N. Taylor, *J. Cereal Sci*. **2005**, *42,* 33.

[53] R.R. Bhattarai, S. Dhital, A. Mense, M.J. Gidley, Y.C. Shi, *Food Hydrocoll*. **2018**, *81*, 139.

[54] A.K. Shrestha, J. Blazek, B.M. Flanagan, S. Dhital, O. Larroque, M.K. Morell, E.P. Gilbert, M.J. Gidley, *Carbohy. Polym*. **2012**, *90*, 23.

[55] M.M.H. Huisman, H.A. Schols, A.G.J. Voragen, *Carbohy. Polym.* **2000**, *43*, 269.

[56] A.P.T. Miafo, B.B. Koubala, G. Kansci, G. Muralikrishna, *J. Cereal Sci*. **2019**, *87*, 124.

[57] C. Chiremba, J.R.N. Taylor, L.W. Rooney, T. Beta, *Food Chem*. **2012**, *134*, 81.

[58] G. Gartaula, S. Dhital, G. Netzel, B.M. Flanagan, G.E. Yakubov, C.T. Beahan, H.M. Collins, R.A. Burton, A. Bacic, A., M.J. Gidley, *Carbohy. Polym*. **2018**, *196*, 199.

[59] A. Stefoska-Needham, E.J. Beck, S.K. Johnson, M.J. Batterham, R. Grant, J. Ashton, L.C. Tapsell, *J. Amer. Coll. Nutr*. **2017**, *36*, 184.

[60] K.C. McCuistion, P.H. Selle, S.Y. Liu, R.D. Goodband, in Sorghum and Millets: Chemistry, Technology and Nutritional Attributes, 2nd ed. (Eds: J.R.N Taylor, K.G. Duodu), Woodhead Publishing, Duxford, UK, **2019**, Ch. 12.

[61] Duodu, K.G., Taylor, J.R.N., Belton, P.S. and Hamaker, B.R., 2003. Factors affecting sorghum protein digestibility. Journal of cereal science, 38(2), pp.117-131.
K.G. Duodu, K.G., J.R.N. Taylor, P.S. Belton, B.R. Hamaker, *J. Cereal Sci*. **2003**, *38*, 117.

[62] M.P. Oria, B.R. Hamaker, J.D. Axtell, C.P. Huang, *Proc. Natl. Acad. Sci. USA,* **2000**, *97*, 5065.

[63] L.S. Da Silva, J. Taylor, J.R.N. Taylor, *J. Agric. Food Chem.* **2011**, *59*, 9265.

[64] M.N. Emmambux, J.R.N. Taylor, *J. Agric. Food Chem.* **2009**, *57*, 1045.

[65] B.R. Hamaker, B.A. Bugusu, in AFRIPRO Workshop on the Proteins of Sorghum and Millets. (Eds: P.S. Belton J.R.N. Taylor, Pretoria, South Africa, **2003**. http://www.afripro.org.uk/

[66] L.I. Ezeogu, K.G. Duodu, M.N. Emmambux, J.R.N. Taylor, *Cereal Chem*. **2008**, *85*, 397.

[67] A.E.O. Elkhalifa, A. Chandrashekar, A., B.E. Mohamed, A.H. El Tinay, *Food Chem.* **1999**, *66*, 323.

[68] H.H. Truong, K.A. Neilson, B.V. McInerney, A. Khoddami, A., T.H. Roberts, S.Y. Liu, P.H. Selle, *Anim. Feed Sci. Technol*. **2016**, *219*, 159.

[69] P.S. Belton, I. Delgadillo, N.G. Halford, P.R. Shewry, *J. Cereal Sci.* **2006**, *44*, 272.

[70] B. Ioerger, S.R. Bean, M.R. Tuinstra, J.F. Pedersen, J. Erpelding, K.M. Lee, T.J. Herrman, *J. Agric. Food Chem*. **2007**, *55*, 10232.

[71] A. Chandrashekar, A.W. Kirleis, *Cereal Chem*. **1988.** *65*, 457.

[72] D.W. Rom, J.M. Shull, A. Chandrashekar, A.W. Kirleis, *Cereal Chem*. **1992**, *69*, 178.

*[73] C.C. Ng'andwe, A.N. Hall, J.R.N. Taylor, *J. Inst. Brew*. **2008**, *114*, 343.

*[74] M.L. Parker, A. Grant, A., N.M. Rigby, P.S. Belton, J.R.N. Taylor, *J. Cereal Sci*. **1999**, *30*, 209.

*[75] C. McDonough, B.J. Anderson, H. Acosta-Zuleta, L.W. Rooney, *Cereal Chem*. **1998**. *75*, 58.

[76] A. Arhaliass, J. Legrand, P, Vauchel, F. Fodil-Pacha, T. Lamer, J.M. Bouvier, *Food Bioprocess Technol.* **2009**, *2*, 186.

[77] M. Jafari, A. Koocheki, E. Milani, *J. Cereal Sci*. **2017**, *75*, 324.

[78] P.C. Anunciação, L. de Mordais Cardoso, V.A.V. Queiroz, C.B. de Menezes, C.W.P. de Carvalho, H.M. Pinheiro-Sant'Ana, R.D.C.G. Alfenas, *Eur. J. Nutr.* **2018**, *57*, 251.

[79] Y.F. Ge, C.H. Wei, W.H. Wang, L.K. Cao, *Food Sci. Nutr*. **2020**, *8,* 4055.

[80] Y.F. Ge, C.H. Wei, W.H. Wang, L.K. Cao, *J. Food Biochem*. **2020**, *44*, e13295.

[81] S.W. Pelpolage, A. Yoshida, R. Nagata, K. Shimada, N. Fukuma, H. Bochimoto, T. Hamamoto, M. Hoshizawa, K. Nakano, K.H. Han, M. Fukushima, *Nutrients* **2020**, *12,* 2412.

[82] O.D.M. Martinez, J.M.V. Theodoro, M. Grancieri, R.C.L. Toledo, V.A.V. Queiroz, F.A. Ribeiro de Barros, H.S.D Martino, *J. Cereal Sci*. **2021**, *99,* 103201.

[83] R. Souilah, D. Djabali, B. Belhadi, H, Mokrane, N. Boudries, B. Nadjemi, *Food Sci. Nutr*. **2014**, *2,* 25.

[84] E.V. Moraes, R. da Silva Marineli, S.A. Lenquiste, C.J. Steel, C.B. de Menezes, V.A.V. Queiroz, M.R.M. Júnior, *Food Chem*. **2015**, *180*, 116.

[85] R. Li, L. Dai, H. Peng, J. Jiang, N. Liu, D. Zhang, C. Wang, Z. Li, *J. Food Process Eng*. **2021**, e13804.

[86] I. Goni, A. Garcia-Alonso, F. Saura-Calixto, *Nutr. Res*. **1997**, *17*, 427.

[87] K.G. Duodu, A. Nunes, I. Delgadillo, M.L. Parker, E.N.C. Mills, P.S. Belton, J.R.N. Taylor, *J. Cereal Sci.* **2002**, *35*, 161.

[88] I. Correia, A. Nunes, A.S. Barros, A.I. Delgadillo, *J. Cereal Sci*. **2010**, *51*, 146.

[89] M. Sharma, D. Mridula, D.N. Yadav, R.K. Gupta, *Proc. Natl. Acad. Sci. India Sect. B: Biol. Sci.* **2015**, *85*, 787.

[90] S.N. Saravanabavan, M.M. Shivanna, S. Bhattacharya, *J. Food Sci. Technol*. **2013**, *50*, 387.

[91] G.H. Al-Rabadi, P.J. Torley, B.A. Williams, W.L. Bryden, M.J. Gidley, *Anim. Feed Sci. Technol.* **2011**, *168*, 267.

[92] D. Mohapatra, A.S. Patel, A. Kar, S.S. Deshpande, M.K. Tripathi, *Food Chem*. **2019**, *271*, 129.

Figure 1: Effects of food processing on sorghum endosperm structure.
A) Wet cooking – confocal laser scanning micrograph (adapted from [73]), PBM = protein body matrix, CW = cell wall; B) Popping –scanning electron micrograph (adapted from [74]), ab = air bubble, CWF – cell wall fragment, SF = starch foam; C) Steam flaking – scanning electron micrograph (adapted from [75]), GSG – gelatinised starch granule, PS = protein strand